OBAFEMI AWOLOWO UNIVERSITY, ILE-IFE, NIGERIA.

Inaugural Lecture Series 87

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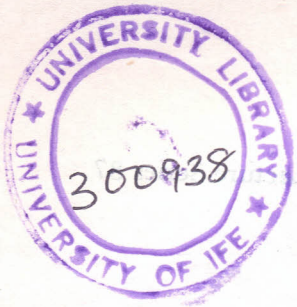

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At a stage in the life of a Professor, he is called upon to deliver an inaugural lecture. A number of things immediately come to his mind – what type of topic is he going to discuss, and more importantly, how does he make himself understandable to such an academically heterogenous audience as we have here today. With all these factors at the back of my mind, I shall attempt to discuss today's chosen topic, "man's unseen allies and their self-synthesized tools".

There is no doubt that the first question that strikes one's mind is, "what are man's unseen allies?" With respect to this lecture, I shall define man's unseen allies as those living creatures which are present in virtually every environment, but which are too small to be perceived by the unaided human eye. This group of organisms is collectively referred to as micriorganisms, and their study is called microbiology.

Microbial Diversity

Microorganisms have a wide taxonomic distribution, and on the basis of their cellular architecture, can be separated into two groups which differ from each other in many fundamental ways. The two groups of microorganisms are called procaryotes and eucaryotes. Microorganisms with the procaryotic cell structure are referred to as the lower protists and they include bacteria, mycoplasmas, rickettsiae, viruses and the blue-green algae. Micro-organisms with the eucaryotic cell structure are called the higher protists and they include fungi, protozoa and all the other algae.

A comparison of the procaryotic and eucaryotic cells is as indicated in Table 1.

The Discoveries of Microorganisms

For quite a long time on man's life, the existence of creatures which are too small to be seen with his naked eye had been suspected. For example, the crafts of food preservation and fermentation have been highly developed for many thousands of years. Early Egyptian records contained the various steps involved in the brewing of beer

and wine. From the Egyptian documents, it was clear that it was necessary to exclude air from the secondary fermentation. In the making of leavened bread, the principle of using a starter culture was a standard practice. The Egyptians also recognized the use of the deposit from fermented beer as an agent for the raising of dough. Thus there is no doubt that the ancient Egyptians were aware of the existence of the agent that produced the afore-mentioned processes, but they were ignorant of the nature of such an agent. In fact, among the ancient Egyptians, numerous mysterious beliefs surrounded the nature of the agent.

The use of microorganisms to produce milk products such as cheese and sour milk drinks such as yoghurt and kamous no doubt goes back to the time of the neolithic agricultural revolution when man first domesticated grazing animals and began to tend them in herbs. The methods of preserving foodstuffs by subjecting them to heat so as to dry them, or through salting and dehydrating by immersing them in strong salt or sugar solutions, seem to have come quite early in the neolithic period, as soon as man had surplus foodstuffs sufficient to harvest.

The birth of microbiology occurred in 1674 when Anton van Leeuwenhoek used microscopes of his own construction to examine a drop of lake water. What Leeuwenhoek examined through the magnifying glasses of his microscope was undoubtedly one of the most startling and amazing sights that man has ever beheld. It was indeed the first look at organisms that we now know as microorganisms. Anton van Leeuwenhock (1632 - 1723) was a merchant in the small town of Delft. He had little informal education and did not at any time attend any higher institution or University. However, his work became widely known in his own lifetime through a series of letters to the Royal Society of London each containing descriptions of his important discoveries. For example, in one of such letters describing for the first time the characteristics of bacteria of the human mouth, he wrote as follows, "I have had several gentle women in my

Table 1. **COMPARISONS OF THE PROCARYOTIC AND EUCARYOTIC CELLS**

		Procaryotes	*Eucaryotes*
1.	Cell wall	Chemically complex cell wall structure (Peptidoglycan)	Where present composed of simple organic or inorganic materials
2.	Composition of cell membranes	Lack sterols	Contain sterols
3.	Cytoplasmic moment	Cytoplasmic streaming rare or absent	Cytoplasmic streaming often occurs
4.	DNA arrangement	Single molecule, not complexed with histones	In several or many chromosomes, usually complexed with histones
5.	Flagella	Submicroscopic size, each flagellum composed of one fibril of molecular dimensions	Microscopic size, each flagellum composed of 20 fibrils in a distinct pattern, 2 x 9 + 2
6.	Asexual reproduction	No mitosis	Mitosis present.
7.	Nuclear body	No nuclear membrane	The nuclear membrane.
8.	Photosynthetic apparatus	Photosynthetic apparatus in organized internal membranes: chloroplasts absent.	Present in membrane-bound organeles, the chloroplasts

Table 1 (CONTD.)

	Procaryotes	*Eucaryotes*
9. Respiratory system	Respiratory system part of plasma membrane or of mesosome. mitochondria absent	Present in membrane-bound organelles, the mitochondria
10. Ribosome size	70 S	80 S, except for ribosomes of mitochondria and chloroplasts which are 70 S
11. Sexual reproduction	Fragmentary process; no meiosis	Regular process, meiosis
12. Vacuoles	Rarely present	Often present.

house, who were keen on seeing the little eels in vinegar: but some of them were so disgusted at the spectacle, that they vowed they'd never use vinegar again. But what if one should tell such people in future that there are more animals living in the scum on the teeth in a man's mouth, than there are men in a whole kingdom? "

Van Leeuwenhoek left no descriptions of the apparatus with which he observed protozoa and bacteria. He kept, for himself alone, the best micriscopes and his technique of using them to observe the 'animacules'. His prized lenses as well as his jealously guarded techniques enabled him to surpass all other microscopists for not less than a century.

Although Leeuwenhoek's contemporaries marvelled at his scientific discoveries, the microscopic studies of micro-organisms which he had so brilliantly started was not appreciably extended for over a century after his death. In fact micro-biology as a science did not develop until late in the nineteenth century. This long delay was due to the fact that certain basic techniques for the study of microorganisms required some time to be fully devised. In the nineteenth century, scientists were faced with two perplexing questions which had to be solved if the science of microbiology was to develop. These questions and their solutions thereof finally laid the foundation of microbiological science. These puzzling questions are as follows:

1. Does spontaneous generation occur?
2. What is the nature of contagious disease?

Investigation of both questions went hand in hand, and sometimes the same people worked on both. By the end of the century, clues to both questions were found, and the science of microbiology became firmly established as a distinct and important field.

