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TOPIC:

THE BUGS THAT REFUSE
TO DIE AND THE SUPER
TAILORS OF LIFE: THEIR IMPACT
ON HEALTH, ECONOMIC AND
SOCIAL WELL-BEING OF MAN

By
PROFESSOR (MRS.) VERONICA IHUAKU OKOCHI



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THE BUGS THAT REFUSE TO DIE AND THE SUPER TAILORS OF LIFE: THEIR IMPACT ON HEALTH, ECONOMIC AND SOCIAL WELL-BEING OF MAN

An Inaugural Lecture Delivered at the University of Lagos
Main Auditorium on Wednesday, 11th November, 2009

By

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I thank God Who has designed and allowed His purpose for me this day to be fulfilled. I thank Him also for the life of each and everyone of us who is and will be present here today.

Mr Vice-Chancellor, sir, as a young girl in secondary school, I had no idea of what I wanted to do in future. My sole ambition was to pass my examinations very well. But the principal, in the manner of missionaries, was a good guardian who wanted the best for her students and her school. So, at the end of the 3rd year, she grouped our courses into two – Science and Arts. She allowed us to choose which side to belong to, though, we would be allowed to keep our choice only if she was convinced that the student concerned was strong enough in the group of her choice. That was how I became a Science student. After sitting for the West African School Certificate, one of my best results was in chemistry. I then decided to study chemistry at Barat College of the Sacred Heart, Lake Forest, Illinois.

At the end of my first degree, I was lucky to be picked up by Professor Norbert Tietz, a renowned pathologist. I worked in his clinical chemistry laboratory at the Mount Sinai Hospital Chicago and he encouraged me to register for Masters Degree in the Biochemistry department of the University of Health Sciences, Chicago, with emphasis in Clinical Biochemistry. I was fascinated to learn about the relationship between Biochemistry and diseases. Then, I decided to continue in the field of Biochemistry. At the end of my Masters degree I came home to Nigeria and was appointed into the then young Department of Biochemistry of the University of Lagos in 1974.

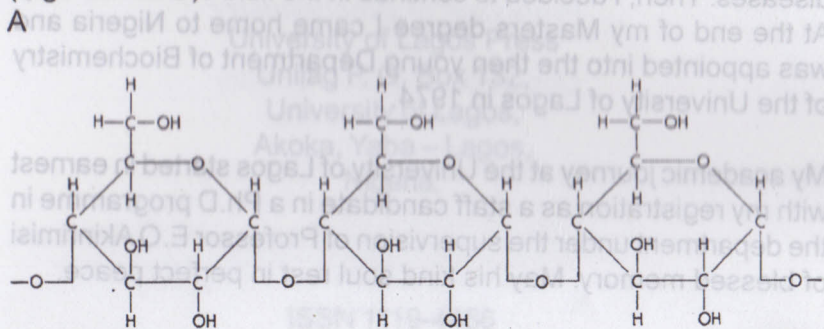
My academic journey at the University of Lagos started in earnest with my registration as a staff candidate in a Ph.D programme in the department under the supervision of Professor E.O Akinrimisi of blessed memory. May his kind soul rest in perfect peace.

But, before I go further, I wish to use this auspicious occasion to answer some common questions I encounter quite often in social interactions. They are: What is Biochemistry and what does the Biochemist do?

What is Biochemistry and What is its goal?

Biochemistry is an offshoot of two great sciences – Biology and Chemistry. Biology deals with the study of the living things – animals, plants and microscopic forms of life (considering how they live, grow and reproduce). Chemistry is the study of the chemical composition of all forms of matter, including rocks, seas and even the stars. The study of the branch of chemistry called organic chemistry gave birth to Biochemistry which deals with the chemistry of living things.

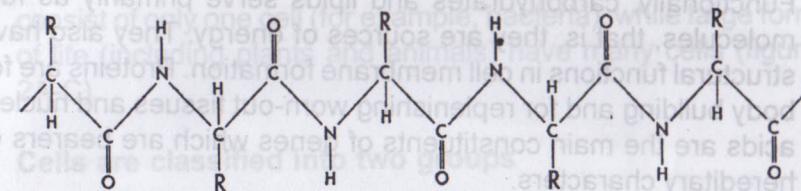
The substances that make up a living organism are water, certain salts and organic compounds. Organic compounds consist, for the most part, of four types of atoms: Carbon (C), Oxygen (O), Nitrogen (N) and Hydrogen (H). These four atoms together constitute about 99% of living matter, including water which is made up of hydrogen and oxygen. The four main organic molecules are: carbohydrates (for example, starch), lipids (commonly called fats), proteins (like albumin that make up the white part of the egg), and the nucleic acids (the genetic material). (Figures 1A-D)



CARBOHYDRATES comprise one of the four principal kinds of carbon compound found in living matter. This structural formula

represents part of a characteristic carbohydrate. It is a polysaccharide consisting of six-carbon sugar units, three of which are shown.

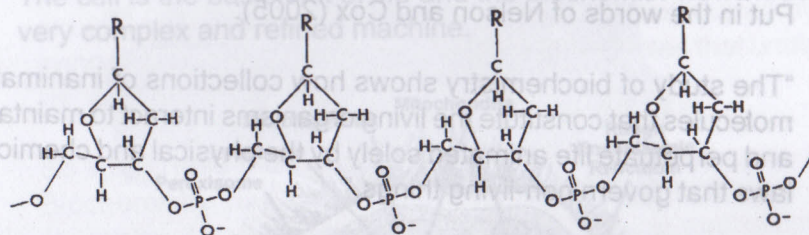
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PROTEINS are a fourth kind of carbon compound found in living matter. This formula represents part of a polypeptide chain, the

backbone of a protein molecule. The chain is made up of amino acids. Here the letter R represents the side chains of these acids.

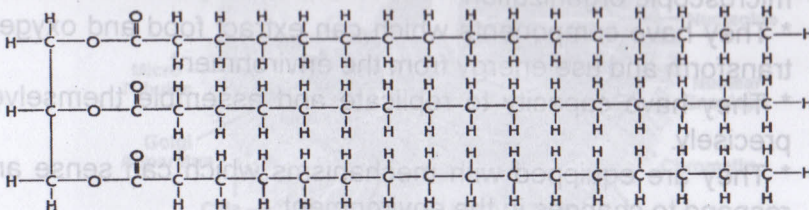
C



NUCLEIC ACIDS are a third kind of carbon compound. This is part of deoxyribonucleic acid, the backbone of which is five-

carbon sugars alternating with phosphoric acid. The letter R is any one of four nitrogenous bases, two purines and two pyrimidines.

D



FATS are a second kind of carbon compound found in living matter. This formula represents the whole molecule of palmitin, one of

the commonest fats. The molecule consists of glycerol (11 atoms at the far left) and fatty acids (hydrocarbon chains at the right).

Figure 1: Structures of organic biomolecules – carbohydrates(A), proteins(B), nucleic acids(C) and lipids (D).

Only nucleic acids have phosphates in their natural carbon skeleton out of the four major macromolecules.

Functionally, carbohydrates and lipids serve primarily as fuel molecules, that is, they are sources of energy. They also have structural functions in cell membrane formation. Proteins are for body building and for replenishing worn-out tissues and nucleic acids are the main constituents of genes which are bearers of hereditary characters.

Biochemistry is simply the study of the molecular basis of life, starting from viruses, bacteria and other microscopic forms of life, to plants and animals including man. The goal of biochemistry is to understand the chemical basis of biological phenomena. Put in the words of Nelson and Cox (2005):

"The study of biochemistry shows how collections of inanimate molecules that constitute the living organisms interact to maintain and perpetuate life animated solely by the physical and chemical laws that govern non-living things."

Living organisms, however, have extraordinary attributes and properties which distinguish them from other types of matter:

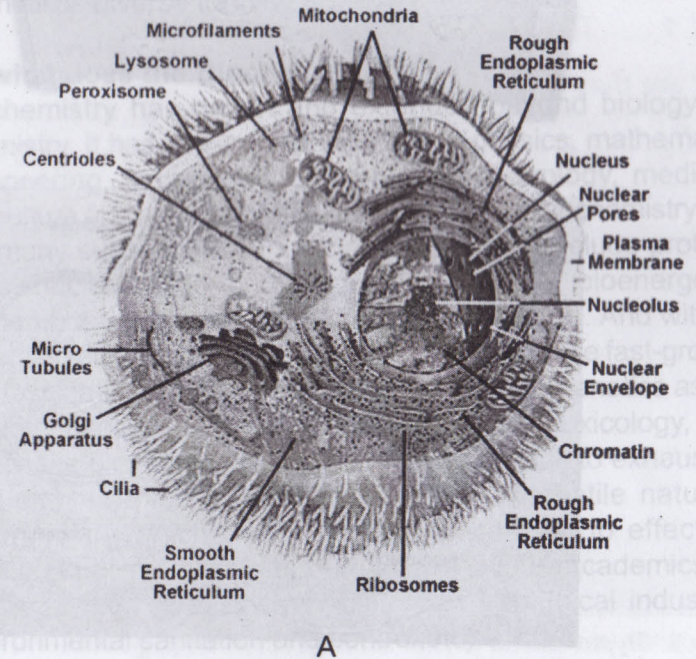
- * They have a high degree of chemical complexity and microscopic organization.
- * They have components which can extract food and oxygen, transform and use energy from the environment.
- * They have capacity to replicate and assemble themselves precisely.
- * They are equipped with mechanisms which can sense and respond to changes in the environment.
- * They have capacity to regulate the functions and interactions of their component microenvironments.

The unity and diversity of living organisms become obvious at the cellular level.

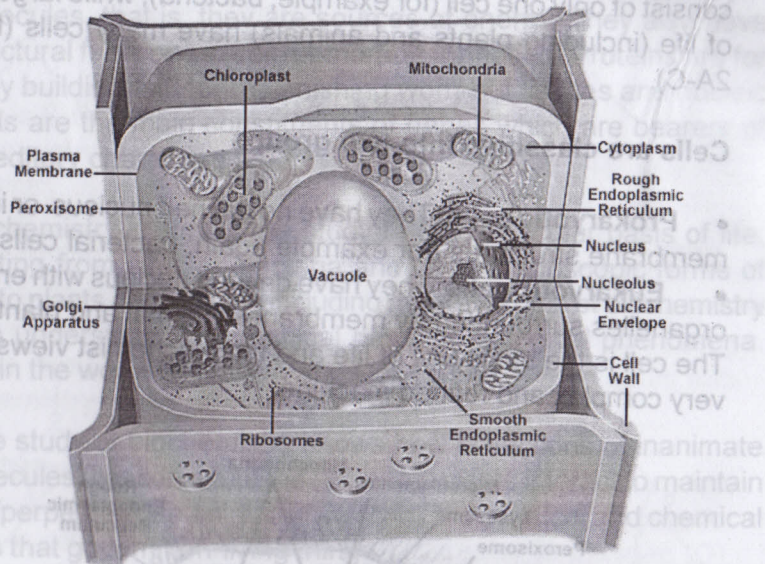
All living things are made up of cells. Some tiny forms of life consist of only one cell (for example, bacteria), while large forms of life (including plants and animals) have many cells (figures 2A-C).

Cells are classified into two groups

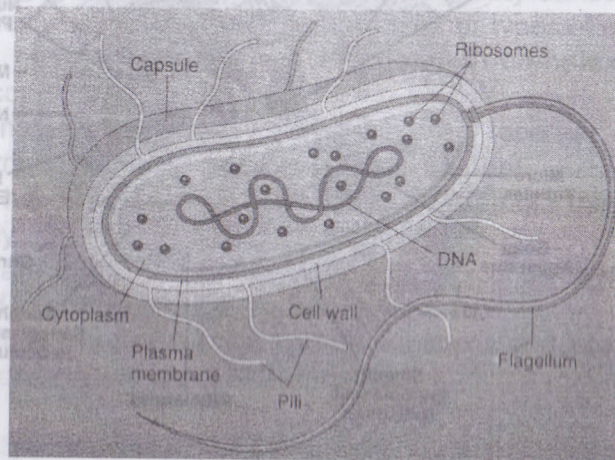
- **Prokaryotic cells:** They have no defined nucleus or internal membrane structures (for example again, bacterial cells),
- **Eukaryotic cells:** They have defined nucleus with enclosed organelles surrounded by membranes (animal and plant cells). The cell is the basic unit of life and the biochemist views it as a very complex and refined machine.



A



B



C

Figure 2: cell structures A: animal, B: plant and C: bacteria. (Information provided by: <http://micro.magnet.fsu.edu>)

In spite of the structural distinctions between prokaryotic and eukaryotic cells, as well as the significant differences in chemical

composition and biochemical activities, many biochemical processes are universal. Indeed, most of the knowledge of biochemistry of living cells of higher organisms come from the studies of prokaryotic and non-mammalian eukaryotic cells. Darwin expressed this fact about two centuries ago in the following words:

"We must, however, acknowledge, as it seems to me, that man with all his noble qualities...still bears in his bodily frame the indelible stamp of his lowly origin" – Charles Darwin, the descent of man 1871 (Nelson and Cox, 2005).

Biochemistry, therefore, explains in molecular terms the structures, mechanisms, and chemical processes shared by all living organisms and provides organizing principles that underlie life in all its diverse forms.

So what does the Biochemist do?

Biochemistry has grown and extended beyond biology and chemistry. It has currently found roots in physics, mathematics, engineering, microbiology, botany, pharmacology, medicine, agriculture and industry. Within the discipline of biochemistry itself are many subsections of specialization which include proteins, lipids, nucleic acids, carbohydrates, enzymes, bioenergetics, biomembranes, hormones, dietetics and nutrition. And with the advent of recombinant DNA technology, we have the fast-growing and fascinating areas of molecular biology and genetics as well as biotechnology. Other special topics include toxicology, plant and tissue biochemistry etc. Indeed, it is difficult to exhaust the list here but this is an illustration of the versatile nature of biochemistry which empowers the biochemist to effectively function in diverse fields of human endeavour (academics and research, medicine, pharmaceutical and chemical industries, environmental sanitation and control, etc).

Where do I come in? - Academics and Research:

My interests:

1. Parasite biochemistry and chemotherapy which led to my studies of membrane characteristics of *Trypanosome vivax* and the subsequent encounter with medicinal plants in search of potential trypanocides.
2. Enzyme chemistry and its application to drug development and biotechnology.

Therefore, my discussion this afternoon is in two parts. It is like telling the story of the good (enzymes) and the bad (trypanosomes), but the common ground linking them is that man, his health and socio-economic well-being remain the focal point.

PART 1 THE BUGS THAT REFUSE TO DIE:

What are they?

They are trypanosomes – a group of parasitic and microscopic creatures which afflict man and his domestic animals, causing the disease known as trypanosomiasis or sleeping sickness.

