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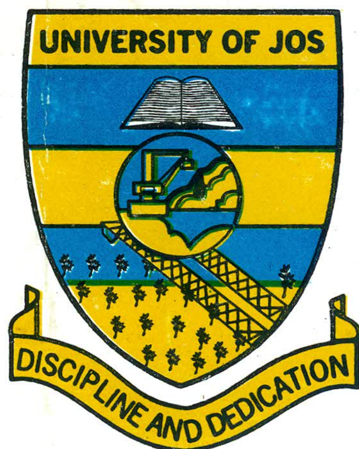

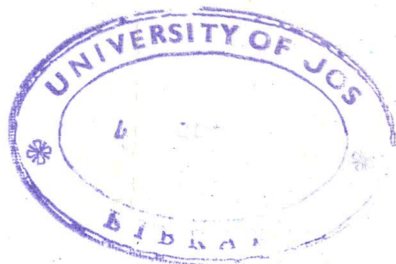

FROM MICROBES TO BIOCHEMICAL BREAKTHROUGHS

INAUGURAL LECTURE

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By

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From Microbes to Biochemical Breakthrough

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By:

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From Microbes to Biochemical Breakthroughs

Introduction

Microbes may be divided into Prokayotes and Ekaryotes. Prokaryotes are those organisms which do not have a nucleus in their cells while eukaryotes are those which possess a nucleus.

By 1977, Woese and Fox had shown that there are two completely distinct types of prokaryotes: the achaebacteria and the eubacteria. The achaebacteria possess a completely unique wall, unique RNA and DNA nucleotide sequences, and a plasma membrane unlike that found in any other living organism.

[figl 1:1]

A plasma membrane consisting of phospholipids and proteins surrounds the protoplasm of all living cells and microbes are no exception. However certain peculiarities exist in bacteria:

1) Steroids such as cholesterol are absent (except in the mycoplasma). In the eukaryotes, these substances help to stabilize the phospholipids.
2) The proportion of protein to phospholipids is high (typically 2:1 in prokaryotes and 1:1 or less in eukaryotes). This reflects the variety of roles played by the prokaryotic plasma membrane, which in eukaryotes are performed by cytoplasmic organelles.

The proteins uniquely present may include those for election transport during respiration and photosynthesis, lipid metabolism transport during respiration and DNA synthesis and chemotaxis. Proteins associated with molecular transport and cell wall synthesis are also present as they are in eukaryotes.

The cytoplasm of most prokaryotes lack chloroplasts and mitochondria as well as all membrane-bound organelles of cytoplamic origin such as endosplamic reticulum and golgi bodies. In prokaryotes, processes with which eukaryotic cytoplasmic organelles are associated take place on the plasma membrane, on structures derived from it or in the fluid matrix.

The nucleoid marks the site of the main bacterial chromosome. The latter is a tightly wound circular loop of DNA attached at one or more points to a mesosome. Sometimes, DNA replication and separation outplace cell division so that two or meore nucleoids may be present within a single cell. Prokaryotic DNA has 3 major features-

a) Histones are absent
b) There are no introks and
c) The genes are organized into operons

In addition to the main bacterial chromosome, bacterial cytoplasm normally contains numerous small circles of DNA called plasmids. These replicate independently of the main chromosome.

[Fig 2]

Reproduction in prokaryotes is asexual – involving simple fussion in which DNA replication is followed by the formation of a septum which divides the cell into two.

[Fig 1.9]

Three mechanisms of gene transfer are known – conjugation, transformation and transduction.

In conjugation, an individual (the donor or male) may produce a small tube of protein called a sex pilus through which it can transfer genes to another individual (the recipient or female).

In transformation, there is an uptake of genes from the surrounding medium by a bacterium.

And in transduction, there is the incorporation of genes into a bacterium by a virus. Viruses which infect bacteria are called bacterio phages.

Eukaryotic microbes consist of fungi, protozoa and algae although not all members of those groups are microbial.

Fungi have 2 common characteristics -

a) They are heterotrophic and so require particular amino acids and vitamins, although some can synthesize their own from inorganic minerals.
b) They are true eukaryotes – they possess not only nuclei, but also the many cytoplasmic organelles commonly found in eukaryotes such as endoplasmic reticulum, cytoskeletal components and mitochondria.

Fig. 3

The main features of the protzoa and algae are summarized in Figs. 4 and 5. They play significant roles in the biosphere, and my discussion today is actually based on work done with protozoa and fungi.