The Controversy over Spontaneous Generation

After Leeuwenhoek had revealed the occurrence and abundance of a variety of microscopic organisms in nature, scientists were now faced with the problem of elucidating the

origin of these creatures. From the beginning there were two schools of thought. Some workers held the belief that the microscopic organisms discovered by Leeuwenhoek must have arisen spontaneously in nature. However, other workers, including Leeuwenhoek himself, believed that such creatures were formed from the 'seeds' or 'germs' of these microscopic creatures, which are always present in the air. The belief in the spontaneous formation of living organisms from non-living matter is known as the doctrine of spontaneous generation (or abiogenesis), and this belief has long been in existence. For example, in the ancient times, it was considered self-evident that many plants and animals can be generated spontaneously under special conditions. The doctrine of spontaneous generation was accepted without question until the Renaissance.

As man's knowledge of living organisms increased, it became quite evident that the spontaneous generation of plants and animals simply does not occur. It became impossible to support the idea for plants and animals. A decisive step in the abandonment of the doctrine as applied to animals took place as a result of the series of experiments carried out about 1665 by an Italian physician, Francesco Redi. He demonstrated beyond reasonable doubt that the maggots that develop in putrefying meat are the larval stages of flies and do not occur if such meat is protected by placing it in a container closed with fine gauze, such that flies are unable to deposit their eggs on the meat. By such a chain of brilliant experiments, Redi destroyed the earlier erroneous belief that maggots found in putrefying meat develop there spontaneously. Consequently, the doctrine of spontaneous generation was already being greatly weakened by exact studies on the development and life cycles of plants and animals at the time when Leeuwenhoek discovered the existence of microorganisms. However, for technical reasons, it is far more difficult to show that microorganisms are not generated spontaneously, and so as time went on, the proponents of the doctrine came to centre their claims more and more on the mysterious appearance of these simplest

forms of life in organic infusions. Thus it became increasingly difficult for those who did not believe in spontaneous generation to prove their case. In fact it was not until the middle of the nineteenth century that the cumulative negative evidence became sufficiently abundant to lead to the general abandonment of the ancient but erroneous doctrine of spontaneous generation.

The Italian naturalist, Lazzaro Spallanzani, was one of the first workers to provide strong evidence that microorganisms do not arise spontaneously in organic infusions. Spallanzani carried out a series of experiments on this problem in the middle of the eighteenth century. He was able to show repeatedly that heating is capable of preventing the appearance of microorganisms in infusions. Judging from the results of his experiments, Spallanzani concluded that microorganisms can be carried into infusions by air and that this is the explanation for their supposed spontaneous generation in well-heated infusions.

There is no doubt that one of the most powerful opponents of spontaneous generation was the French Chemist, Louis Pasteur (1822-1855). His work was extremely interesting, and proved conclusively that there were structures normally present in the air that very closely resembled the microorganisms which usually occur in putrefying materials. Louis Pasteur discovered that the ordinary air contains a number of organized solid structures which resemble fungal spores, protozoan cysts and several other microbial cells. Since these organized bodies present in the air were identical to those microorganisms found in much larger numbers in putrefying materials, Pasteur concluded that the organisms found in putrefying materials orginated from the organized bodies present in the air which are constantly being deposited on all objects. If this conclusion was correct, it would mean that if a food sample or organic infusion was so treated as to destroy all the living organisms contaminating it, then it should not putrefy.

In order to eliminate contaminants, Pasteur employed

heat, since it had already been proved that heat effectively killed living organisms. Also during this period, many other workers showed that if an organic infusion was sealed in a glass flask and heated to boiling, it never putrefied. However, the proponents of spontaneous generation criticized such experiments by explaining that fresh air was necessary for spontaneous generation to occur, and that the air itself inside the sealed flask was affected, in one way or the other, by heating, so that it would no longer support spontaneous generation. In a brilliant manner, Pasteur was able to overcome this objection by constructing a swan-neck flask, now called 'Pasteur flask'. In such a flask, putrefying materials can be heated to boiling. After cooling, air can re enter the Pasteur flasks although the bends in the neck prevent particulate matter such as bacteria or other micro-organisms from re-entering. The materials sterilized in such a flask did not putrefy, and no microorganisms ever appeared as long as the neck of the flask remained intact. However, if the neck is broken, putre-faction occurs and the nutrient content of the flask becomes full of living microorganisms of all kinds. This simple but brilliant experiment of Pasteur effectively settled the controversy of spontaneous generation.

Killing all bacteria or germs is a process we now call 'sterilization', and the procedures that Pasteur and other workers used eventually formed the basis of sterilization techniques in microbiological research. Thus, the study of spontaneous generation led to the development of effective sterilization techniques, without which it would have been impossible for microbiology as a science to develop and become properly established.

Subsequent workers were able to show that test tubes, flasks and other vessels could be protected from contamination by cotton wool plugs or stoppers which still permit the exchange of air. The principles of aseptic technique, developed so effectively by Pasteur, are the first procedures usually learned by the novice microbiologist. Food science and the food industry owe a debt to Pasteur since his principles are applied in the canning and preservation of many foods as well

as in the sterilization (pasteurization) of milk and many milk products.

Another important scientist who played a prominent role in disproving the doctrine of spontaneous generation is the Egnlish physicist, John Tyndall. Judging from the results of his experiments on a variety of infusions, he concluded that bacteria have at least two phases, one relatively thermolabile (i.e. destroyed by boiling for 5 min), and one thermoresistant to an almost incredible extent. These conclusions were almost immediately confirmed by the German Botanist, Ferdinand Cohn, who demonstrated that the hay bacteria can produce microscopically distinguishable resting bodies, the endospores which are highly heat resistant.

Thereafter, Tyndall began to develop a method of sterilization by discontinuous heating which could kill all bacteria in infusions. This method is now referred to as tyndallization. The principle of the technique was simple and efficient. Since growing bacteria are easily killed by brief boiling, all that is necessary is to allow the infusion to stand for a certain period before applying heat so as to enable the germinaation of the spores with a consequent loss of their heat resistance. A very brief period of boiling can then be used, and repeated, if need be, several times at intervals to catch any spores late in germination. Tyndall discovered that discontinuous boiling for 1 min on five successive occasions would make an infusion sterile, whereas a single continuous boiling for as much as a 1 hour period would not.