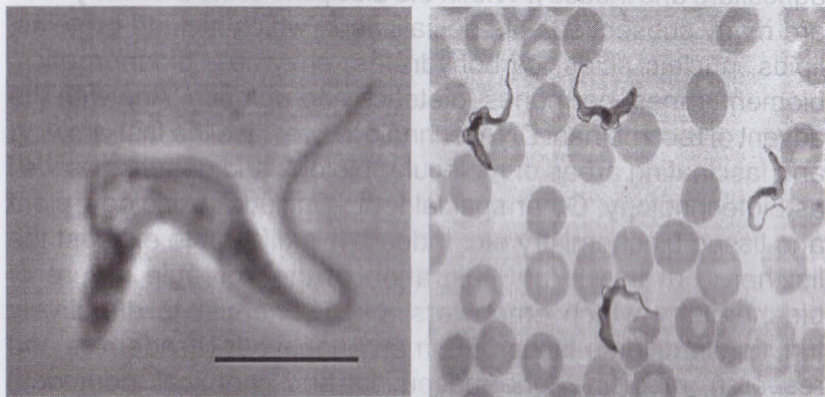


Figure 3: *Trypanosoma Brucei Brucei* TREU667 (Bloodstream form, phase contrast picture. Black bar indicates 10 μm .), and on the right side is a picture of blood sample infected by trypanosomes. (From Wikipedia, the free encyclopedia)

Trypanosomes are grouped either as starcorarian or as salivarian based on differences in their modes of infectivity, biochemistry and morphology. We are interested in the salivarian subgroup which is found predominantly in the sub-Saharan African region.

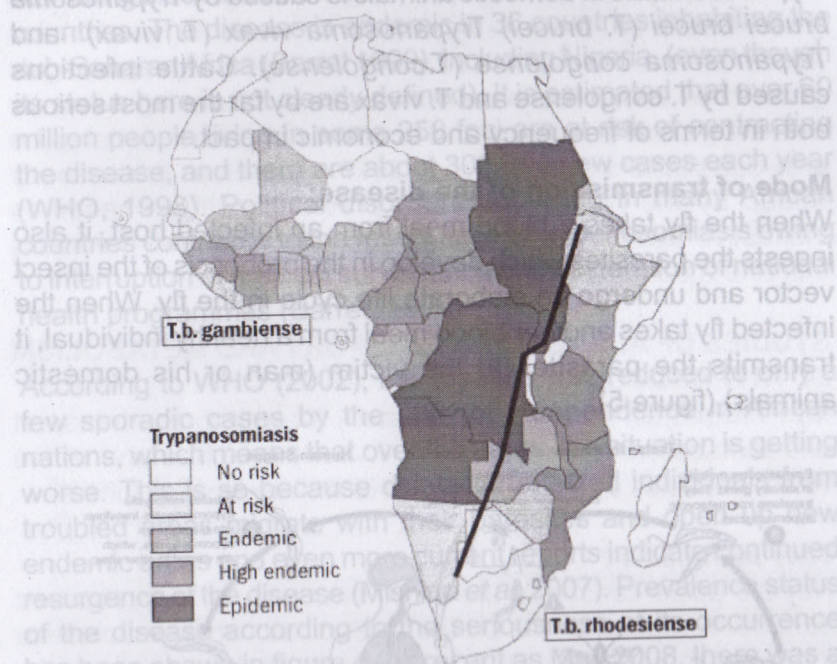


Figure 4: Distribution of gambiense and rhodesiense sleeping sickness in sub-Saharan Africa, 1999 (WHO Report on Global Surveillance of Epidemic-prone Infectious Diseases – African trypanosomiasis: 1999)

African trypanosomiasis is caused by the salivarian trypanosomes which are transmitted by the blood sucking tsetse fly called glossina.

The human type of the disease is caused by two different parasites consisting of:

- a) *Trypanosoma brucei gambiense* which causes a chronic infection lasting for years and infects human beings in countries in the Western and Central Africa.

b) *Trypanosoma brucei rhodesiense* which causes an acute illness lasting only several weeks in countries of Eastern and Southern Africa (WHO 1999) (figure 4).

Trypanosomiasis in domestic animals is caused by *Trypanosoma brucei brucei* (*T. brucei*) *Trypanosoma vivax* (*T. vivax*) and *Trypanosoma congolense* (*T. congolense*). Cattle infections caused by *T. congolense* and *T. vivax* are by far the most serious both in terms of frequency and economic impact.

Mode of transmission of the disease:

When the fly takes a blood meal from an infected host, it also ingests the parasites which develop in the proboscis of the insect vector and undergo an elaborate life cycle in the fly. When the infected fly takes another blood meal from a healthy individual, it transmits the parasites to the victim (man or his domestic animals) (figure 5).

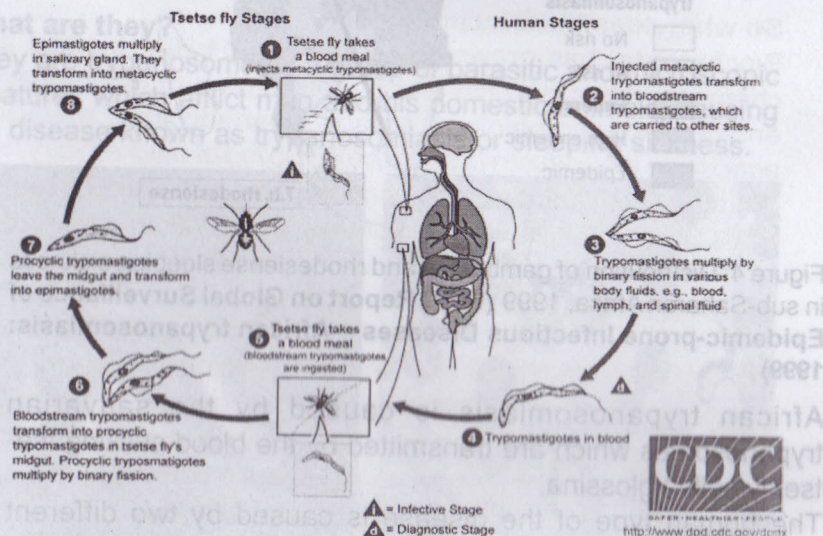


Figure 5: Life cycle of trypanosomes (from center of disease control and prevention.)

Socio-economic Implications of the Disease:

Trypanosomiasis, first described in the 14th century in the country now called Mali (WHO 1999, 2002) continues to constitute a stumbling block to socio-economic developments in many African countries. The disease is endemic in 36 countries inhabiting the sub-Saharan Africa (Barret 1999), including Nigeria, (even though its status here is not clearly defined). It is estimated that over 60 million people living in some 250 foci are at risk of contracting the disease, and there are about 300,000 new cases each year (WHO, 1998). Political disorders occurring in many African countries contribute to the resurgence of trypanosomiasis owing to interruption of medical surveillance and obliteration of national health programmes (Barret, 1999).

According to WHO (2002), this disease was reduced to only a few sporadic cases by the start of independence in African nations, which means that over the years, the situation is getting worse. This is so because displaced infected individuals from troubled areas migrate with their parasites and open up new endemic areas and even more current reports indicate continued resurgence of the disease (Mishina *et al*, 2007). Prevalence status of the disease according to the seriousness of its occurrence has been shown in figure 4. As recent as May 2008, there was a publication in Ugandan New Vision that there was an epidemic in Uganda which was "reaching alarming levels".

Besides the tremendous suffering the disease causes the human person in endemic areas, it has even greater devastating impact at the agricultural level. In the first case, there is loss of time for cultivation and harvesting of crops. Secondly it renders vast areas of grazing land in the affected regions unsuitable for raising domestic animals such as cows, cattle, sheep and goats. The disease reduces growth rate, milk productivity and the ability of the animals to thrive. This has grave economic consequences in terms of viable livelihood as well as sources of the much needed quality protein for proper nutrition and good health.

How do the parasites survive in their hosts?

They evade their hosts' immune response because they possess variable antigens on the surface of their membranes which are coded for by multiple genes. These antigens, which are glycoproteins by nature, are called variant surface glycoproteins (VSG). The parasites may switch the VSG either by replacing its gene in an active expression site with a different gene or by activating another expression site at the same time silencing the previous active site (Borst *et al* 1997). Therefore the antibody raised by the host against the previous antigen becomes ineffective against the new one. This continues until the host's immune mechanism is overwhelmed.

STUDIES OF ADENOSINE TRANSPORT AND METABOLISM IN *TRYPANOSOMA VIVAX*:

My Ph.D project was designed to study the membrane transport and metabolism of adenosine in *T.vivax*, (which is one of the virulent species of the animal form of African trypanosomes).

Why adenosine?

Nucleic acids constitute the basic structure of the gene and adenosine (figure 6) is a purine nucleoside, whose nucleotide form constitutes one of the components of the nucleic acids. Adenosine became of particular interest to us because it has been documented as an essential nutrient for the bloodstream forms of African trypanosomes (Little and Oleson, 1951; Williamson and Rollo 1952; Fernandes and Castellani 1958) because they lack the ability to make the amino substitution on the carbon atom six of the purine ring (James and Born, 1980). A nutrient becomes essential when it is needed for normal cell function yet it cannot be synthesized *in vivo* (in the body) and must be supplied in the diet or obtained preformed from another source.

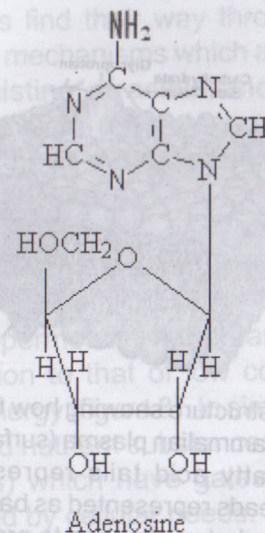


Figure 6: Diagram of adenosine showing the amino substitution on carbon atom six.

Since these parasites must compulsorily obtain adenosine from their host in order to synthesize their own nucleic acids, we reasoned that knowledge of adenosine uptake and metabolism might reveal targets for metabolic inhibition. As at that time no detailed studies of such type had been carried out on *T. vivax*.

What is transport?

Transport as it is used here means "uptake" or "passage" across a biological membrane. A biological membrane is a thin sheet-like structure which encloses the inner contents of the cell and functions as a permeability barrier to extracellular (nutrient substances outside the cell) molecules (figure 7).

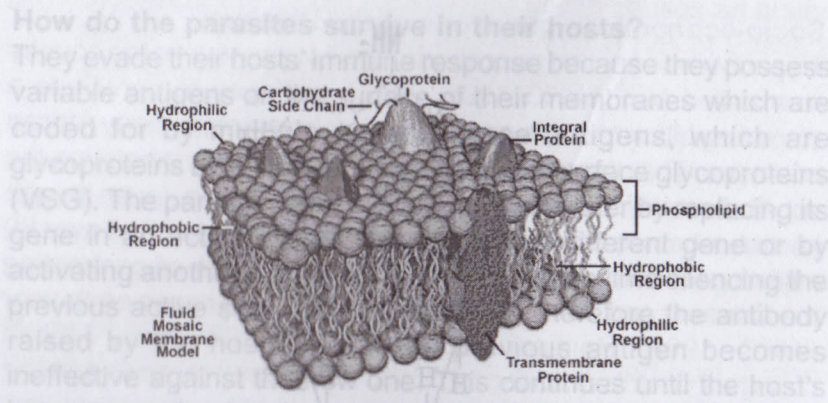


Figure 7: Membrane structure showing how the lipids and proteins are arranged in a typical mammalian plasma (surface) membrane (the lipids have hydrophobic fatty acid tails represented as strands and phospholipids polar heads represented as balls, while the proteins are represented). Carbohydrates attached to proteins (glycoproteins) are shown (Source: www.molecularexpressions.com/cells/plasma-membrane.com)

The mammalian cell membrane is a selective permeability barrier, made up of the lipid bilayer in weak association with proteins. The fatty acids in the bilayer associate with each other and form a highly hydrophobic core (water-hating medium), which keeps off all water-soluble substances.

Some of the proteins are on top of the bilayer (peripheral proteins), others are buried within the bilayer or run transverse the bilayer (integral proteins). The lipids constitute the structural framework of the membrane that houses the proteins which are the functional units. (Some proteins are receptors (just like doors), on which extracellular molecules must bind before entering into the membrane matrix, others are enzymes, immune proteins etc). The outermost surface membrane which encloses the cell organelles is called the plasma membrane. When carbohydrates are covalently attached to lipids or proteins on the plasma membrane, glycolipids or glycoproteins are formed. Trypanosomes possess glycoproteins on their plasma membranes, as already mentioned, by which they evade their host's immune response.

Mechanisms of membrane transport:

Extracellular molecules find their way through the membrane into the cell by different mechanisms which are broadly classified into two groups consisting of active and passive transport processes. The mechanism used depends on the chemical nature of the molecule (that is, whether it is polar (water-soluble) or non-polar (insoluble in water))

Passive transport mechanisms include:

- Simple diffusion:** This is movement of solute molecules across a semi-permeable membrane from a region of high concentration to that of low concentration without expenditure of energy (figure 8). In simple diffusion, solutes (that is, dissolved nutrient substances) are transported by channels (pores) which have gates that can open and close, as dictated by cellular needs.
- Facilitated diffusion:** This, like simple diffusion, does not require energy input but solute movement is facilitated by the presence of a specific carrier which is usually a protein and the process is saturable.

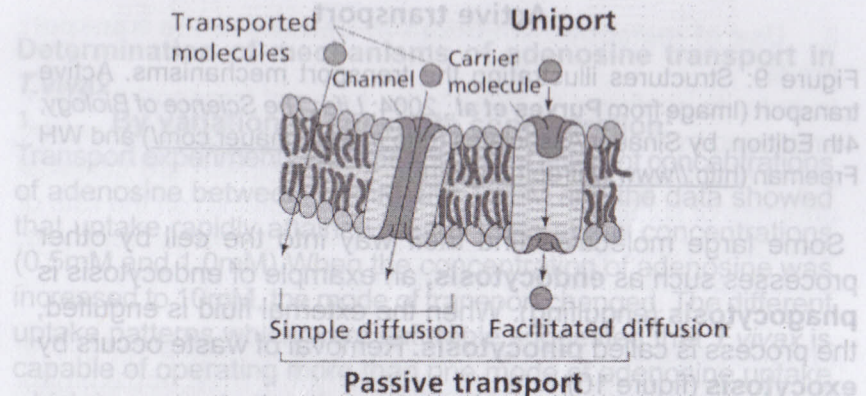


Figure 8: Structures illustrating the transport mechanisms. (Image from Purves et al., 2004 *Life: The Science of Biology*, 4th Edition, by Sinauer Associates (<http://www.sinauer.com/>) and WH Freeman (<http://www.whfreeman.com/>))

Active transport: This process involves movement of molecules from a region of low concentration to that of high concentration, that is, against concentration gradient (going up hill). Energy is required for such a movement (figure 9). The process shares some similarities with facilitated diffusion because a specific carrier is involved and it (the active transport process) is also saturable. A transport protein becomes saturated when all the points of attachment of the solute molecule become fully occupied.

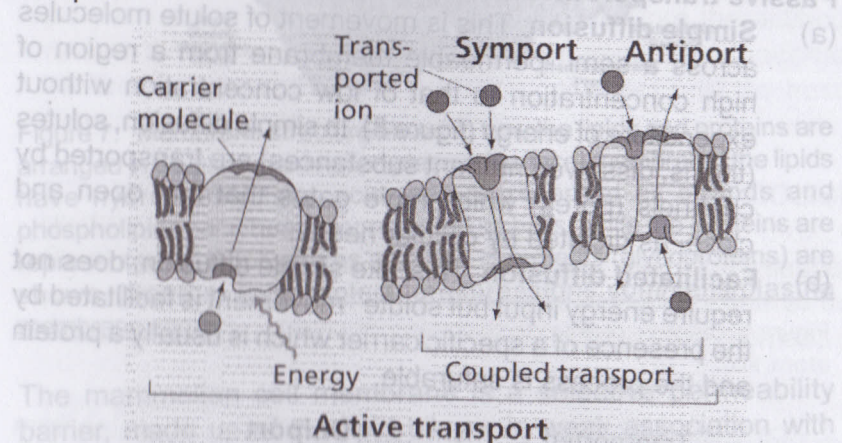


Figure 9: Structures illustrating the transport mechanisms. Active transport (Image from Purves et al., 2004; *Life: The Science of Biology*, 4th Edition, by Sinauer Associates (<http://www.sinauer.com/>) and WH Freeman (<http://www.whfreeman.com/>))

Some large molecules find their way into the cell by other processes such as **endocytosis**, an example of endocytosis is **phagocytosis** (engulfing). When the external fluid is engulfed, the process is called **pinocytosis**. Removal of waste occurs by **exocytosis** (figure 10).