Studies With The Fungus

My first contact with microbes was during my postgraduate studies in the laboratories of Professor R. Cecil Jack in New York. As a pure chemist, I did not quite like the idea of taking a course in microbiology before embarking on research with microbes, but my major Professor managed to convince me that a good grounding in

microbiology will be a great asset to me in my research in his laboratories. He was right. We started our studies on the biosynthesis of Triacylglycerides and Phosphoglycerides in the fungus Glomerella cingulata. Fungi produce large quantities of triacylglycerides and phosphoglycerides of qualitatively similar fatty acid composition[1]. Therefore it appeared that fungi might be useful organisms for investigating problems bearing upon the regulation of glyceride metabolism. Thus we set out to investigate:

a) The incorporation of labeled precursors into fungal triacylglycerides and phosphoglycerides in relation to accumulation of these lipids at selected stages of growth and
b) To investigate the changes in radioactivity of the triacylglycerides and phosphoglycerides (i.e. their apparent turnover) when the labeled mycelium was transferred from nutrient medium supplemented with unlabeled precursor.

Data from this study showed there was greater incorporation of
[2 ^{14}C] acetate and of [6 – ^{14}C] glucose into phosphoglycerides than into triacylglycerides of $1^1/_2$, 2, 3, 4, and 6 day old mycelial sample of the fungus. Maximum incorporation into both classes of lipids occurred in young mycelial samples (2 to 3 days of age) which had a high content of total nitrogen. The five sets of mycelial samples all contained somewhat larger quantities of phosphoglycerides that triacylglycerides and changes in content of both classes of lipids were similar in pattern to changes in content of total nitrogen. Incorporation, accumulation and total nitrogen of the mycelial samples decreased at 4 days, but increased again by 6 days. The apparent turnover of the triacylglycerides and phosphoglycerides was qualitatively similar although there was greater apparent turnover of phosphoglycerides than triacylglycerides. The similarity in patterns of apparent turnover was inferred to be a consequence of acyl exchange between the labeled and unlabeled triacylglycerides and phosphoglycerides. The overall observation from this study was that it is now apparent that lipid biosynthesis follows the same pattern in microorganisms as in mammalian cells, thus making lipids the only biomolecules which exhibit such tendency.

In furtherance of these studies, Anekwe and Dubal[2] tried to have an idea of the types of fatty acids contained in the triacylglycerides and phosphoglycerides. To this effect the mycelia of Glomerella Cingulata was grown as described before. The triacylglycerol and the phosphoglycerides were separated from the rest of the lipids and from each other by column chromatography on silicic acid. The result from these studies for the first time showed that the triacylglycerols and phosphoglycerides contain the same major classes of fatty acids at the selected stages of growth, a factor which eventually proved significant in diagnosing lipidoses.

Table I

In 1973, Anekwe and Lee went in search of sulfolipids in fungi. Sulfolipids are a group of sphingolipids generally associated with the cerebellum. Indeed the entire cerebellum is bound together by disulfide bonds derived from sulfolipids. [fig 6] For this experiment the culture of Glomerella cingulata was obtained from the United States Department of Agriculture, Crops Research Division, Beltsville, Maryland, and grown in a nutrient medium consisting of D-glucose, 10 g; L-Asparagine, 4g; KH_2 PO_4 (monobasic), 1g; Mg SO_4 .$7H_2O$, 0.5g; $cacl_2$ $2H_2O$, 0.1g; Biotin, 0.005g. Approximately 60mg of spores of the Glomerella cingulata were used to inoculate 1000ml. of the nutrient medium. The spores were then incubated with shaking at 22^0C. Growth was discontinued at the end of a 3-day period. Data from this study showed that Glomerella cingulata contains a sulfated cerbroside fraction among its lipid, which amounts to 0.6% of the total lipid of the fungus. This is the first time this has been shown in fungi. This lipid seems to be composed of a sphingosine, carbohydrate (aldohexose) and fatty acids mainly, and apparently contains only one type of fatty acid group – C_{16}. [fig7]

These studies with fungi demonstrated four major points:

1) That lipid biosynthesis follows the same pattern in micro organisms as in higher organisms, thereby making lipids the only macromolecule which displays this characteristic, thus making it easy to study lipid disorders in higher organisms such as mammals by simply using much lower organisms such as fungi.
2) That the fatty acid composition of the two major glycerides found in fungi – triacylglycerides and phosphoglycerides contain the same types of fatty acids thus suggesting that the same glycerides in higher organisms also contain identical fatty acids. and
3) The presence of sulfatides in fungi is novel and is a major breakthrough in the understanding of the etiology of metachromatic leuckodystrophy.