With the application of Tyndall's findings, coupled with the findings of Pasteur and other similar workers, the scientific world acknowledged the demise of the doctrine of spontaneous generation.

The Germ Theory of Disease

Right from the ancient times, man had begun to suspect that certain 'agents' or 'factors' could cause disease, but the nature of such agents or factors was unknown to him. For example, as far back as in the sixteenth century, it was

thought that something could be transmitted from a diseased person to a healthy person to induce in the latter the disease of the former. Many diseases appeared to spread from one population to another and were called 'contagious', and the 'agent' which did the spreading was called the 'contagion'. Soon after the discovery of microorganisms by Leeuwenhoek it became widely held that these organisms might be responsible for contagious diseases, although there was as yet no proof to support the idea. In 1845, M. J. Berkeley provided the first clear demonstration that microorganisms caused diseases by showing that a fungus was responsible for irish potato blight. Discoveries by Ignaz Semmelweis and Joseph Lister provided some evidence for the importance of microorganisms in causing human disease, but conclusive evidence was not provided until 1876 when Robert Koch proved the etiological role of bacteria in the anthrax disease of cattle. Anthrax is caused by a spore-forming bacterium now called *Bacillus anthracis*. Koch established, by careful microscopy, that the bacteria were always present in the blood of an animal that had the disease. However, Koch was also aware that the mere association of the bacterium with the disease did not prove unequivocally that it caused the disease, and that it might in fact be a result of the disease. Therefore, Koch demonstrated that if a small amount of blood was taken from the diseased animal and injected into an apparently healthy animal, the healthy animal also developed the disease and died. By repeating this process many times, each time transferring small amounts of blood containing bacteria from one animal to another, he proved that the bacteria actually caused anthrax. Each subsequent animal died just as rapidly as the first, and in each case Koch was able to demonstrate by microscopy that the blood of each dying animal contained large numbers of the bacterium.

Koch was able to carry these experiments further. He found that the bacteria could also be cultivated in nutrient fluids outside the animal body Koch also found that even after many transfers in culture, the bacteria could still cause the disease when re-inoculated into an animal. Bacteria from

a diseased animal and bacteria in culture broth were found to induce the same disease symptoms upon injection. On the basis of these and other experiments, Koch formulated the following criteria for proving that a specific type of bacterium causes a specific disease. These criteria, now commonly referred to as "Koch's postulates", are as follows:

1. The organism should always be found in animals suffering from the disease and should not be present in healthy individuals.
2. The organisms must be cultivated in pure culture away from the animal body.
3. Such a culture, when inoculated into susceptible animals, should initiate the chracteristic disease symptoms.
4. The organism should be re-isolated from these experimental animals and cultured again in the laboratory, after which it should still be the same as the original organism.

Koch's postulates helped to demonstrate the fact that specific organisms are responsible for specific diseases, and also provided a tremendous impetus for the development of the science of microbiology by emphasizing laboratory culture techniques. Following Koch's discoverires, the causal agents of a large number of contagious disease were discovered and isolated. This led to the development of successful treatments for the prevention and care of contagious diseases, and contributed tremendously to the development of modern medical practice.

However, it is also necessary to realise that not all diseases are caused by microorganisms. For example, a number of diseases are inherited or are due to deficiencies in diet, and to other deleterious influences of the environment. Thus, the microorganism is only one factor in the disease, and although it is a necessary cause, it is not sufficient in itself. To produce disease, the microorganism must infect a sensitive host, and not all hosts are equally susceptible. The outcome of an infection is greatly influenced by the state of health of the host, its general vigour and the presence or absence of

specific immunity.

From the discussion so far, it is easy to get the idea that, because some microorganisms cause diseases, all microorganisms are harmful. This is far from the truth as most microorganisms are probably beneficial to man, or are at least harmless, and it is only the rare organisms that cause disease.

Enzymes

So far I have discussed very briefly some of the characteristics of "man's invisible allies' and how they were discovered. It therefore appears appropriate at this time to discuss the second aspect of today's topic, i.e. "tools synthesized by man's unseen allies".

According to *Webster New World Dictionary* (1975 edition), a tool may be defined as 'anything that serves as a means to get soemthing done'. For the sake of this lecture, the term 'tools' refers to those biological catalysts which enable microorganisms to carry out the basic functions of life necessary for their existence, or in other words 'to carry out metabolic activities' These biological catalysts are called 'enzymes'. The catalytic activity of enzymes is extraordinary, not only in that they are far more efficient than any other catalysts so far studied, but also in that many of them exhibit exceptional properties of specificity. Enzymes derive their greatest importance from the fact that life is intimately bound up with enzyme catalysts.

The Origin of the Term 'Enzyme'

Although the phenomenon of fermentation and digestion had long been known, what was probably the first clear recognition of an enzyme was made by Payen and Persoz in 1883, when they discovered that an alcohol precipitate of malt extract contained a thermolabile substance which converted starch into sugar. The substance was then termed diastase because of its ability to separate soluble dextrin from the insoluble envelopes of the starch grains. Subsequently

the name diastase came to be used as a general term for enzymes. However in 1898, Ducłaux proposed the use of the last three letters of this name (diastase) as a suffix '-ase' to be attached to a root indicating the nature of the substance on which the enzyme acts. This provided a basis for the systematic nomenclature of enzymes, which is still in use. However, a few names ending in 'in' had earlier been given to the digestive enzymes and such names have persisted till today. As more work on enzymes, continued, more enzymes were known, and it thus became necessary to indicate in the name not only the nature of the substance acted upon (i.e. substrate), but also the nature of the reaction, although the suffix '-ase' has been retained, e.g. lactate dehydrogenase.

When enzymes were first discovered, many workers observed that there was a sort of similarity between enzyme action and the action of yeasts in fermentation. The name 'ferment' was therefore used to denote enzymes. During the second half of the nineteenth century, there was much controversy over the views of two great workers, Liebig and Pasteur. While Liebig believed that fermentation and similar processes were due to the action of chemical substances, Pasteur held the view that fermentation was inseparable from living cells. The names 'unorganized ferments' and 'organized ferments' were therefore used to denote what we may now call extracted enzymes and microorganisms respectively. In 1878, and in order to avoid these unsatisfactory names, Kuhne introduced the term 'enzyme' to replace 'ferment'. However, the Pausteur-Liebig controversy came to an end when Buchner succeeded in obtaining the fermentation system from yeast in a cell-free extract, although even up to the present time, the name 'ferments' for enzymes has persisted in some parts of the world, especially Germany.