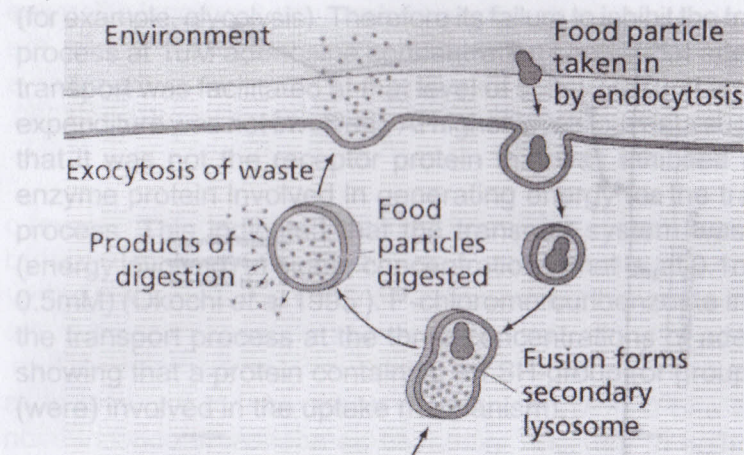


Figure 10: Illustration of endocytosis and exocytosis modes of uptake. (Image from Purves et al., *Life: The Science of Biology*, 4th Edition, by Sinauer Associates (<http://www.sinauer.com/>) and WH Freeman (<http://www.whfreeman.com/>))

MY STUDIES:

Determination of mechanisms of adenosine transport in *T.vivax*

1. By variation of substrate concentration:

Transport experiments were performed at different concentrations of adenosine between 0.5mM and 10mM and the data showed that uptake rapidly attained steady state at low concentrations (0.5mM and 1.0mM). When the concentration of adenosine was increased to 10mM, the mode of transport changed. The different uptake patterns which were saturable suggested that *T.vivax* is capable of operating more than one mode of adenosine uptake which is concentration dependent (Okochi et al, 1983).

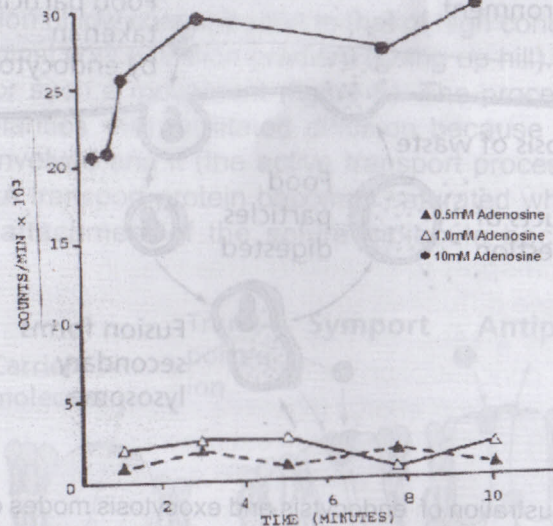


Figure 11: Saturable mechanisms of adenosine transport which were concentration dependent. (Okochi *et al*, 1983, Okochi, 1986).

2. Use of metabolic inhibitors to determine the transport mode:

In order to confirm and classify the types of transport systems employed by the parasite for adenosine uptake, we looked at the effect of a group of reagents called thiols (which are classical effectors of sulfur-containing residues in proteins) on the rate of adenosine transport. They include para - chloromercuribenzoate, α -mercaptoethanol and iodoacetate (Okochi *et al* 1995).

These reagents were, respectively, used in the presence of different concentrations of adenosine (1uM, 0.1mM, and 0.5mM). Our data showed that 5mM iodoacetate did not inhibit the transport process at 1uM substrate (very low concentration of adenosine) while it inhibited at 0.1mM and 0.5mM, respectively. This confirmed that multiple modes of adenosine transport exist in *T. vivax*. Iodoacetate is an inhibitor of energy generating systems

(for example, glycolysis). Therefore its failure to inhibit the transport process at 1uM adenosine concentration shows that adenosine transport was facilitated at that level of substrate (that is, energy expenditure was not involved). At higher levels our data suggested that it was not the receptor protein that was inhibited but the enzyme protein involved in generating energy for the transport process. This indicated that the transport system was active (energy involved) at higher concentrations (that is, at 0.1mM and 0.5mM) (Okochi *et al* 1995). P-chloromercuribenzoate inhibited the transport process at the three concentrations of adenosine showing that a protein containing an SH-group (or groups) was (were) involved in the uptake mechanism).

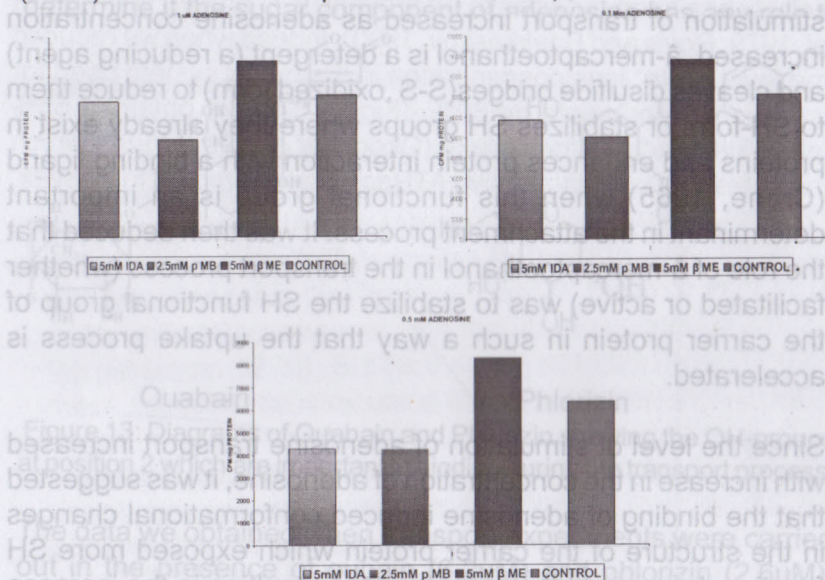


Figure 12: Shows the pattern of the effects of the protein inhibitors on adenosine transport.

KEYS

IDA = Iodoacetate

α ME = α -mercaptoethanol

pMB = Para- chloromercuribenzoate

These observations demonstrate clearly the functional difference between iodoacetate and para-chloromercuribenzoate, the former identifies energy-requiring processes while the latter is unspecific and binds all SH group-containing proteins (Jain, 1980, Okochi *et al*, 1983).

Contrary to the inhibitory effects produced by iodoacetate and p chloromercuribenzoate, β -mercapto- ethanol stimulated adenosine transport in all cases, confirming that a protein carrier containing SH functional groups was, indeed, involved in the transport process at the three concentrations of adenosine (1 μ M, 0.1 mM and 0.5 mM). It was also observed that the level of its stimulation of transport increased as adenosine concentration increased. β -mercaptoethanol is a detergent (a reducing agent) and cleaves disulfide bridges (S-S, oxidized form) to reduce them to SH-form or stabilizes SH groups where they already exist in proteins and enhances protein interaction with a binding ligand (Crane, 1965) when this functional group is an important determinant in the attachment process. It was then deduced that the role of β -mercaptoethanol in the transport process (whether facilitated or active) was to stabilize the SH functional group of the carrier protein in such a way that the uptake process is accelerated.

Since the level of stimulation of adenosine transport increased with increase in the concentration of adenosine, it was suggested that the binding of adenosine induced conformational changes in the structure of the carrier protein which exposed more SH functional groups at the receptor sites resulting in the increase in the rate of transport.

With the concentrations of adenosine we used in these studies we were able to define experimentally two modes of adenosine uptake in *T.vivax*, viz: facilitated diffusion and active transport mechanisms. Current information on this topic reveal that trypanosomes salvage purines from their hosts through a variety

of transporters (Gieser *et al*, 2005). The ability to change transport mode illustrates the typical resilience of these parasites to adapt to even extreme conditions which is their strong weapon for survival in their hosts (Okochi *et al*, 1983; Okochi, 1986).

3. Determination of carrier-substrate interaction at the membrane surface:

Our next quest was to understand how adenosine interacted with the carrier protein during the transport process. Since adenosine has a sugar unit attached to the pyridine ring, we used classical inhibitors of sugar transport (ouabain and phlorizin) to determine if the sugar component of adenosine has any role in

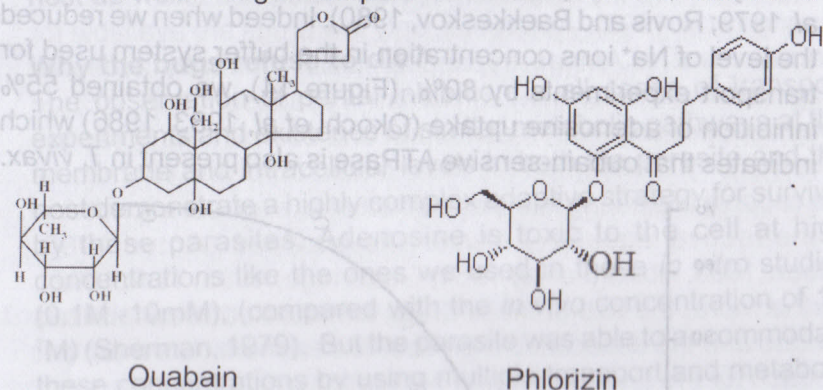


Figure 13: Diagrams of Ouabain and Phlorizin showing the OH-groups at position 2 which are important in binding during the transport process.

The data we obtained when transport experiments were carried out in the presence of ouabain (6.2 μ M) and phlorizin (2.6 μ M), showed that they also inhibited transport by 49% for ouabain and 50% - 75% for phlorizin, (depending on the length of preincubation for phlorizin) (Okochi, 1986).

Phlorizin would inhibit sugar transport because, like sugars, it possesses OH group at carbon atom 2 of the pyranose ring of its sugar part structure, thus it would bind to the receptor site of the carrier protein just like sugars but would not be transported into the cell. In so doing it limits the number of receptor sites

available for sugar binding. In a similar fashion, it would block the binding of the sugar component of adenosine (which also has OH-group at the carbon atom 2 of the ribose unit) from binding at the receptor site and hence limits its (adenosine) uptake into the cell.

Oubain inhibition of the transport process is linked to the involvement of Na^+ ions in the uptake mechanism. Oubain is a toxic glycoside that specifically inhibits $\text{Na}^+ \text{K}^+$ ATPases which are ATP-driven cation transporters found on the membrane surface (Nelson and Cox, 2005). Oubain-sensitive ATPase activity has been reported to be present in *T. brucei* (Voorheis *et al*, 1979; Rovis and Baekkeskov, 1980). Indeed when we reduced the level of Na^+ ions concentration in the buffer system used for transport experiments by 80%, (Figure 14), we obtained 55% inhibition of adenosine uptake (Okochi *et al*, 1983, 1986) which indicates that oubain-sensitive ATPase is also present in *T. vivax*.

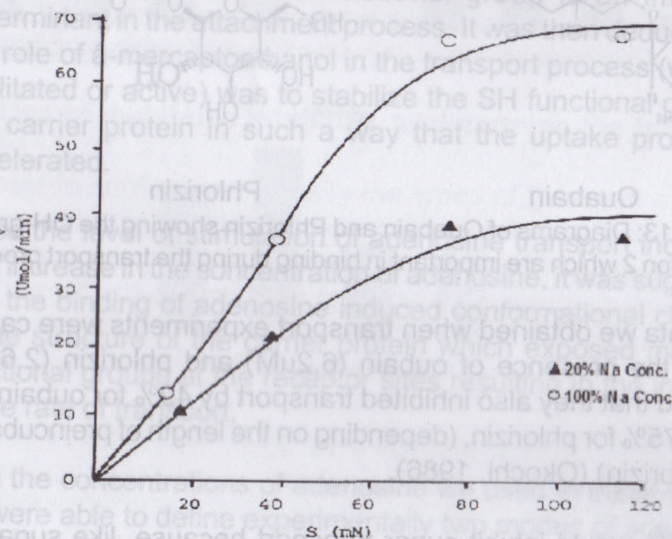


Figure 14: Inhibition of transport by reduction of Na^+ ion concentration.

When we repeated the same experiments with adenine which does not have the sugar unit in its structure, neither oubain nor

phlorizin had any effect, showing that the sugar component of adenosine was a determinant in the transport mechanism. This information could be a useful tool in the design of metabolic inhibitors against these parasites. But this will be possible when only the adenosine inside the parasite can be the target of drug action.

Further studies carried out on adenosine metabolism in *T. vivax* revealed that both the parasite and its mammalian host share similar enzyme pathways (Okochi *et al* 1983, 1986, 2002). This constitutes one of the serious difficulties encountered in the development of rational chemotherapy against trypanosomiasis since any drug produced against the parasite will be toxic to the host as well.

Why the bugs refuse to die?

The observation of partial inhibition in all cases of transport experiments and existence of similar metabolic pathways at the membrane and intracellular levels in both the parasite and the host demonstrate a highly complex adaptive strategy for survival by these parasites. Adenosine is toxic to the cell at high concentrations like the ones we used in these *in vitro* studies (0.1M -10mM), (compared with the *in vivo* concentration of 10⁻⁷M) (Sherman, 1979). But the parasite was able to accommodate these concentrations by using multiple transport and metabolic routes.

Therefore, it had access to the essential nutrient even at extreme conditions and was able to synthesize its own nucleic acids which are translated to the required proteins for different metabolic needs as well as for membrane synthesis. Consequently, it remained viable in the "switch" of its surface glycoprotein (VSG) by which means it evades its host's immune response.

Challenges in the chemotherapy of trypanosomiasis

Chemotherapy of African trypanosomiasis remains a major health challenge in sub-Saharan Africa. Drug is the most important means of intervention for now since development of vaccines remains evasive due to antigenic variation.

An adequate chemotherapeutic measure must not only be effective but must also be discriminatory with respect to its interaction with cellular processes which are common in both the parasite and its host.

Even though potentially good biochemical targets have been identified (Mottram and Coombs, 1999), the major handicap in trypanosomiasis chemotherapy has been lack of effective drugs which discriminate sufficiently and clearly between the host's and the parasite's metabolic pathways. The consequence is that many of the drugs developed for chemotherapy of African human and animal trypanosomiasis are unreliable because of toxicity, adverse side effects coupled with drug resistance and refraction (Enanga *et al*, 1998). As a result, only few drugs are available for the treatment of African trypanosomiasis. Currently accepted drugs used for treatment of human trypanosomiasis include suramine, pentamidine, melarsoprol and eflornithine. Suramine is useful only for early stages while melarsoprol and eflornithine are capable of treating late stage infections, even so, eflornithine is ineffective against *T. brucei rhodesiense* (Apted, 1980; Gilbert, 1983, Abaaru *et al*, 1984; Gutteridge, 1985; TDR, 1992). Furthermore, the use of these drugs is restricted to specialized centers and the associated high cost of treatment puts them out of reach for the underprivileged populations.