Studies With the Protozoa

By the end of 1973, I was beginning to shift to other micro organisms this time the protozoa. My first foray in this direction was with the Tripanosomatidae. In this family, two species were specifically targeted – the Leishmania and the trypanosomes, primarily because of their established resistance to chemotherapy. Thus, we set out to find out why these organisms which cause a sizeable amount of damage to both humans and livestock are characteristically resistant to chemotherapy.

Mild alkaline hydrolysis followed by mild acid hydrolysis of the phosphoglyceride fraction of Trypanosma vivax and subsequent paper chromatography of the product of this hydrolysis was used to identify a monovinyl – ether, mono fatty – acyl – phosphoglyceride (plasmalogen) in Trypanosma vivax. The

data suggested an ethanolamine plasmologen. [fig. 7] Similarly a chromatographic lipid analysis was performed on the promastigotes of Leishimania tarentolae. Total lipids were 11 – 15% of the dry weight, and neutral and polar lipids were 23 – 37% and 63 - 77% of total lipids respectively. The major lipids were phosphatidylcholine, phosphatidyl, ethanol-amine, sterols, triacylglycerols, phosphatidylinositol, diphosphatidylglycerol, an unknown phospholipid, and sterol esters. Alkoxy forms were found in the phosphatidyl ethanol-amine and phosphatidylinositol fractions. Fatty acids of the total lipids of the Leishmania were 18:2 (n-6), 18:3 (n-3), 18:1 (n-9), 18:0, 22:6 (n-3), 22:5 (n-6), 16:0, 14:0, and 20:3 (n-3). Present also were branched chain fatty acids (ISO - $^{<}$18) and a cyclopropane fatty acid (cis – 9, 10 – methylene octadecanoic acid). The bulk of our studies for the next 10 years centered on the plasmalogens and the cyclopropane fatty acid.

Plasmalogens are a group of phosphoglyceride sub-classes in which the alpha 1 position is in vinyl linkage with an aldehydogenic unit and the Beta position in ester linkage with the fatty acid. There are many sub-classes of plasmalogens but the ethanol-amine containing one is generally considered the most abundant in mammalian tissues. In the liver the quantity of plasmalogen is small; but in the brain, heart, skeletal muscle and semen, plasmalogen makes up about 20 moles % of the phosphoglycerides[3]. It is very probable that all parasitic protozoa possess phosphoglycerides, but the actual types present have been investigated in only a few cases. Dixon and Williamson have pointed out the existence of some of these in various classes of Trypanosomes.

But the amount of information pertaining to other lipids in the phylum protozoa is scant. Only one protozoa examined so far – Tetrahymena pyri formis is known to have a high concentration of alkyl glyceryl ether among its phospholipids. Hack et. al.[4] showed that crithidia fasciculate, C. Tricilae, Harpetomanas culicis, Trypanosoma cruzi and Lishimania donovani exhibited positive reactions for traces of ethanolamine plasmalogen. Two other protozoa – Acanthomoeba sp. and Tetrahymena pyriformis showed faint suggestive reactions to the presence of plasmalogens.

But knowledge of the presence of this group of ether lipids in the trypanosome is very essential to an understanding of their general reactivity to chemotherapy. Preliminary evidence has been reported to the effect that the ether lipids are localized to some extent in the surface membranes providing the interface between the cell contents and the environment[5]. It is not altogether coincidental that lipids containing the ether linkage and or the carbonphosphorus bond are markedly resistant to lipolytic enzymes, Thompson[6]. It is quite possible that they serve as specialized protective devise for a cell exposed to chemical and enzymic agents.

Accordingly, we thought it fit to examine as a start a salivarian trypanosome commonly available in Nigeria and which is responsible nagana and similar diseases in cattle in Nigeria for the presence of this group of ether-linked lipids. It was hoped that information obtained from such study could be helpful in understanding problems attributatble to Trypanosma vivax, which causes an estimated 3 x 10^6 deaths of cattle in this region.

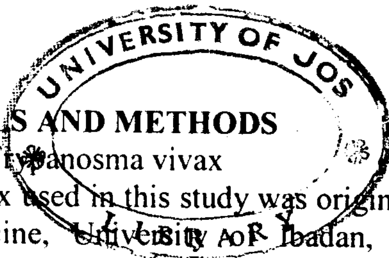
In addition, knowledge of the lipids of leishmanias is fragmentary and datas from a period of interest in the comparative biochemistry of fatty acid and sterol biosynthesis (Hack[4], Halevy and Avivi[7], Korn and Greenblatt[8], Korn et. al.[8], Meyer and Hols[9],). It has become increasingly apparent that a systematic analysis of the composition and distribution of leishmania lipids would be requisite to the investigation of a number of important associations of lipids and the host-parasite relationships of leishmanias.