Enzyme Structure

An enzyme may be pictured as a protein containing a special structure, the active centre, with sometimes an additional group, the prosthetic group, attached. The subject

of enzyme structure therefore includes:

(1) the structure of the protein;
(2) the nature of the active centre;
(3) structure of the prothetic group.

Structure of Protein

There are 20 α–amino acids commonly found in proteins, and are referred to as standard amino acids. These amino acids are alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, methionine, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine and histidine. All the amino acids, except proline have as common denominators, a free carboxyl group and a free unsubstituted amino group on the α–carbon atom. The amino acids differ from each other in the structure of their distinctive side chains, called the R groups. Amino acids may be classified on the basis of their R groups into four main classes as follows:

(1) non-polar or hydrophobic R groups;
(2) neutral, uncharged polar R group;
(3) positively charged R groups and;
(4) negatively charged R groups;

Besides the 20 standard amino acids, several other amino acids of relatively rare occurrence have been isolated from hydrolyzates of some specialized types of proteins. All are derivates of some standard amino acids. Such rare amino acids include hydroxylysine, desmosine and isodesmosine.

In addition to the 20 common and several rare amino acids of proteins, over 150 other amino acids are known to occur biologically in free or combined form, but never in proteins. Such amino acids include homocysteine, homoserine, citrulline and ornithine.

In its native state, each type of protein molecule has a characteristic three dimensional shape referred to as its confornation. Depending on their conformation, proteins may be either fibrous or globular. The fibrous proteins consist of polypeptide chains arranged in parallel along a

single axis to yield long fibres or sheets. In globular proteins, on the other hand, the polypeptide chains are tightly folded into compact spherical or globular shapes. Of the nearly 2,000 different enzymes known to date, nearly all are globular proteins.

The levels of protein structure may be primary, secondary, tertiary or quaternary. Primary structure refers to the covalent backbone of the polypeptide chain and the sequence of its amino acid residues. Although the primary structure of almost all intracellular proteins consists of linear polypeptide chains, many extracellular proteins contain covalent -S-S- crossbridges from having two cysteine resides linked by their thiols. In the secondary structure, the peptide chain twists into a helix in which the carboxyl from one turn forms a hydrogen bond with the amino group of the turn below. The tertiary structure is achieved by the helix folding into a globular configuration. Several globular subunits combine to form larger aggregates that repre nt the completed and functional enzyme with the quaternary structure. Most of these larger proteins contain two or more polypeptide chains, between which there are usually no covalent linkages.

Enzyme Cofactors

A number of enzymes depend for activity on their structure as proteins while others also require one or more non-protein components called cofactors. The cofactor may be a metal ion or an organic molecule called a coenzyme. There are enzymes which require both types of cofactors. The enzyme-cofactor complex which is catalytically active is referred to as a holoenzyme. If the cofactor moiety is removed, the catalytically inactive protein which is left is called an apoenzyme.

Metal ions commonly required by enzymes include Zn^{++}, Ca^{++}, Mg^{++}, K^{+} and Na^{+}. Coenzymes include nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate (NADP), Coenzyme O, Coenzyme A, biocytin and flavin adenine dinucleotide (FAD).

When the coenzyme is very tightly bound to the enzyme molecule, it is usually called a prosthetic group, e.g. the biocytin group of acetyl CoA carboxylase, which is covalently incorporated in the polypeptide chain.

Active Centre and Enzyme Action

(a) *Active Centre:* The knowledge of the active centre of enzymes, as distinct from that of the prosthetic group, is scanty, and it is usually very difficult to give a complete picture of an active centre. The active centre may be defined as that special part of the protein strucutre which combines with the substrate and is responsible for the enzymatic properties of the molecule. The active centre determines not only the catalytic activity but also the specificity of the molecule. The active centre is usually of a complex structure, adapted to a fairly close fitting of either the substrate molecule or of those parts of the substrate molecule which are concerned in the reaction. The general nature of the fit and the facts of specificity show that there must exist a number of different groups in the active centre capable of combining with different parts of the substrate.

(b) *Enzyme Activity.* An essential characteristic of the enzymic reaction is that the first step in the process is the association of the enzyme and the substrate into an enzyme-substrate complex. The formation of this complex is reversible, depending on an equilibrium constant, Ks. The binding between enzyme and substrate is generally non-covalent, involving ionic and non-polar forces in addition to hydrogen bonds. These binding forces also, to some extent, reflect the specificity of the enzyme protein. For example, chymotripsin possesses a non-polar region which is available at the active site to accommodate the aromatic side-chain of the substrate.

However it is now well known that substrate specificity is due not only to the complementarity between the enzyme and the substrate but also to some other factors. This prompted Koshland (1959) to propose the 'induced-fit'

theory. This theory implies that the binding of substrate to the enzyme might cause a conformational change in the enzyme and bring about a delicately-controlled geometrrical arrangement of the catalytic site. By the induced-fit hypothesis, it is assumed that the essential functional groups on the active site of the free enzyme are not in their optimal positions for promoting catalysis when the active site is unoccupied by the substrate, but as soon as the substrate molecule is bound by the enzyme, the binding affinity forces the active site (postulated to be flexible) to adjust its conformation to that of the substrate, i.e. there is an 'induced-fit'.

There are several different views about the significance of these conformation differences between the 'free' and the 'combined' enzyme. One of such views, 'the strain theory', is the classical concept of Haldane and Pauline (Fersht, 1977). By this theory, it is assumed that the enzyme has an active site which is complementary, not to the structure of the substrate itself, but to the transition state of the substrate. On binding, the substrate is 'strained' or distorted. In Haldane's words, 'using Fischer's lock and key simile, the key does not fit the lock perfectly but exercises a certain strain on it. Thus the binding site on the enzyme stretches the substrate towards products and simultaneously compresses the products towards the structure of the substrate.