Prohibitive high cost of importing drugs and producing new ones remain another clog in the wheel of progress in the development of drugs against trypanosomiasis and indeed general healthcare system. These are challenges which demand local strategy for rational management of this disease, particularly, for our domestic flock.

So what effort have I made?

Screening medicinal plants for potential trypanocides:

The above considerations spurred me on to look at the attractive option of exploring our abundant forest reserves of medicinal plants for potential antitrypanosomal agents.

Mr. Vice-Chancellor, sir, I acknowledge with appreciation that the proposal written for executing this project was approved and the work was funded by the University through the recommendation of the Central Research Committee of this great institution.

Some of the plants investigated included the following:

Mangifera indica – mango (leaf); *Tetrapleura tetraptera* – aidon (Yoruba) (fruit); *Carica papaya* – pawpaw (leaf, seed); *Enantia chloranta* – awopa (Yoruba) (fruit), *Morinda lucida* – oruwo (Yoruba) (leaf) and *Mitragyna ciliata* – Abura (root). We also included two commonly used herbs – *Allium sativum* – garlic (bulb) and *Allium cepa* – onions (bulb).

Many of the plants were chosen because of their traditional uses. (Some are used as worm expellers, antimalarial agents or as trypanocides). For example, *Allium sativum* has been used in *in vitro* studies and was found to have inhibited the proliferation of *Trypanosoma cruzi* (A South American type of Trypanosomes) by disrupting membrane development (Urbina *et al*, 1993). Nok *et al*, (1996) had also reported its antitrypanosomal activity *in vitro*.

Experimental procedures with medicinal plants:

Powdered and measured quantities of the plant materials were subjected to either Soxhlet extraction of the active components in organic solvents or by boiling in water. After drying off the solvents and determining the yield, appropriate quantities were redissolved in suitable solvents according to the nature of the solutes. In vivo screening of plant extracts for trypanocidal efficacy was carried out by administering them to experimental animals (either rats or mice, as the case may be), which had been infected with *Trypanosoma brucei*. In order to check for possible side effects of the plant extracts, control groups were set up in which the experimental animals were not infected with the parasites but were administered with the respective plant extracts. In addition, there was another group containing experimental animals designated as "neat" rats. The animals in this group were given neither the extracts nor infected with the parasites.

All experimental animals were maintained under the same conditions and were allowed free access (continuously) to animal chow (feeds) and water throughout the duration of the experiments. The progress of infectivity and its effect were followed by monitoring the increase or decrease in the parasite load in the blood by microscopy and by analyses of changes in blood chemistries (plasma glucose, protein, creatinine, bilirubin and uric acid). We also monitored the haematological changes (the red blood cells (RBC), white blood cells (WBC) and differentials, hemoglobin (Hb), packed cell volume (PCV)), and measured changes in the body weight of the experimental animals.

The data obtained from our experiments with medicinal plants indicate that *Allium sativum* (garlic), and *Tetrapleura tetraptera* (aidon) gave the best results. *Mangifera indica* (mango) and *Carica papaya* (pawpaw) gave us some encouraging results but were weaker than garlic and aidon with respect to their antiparasitic properties. Some of our observations in these studies have been published (Okochi *et al*, 1999; 2001) but representative data are presented here.

a): *Tetrapleura tetraptera*

Tetrapleura tetraptera (aqueous extract) administered to experimental rats significantly reduced parasite load in the blood (figure 15).

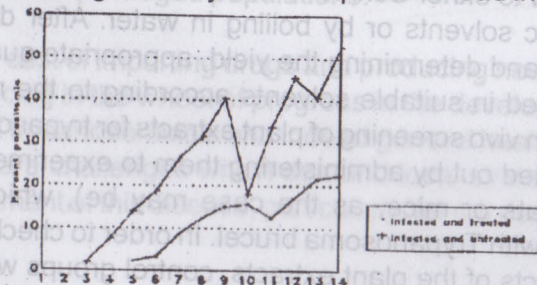


Figure 15: Changes in parasite load after treatment with *Tetrapleura tetraptera* aqueous extract.

Also weight loss was moderately reduced in the infected and treated rats when compared with the infected but untreated ones (figure 16).

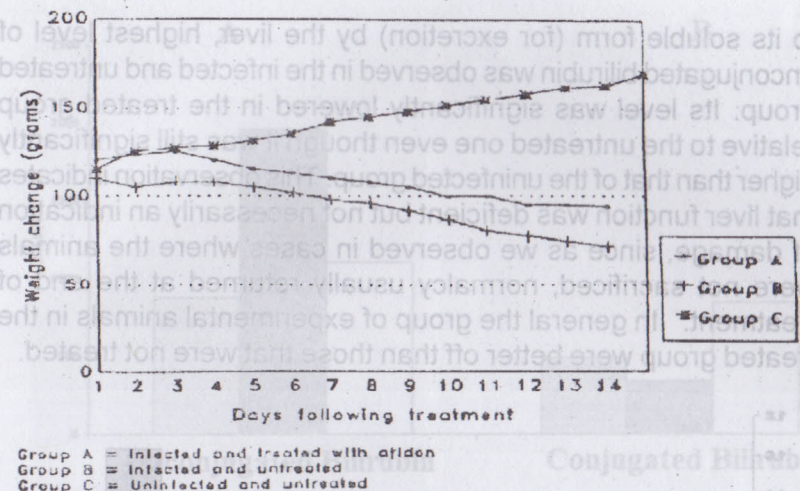


Figure 16: Weight profile of experimental animals in the different groups in the studies with *Tetrapleura tetraptera*.

Values obtained in the determination of the blood chemistries showed significant increases in the values of total plasma protein, uric acid and creatinine in the infected animals relative to control (uninfected and untreated). The increase in the total plasma protein is understandable because it includes some of the immune proteins which the animals needed to resist survival of the parasites in their blood. The increased change observed in the value of uric acid was not statistically significant. Uric acid is one of the parameters used in monitoring kidney function, therefore, this observation indicates that there is no threat to kidney function under our experimental conditions. The high values observed in some of the parameters decreased significantly after the infected animals were treated with the plant extract (figures 17-18A and B). Glucose level decreased in the infected animals, whether treated or not. This is expected because the parasites have high metabolic rate and increased energy demand would deplete available sugar content in the blood of the infected animals. This also explains the increase in creatinine level which is a breakdown product of phosphocreatine, an important source of energy in the muscle. Bilirubin is conjugated

to its soluble form (for excretion) by the liver, highest level of unconjugated bilirubin was observed in the infected and untreated group. Its level was significantly lowered in the treated group relative to the untreated one even though it was still significantly higher than that of the uninfected group. This observation indicates that liver function was deficient but not necessarily an indication of damage, since as we observed in cases where the animals were not sacrificed, normalcy usually returned at the end of treatment. In general the group of experimental animals in the treated group were better off than those that were not treated.

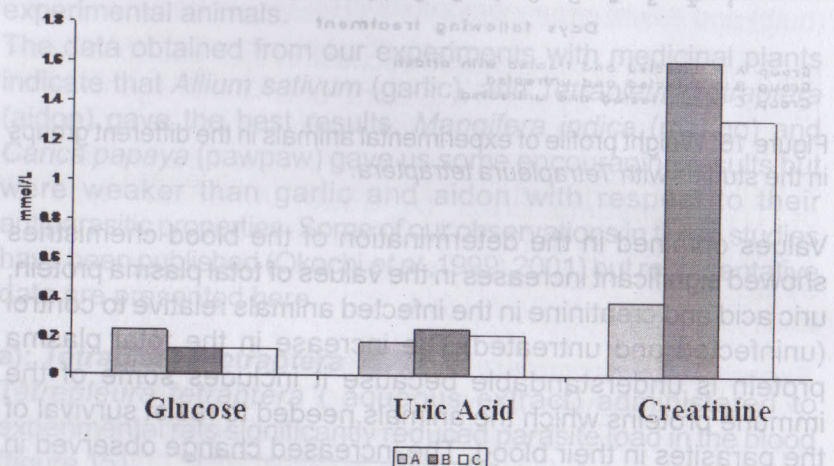


Figure 17: Changes in blood glucose, uric acid and creatinine.

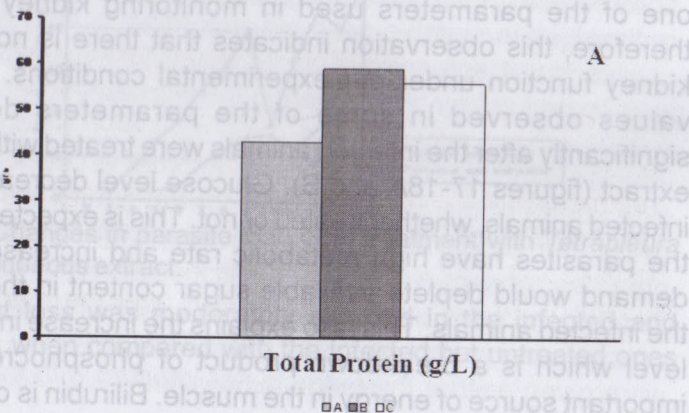


Figure 18A

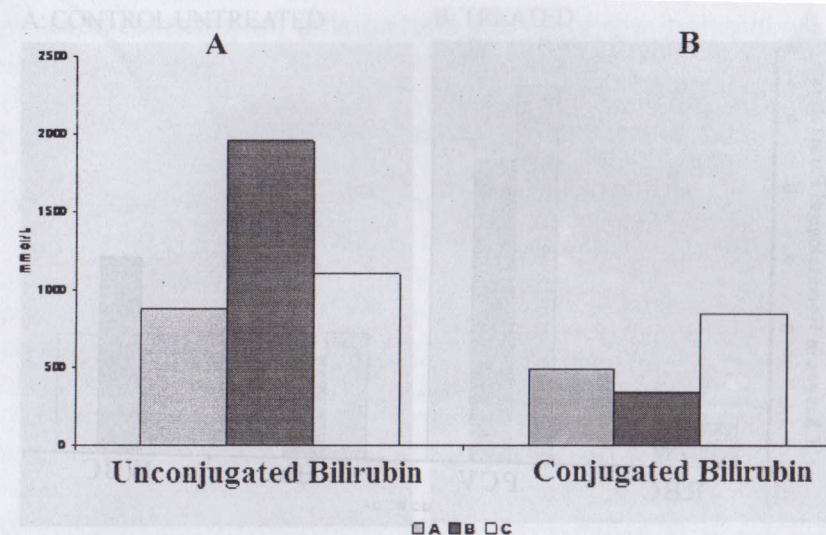


Figure 18B: changes in A: Protein and B: unconjugated and conjugated bilirubin profile.

When the animals were given the extract without infection, results obtained from the determination of the blood chemistries revealed that there were no significant differences in the concentrations of the blood glucose, uric acid and conjugated bilirubin compared to the neat rats which were neither infected nor treated. This indicates that the plant extract had no adverse effect on the experimental animals.

In the case of the haematological parameters, the red blood cell (RBC), packed cell volume (PCV) and haemoglobin (Hb) of the infected and untreated group (group C) decreased significantly relative to the uninfected and untreated group (group D) but after treatment with the plant extract the values improved. The white blood cells (WBC) increased significantly after infection with the parasites (because these are the components of the defense system of the animals), but decreased after the infected animals were treated with the plant extract (figure 19).

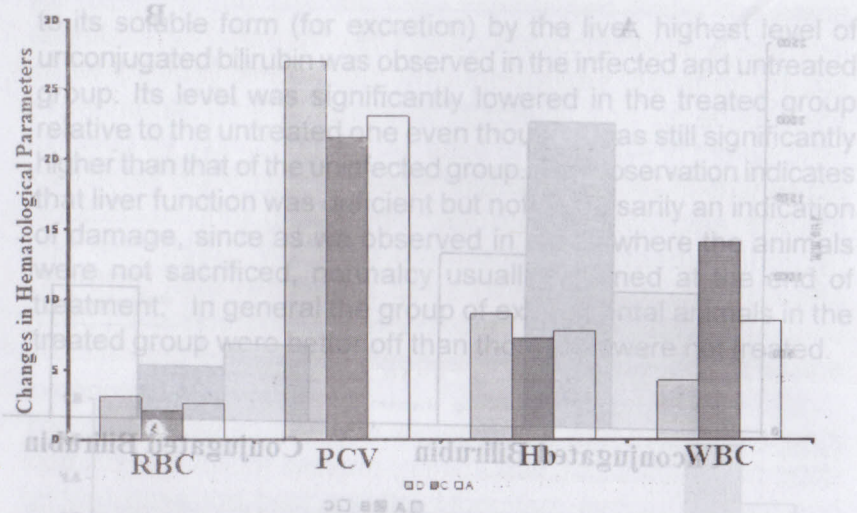


Figure 19: Changes in the profile of the haematological parameters.

These positive observations: reduced leukocytosis (which means that the immune system of the treated animals was more competent in facing the challenge of infection with parasites than that of the untreated ones), improved state of anemia (less breakdown of the red cells), as well as increased level of packed cell volume, are all indices of relief of the state of cell toxicity arising from the infection with parasites and suggest that *T. tetraptera* contains active agents against the parasites that cause sleeping sickness which should be further investigated and exploited Okochi *et al* 1999).

b). *Allium sativum* (garlic)

In the case of *Allium sativum* (garlic), after treatment of the experimental rats for 14 days, it was found to reduce the circulating load of parasites in the blood significantly (figures 20a and b)

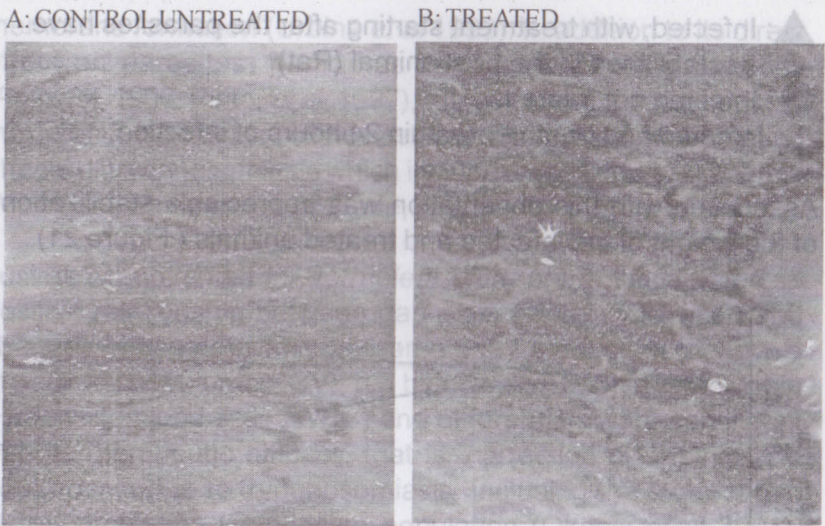


Figure 20a: Shows pictures of slides (under microscope) prepared with blood film from our experimental animals. A: control where the animal was infected but not treated and B is the test showing the disappearance of parasites after treatment with the garlic extract.