It is possible that preferred lipids are nutritional requirements of species that parasitize homoeothermic vertebrates, and that these requirements are heightened by growth at the elevated body temperatures of the hosts. This is inferred from the nature of the culture media necessary to support the reproduction of the promastigote form characteristic of the phlebotomine vector, the inadequacy of such media for growth of the amastigate form that colonizes the vertebrate macrophages, and the temperature sensitivity of fatty acid biosynthesis in leishmanias. Instructive in this regard are the reports that bloodstream forms of salivarian trypanosomes incorporate complex host lipids which they are able to synthesize in culture at vector body temperature. The classic example of this phenomenon is the finding of cholesterol in bloodstream. T. rhodesiense, and ergosterol in the culture form[11].

Study of the membrane biology of leishmanias will also require a knowledge of their lipids. Nothing is known of the nature and organization of the lipid bilayer of the plasma membrane of the amastigote, across which are carried out the transactions with the host macrophage that regulate the nature of the host-parasite relationship.

Finally, entry of a leishmania into a macrophage is accompanied by a regression of the mitochondrial component of the kinetoplast-mitochondrion complex. Conversely, transformation from amastigote to promastigote in the vector is attended by realaboration of that organelle. The importance of membrane lipids in these structural and functional transformation are self-evident, but unexplored.

Therefore, we initiated chromatographic analysis of the lipids of intact promastigotes of L. tarentolae, a primitive species isolated from the blood of a pikilothermic vertebrate (gheko), and cultivatable in a chemically-defined, lipid-free medium[12].



MATERIALS AND METHODS

1. Analysis of the Plasmalogen of Trypanosma vivax

The species of Trypanosma vivax used in this study was originally obtained from the Department of Veterinary Medicine, University of Ibadan, Nigeria and was subsequently maintained in rats. The organism was then separated from the rat blood by the method of Lanham[13]. Phosphate Saline glucose (PSg) pH 8, was found more suitable for this work. The sample was centrifuged at 1800g for 20 min and the residue examined microscopically for red cells. An aliquot of the separated sample was then dried and weighed and stored in the PSg buffer.

Lipid extraction was carried out immediately thereafter in the following manner: the wet paste was diluted to 1ml. with the PSg buffer. To this 3.75ml. of methanol/chloroform (2/1 v/v) was added and the material shaken and left at room temperature for 6 hours with occasional shaking thereafter. At the end of this period, the sample was centrifuged for 15 min. at 5000g. and the supernatant decanted and kept. The residue was resuspended in 4.75ml. methanol/chloroform/H_2O (2/1/0.8 v/v/v/) shaken and centrifuged again for 15 mins. at 5000g. The supernatant was decanted and combined with the previous one. To this combined supernatant, 2.5.ml. each of chloroform and H_2O were added, and the material centrifuged. The lower chloroform phase was withdrawn, diluted with benzene and dried under H_2 . The lipid extract obtained was dissolved in chloroform/methanol, (1/1, v/v). Lipid purification was carried out as described before[2].

The purified lipid was dried under N_2, weighed and separated into neutral and polar lipids, by silicic acid column chromatography[1]; neutral lipids with chloroform, polar lipids with chloroform/methanol 2/1, followed by methanol. The fractions were dried in a rotor evaporator under N_2, and weighed.

The polar lipid was separated into its major fractions and each fraction weighed. The phosphoglyceride fraction was then subjected to mild alkaline hydrolysis according to Dawson[5], and water soluble backbone upper layer identified by paper chromatography, employing standards. Since plasmalogens are resistant to mild alkaline hydrolysis, this first procedure ensured separation from its lecithin analogue. The acid labile fraction (the lower organic phase) was neutralized by passing it through Bio-Rex-70 'H' form (Bio-Rad), pH 5, and eluent collected. After the sample had passed through the column, the elution was completed by passing two aliquots of 80% ethanol through the column. The total eluent was dried in a rotorevaporator at below 60^0C.

To hydrolyse this layer, 0.8ml. of 10% (v/v) trichloroascetic acid was added, and the material shaken for 30mins in a 37^0C water bath. The sample was cooled and 2ml of petroleum ether added. It was then shaken, and centrifuged. The upper solvent layer removed. The lower layer was washed by adding 5ml of chloroform-ether

(1/4 v/v) shaken, and centrifuged. This procedure was repeated twice. This was then collected and prepared for paper chromatography.