Rationale for Microbial Synthesis of Enzymes

For a microorganism to be alive, it must be able to carry out metabolic activities. In this regard, metabolism may be defined as the totality of chemical reactions which occur in living cells. By means of these reactions, energy is extracted from the environment and expended for biosynthesis, growth and such secondary activities as motility, luminescence and heat. Energy may be obtained from the environment in the form of light (photosynthesis) or by the oxidation of chemical light (chemosynthesis). In both photosynthetic and chemosynthetic organisms, the pathways by means of which

the acquired energy is used for cell synthesis are similar and are collectively known as anabolism. Conversely, the energy-liberating pathways involved in the breakdown of chemical substances are called catabolism.

All these processes are made possible through the action of hundreds of different enzymes. These enzymes allow the cell to carry out its reactions at relatively low temperatures. In a chemical laboratory, these same reactions cannot be carried out without employing considerably higher temperatures. One fantastic aspect of cell metabolism is the unbelievable speed with which cell components are synthesized. For example, a single *Escherichia coli* cell, dividing every hour, synthesizes 4,000 molecules of lipid, almost 1,000 protein molecules (each containing about 300 amino acids), and 4 molecules of ribonucleic acid (RNA) per second.

Besides producing the anabolic and catabolic pathway enzymes (e.g. aldolase, transketolase, aconitase, phosphoribulokinase and ribulose diphosphate carboxylase). Stainer *et al.*, 1976), microorganisms also synthesize a variety of other enzymes, some of which may be associated with pathogenicity. Many phytopathogens produce a series of enzymes which aid in hydrolysing the cell components of the host tissue (Jawetz *et al.*, 1974; Spalding, 1963). Prominent among such enzymes are pectinases (Wood, 1960), cellulases (Hunter and Elkan, 1975), and proteases (Reddy *et al.*, 1971) which aid in breaking down the pectic, cellulosic and proteinous components of the host tissue respectively. Many bacteria pathogenic to man secret some specific enzymes which aid in their infection processes. These include collagenase, coagulase, hyaluronase and streptokinase.

Collagenase is responsible for the disintegration of collagen and this helps to promote the spread of pathogenic bacilli. Coagulase contributes to the formation of fibrin walls around staphylococcal lesions which protect the organisms from the defences of the body and aid in their persistence. Coagulase also causes a deposit of fibrin on the surface of individual staphylococci. which may protect them from phagocytosis,

or from destruction within phagocytic cells. Hyaluronidase is an enzyme capable of hydrolyzing hyaluronic acid, a constituent of the ground substance of connective tissue. This enzyme is produced by many microorganisms which aid in their spread through tissues. Many haemolytic streptococci produce the enzyme streptokinase which activates a proteolytic ensyme of the plasma (plasminogen -> plasma) This enzyme (also called fibrinolysin) is then able to dissolve coagulated plasma and probably aids in the spread of streptococci through tissues.

Synthesis of Enzymes

The synthesis of enzymes in living cells is under two different kinds of control. In the first place, their production, like that of protein in general, is under genetic control, and a given enzyme can only be formed if the corresponding gene is present in the cell. However, if the gene is absent or damaged (e.g. by a mutation), the enzyme will also be lacking. Thus, the system of genes (genotype) of the cell fixes its enzyme-forming potentialities; the genes act by producing enzymes, and they contain the plan of the cell's metabolism. Thus, the cell can not produce an unlimited variety of different enzymes, which would lead to chaos, but only those which fall within the range of the genotype.

In the second place, the production of many enzymes is strongly influenced by the presence of metabolites. The mere presence of a gene does not guarantee that the corresponding active enzyme will be produced in significant amounts; the presence of an 'inducer' may also be required, and may in many cases, cause a considerable increase in the amount of the corresponding enzyme.

The Biochemical Basis of Regulation

There are two different regulatory mechanisms in the microbial cell: the regulation of enzyme activity, and the regulation of enzyme synthesis. Both are mediated by compounds of low molecular weight, which are either formed in

the cell as intermediary metabolites or enter it from the environment. Both regulatory mechanisms involve the operation of a special class of proteins, called allosteric proteins. Allosteric proteins are proteins whose properties change if certain specific small molecules, referred to as 'efectors', are bound to them.

Regulation of enzyme activity

The genome controls the enzymic composition of a microorganism in that an organism can synthesize only those enzymes for which it has appropriate genes. However, if a microorganism is to respond rapidly to changes in the chemical and physical properties of the environment, it is clearly essential for it to possess mechanisms by which the activity of enzymes can be regulated so that the reactions which they catalyse produce just a sufficient amount of the various metabolic end-products.

The most extensively studied of the mechanisms of regulating enzyme activity is end-product inhibition. End-product inhibition is a process that microorganisms use to prevent overproduction of low molecular weight intermediates such as amino acids, purine and pyrimidine nucleotides. If a metabolic product, D, is synthesized through a series of reactions in which A is converted to B, B to C, and C to D, then by a process of feedback inhibition, the end-product D can inhibit the action of enzymes that catalyse one (or sometimes more) of the earlier reactions in the sequence, and thereby prevent overproduction of D.

One of the first examples of end-product inhibition was discovered during work on the pathway used by *Escherichia coli* for synthesizing isoleucine. Initially, it was shown that threonine was an intermediate on the pathway to isoleucine, since a mutant strain of the bacterium that was auxotrophic for threonine was found to utilize exogenous threonine to synthesize isoleucine. When this mutant was grown in a medium containing isoleucine, threonine was less efficiently utilized than in a medium lacking isoleucine. A possible

explanation of this sparing effect was that isolevcine was inhibiting the action of the enzyme that catalyses the first reaction in the conversion of threonine to isolencine, namely threonine hydrolyase. When cell-free extracts of the mutant bacterium were examined, it was indeed found that the action of threonine hydrolyase was inhibited by isoleucine.

Experiments have also shown that the activity of aspartic transcarbamylase (ATCase), which catalyses the first reaction in the pathway of pyrimidine hiosynthesis, is inhibited by cystidine triphosphate (CTP) which is an end-product of the pathway. It has been shown that elevated intracellular concentrations of CTP inhibit the functioning of ATCase, and consequently the formation of more CTP until its concentration deceases to an optimal level. ATP, a second effector of ATCase, activates the enzyme and thus serves to co-ordinate the synthesis of purine and pyrimidine nucleotides.