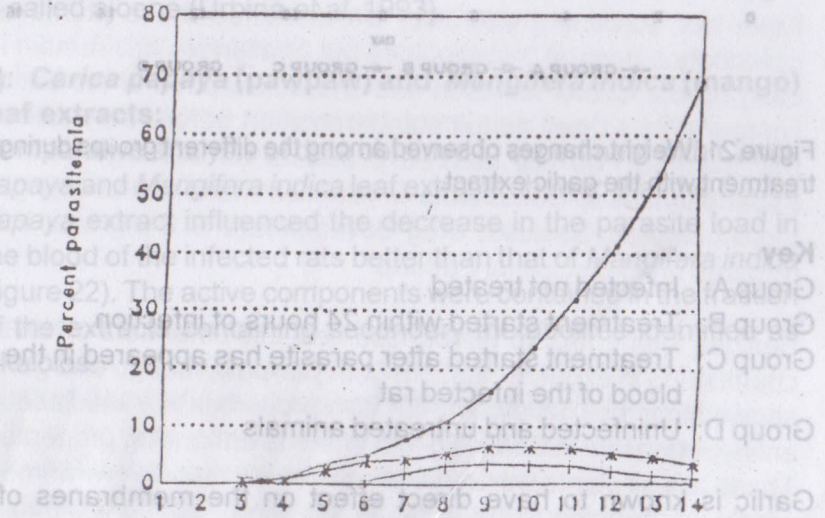


Figure 20b: Shows the plot of percentage parasitemia over the control obtained by counting the number of parasites per 1000 red cells

▲ : Infected, with treatment starting after the parasites have established in the host animal (Rat).

● : Infected not treated.

I : Infected and treatment within 24 hours of infection.

Associated with this observation was appreciable stabilization of the weight of the infected and treated animals (Figure 21).

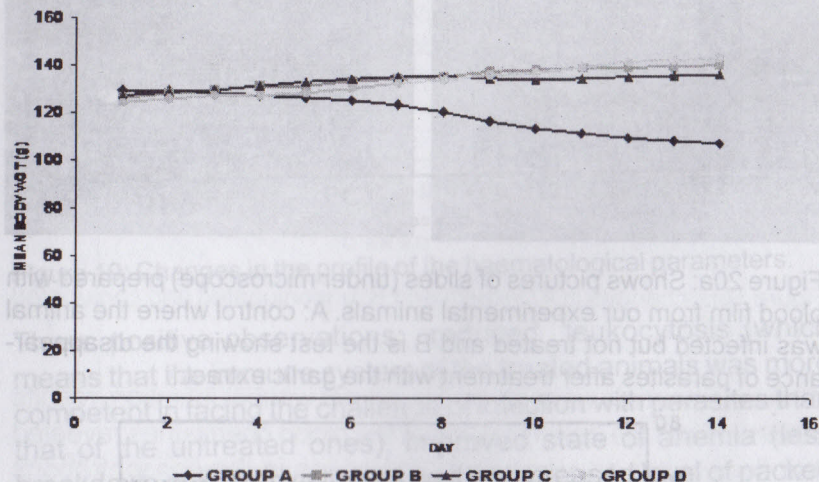


Figure 21: Weight changes observed among the different groups during treatment with the garlic extract.

Key

Group A: Infected not treated

Group B: Treatment started within 24 hours of infection

Group C: Treatment started after parasite has appeared in the blood of the infected rat

Group D: Uninfected and untreated animals

Garlic is known to have direct effect on the membranes of *Trypanosoma cruzi* by disrupting the insertion of the appropriate fatty acids into the phospholipids thereby preventing proper

membrane formation (Urbina *et al* 1993). In addition to its direct effect on parasites, garlic has immune-enhancing capacity (Agarwal, 1996, Pinto, *et al*, 1997), therefore it must have boosted the defence mechanism of the host animals against the debilitating effects of trypanosomiasis which include weight loss and anemia (Murray, 1979; Lososo and Ikede, 1973).

Just as we observed for *Tetrapleura tetraoptera*, there was also positive relationship between garlic extract administration and changes in haematological parameters. Its effect on the levels of packed cell volume(PCV), red blood cells(RBC), haemoglobin (Hb), white blood cells (WBC) and differentials were consistent with its therapeutic efficacy, that is, correction of anemia and neutropenia due to trypanosomiasis, including the stabilization of the circulating lymphocyte population (Okochi *et al* 2001). Former work reported on the trypanocidal effect of garlic was carried out *in vitro*, therefore, our work confirmed its antitrypanosomal properties *in vivo*. The active component in garlic which has been associated with anti-trypanosomal activity is called ajoene (Urbina *et al*, 1993).

c): *Carica papaya* (pawpaw) and *Mangifera indica* (mango) leaf extracts:

Comparative analysis of data obtained in experiment with *Carica papaya* and *Mangifera indica* leaf extracts showed that the *Carica papaya* extract influenced the decrease in the parasite load in the blood of the infected rats better than that of *Mangifera indica* (figure 22). The active components were contained in the fraction of the extracts containing secondary metabolites identified as alkaloids.

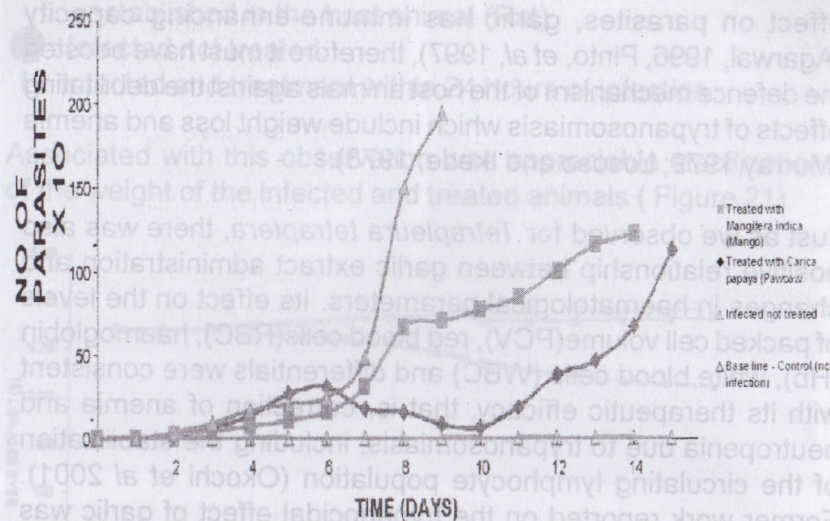


Figure 22: Changes in parasite load during treatment with organic extract of mango and pawpaw.

From our screening exercise, we identified these plants as potential sources of trypanocides but challenges still remain in carrying out further isolation and characterization of the active components as well as full standardization before they can be transformed into the desired products for management of trypanosomiasis. Constraints in facilities make the challenges more complex. Besides, drug development is a big task that requires multi-disciplinary efforts.

Besides their effects on trypanosomes, our other data on blood chemistries in experimentations with pawpaw, mango and garlic showed that they have many curative properties like antidiabetic, antihypertensive, antisickling and immune enhancing properties. These are all plants we are very familiar with in our environment which are begging to be exploited for the benefit of humanity. *Mytragyna ciliata*, another medicinal plant with potential anti-trypanosomal activity, was investigated in detail at Ph. D level and the data obtained was recently defended successfully for

the award of the Doctor of Philosophy by the Postgraduate School of the University of Lagos (Ogbunugafor, 2009). Ogbunugafor was able to demonstrate that the butanol extract of the root of this plant is the best active fraction and the active component was identified as an alkaloid. The study also provided evidence for the biochemical basis for trypanocidal action of *M. ciliata* which included the creation of oxidative stress as well as negative modulation of the Ca^{++} level in the infected animals, both conditions of which are unfavourable to the proliferation of the parasites in their hosts.

These are important documented explanations that justify the use of this plant in folk medicine for the treatment of trypanosomiasis. However, the product needs to be standardized as stated earlier, for better and effective utilization.

Part II: The Super Tailors of Life

Mr. Vice-Chancellor, sir, I was privileged to travel with my husband to the diplomatic missions during his active years in the Federal Civil Service of Nigeria. And I was able to work in the laboratories of some renowned biochemists such as Professor Hiroshi Oya at Juntendo University, Tokyo, Japan and Professor Francois Chapeville at the famous Jacques Monod Institute, Paris, France. They awakened my interest in enzymology, for which I am very grateful.

Enzymes: The Biological Catalysts of Life

In chemical laboratories, chemists have employed blocking groups and powerful reagents and often obtained only very limited yields in the synthesis of biomolecules. The same synthesis is achieved in the cells smoothly and routinely, with speed and accuracy, principally through the action of enzymes (the proteinacious catalysts of the biosphere).

I call enzymes "the super tailors of life" because they have greater dexterity in maintaining the machinery of life than tailors have in

cutting and sewing materials into beautiful clothes. In the cell, enzymes mediate meticulously the coupling of small biomolecules into large ones like proteins for growth and repair of worn out tissues; carbohydrates and lipids for energy generation, both for utilization and storage, as well as for membrane synthesis, and nucleic acids for the preservation and transmission of hereditary characters from parents to offsprings. Just as tailors use buttons to decorate clothes, enzymes pick up micronutrients (minerals and vitamins) to nourish life. They insert the food supplements into the appropriate positions in the macromolecules to make them acquire proper structures for appropriate functions. Sometimes they use the micronutrients (where necessary) in the process of metabolic synthesis or breakdown of biomolecules.

In the course of chemical synthesis, some enzymes, sometimes behave like journalists (for example, the nucleic acid polymerases in the synthesis of genetic materials) because they possess the capacity to retrace their steps, edit what they have synthesized, and like tailors, they nip off unwanted additions and replace them as appropriate.

They mediate not just the synthesis of macromolecules, they also catalyze the breakdown of the foods we eat, for the supply of energy to the cell, and for producing small molecules which are essential precursors of other required cellular metabolites.

Practical Applications of Enzymes: Enzymes function as diagnostic tools:

Their specificity and ability to catalyze reactions that otherwise would be immeasurably slow, allow quantitative analysis of individual substances in biological fluids. Assays of some of the enzymes present in blood plasma or serum are carried out routinely in most clinical laboratories. Enzymes in plasma are of two types - those that are normally present in plasma and have functional roles there and those released from tissues which

can be present in the plasma, normally at very low concentrations, but have no metabolic functions there, however, they are most important for diagnostic purposes. The reason for this is that if the cells of a particular tissue are affected by disease in such a way that many of them (the cells) no longer have intact membranes, their contents will leak out into the bloodstream at an increased rate and the enzymes associated with such cells of the ailing tissue will be found in plasma in elevated amounts. Since many enzymes or isoenzymes are usually associated with cells of certain tissues, assay of their activities in plasma are used as diagnostic tools in the identification of the source of tissue damage. For example, in the case of heart failure (myocardial infarction), time course assay of some cardiac enzymes will assist the doctor in monitoring the state of the patient as time progresses (figure 23).

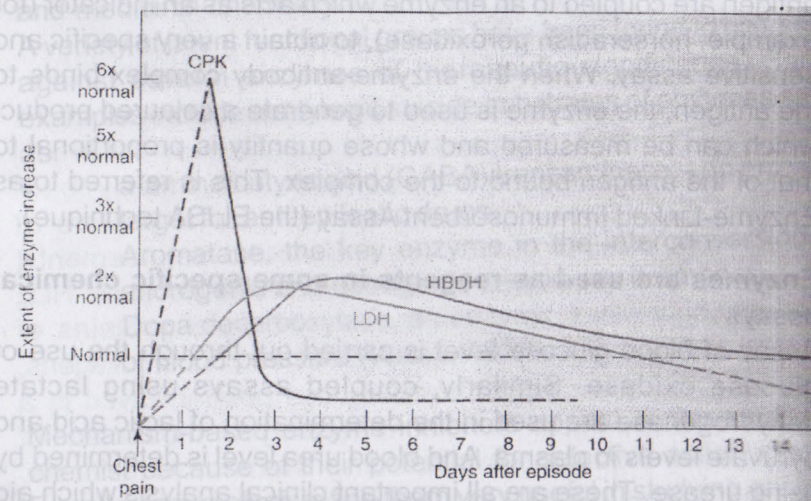


Figure 23: The Figure illustrates the changes observed in some cardiac enzymes - creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and α -hydroxy butyric acid dehydrogenase (HBDH). (Palmer, 2001)

And in the case of an inborn error of metabolism (characterized by the loss of activity of a specific enzyme as a result of genetic mutation), the preliminary diagnosis is usually made by observing

a build up in plasma or urine of the metabolic intermediate which is the substrate of the defective enzyme. Then confirmation of diagnosis may be made by assaying for the activity of the defective enzyme in the tissue where it is metabolically most important.

Enzymes are used as Drugs

An example of this type of enzyme application is the use of streptokinase (an enzyme mixture prepared from streptococcus) in the activation of plasminogen to plasmin which cleaves insoluble fibrin in blood clots into soluble products. In this way it clears the blood clots formed in myocardial infarcts and those that accumulate in the extremities of affected patients.

They are also used as indicators

In some immunoassay methods, antibodies specific for a protein antigen are coupled to an enzyme which acts as an indicator (for example, horseradish peroxidase), to obtain a very specific and sensitive assay. When the enzyme-antibody complex binds to the antigen, the enzyme is used to generate a coloured product which can be measured and whose quantity is proportional to that of the antigen bound to the complex. This is referred to as Enzyme-Linked Immunosorbent Assay (the ELISA technique).

Enzymes are used as reagents in some specific chemical assays

Assay of blood glucose level is carried out through the use of glucose oxidase. Similarly, coupled assays using lactate dehydrogenase are used in the determination of lactic acid and pyruvate levels in plasma. And blood urea level is determined by using urease. These are all important clinical analysis which aid physicians to make proper diagnosis in health-care delivery.

Industrial Application of Enzymes

Enzymes are used industrially for the production of chemicals and pharmaceuticals, laundering detergents as well as for food processing. They also find useful application in environmental sanitation through bioprocessing of waste materials. Modern

techniques for gene manipulation leading to easy and increased synthesis of enzymes and the use of immobilized enzymes in product isolation and purification is bound to boost industrial application of enzymes.

My activities in this aspect of enzyme application include drug development and biotechnology.

Enzymes in Drug Development

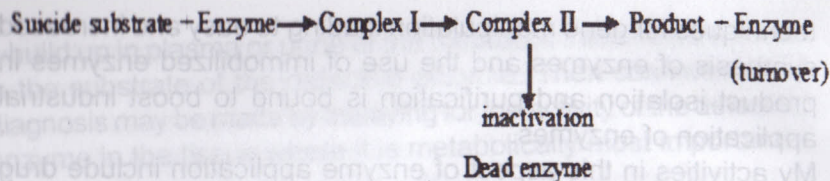
There is growing interest in the study of the kinetics of irreversible inhibitors of some enzymes, not only because of their importance in understanding the nature of catalysis, but also, their increasing application in pharmaceutical chemistry, pharmacology and toxicology. In particular, studies with mechanism-based enzyme inhibitors have opened up a new area of interdisciplinary relationship with regards to the study of enzyme mechanisms and medicinal chemistry.

A variety of latent functional groups have been found to be useful against several enzymes of therapeutic significance. Some examples include acetylenic suicide substrates of enzymes such as:

- α -amino butyric acid (GABA) transaminase – an enzyme target for anti-epileptic drugs.
- Aromatase, the key enzyme in the interconversion of androgenic and estrogenic steroid sex hormones.
- Dopa decarboxylase, an enzyme involved in regulation of blood pressure (Walsh, 1983).