For this process, the sample was dissolved in 0.2ml of Solution A (prepared thus: one volume of H_2O to two volumes of Isobutanol: Chloroform (1/2 v/v), and shaken for 5 mins.). the upper phase is solution A, the lower phase is solution B, spotted on Whatman No. 1 filter paper, and chromatographed using the descending technique in redistilled phenol saturated with aqueous 0.1 ammonia (v/v) overnight; phenol H_2O (100/38, v/v), and butanol/acetic acid/H_2O (5/3/1, v/v/v). detection was carried out with Rhodamine 6-9, ninhydrin, dragendorf and Molybdate reagent, [fig. 3].

To confirm the presence of the plasmalogens, a series of indicator reactions were carried out.

1. The Procedure of Hack and Ferrans

The dried paper chromatogram was immersed in 500ml metabisulfite – $Hgcl_2$ solution, and 5 ml Schiff reagent were added to it. This was mixed by gentle rocking and allowed to stay for 10 mins. at the end of this period, the paper was rinsed four times in 0.05 bisulfite solution, and again stained with Rhodamine to locate other lipid components. To check for non-specific Schiff reagent staining, the chromatogram was first immersed in the Schiff reagent diluted 1:100 (v/v) with 0.05 metabisulfite but without the $Hgcl_2$; any spots were outlined in pencil. The $Hgcl_2$ was then added and the paper left for 10 mins.

2. The Procedure of Marinetti (1964)

The paper chromatogram was washed in distilled water and dried. It was then immersed in 2, 4-dinitrophenylhydrazine solution for 2 mins. The paper was washed four times, 10 mins. each time, in distilled water, to remove excess reagent, and then viewed under u-v light.

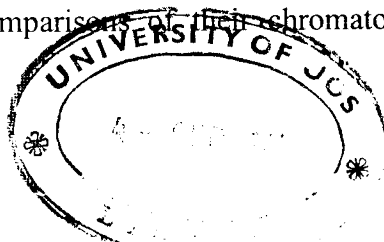
3. Phosphate Test

The procedure of Hack and Ferrans was again adopted. The dried chromatogram was sprayed with ammonium molybadate solution, and heated for 1.5 min. at 100^0C. it was then sprayed with dilute stannous chloride solution and left at room temperature. The spots were later viewed under u-v light, since the blue background could mask the blue spot.

The procedure of Feiss utilizing Zinzade Reagent wa also employed. The dried chromatogram was immersed in Zinzade reagent for several minutes. Excess reagent was washed off with running water, followed by methanol. The chromatogram was dried in a fume hood. Finally, the procedure of Rosenberg for acid-labile phosphates, utilizing molybdate reagent was also employed. 2. Analysis of the Cyclopropane and other Lipids of L. tarentolae

Leishimania tarentolae ATCC 30143 was grown in Brain Heart Infusion (BHI), (Difco Labs.) plus hemin 1mg% (w/v) (Simpson and Braly, 1970), and in chemically-defined medium of C, Trager[11], (Components from Calbiochem.) Inoculum for BHI was 2 x 10^6/ml, incubation 70 hrs at – 25^0C and yield 175 x 10^6/ml. Inoculum for medium C was 2 x 10^6/ml, incubation 168hrs at – 25^0C, and yield was 50 x 10^6/ml. Cultures were harvested by centrifugation (15 min, 5000 rpm, Sorvall RC-2B, GSA rotor) and were washed twice with Lock's solution.