Regulation of enzyme synthesis

Some enzymes are synthesized by microorganisms irrespective of the chemical composition of the environment. These are referred to as constitutive enzymes. Other enzymes are synthesized only in response to the presence in the environment of an inducer and are called inductive enzymes. The inducer is usually the substrate for the enzyme or some structurally related compound. When an inducer is present in the environment, not one but a number of inducible enzymes may be formed by the microorganism. Although the end-product inhibition provides a rapid and sensitive mechanism for preventing the overproduction of low molecular weight compounds by microorganisms, it is fundamentally an inefficient mechanism in that enzymes are still synthesized but are only prevented from functioning.

It has been shown, however, that in many microorganisms, there exists another more efficient regulatory mechanism which acts by stopping the synthesis of enzymes in response to the presence of certain concentrations of low molecular

weight compounds. This process is known as end-product repression of enzyme synthesis. Compared with end-product inhibition, it is a less rapid mechanism for preventing the overproduction of low molecular weight compounds, but it has the advantage that it helps to conserve cell protein by ensuring that microorganisms stop synthesizing those enzymes which are no longer required by them.

Proposed mechanism for induction and repression of enzyme synthesis

(a) *End-product inhibition:* The fact that the synthesis of enzymes of quite different catalytic activities is frequently co-induced and co-repressed to the same extent by a low molecular weight compound suggests that the element controlling synthesis of the enzymes on a pathway is not represented by the structural genes themselves. This prompted Jacob and Monod (1961) to propose a mechanism in which it is postulated that in a microorganism, there are separate 'regulator genes' responsible for the production of 'repressor substances', each of which acts specifically on a system for synthesizing a group of enzymes. Genetic studies and experiments using metabolic inhibitors suggest that repressor substances are proteins. It is believed that the repressor substances act directly on the genes. In their theory, Jacob and Monod suggested that the site of action of the repressor substance is a single gene known as the operator gene which controls the transcription of structual genes. The operator and structural gene complex is known as operon.

Repression of enzyme synthesis can then be explained by assuming that the repressor substance is an allosteric protein with at least two recognition sites, one of which combines with the operator gene and the other with co-repressor (such as the end-product of a reaction sequence). When the co-repressor combines with the repressor protein, it is thought to produce a

conformational change in the protein, with the result that the repressor substance has a greater affinity for the operator gene at the other recognition site. Blocking of the operator gene then prevents transcription of the adjacent structural genes. Inducer compounds (such as the substrate for the enzyme) are also thought to combine with the repressor substance. As a result of this combination, conformational changes are produced in the repressor substance, resulting in a decrease in the affinity of the repressor substance for the operator gene. Thus, the operator gene becomes free to initiate transcription of the structual genes.

(b) *Catabolite Repression:* A less specific type of enzyme repression is that called catabolite repression. In this phenomenon, the syntheses of a variety of unrelated enzymes are inhibited when cells grow in a medium that contains a carbohydrate such as glucose as the main energy source. One consequence of catabolite repression is that it can lead to the so-called diauxic growth. This phenomenon was first discovered by J. Monod (Stanier *et al.*, 1979) who observed that *Escherichia coli* in a medium containing glucose and lactose underwent two distinct growth cycles, characteriszed by two exponential phases of growth separated by a distinct lag phase. Glucose is utilized during the first growth cycle and lactose is utilized during the second. The enzymes necessary for the metabolism of lactose are not synthesized (even though the inducer is always present) until the glucose in the medium has been exhausted. While glucose is being metabolized, the induction of β-galactosidase and galactoside permease is prevented. Subsequent work has shown that all rapidly metabolizable energy sources (and not glucose alone) repress the formation of enzymes necessary for the dissimilation of energy sources that are more slowly attacked, and the phenomenon is known as catabolite repression.

Some Uses of Microbial Enzymes

With the discovery of the microbial world, man began to utilize not only the microorganisms, but also their metabolic products for his needs, especially in the industry and the field of medicine.

(a) *Industrial Application*

The microbial enzymes most used so far include amylases, proteases, pectinases and cellulases. A major use of microbial amylase is in starch saccharification, distilling and wine industry (Stentebjerg-Olesen, 1971; Lambert & Meers, 1983). Proteases are employed in the industrial manufacture of cheese (Keay, 1971; Sizler, 1964). By far the greatest development in the use of microbial proteases has been the introduction of enzyme-containing detergents where alkaline proteases are employed. Other potential markets for microbial protease is in toothpaste or mouth-wash. The main use of pectinase is in the clearing of fruit juices by hydrolysing any pectic polymers which are liable to course cloudiness; they are also useful in the wine industry (Lambert & Meers, 1983). Cellulases are being used extensively to produce concentrations of glucose as high as 30% from cellulosic materials. Cellulases are also employed in the extraction of essential oils and flavouring materials, tenderization of fruits and vegetables and clarification of fruit juices.

(b) *Medical Application*

Many enzymes from a variety of sources are now used as diagnostic reagents in clinical chemistry for the estimation of, in particular, metabolites in blood or urine. Such metabolites as glucose, triglycerides and cholesterol are routinely measured in many clinical laboratories, employing enzyme systems or kits to effect the analysis. Glucose analysis can be performed by using several different enzyme systems, including direct measurement employing glucose dehydrogenase or glucose oxidase, or in coupled systems with hexokinase.

Glycerokinase is widely used in the determination of seerum triglyceride levels. Concentrations of triglycerides outside the normal range have important clinical significance in the idagnosis of lipid disorders and arteriosclerosis.

Many species of bacteria produce enzymes that inactivate or degrade antibiotics, the genes coding for the synthesis of these enzymes being carried mainly on resistance plamids (Broda, 1979). Both chloramphenicol acetyl transferase and gentamycin acetyl transferase, which modify their respective antibiotic substrates by acetylation (by using acetyl CoA as a source of acetyl groups) have been used to determine chloramphenicol and gentamycin concentrations respectively in plasma and urine.

The antibiotic-degrading β–lactamases are specifically able to hydrolyse penicillin and cephalosporin and are therefore used to determine the concentrations of both antibiotics in clinical plasma and urine. β–lactamases are also used in the sterility testing of sensitive antibiotic preparations.

Summary of my Research Activities

First, I must express my sincere gratitude to this University for being generous enough to award me grants over the past twelve years. With these substantial grants, I was able to set-up, what I will honestly term one of the best microbial physiology laboratories in the country comparing favourably with similar laboratories in other parts of the world. It is equipped with facilities for carrying out research work in biochemical, industrial, medical and general aspects of microbiology. Over the years, the laboratory has also been made available to researchers from other universities.