Mechanism-based enzyme inhibitors appeal to the medicinal chemist because of their potential for selective action *in vivo*. Equally, enzymologists find them very useful in studying enzyme active sites, particularly as it relates to specificity.

Mechanism-based enzyme inhibitors or suicide substrates may be defined in terms of their dual role as compounds which interact with enzymes by a mechanism with branched pathway, the branches representing turnover of substrate and inactivation of enzyme.



The essential and unique feature of a mechanism-based enzyme inhibitor is that the target enzyme must chemically transform the molecule into the actual inactivating species and that inactivation must occur by the species prior to its release from the active site.

Work at the Institute Jacques Monod, Paris

At the Institute, the laboratory of Bioactivation of peptides was working with a series of newly designed functionalized N-aryl β -lactam and some coumarine derivatives as mechanism-based inhibitors of some enzymes belonging to the serine protease family, namely: human leukocyte elastase (HLE), and porcine pancreatic elastase (PPE).

Why are these enzymes important subjects of research?

Leukocyte elastase, expressed by polymorphonuclear (PMN) leukocytes, mainly neutrophils, acts both intracellularly to kill engulfed pathogens and extracellularly as mediators of coagulation, immune responses and wound debridement (Dell'aica *et al*, 2006; Sternlich and Werb, 1999). Because HLE has the potential to degrade some structural proteins of extracellular matrix such as elastin, fibronectin and collagens, excess of leukocyte elastase activity has been involved in a number of pathological conditions leading to impairment of extracellular organization, including rheumatoid arthritis, emphysema, cystic fibrosis, acute respiratory distress syndrome and tumor progression (Wakselman *et al* 1991; Balkwill and Mantovanic, 2001). Pancreatic elastase is implicated in the pathogenesis of pancreatitis (Vilain *et al* 1991).

Under normal physiological conditions, these enzymes are regulated by compartmentation (that is, isolation of activities e.g. by membranes, to tightly controlled microenvironments) and by

plasma proteinase inhibitors such as α_1 -antiproteinase (Trainor, 1987, Powers and Zimmerman, 1989) as well as α_2 -macroglobulin (Robert *et al*, 1980). These enzymes may escape control by their naturally-occurring inhibitors because of genetic or functional deficiencies (Hornebeck *et al*, 1987).

Under such conditions it becomes imperative to use synthetic specific inhibitors as therapeutic agents to stem the harmful effects of uncontrolled action of these proteinases. This was the basis for the work with the newly synthesized β -lactam compounds in which I was involved.

Most β -lactams inhibit bacterial cell wall biosynthesis but others behave as β -lactamase inhibitors. Recently, a number of β -lactam compounds widely used as antimicrobial drugs have been identified as inhibitors of serine enzymes, in particular, leukocyte elastase (Konaklieva, 2002), and prostate specific antigen (Adlington *et al*, 1997; Wright *et al*, 2000).

Our experiments were aimed at determining the functional efficiency and specificity of the newly synthesized drugs as suicide substrates by investigating their kinetic properties.

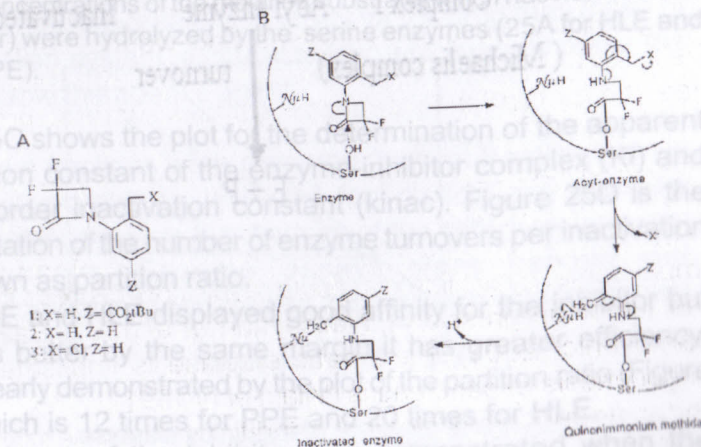
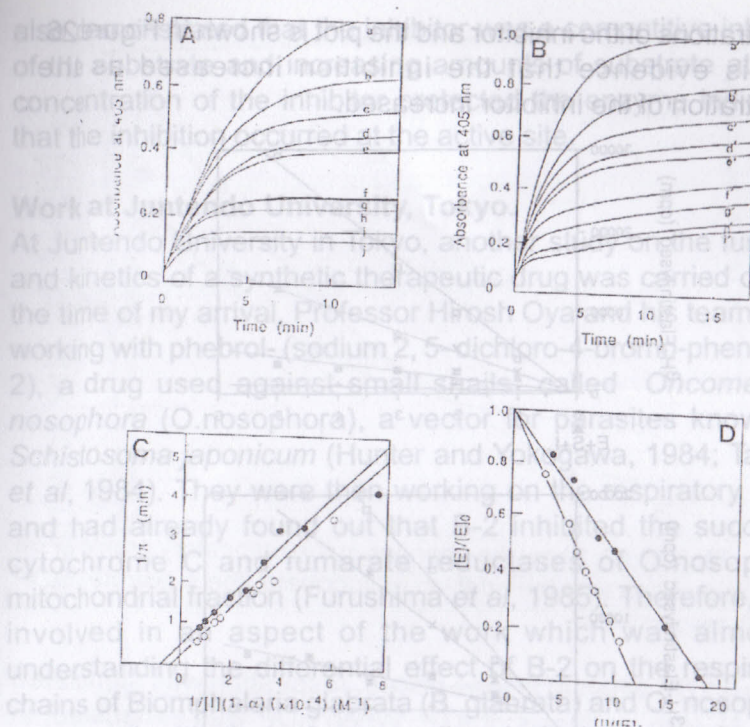
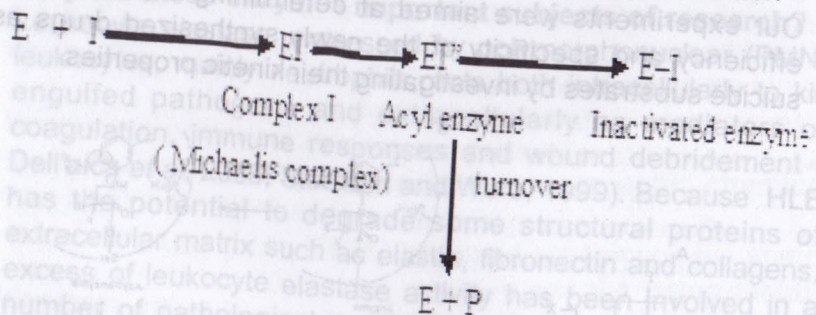


Figure 24: Shows representative structures of the molecules studied and mechanism of their interaction with a serine enzyme.

As shown in figure 24, structure A was well hydrolyzed by HLE and PPE but it was not an inactivator (when modified as shown in numbers 1 and 2). Structure A becomes a suicide substrate (structure B) by substitution of a good-leaving group (in our studies, it was a halogen as shown in 3 above) attached to a methylphenyl-ring. The initial binding of the enzyme (through the interaction of the OH-group of the serine residue with the β -lactam ring) to the substrate (its potential inactivator) led to rearrangement of electrons within the substrate molecule resulting in the breaking of the highly strained β -lactam ring and subsequent activation of the methylphenyl ring. The preceding intramolecular events resulted in the exit of the substituted good-leaving group (the Cl-group, as shown in 3 above), with the enzyme still held onto the substrate. The exit of the leaving group created a highly electrophilic center for attack by another nucleophilic functional group on the enzyme. The fate of the enzyme as it binds follows the general scheme of the reaction of mechanism-based enzyme inactivators as explained earlier:



Figures 25: A and B show the inhibition pattern in time, obtained when different concentrations of the modified substrate (which has now become an inhibitor) were hydrolyzed by the serine enzymes (25A for HLE and 25B for PPE).

Figure 25C shows the plot for the determination of the apparent dissociation constant of the enzyme-inhibitor complex (K_i) and the first order inactivation constant (k_{inac}). Figure 25D is the representation of the number of enzyme turnovers per inactivation also known as partition ratio.

Both PPE and HLE displayed good affinity for the inhibitor but HLE was better by the same margin it has greater efficiency. This is clearly demonstrated by the plot of the partition ratio (Figure 27D), which is 12 times for PPE and 20 times for HLE. The efficiency of the inhibitor was demonstrated when the inhibition of elastin digestion was carried out at different

concentrations of the inhibitor and the plot is shown in Figure 26. There is evidence that the inhibition increased as the concentration of the inhibitor increased.

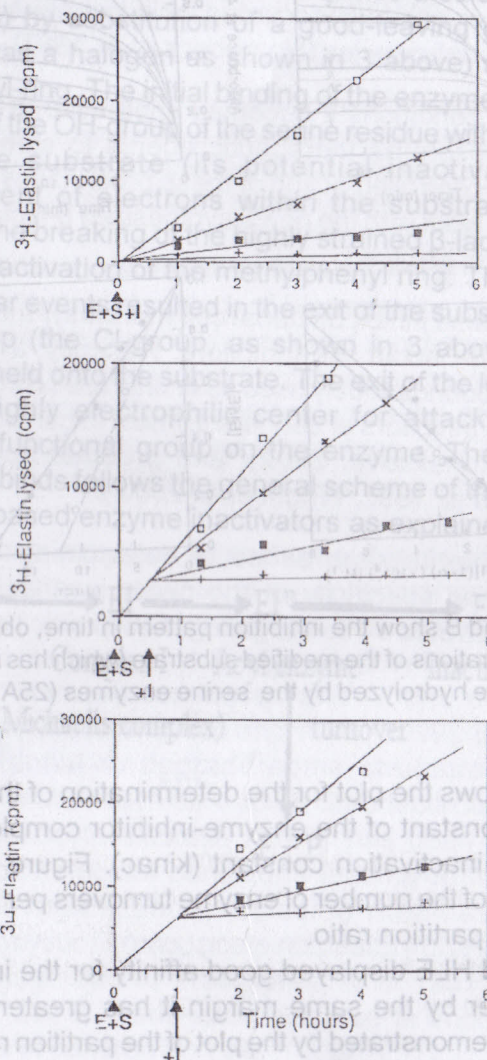


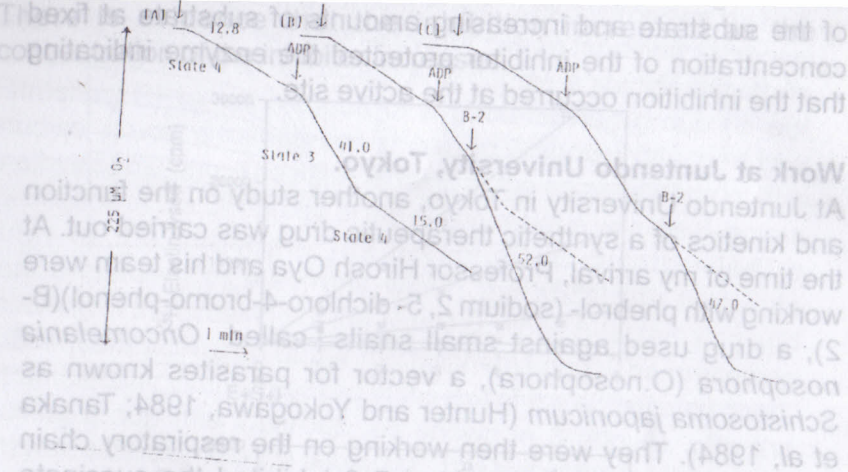
Figure 26: Inhibition of digestion of elastin by elastase. This study succeeded in showing that the newly synthesized molecule was an efficient suicide substrate. Our experiments

also demonstrated that the inhibitor was a competitive inhibitor of the substrate and increasing amounts of substrate at fixed concentration of the inhibitor protected the enzyme indicating that the inhibition occurred at the active site.

Work at Juntendo University, Tokyo.

At Juntendo University in Tokyo, another study on the function and kinetics of a synthetic therapeutic drug was carried out. At the time of my arrival, Professor Hirosh Oya and his team were working with phebol- (sodium 2, 5- dichloro-4-bromo-phenol)(B-2), a drug used against small snails called *Oncomelania nosophora* (*O.nosophora*), a vector for parasites known as *Schistosoma japonicum* (Hunter and Yokogawa, 1984; Tanaka *et al*, 1984). They were then working on the respiratory chain and had already found out that B-2 inhibited the succinate cytochrome C and fumarate reductases of *O.nosophora* mitochondrial fraction (Furushima *et al*, 1985). Therefore, I got involved in an aspect of the work which was aimed at understanding the differential effect of B-2 on the respiratory chains of *Biomphalaria glabrata* (*B. glabrata*) and *O. nosophora* compared with that of mammalian (rat) liver mitochondria (Furushima *et al*, 1991). The objective was to understand if the snails' enzymes had greater sensitivity for the drug than those of the mammalian system, a characteristic which would make the drug a valuable therapeutic agent.

Mitochondrial fractions were prepared from rat liver and whole tissues of *O. nosophora*, while the foot muscle and digestive gland of *B.glabrata* were the sources of the mitochondria used for the assays. Enzyme assays were carried out spectrophotometrically and oxygen consumption was measured polarographically.



Figures 27: (A,B,C): Show the changes in the respiratory profile of rat liver mitochondria with the addition of succinate (the substrate), ADP and B-2, respectively.

In figure 27A, when ADP was added, respiration was stimulated (state 3), with synthesis of ATP. When ADP was exhausted, in spite of the presence of adequate substrate (succinate), as well as inorganic phosphate, rate of respiration decreased again (state 4). In figure 27B, addition of B-2 to state 3, prevented the conversion of ADP to ATP, even though oxygen consumption continued which was an indication that respiration was going on but was not coupled to oxidative respiration. The same was the case when B-2 was added as is shown in figure 27C.

The maximum stimulation of respiration occurred at 10μM of B-2 in both states 3 and 4 (figure 28). After this concentration, inhibition starts, therefore, B-2 has dual effect on respiration in rat liver mitochondria, acting as uncoupler at low concentration and as an inhibitor at higher concentration.

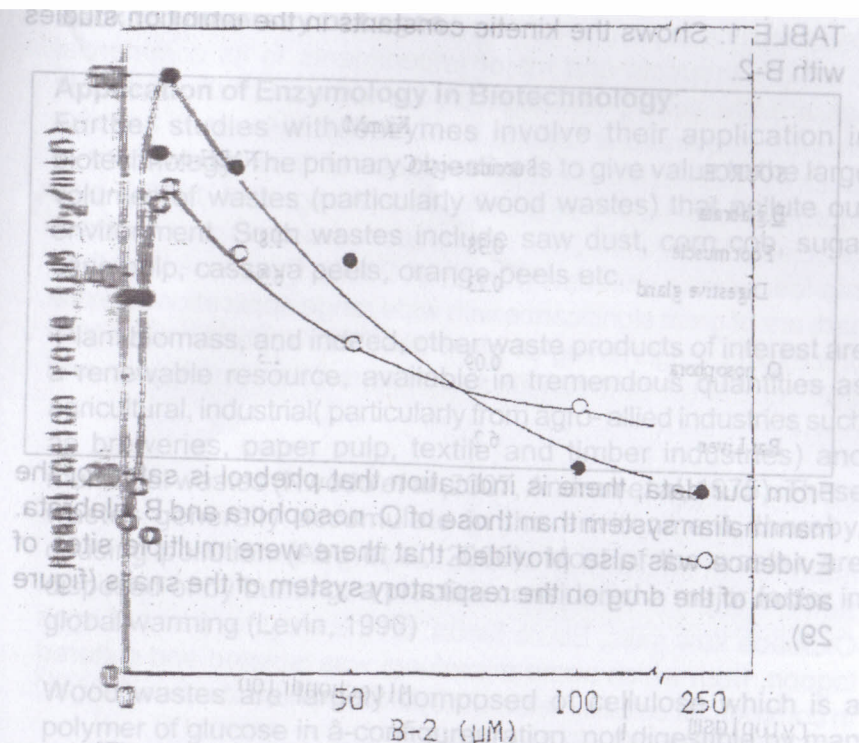


Figure 28: Shows how different concentrations of B-2 affect electron transport chain in the rat liver mitochondria.