To monitor the fractionation of lipids and assist in their characterization, promastigotes were grown with $H_3{}^{32}PO_4$ (carrier-free 0.75 uCi/ml medium) and with sodium (1-^{14}C) acetate (sp. act. 50 Ci/mole, 0.25 uCi/ml medium) (New England Nuclear), added as Milliporefiltered aqueous solutions. To enhance the incorporation of ^{32}p, L. tarentolae was grown in a phosphorus-poor medium (%w/v), Neopeptone 1 (Difco Labs.); yeast extract 0.05 (Difco Labs.) NaCl 0.9, morpholinopropane-sulfonic acid 0.4 (Calibiochem), glucose 0.5, hemin 0.001 (Sigma Chemical Co.), pH 7.9. inoculum was 3 x 10^6/ml, incubation 75h at 25^0C, and yield 70 x 10^6/ml. Harvested cells were extracted three times (3, 2 and 1 hr extractions), with chloroform/methanol (2/1, v/v) under N_2. the pooled lipid extracts were filtered, taken to dryness with a rotary, flashevaporator at about 40^0C and redissolved in chloroform. Samples of cells and lipids were taken for fatty acid analysis and for phosphorus determination. For radioactivity measurement, samples were mixed with Aquasol (New England Nuclear), and assayed by standard liquid scintillation counting procedures. Appropriate aliquots of cell-suspensions and extracted lipids were also transferred to tared (1-g) thin-walled glass cups weighed to constant weight. Cells were heat-dried, and lipids were dried by evaporation in a stream of N_2.Total lipids were freed of any non-lipids and highly polar lipids (gangliosides) were collected, by sephadex G-25 (Pharmacial) column chromatography. The remaining lipids were then fractionated by triethylaminoethylcellulose (TEAE cellulose) (Brown Co.) column chromatography[1], or the diethylaminoethycellulse column chromatography method of Anekwe and Lee[14]. Fractions from column chromatography were quantified as described for total lipids.
Each column fraction was subfractionated by analytical, silica gel H (Brinkman Instr. Inc.) thin-layer chromatography; neutral lipids in one dimension[15], and polar lipids in two dimensions[16]. The locations and tentative identifications of the individual lipids so separated were made on thin-layer plates by comparisons of their chromatographic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properties with those of standard, by autoradiography (^{14}C and ^{32}P), and by their reactions to indictor reagents[17]; ninhydrin (amino groups), Dittermer-Lester (P), Liebermann-Burchard (Sterols); anthrone-H_2SO_4, periodate Schiff's diphenylamine (glycolipids); Cis-aconitate-acetate anhydride (choline), acid hydrolysis, 4-amino-5-hydrazino-1, 2,4-tiazole-3-thiol (aldehydes). Commercial standards (Applied Science Lab. Inc.) were purified where necessary by preparative, silica gel h thin layer chromatography.

The major lipids identified by analytical thin-layer chromatography were then separated and collected by preparative, silica gel h thin-layer chromatography of column chromatography fractions, neutral lipid in one dimension with the solvent systems pentane/diethyl ether/acetic acid (85/15/1 and 80/20/1, v/v/v/)[15], and polar lipids in one dimension with chloroform/methanol/acetic acid/H_20 (50/30/8/4, v/v/v/v/)[15] or in two dimensions with the solvent systems chloroform/acetane/methanol/ammonium hydroxide (28%) (65/25/5, v/v/v/) and chloroform/acetane/methanol/acetic acid/water (6/8/2/2/1, v/v/v/v/v/)[16]. Lipids were visualized with 2, 7-dichlocrofluoroscein in marker lanes. Neutral lipids were eluted with chloroform and phospholipids with chloroform/methanol 2/1. Eluates were reduced in volume and residual water removed by solvent replacement with ethanol/benzene (1/1, v/v) saturated with H_2O, placed in Sephadex G-25 column and saturated with the same solvent. Neutral and phospholipids were examined for homogeneity by analytical, silca gel H thin-layer chromatography and then were dried, weighed and assayed for phosphorus content and for radioactivity as described for total lipids. Phosphatidlethatnol-aminonin 1-dimensional development, but was separated by thin-layer chromatography on silica gel G-25 (Brinkmann Instr. Inc.) impregnated with $(NH_4)_2$ SO_4, after Kaulen[18]. Phospholipids were further characterized by paper chromatography and ^{32}p autoradiagraphy of the water-soluble glycerylphosphory products of their deacylation[14], and by sequential degradation to diacyl, alk-l-enyl-acyl, and alkyl-acyl forms Sheltawy's modifications[19] of the methods of Dawson et al.[12] and Brockerhoff[20].

The total lipids and the major neutral and phospholipids collected by preparative thin-layer chromatography were saponified under N_2 with KGH (5% in 70% methanol, final concentration) for 3 hrs. Unsaponifiables were removed with petroleum ether, and solution was acidified, and the fatty acids were extracted with diethyl ether, dried over an hydrous Na_2SO_4, and converted to their methyl esters by reaction with diazomethane. Fatty acids were also freed and methylated by reaction with boron trifluride-methanol-benzene. Saponifiecation and methanolysis were monitored by analytical thin-layer chromatography of the reaction products. Fatty acid methyl ester mixtures were freed of any materials of different polarity by silicic acid (Clarkson Chem. Co.) column chromatography, with 5% diethyl ether in pentane as the eluent, or by preparative, silica gel G thin-layer chromatography, with the solvent system pentane/diethylether/acetic acid (80/20/1, v/v/v/).