I also wish to seize this occasion to sincerely thank Mr. O. O. Cole, a Chief Technologist in the Department of Microbiology, who contributed immensely to the success of my research work.

My research activities may be summarised as follows:

(a) *Environmental Factors Affecting Microbial Life*

The environmental factors which affect the activities of microorganisms may be grouped into two broad categories, namely, chemical and physical.

A chemical compound may be beneficial to an organism, or in other words, it may act as a nutrient. On the other hand, a chemical may have an adverse effect on the organism and so acts as an antimicrobial agent. Sometimes, a microorganism may be completely indifferent to the presence of the compound, e.g. agar and silica gel which are often used to solidify nutrient media. However, microorganisms differ widely in their reaction to a particular compound, such that a chemical compound which serves as a nutrient to one organism may act as an antimicrobial agent to another organism.

Chemical compounds which I have worked upon so far include a variety of carbohydrates and nitrogen compounds. The results show that virtually all the microorganisms tested were able to utilize many types of carbohydrates for grwoth and reproduction (Olutiola & Cole, 1977a; Olutiola & Nwaogwugwu, 1982; Olutiola & Ayres, 1973a). During metabolism of the carbohydrates, a number of other carbohydrates were synthesized in the microbial cell and these include mannose, arabitol, mannitol, myoinositol, ribose trehalose, rhamnose and fructose (Olutiola & Cole, 1977b; Olutiola & Okonkwo, 1982; Ayres & Olutiola, 1973; Olutiola, 1976a). The uptake mechanism in some of these microorganisms was also investigated. For example, the uptake of galactose in *Ceratocystis paradoxa* obeyed the Michaelis – Menten principle, with an apparent Km of approximately 33 mM and a Vmax of 0.25 μmol/min/mg dry wt (Olutiola, 1983a). Uptake of galactose was competitively inhibited by glucose and sucrose respectively, and was affected by thiol reagents and metabolic poisons. In *Rhynchosporium secalis*, uptake of galacturonic acid was competitively inhibited by glucose and had an apparent Km of 3.1mM (Ayres & Olutiola, 1973). The apparent Km values for the uptake of glucose and galactose were 2.76 mM and

29.67mM respectively. In *Aspergillus flavus*, the apparent kM for the absorption of galacturonic acid was 1.3×10^{-2}M, and the absorption was inhibited by p-chloromercuribenzoate (1 mM), mercuric chloride (30 mM) and 2,4-dinitrophenol (1mM) (Olutiola & Okonkwo, 1982).

Both inorganic and organic sources of nitrogen were utilized for growth and sporulation by a number of the microorganisms tested (Olutiola, 1978; Olutiola & Cole, 1977a; Olutiola & Okonkwo, 1982). In most cases, the organic sources were usually better for microbial growth than the inorganic sources (Olutiola & Cole, 1977b; Olutiola, 1976a).

The effects of a number factors on the growth and reproduction of the organisms were examined. Both processes were affected by pH (Olutiola & Nwaogwugwu, 1982; Olutiola & Cole, 1977b), light (Olutiola & Cole, 1977a; Olutiola, 1978), agitation (Olutiola, 1976a) and temperature (Olutiola & Cole, 1977b; Olutiola & Nwaogwugwu, 1982).

(b) *Synthesis of Enzymes by Mcroorganisms*

Most of the work done in this regard was in connection with microorganisms pathogenic to crops of economic importance in Nigeria. The cell walls of plants are fundamentally involved in many aspects of plant biology including morphology, growth and development of plant cells, as well as the interactions between plant hosts and their pathogens (Albersheim *et al.*, 1969; English *et al.*, 1969). Cellulose, hemicellulose, pectic polysaccharide, structural protein and lignin have been identified as the major components of the plant cell wall (Albersteim, 1985; Cleland, 1971). The ability of phytopathogens to produce enzymes necessary for the degradation of these cell wall components will be an advantage in infection (Wood, 1960; Hunter and Elkan, 1975).

A complex of celluloytic enzymes (including C_x and X cellulases) were isolated from a number of microorganisms. These enzymes were purified by using standard biochemical

techniques and then characterized (Olutiola, 1976b; Olutiola, 1982a; Olutiola, 1976c; Olutiola & Cole, 1977c; Olutiola & Cole, 1976; Olutiola, 1976d; Olutiola & Ayres, 1973b). The activities of the cellulases were affected by a number of factors including substrate concentration, pH, temperature, cations, metabolic poisons and salts of heavy metals.

The ability of the microorganisms to synthesize pectin-degrading enzymes was also investigated. The results showed that most of the phytopathogens tested were able to synthesize a variety of pectinases including polygalacturonase, pectin methylesterase and pectin transeliminase (Olutiola & Akintunde, 1979; Olutiola, 1982b; Olutiola, 198c; Olutiola, 1983b). The enzymes were also purified and charactized. Other microbial enzymes which I studied included amylase (Olutiola, 1981), protease (Olutiola *et al.*, 1982b), invertase (Olutiola & Cole, 1982) and maltase (Olutiola, 1981).

Besides the investigation on phytopathogens, some work was also carried out on the synthesis, nature and characteristics of enzymes from human pathogens (Olusanya & Olutiola, 1986; Olutiola & Nwaogwugwu, 1982).

(c) *Water bacteriology*

I have carried out a lot of research into the bacteriology of water and sewage. Many of the reports have been presented at various scientific conferences, while others have been published (Olutiola *et al.*, 1982a; Olutiola & Cole, 1981). With regard to pipe-borne water supply, the results showed that most of the pipe-borne water supplies in this country are microbiologically unsafe for human consumption, and show evidence of inadequate treatment.

Conclusion

As a microbiologist, a most serious problem that has consistently troubled my mind for a long time has been the unacceptable level of the treatment of pipe-borne water supplies in Nigeria. It is ordinarily assumed by the non-

microbiologist that potable water is synonymous with pipe-borne water supply. Water for human consumption must be free from disease organisms, poisonous substances and excessive amounts of mineral organic matter (Fiar *et al.*, 1966). From the health point of view, the most important characteristic of good water is obviously an absence of pathogenic organisms (Feachem *et al.*, 1978).