Kinetic studies showed that succinate cytochrome C reductase of *B. glabrata* and *O. nosophora* are more sensitive to phebrol than that of the rat liver mitochondria (Table 1)

TABLE 1: Shows the kinetic constants in the inhibition studies with B-2.

SOURCE	K _i (mM)	
	Succinate-cyt C	NADH-cyt C
<i>B. glabrata</i>		
Foot muscle	0.38	1.8
Digestive gland	0.23	6.5
<i>O. nosophora</i>	0.09	1.3
Rat Liver	6.2	

From our data, there is indication that phebolol is safer for the mammalian system than those of *O. nosophora* and *B. glabrata*. Evidence was also provided that there were multiple sites of action of the drug on the respiratory system of the snails (figure 29).

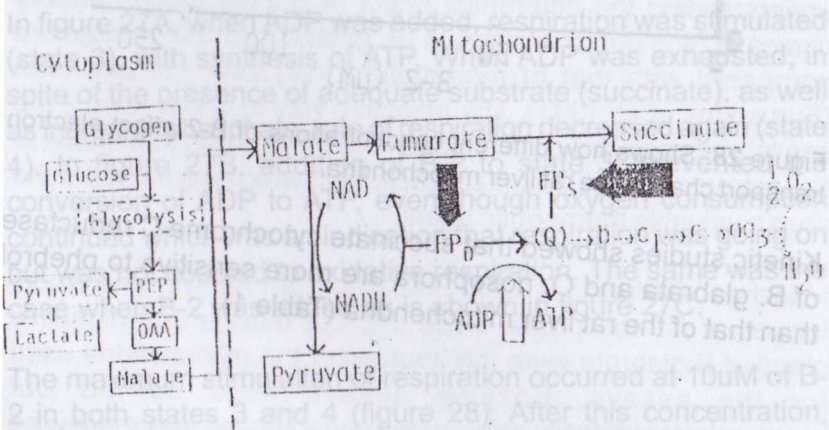


Figure 29: Shows electron flow during respiration and the points of inhibition by B-2 (the big arrows).

Back to University of Lagos

Application of Enzymology in Biotechnology:

Further studies with enzymes involve their application in biotechnology. The primary objective is to give value to the large volumes of wastes (particularly wood wastes) that pollute our environment. Such wastes include saw dust, corn cob, sugar cane pulp, cassava peels, orange peels etc.

Plant biomass, and indeed, other waste products of interest are a renewable resource, available in tremendous quantities as agricultural, industrial (particularly from agro-allied industries such as breweries, paper pulp, textile and timber industries) and municipal wastes (Nwodo *et al*, 2007, Andren *et al*, 1975). These wastes generally accumulate in the environment, thereby, causing pollution (Abu *et al*, 2000). Most of the wastes are disposed of by burning, a practice considered a major factor in global warming (Levin, 1996).

Wood wastes are largely composed of cellulose which is a polymer of glucose in α -configuration, not digestible by man and monogastric animals like birds. But they are degraded by bacteria and fungi which are capable of producing complex array of enzymes for degrading cellulose and other cell-wall polymers (Grant and Long, 1981). Fungal enzymes are, however, usually preferred (to bacterial enzymes) because they are extracellular, adaptive and usually secreted in large quantities. This is in sharp contrast to many bacterial enzymes which exist as tight multi-enzyme complexes, often membrane-bound, from which it is difficult to recover individual active enzyme groups (Berry and Paterson, 1990). Therefore, our focus in this study is on microfungi which produce enzymes that degrade wood wastes.

Enzymes of interest in these studies:

There is growing interest in the production of industrial enzymes such as cellulases, xylanases and pectinases because they are largely responsible for breaking down wood wastes.

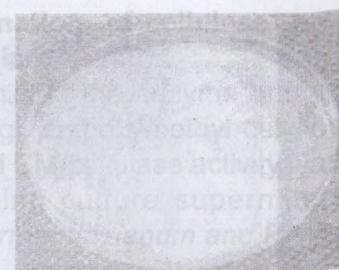
Cellulase is a key enzyme in the bioconversion of plant biomass to simple products and major impediments to its commercial use are low activity and high production cost of the available enzyme preparations (Spano *et al*, 1978). Xylanases are also receiving increasing attention because of their applications in improving digestibility of animal feed (Wong *et al*, 1988), pulp bleaching (Rifaat *et al*, 2005) and bioconversion of lignocelluloses into feed stocks and fuels (Kim *et al*, 2000). Pectinases, on their part, are of great significance with wide range application in textile processing, degumming of plant bast fibers, paper making as well as coffee and tea fermentations (Sarvamangala and Dayanand, 2000).

My Contributions

Isolation of Microfungi from decaying saw dust:

Some sample of decaying saw dust was taken from the site at Okobaba saw mills, Ebute Metta, on the western part of Lagos lagoon, from which some microfungi was isolated and cultured in commercial culture media (Czapek dox and Sabourauds agar) (Nwodo *et al* 2005).

Stock culture of the waste materials was plated on basal mineral salt agar (pH 5.6) containing cellulose as sole carbon source and different types of fungi colonies grew on the plate. These colonies of fungi were subcultured on potato dextrose agar (PDA) plates. Pure fungal isolates were identified based on the colonial and morphological properties as shown in figures 30–35, A and B, respectively, identify the pure culture colony and the spore image as viewed under the microscope.



A



B

Figure 30: *Trichoderma harzianum*

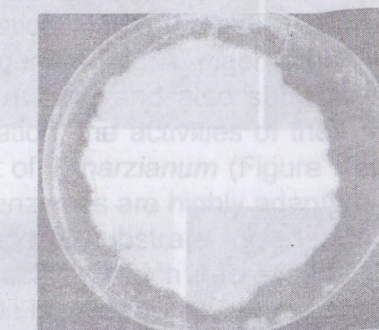


A



B

Figure 31: *Penicillium chrysogenum*



A



B

Figure 32: *Fusarium solani*



Figure 33: *Aspergillus niger*.



Figure 34: *Aspergillus fumigatus*

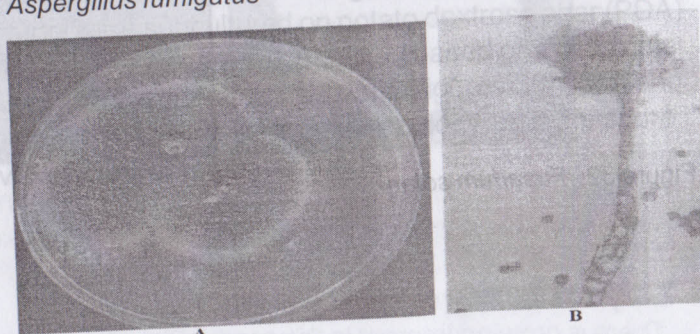


Figure 35: *Aspergillus flavus*.

Aspergillus fumigatus and *Aspergillus flavus* are pathogenic so we did not continue any work with them.

Determination of cellulase production potential of some of the microfungi:

In examining the enzyme producing capacity of the isolated microfungi, carboxymethyl cellulose (CMC) was the substrate used and CM-cellulase activity was determined for a period of 3 days using culture supernatant of *Aspergillus niger*1, *Trichoderma harzianum* and *Penicillium* species.

Cellulose is a homopolysaccharide made up of glucose units (joined by glycosidic single bonds) which are easily hydrolyzed to simple sugars by the enzymes secreted into the culture medium by the microfungi. We used a chemical method in which the sugars liberated formed colours when treated with appropriate reagents to measure (using spectrophotometer) the quantity of sugar produced by each microorganism.

From the data obtained, it was realized that *Trichoderma harzianum* gave the highest enzyme activity at the shortest possible time of incubation, however, the enzyme activity was easily repressed by feedback inhibition because of product accumulation. While the activities of the other two microfungi (*P. chrysogenum* and *A. niger*) were lower at the first optimum point of activity and also subject to repression by product accumulation, the activities of their enzymes recovered faster than that of *T. harzianum* (Figure 36). The data really showed that the enzymes are highly adaptive and complex, responding to absence of substrate for energy production by increased enzyme activity which decreased when substrate for energy production is present.

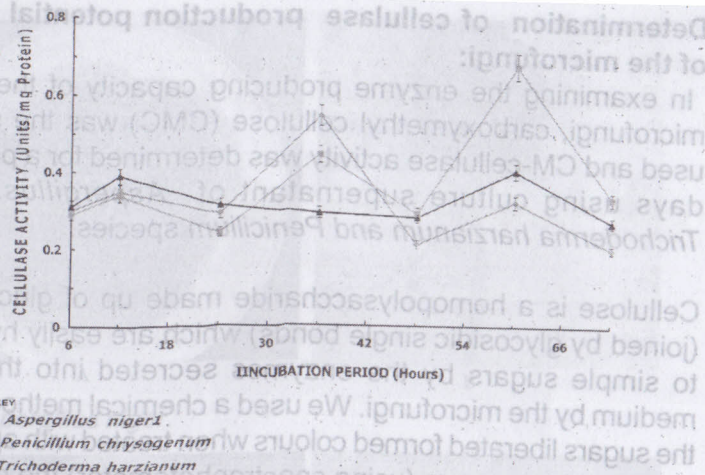


Figure 36: Graph showing cellulase activities produced by the three isolates

Modification of the Commercial Culture medium for cost effective fungal growth and enzyme production:

In order to save costs from purchase of commercial culture media, we compared the growth and cellulase production by *P. chrysogenum*, *T. harzianum* and *A. niger* 1 in modified media containing corn cob, sugar cane pulp and saw dust as sole carbon sources (Nwodo *et al* 2007). The data shows that *P. chrysogenum* does equally well with sugarcane pulps and saw dust, *A. niger* is best with sugarcane pulps and *T. harzianum* is best with sawdust than corncob and sugarcane pulp as sole carbon source in the culture media (figure 37).

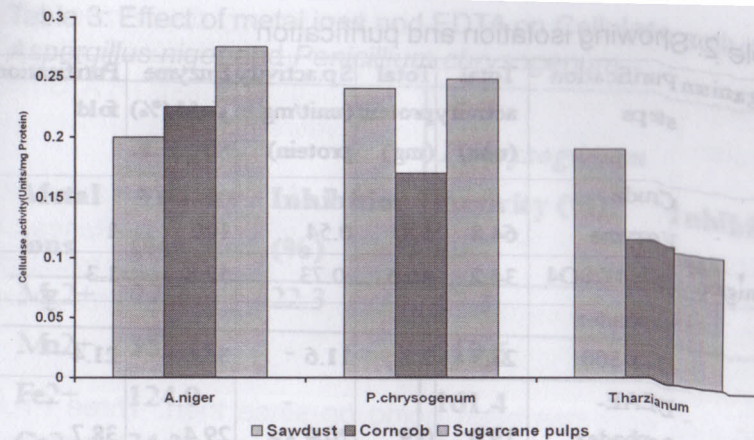


Figure 37: The bar chart shows the cellulase activities produced by the three isolates on various agro-wastes studied.

Isolation and Purification of Cellulase

Having observed that the microfungi can grow and produce enzymes efficiently in our own cheap selective media, we isolated and characterized cellulase from *A. niger* and *P. chrysogenum* following typical established techniques in enzyme studies. The data obtained in these experiments are presented in the table 2.

Table 2: Showing isolation and purification

Organism	Purification steps	Total activity (unit)	Total protein (mg)	Sp activity (unit/mg protein)	Enzyme yield (%)	Purification fold
A.niger	Crude Enzyme	64.8	120	0.54	100	1
	(NH ₄) ₂ SO ₄	34.2	46.6	0.73	52.8	1.3
	Sephadex G25-300	23.7	2.1	11.6	36.6	21.4
	DEAE-Sephadex	19.0	0.9	20.9	29.4	38.7
	Crude Enzyme	59.3	88.5	0.67	100	1
P.chryso- genum	(NH ₄) ₂ SO ₄	28.3	30.9	0.9	47.8	1.4
	Sephadex G25-300	12.3	0.57	21.6	20.9	32.2
	DEAE-Sephadex	11.7	0.20	58.4	20.0	87.2

The cellulase isolated from A.niger was purified to 38.7% fold and that of P.chrysogenum to 87.2% fold.

Characterization of Cellulase

The effect of metal ions and EDTA on cellulase activity of A.niger and P.chrysogenum were examined. Our data show that Mn⁺⁺ ion stimulated the enzyme activity by 353.4 and 319.6 % respectively for A.niger and P.chrysogenum (table 3) (Nwodo 2007). Finally, we determined the optimum temperature, pH and the effect of substrate concentration on cellulase activity. We observed that an optimal temperature of 45°C and 50°C were obtained for A.niger and P.chrysogenum respectively. A.niger showed a broad spectrum in terms of optima pH over a range of 3.5 to 7.5 while P.chrysogenum has an optima pH of 5.0. The result on substrate concentration shows that P.chrysogenum has better affinity and higher turnover for the substrate. (Table 4).

Table 3: Effect of metal ions and EDTA on Cellulase activity of *Aspergillus niger* and *Penicillium chrysogenum*

Metal ions	<i>A.niger</i>		<i>P.chrysogenum</i>	
	Activity (%)	Inhibition (%)	Activity (%)	Inhibition (%)
Mg ²⁺	77.7	22.3	67.3	32.7
Mn ²⁺	353.4	-	319.6	-
Fe ²⁺	124.0	-	161.4	-
Ca ²⁺	74.3	25.7	92.2	7.8
Cu ²⁺	72.0	28.0	63.1	36.9
Zn ²⁺	70.6	29.4	58.9	41.1
Hg ²⁺	25.0	75.0	43.1	65.9
EDTA	28.7	71.3	20.3	79.7

Table 4: Determination of the kinetic characteristics of Cellulase of *A.niger* and *Penicillium chrysogenum*

Enzyme Characterization	<i>A.niger</i>	<i>P. chrysogenum</i>
Optimal Temperature °C	45.0	50.0
Optimal pH	3.5, 5.5, 7.5	5.0
Time course (min)	> 50.0	> 50.0
K _m (g/l)	12.5	11.8
V _{max} (μ mol min ⁻¹ mg protein ⁻¹)	4.4	10.0

Mr. Vice-Chancellor, sir, I am happy to mention that these studies were successfully carried through the dedicated efforts of Dr. Nwodo, S. Chinedu who was then a Ph. D student under my supervision in collaboration with Professor O. Omidiji of the Department of Cell Biology and Genetics. I wish to appreciate

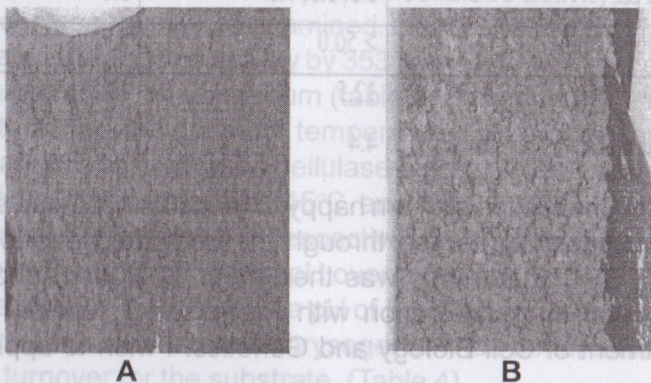
Dr. A. A Adekunle of Botany and Microbiology department who put us through in microbial culture methods and identification of the different micro fungi in the growth media. Dr. (Mrs.) H.A. Smith was also a co-supervisor before her retirement.