The fatty acid methyl ester (FAME) mixtures were analyzed by gasliquid chromatography with an F & H model 402 Gas chromatograph equipped with dual, flame-ionization detectors, and glass xolumns containing 6 diethylene glycol succinate on 80-100 mesh Diatoport S (Hewlett-Packard Co.). columns were operated isothermally at 170^0C and were programmed over the temperature range $100\text{-}200^0$ at 2^0 per min in dual column mode. The He carrier gas flow rate was 60ml/min and pressure 40 p.s.i. FAMEs were tentatively identified by direct comparisms of their relative retention times (RRT) with standard values, by plots of the logarithms of the RRT after Ackman, and by cochromatography with standards. They were quantitated by measurements of the areas of peaks on recorder tracings by the width at ½ height method, and by the use of a mechanical intergrater (Disc).

Individual, presumptive unsaturated fatty acid methyl esters were collected by preparative gas-liquid chromatography with an F & M model 720 Gas chromatograph equipped with a thermal conductivity detector and a ceiled copper column (9ft x1/4 inch ID) packed with diethylene glycol succinate on Gas chrom P (80-100 mesh, Applied Science Lab. Inc.) and operated isothermally at 200^0. The He carrier gas flow rate was 60ml/min and pressure 40 p.s.i. Methyl esters were collected in cooled glass tubes at the exit port of the chromatograph at the time intervals coincident with the recorded responses of the detector to their passage. Collection recovery was 95%of injected sample. Homogeneity of the collected methyl ester samples was confirmed by analytical gas-liquid chromatography. The chain length was established by gas-liquid chromatography analysis of the products of hydrogenation, and locations of the double bonds in the methyl esters were determined by gas-liquid chromatographic analysis of the fragments by osmonization-reduction[21]. Aldehyde ester and aldehyde fragments, and standards were compared with the F & H model 402 Gas Chromatograph. Glass columns contained 5 carboware 20M on Gas ChromP (60-80 mesh Applied Science Lab. Inc.) and were temperature-progammed from 50^0 (6 min) to 200^0, at 5^0/min. The he carrier gas flow rate was 60ml/min and pressure 40 p.s.i. Presumptive cyclorpropane and branched chain fatty acids were collected by preparative gas-liquid chromatography of appropriate fractions from preparative, silica gel G, argentation ($AgNO_3$5% w/w) thin-layer chromatography (solvent-benzene) of the total fatty acid methyl esters. The compositions of the fractions from thin-layer chromatography was monitored by analytical gas-liquid chromatography. The presumptive cyclopropane and branched chain fatty acids and reference compounds were brominated[22] and were exposed to catalytic hydrogenation. Products of bromination and catalytic hydrogenation were examined by analytical gas-liquid chromatography as for fatty acid methyl esters.

Mass spectra of purified presumptive cyclopropane fatty acid and cis-9, 10-methylen octadecanoic acid standard showed the M ion at N/e 310, and M-32 at m/278, M-74 at m/e 236, and M-116 at m/e 194 [23]. The 100 MHz proton magnetic

resonance spectra of the two samples were identical with respect to the unique resonance at 0.6 ppm and also identical in the cyclopropane region (0 to – 0.3 ppm) [fig 2]