The analyses of water supplies from different parts of the country, which I have performed for the past ten years, indicate that things have not been well. Irrespective of the source, the results have one thing in common, namely, they are microbiologically unsafe for human consumption. They at no time satisfy the standard conditions laid down by the world Health Organisation (WHO, 1971). Such conditions make it mandatory for distribution systems carrying treated water to contain a residual chlorine concentration of at least 0.2mg/l after 20 minutes contact (Feachem *et al.*, 1978). The water supply should not contain more than one coliform bacteria per 100 ml of water sample (Hammer, 1977). For most of the time, residual chlorine is virtually absent, and even when present, it is usually much lower than the recommended minimum level. As for the coliform bacteria, these are usually in hundreds per 100 ml of water. It appears to me that the various chief executives in charge of our water supply, who are mostly engineers and constitute the special advisers to the Government on such matters, are either intentionally or perhaps unconsciously unaware that one of the greatest dangers to human health is the type of water he consumes. This is because certain genera of bacteria, especially *Salmonella, Shigella* and *Vibrio*, are potentially capable of contaminating drinking water supplies and causing diseases in man.

Contamination of drinking water with *Salmonella* may cause outbreaks of gastroenteritis or typhoid fever. Contamination of drinking water or food with faeces containing *Shigella* causes epidemics of bacillary dysentery. Presence of *Vibrio cholerae* in drinking water may result in the disease

commonly referred to as cholera. Cholera is a severe gastro-intestinal disease of humans and is caused by eating food or drinking water contaminated by faeces containing the vibrio. Other water-borne diseases include infectious hepatitis caused by viruses and the amoebic dysentery caused by the protozoa, *Entamoeba histolytica*.

In developed countries of the world, the incidence of water-borne diseases is on the decease while in the developing countries, it is on the increase. Perhaps at this stage, I should speak on the specific case of Nigeria. Analyses of the various pipe-borne water supplies indicate that the quality of the water samples appear to decline with each succeeding year, even though one would have expected an improvement. Reasons given at the various water treatment plants appear to be the same, namely, no money to purchase the required chemicals, especially alum and chlorine. One then wonders at the rationale behind the step being taken recently by a State Government in Nigeria to disconnect public water taps. As a matter of fact, the water executives have started implementing this decision in that State capital. This is a very serious problem indicating a lack of concern for the health of the common man, in that even when the pipe-borne water supply is available, it is still not too safe for human consumption. One then wonders at the type of water which the citizens in that part of our country will have to consume. Definitely they will be forced to fetch untreated or highly polluted water, well-loaded with pathogens. This to me is a great disservice to the health care delivery system and the slogan of 'health for all by the year 2000'. Therefore, in the interest of the public which that Government is supposed to serve, the decision should be immediately reversed.

Another interesting aspect of this retrogressive decision is that, as if it were a step in the right direction, few neighbouring State Governments are also contemplating to eliminate public water taps. Perhaps it will be of interest for them to know that the series of analyses which I made recently, i.e. after a number of the public taps have been disconnected,

have shown no evidence of improvement, and, in fact, I am bold to say that some of the results indicate a worsening of the water quality. Thus, the excuse, as adduced by the State Government in question, of saving costs and improving water quality by disconnecting public water taps has not been, and definitely can not be justified.

I wish to use this forum to appeal to the Federal Government to make it imperative for all State Governments to make safe drinking water available to the public. Thus, each state Government should not only ensure proper treatment of its water supply, but must install more public water taps to serve the daily needs of the people. However, until such a time that the Government is able to supply safe drinking water, members of the public should always ensure that water samples, especially those meant for the infants, are boiled properly before consumption. The Federal Government must therefore, as a matter of urgency, set up a water control unit, placed directly under the auspices of the President, with branches in each state capital. Such a unit should constantly monitor the activity of the treatment plants to ensure that the WHO Standards for drinking water are strictly adhered to.

Perhaps at this stage, it is not out of place to commend the Federal Military Government for not only introducing, but also enforcing the monthly sanitation day. One problem which seems most intractable in Nigeria is the safe disposal of our domestic wastes. Around the various homes, offices, market places, streets and the like, piles of decaying food wastes, rotting vegetables and other domestic wastes in various stages of putrefaction. It is also a common site to see hundreds of animals including goats, dogs, pigs and even over-sized rats feeding on such wastes. Their number is only exceeded by the number of flies who swarm in their thousands around everyone and everything. A common transmission route of bacillary dysentery, amoebic dysentery and diarrhoea is from man's faeces by these flies to food or water and thence to man (Rajagopalan, 1974). The diseases for which the rats may be a reservoir are numerous and

include plague, murine typnus, leptospirosis, histoplasmosis, rat bite fever and salmonellosis (WHO Expert Committee, 1971).

These decaying wastes are rich in organic matter and provide good nutrients for the growth and multiplication of microorganisms. Thus, besides the unpleasant sight and the objectionable odour emanating from such wastes, they also provide adequate conditions for microbial activities. Such activities result in the production of air pollutants. For example, microbiological degradation of organic matter under anaerobic conditions yields high concentrations of hydrogen sulphide. Also anaerobic processes in swampy decaying organic wastes produce large quantities of methane. Denitrification of organic wastes by microorganisms lead to production of nitrous oxides. Such air pollutants produced by microbial activity, in addition to those produced by chemical processes in nature, constitute serious danger to our health. Many air pollutants are carcinogenic. There is available evidence to show that exposure to air pollutants predisposes individuals to deep lung microbial infections. Both bacterial and viral infections are increased in the presence of pollutants. For example, bronchitis-emphysema is a chronic lung disease that appears accentuated by air polluants.

Considering the rapid rate of growth and multiplication of microorganisms and the rapidity with which pathogenic ones may cause disease (e.g. sometimes within a few hours or days), the one day set aside in a month for environmental sanitation is obviously inadequate. I therefore wish to use this forum to appeal to the Federal Military Government to compel all state Governments to introduce (or re-introduce) the sanitary inspector unit. The sanitary inspectors will then be able to monitor, on a daily basis, the sanitary condition of our environment. Such sanitary inspectors must be men and women of high integrity who will be empowered to enforce sanitary laws, if and when necessary. Thus, with the provision of safe drinking water, and the maintenance of a clean (or

pathogen-free) environment, Nigeria will be nearing her much desired goal of 'health for all by the year 2000'.

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