I am also happy to mention that some of the data from this work presented on poster at the first edition of the Annual Research Conference of the University of Lagos won a prize for my research team as Best Researcher, College of Medicine of this great institution, in 2005.

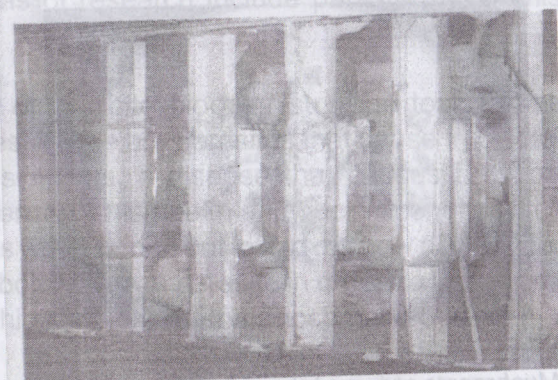
Extensive work has been going on since then. Three Ph.D students are currently working on various aspects of this project which include:

- Pectinase isolation, characterization and molecular modification for higher yield of the enzyme for industrial application.
- Isolation and characterization of a secondary metabolite with potent antibacterial and antithrombic activities using *P.chrysogenum* grown on agrowastes.

We have also used our isolated microfungi to hydrolyze some agrowastes to simple sugars which we have used to prepare feed for chicks (monogastric animals which cannot use the cellulose in the wastes directly as energy source.) figure 38.



B Experimental Birds feeding on formulated feed.



C

Figure 38: [Showing A; untreated Agrowastes, B: Agrowastes treated with microfungi, C: Chicks grown on formulated feeds from agrowastes

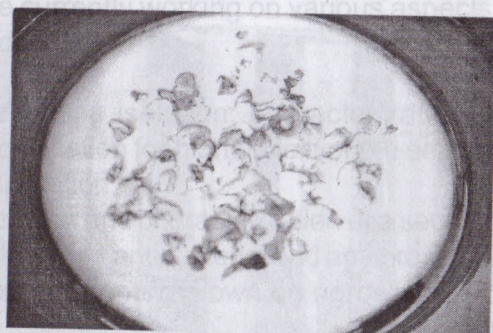
Work continues on this aspect at Ph.D level. It is our ambition to modify these wastes in order to formulate feeds for breeding special types of chicks with reduced fat content. That will be of immense benefit in terms of nutrition and health as well as making our environment more pleasant in outlook.

Furthermore we have used these agrowastes for cultivation of edible mushroom (figure 39).



A

B



C

Figure 39: Mushrooms grown on agrowastes.

What is unique about this project is that we are working on species of microfungi from the wild which gives us the opportunity to explore widely at the molecular level for the purpose of solving our local problems (environmental control and industrial application) and our methodology is adaptable to various environments in our country.

I wish to express my profound gratitude to the University Authorities for encouraging me and my research team with Research Grants from 2006/2007 session to date. Other aspects of my research activities which cannot be discussed here (because of constraints of time) include dietary lipids and enzyme

function, medicinal plants and membrane function in sickle cell disease as well as their effects on enzymes of heart and liver functions.

SUMMARY

The lecture started with the definition of biochemistry as the study of the molecular basis of life in all forms of living organisms. The cell is also defined as the basic unit of life viewed by the biochemist as a complex machine where all chemical processes that maintain life take place.

My core areas of research include parasite biochemistry and enzymology.

In the study of parasite biochemistry, the topic of interest is trypanosomes. These are parasites that cause trypanosomiasis or sleeping sickness, a disease that causes a lot of human suffering in terms of his health and socio-economic well-being. Investigations were carried out to find out how adenosine is taken up and metabolized in the parasite in order to identify metabolic targets for inhibition with antitrypanosomal drugs.

Adenosine is a component of nucleic acids that make up the gene which is essential for the parasite to carry out its metabolic activities for survival. It was of interest to understand how the parasite obtains adenosine from its host since it does not have all the requisite constituents to synthesize its own adenosine and must depend on the host for its supply.

Our studies revealed that the parasite has multiple routes of adenosine uptake and shares similar metabolic pathways for adenosine with the host. These characteristics pose serious challenges to the development of rational chemotherapy against trypanosomiasis since any drug aimed at the parasite will also affect the host. Indeed few of the drugs currently available for treatment of trypanosomiasis have problems of toxicity,

resistance and other complications. Another major challenge to this effect is the prohibitive high cost of the available drugs which puts them out of reach to the poor populations who are usually the victims of this disease. This awareness spurred me to explore our vast forest reserve of medicinal plants in search of trypanocides.

The result of this search is the identification of five medicinal plants that have active components with very promising antitrypanosomal effects viz: *Allium sativum* (garlic), *Mitragyna ciliata* (Rubiaceae, local name, "abura"), *Tetrapleura tetraptera* (aidon- local name), *Carica papaya* (pawpaw), and *Mangifera indica* (mango). Biological studies on them so far indicate that they are safe to be developed as drugs.

My second area of research is enzymology. Enzymes are defined as the proteinaceous catalysts of the biosphere characterized by efficiency, speed and accuracy in its functions. Various applications of enzymes are discussed which include clinical and industrial applications as well as uses as drugs, indicators and reagents in some specific chemical assays.

Experimental studies involve its application to drug development and biotechnology. Characteristics of newly synthesized drugs (with derived β -lactam nucleus) were defined in terms of their kinetic properties which revealed that they are mechanism-based inhibitors specific for serine protease. Mechanism-based enzyme inhibitors are also called suicide substrates because their initial interactions with their target enzymes activate them to products that inactivate the enzymes. The target serine proteases are those that are potential agents of pathological conditions leading to impairment of extra cellular organization, examples, rheumatoid arthritis, emphysema, cystic fibrosis etc. Another study in this aspect examined the differential effect of phebrool (an anti-parasitic drug against schistosomiasis) on the respiratory systems of snails (the vectors of the parasite) and those of the

mammals (experimental rats). Results revealed that the respiratory systems of the snails were more sensitive to the drug than those of the experimental animals, an evidence of its usefulness as a potential curative agent.

Finally, studies applying enzymes to biotechnology were carried out in order to give value to the heaps of agricultural and industrial wastes that constitute health hazards and an eye-sore in our environment. Pure cultures of microfungi isolated from decayed wood wastes (saw dust, corn cob, sugar cane pulp, etc) were used to produce enzymes which were purified and characterized. Using the waste materials as sole carbon sources in the culture media, cost-effective method of producing different enzymes involved in hydrolysis of the wastes to simple sugars was developed. The simple sugars so produced have many industrial applications such as animal feed production and fermentation to ethanol. The enzymes produced (cellulases, pectinases and xylanases) also have important industrial applications.

RECOMMENDATIONS

The threat to economic and social well-being of man posed by trypanosomiasis demands that greater attention be paid to its management and eradication. Though, the status of human trypanosomiasis is not clear in Nigeria, there is evidence that the animal disease is here with us because the isolates we use in our studies are obtained from the domestic flock that eventually land on our dining tables as rich protein source. Therefore, scientists should not "look the other way" because the quality of live stock produced at the agricultural level impacts on the general health and economic well-being of the populace. Besides, the disease is resurgent and continues to pose serious debilitating problems in some countries of South East Africa. Our research findings on medicinal plants point to the fact that, with concerted effort, local strategies can be put in place for cheap and sustained management of animal and human trypanosomiasis. Efficacious medicinal plant preparation can be packaged in animal feed formulation for maintenance of more viable livestock and reduction in economic loss.

The biological experiments we carried out during the screening of medicinal plants for antitrypanosomal activity have revealed the inestimable health-giving treasures locked up in plants to me. Currently, voluminous work is going on in many departments and in many universities in this country, but all are in an uncoordinated and uncollaborative manner, therefore, there is not much at hand presently to show for it. So far it is only at the Obafemi Awolowo University Ile-Ife that something concrete can be seen in terms of formulation of herbal products from our medicinal plants. The fact is that medicinal plants remain veritable, safe, sustainable and cheap sources of remedies. The problem with our local preparations is not in lack of efficacy but in lack of standardization and hygienic packaging. There lies our challenge. The benefit in herbal remedies is, according to *Encyclopedia of Medicinal Plants*, "The human body is much better suited to treatment with herbal remedies than the isolated chemical

medicines for the very reason that man has evolved with plants over the centuries, therefore, his digestive system and physiology as a whole are geared to digesting and utilizing plant-based foods, which often have medicinal values as well as providing sustenance."

Currently in industrialized countries, conventional medicines are becoming more and more ineffective owing to drug resistance, toxicity, adverse side effects and prohibitive cost of production, hence the use of herbal medicines is gaining prominence. It is well documented that in China traditional and orthodox medical practice have been integrated within the framework of official health services, thereby, taking advantage of the positive features of each. (Farnsworth, 1966).

The surprising aspect of us is our penchant for the numerous imported packaged medicinal products, for example, ginger-honey tea, when our local markets are awash with ginger and other health-giving products from which beverages can be easily prepared.

I think it is about time we look down and pick up the treasures divinely laid down at our feet instead of looking up to the sky where we can't reach easily. We have the manpower and sustainable resources to effect maximum use of our medicinal plants thereby empowering our people and conserving our scarce financial resources for other judicious uses.

Considering the many applications of enzymology in health, industries and environment, I think there is need to encourage the younger generation to develop interest in enzyme studies. This will be of benefit to national development, because producing our enzymes for the various applications will not only reduce importation costs but create more jobs.

I think we (academic) should be more generous with interdepartmental and interdisciplinary collaboration. Sometimes

we frown when more than four or five people genuinely publish a paper, but I think that the more the interaction the wider the intellectual horizon and the better the scope and quality of output for the benefit of humanity.

ACKNOWLEDGEMENTS

First and foremost, I wish to give all praise, honor and glory to God our Father for His bountiful mercies. Who else could have given me the strength for this long and difficult academic journey?

I am so much indebted to my parents, papa George Aguguo and mama Susanna Ada Ohaya, both of blessed memory. They were my first teachers in little things of life that matter. I also thank our step-mother, Mrs. Josephine Nwaobani Ohaya who passed on early this year, for her care after our mother's death.

To my brothers and sisters, I know no greater word to say than "thank you". You are simply the greatest and the best. You motivated and mentored me, the sixth child of our parents, and your love kept my spirits undaunted when the going was tough. I appreciate the love and support from my uncles, aunties, cousins, nephews and nieces.

To my in-laws, the Eziachi community based in Lagos, particularly, the Umuoba kindred (men and women) I say a big thank you for your wonderful support. I also thank the Amaigbo community present here today for the honour you have given me. Dr. Umello Oguike, I wish to use this august occasion to thank you for the love and care over the years not only for me but my family as a whole.

I thank all my teachers from the primary school to Ph.D. level, especially, Mr. Adams, Mr Akano and Mr. Emeafu, who encouraged our father, after our mother's death, never to relent in sending all his children to school.

I thank my teachers at Barat College of The Sacred Heart, Lake Forest, Illinois, whose good work enabled me make the dean's list which earned me the U.S.A National Science Foundation Fellowship for undergraduates in 1970. This gave me the opportunity to have my first experience of the goings-on in an

advanced research laboratory and I was able to undergo a research training for undergraduates at Northern Illinois University at Dekalb, Illinois.

I am indebted to Professor Nobert Tietz, who trained me further in research and triggered off my interest in the academic world and Professor E.O Akinrimisi, of the blessed memory, who continued from where he stopped. Professor Akinrimisi was able to secure some grants from The International Agency for Atomic Energy Research (African sub-region) with which my Ph.D. project was financed.

I am indebted to Professors Francois Chapeville and Hiroshi Oya who welcomed me into their laboratories (as I mentioned earlier), which enabled me gain more insights into enzyme characteristics and functions.

To my colleagues (academic, technical and secretarial staff) in the department of Biochemistry, I say "big thank you" for you have all contributed in no small way in building me up. I am particularly indebted to Professor (Mrs.) A.M. Abaelu for her invaluable moral and intellectual support while working on my Ph.D. I appreciate the collaborations with Professor G.O. Gbenle, Dr. O.A. Ebuehi and Dr. O.S. Odesanmi in the supervision of some of our Ph.D students.

I also thank sincerely other colleagues at Idi-Araba and Akoka campuses with whom I collaborated in different rewarding projects. They include Professor A.A Kazeem of Clinical Pathology Department, with whom I collaborated in supervising a Ph.D and a Master's Degree Theses and I learnt a lot more in immunology. Also Dr. (Mrs.) Joy Okpuzor of Cell Biology and Genetics, whose academic interaction with me led to family friendship. I thank her husband, Mr. P. Okpuzor for his moral support. From the same department, I wish to express my sincere thanks to Professor O.O.Omidiji, with whom I supervised another Ph.D. thesis, along with Dr. (Mrs). H.A. Smith, formerly of my

department. My appreciations also go to Drs. A.A. Adekunle of Microbiology and Botany Department and S.O.Ogbonnia of Pharmacognosy Department for invaluable contributions to my academic package.

I appreciate my students, the undergraduates, who under the auspices of the National Association of Biochemistry Students, Lagos, recognized my humble contributions to their academic advancement as their course adviser. I was given certificates for selfless and distinguished service three times. One of certificates gave me the title of "Iya ewe" which I understand to mean "the mother of school children". I feel fulfilled and thank you so much for that lovely title.

Also I am grateful for the contributions of my postgraduate students at the Master's Degree level who are too many to mention individually here. Then my postgraduate students at the Ph.D. level: Dr Nwodo Chinedu, Dr. (Mrs). Ngozi Imaga, Dr. (Mrs.) Henrietta Ogbunugafor, Mr.Uzoma Anthony Okafor, Mrs Blessing Onyegeme, Mr. M.O Babalola, Mr. T.N.Emezue and Dr. A.A.Adiega. You have all been wonderful. Thank you all.

I am greatly indebted to the authorities of the University of Lagos for the opportunities given to me to contribute to building up the future generation of our great nation. I am particularly grateful for the research grants given to me to support my academic advancement. Worthy of mention also are the awards for long and distinguished service to the University by the Authorities at the College of Medicine and the mother University at Akoka. I thank the University authorities for those recognitions.

Then, to my big brother, friend, confidant, companion and treasured husband, Dr. Ibe Agu Okochi, and our lovely children Dr. Chimezie and Emeka, I say "thank God" for putting me in your midst, a congenial home that kept my heart at peace to keep moving on with the academic struggle, no matter the odds!

Then to my beautiful daughters-in-law I say welcome to a beautiful home. You are well appreciated and I thank the families of the young ladies, the Somolu family of Abeokuta and the Iguare family of Eme-ora, Owan West Local Government area, Edo state, for the good friendship we have all shared so far.

Finally, to my wonderful audience I say thank you and may God bless you all.

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