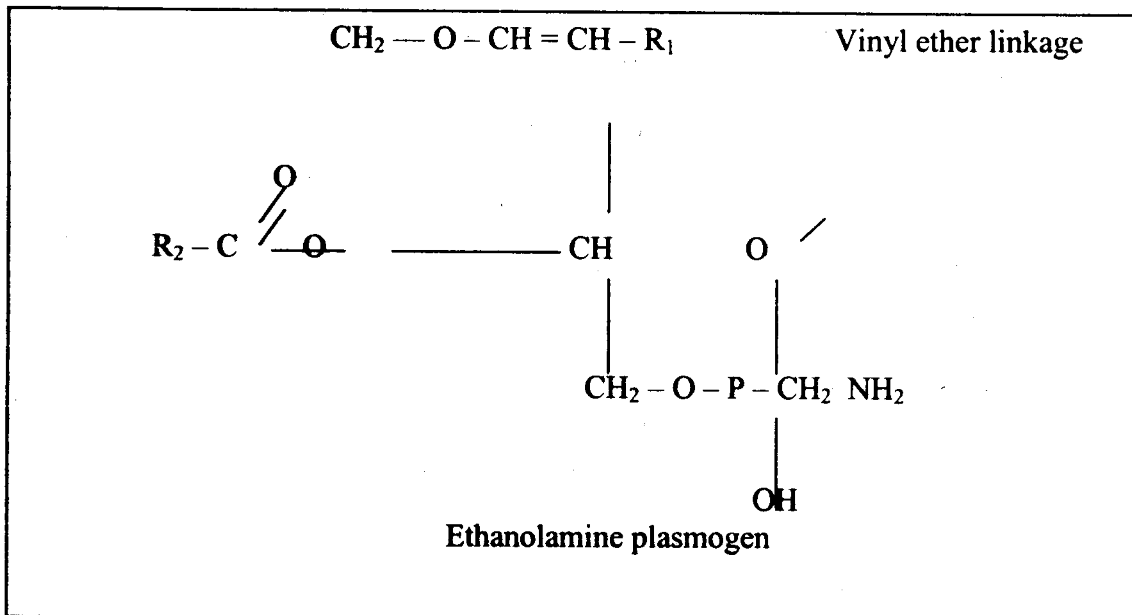
RESULTS AND DISCUSSION

1. The Plasmologen of Trypanosma vivax

The % total lipid yield of Trypanosma vivax was about 12% of the dry sample. Neutral lipids made up about 38% of the total lipids, and polar lipids about 60%. Phosphotidylethanolamine made up about 24% of the polar lipid fraction, and phosphatidylcholine about 46%. Sphingomyelin made up about 20%. The other polar lipids appeared only as traces, and there was a small quantity of lysophosphotidyl choline (about 4%).

The RF value of the glyceryl phosphoryl hydrolysis product was 0.47 in phenol/H_2O (100/38, v/v) and 0.2 in Diethanol/acetic acid/H_2O (5/3/1,v/v/v). this seems to correspond well with observed values for ethanolamine plasmologene (Anekwe, 1978- also unpublished data).

The results of the indicator reaction showed that they hydrolysis product was ninhydrin positive, Rhodamin positive dragendorf negative and molybdate positive. Table 1 shows the reaction to the various indicators. A positive ninhydrin reaction coupled with a negative dragendorf reaction suggested the presence of an ethanolamine plasmalogen. The other lipid fraction which could have been suspected is serine plasmalogen, but phosphotidylserine was not noticed in the original total polar lipids [3].



Ethanolamine plasmogen

The role played by ether lipids has long been the concern of investigators. Debuch and Seng[24] noted that Hadju et al. identified a plamitoyl lysolecithin in mammalian blood which had a digitalis-like activity and believe it originated from cholin plasmoalogen precursor; that Ruddecke and Andersen investigated the plasmal content in human oartas and found that arteriosalerotic aortas contained much less than normals, and Hack Ferrans observed a nitrogen-free plasmalogen in infacted myocardium of dog. They also reported that Berger showed that ether lipids possess a central depressant action and that a radio-protective effect by ether-linked lipids was demonstrated by Brohult. But Thompson's[25] suggestion that plasmalogens could play a very significant role in the protection of certain protozoa from chemical and enzymic attacks is most pertinent in our observation. It could prove very useful in understanding the behavior of this trypanosome to chemotherapy and thus aid in finding plausible solution to the problem.

2. The cyclopropane and other Lipids of L. tarentolae The total lipid yield of L. tarentolae was between 11-15 dry weight neutral lipids 23-37 of total lipids, polar lipids 63-77 of total lipids. The more vigorous the growth, as judged by short generation times and high generation yields at harvest, the higher the percentage of polar lipids, (almost exclusively phospholipids). Scant information exists on the properties of the lipid classes in L. tarentolae but comparisms can be made with other trypanosmatide; vertebrate trypanosomes and insect gut flagellate. Among a variety of forms, cultured or isolated from a host, the total lipids as dry weight ranged from 12-20, neutral lipids as total lipids 21-35, and polar lipids as total lipids 64-97. [table 2] In general, the polar lipid content, mostly phospholipids, was about 2-3 times that of the neutral lipids (Dixon[10]; Meyer[9]; Korn[8]; Hunt[26]). Two-dimensional, thin-layer chromatography of the fractions from TEAE cellulose column chromatography of the ^{14}C-and ^{32}P-labelled lipids of L. tarentolae revealed partial glycerides, sterols, unesterified fatty acids, triancylglycerols, diacylalkoxy lipids, sterolester, diphosphatidyl-glycero., phosphtidyl-lethanlamine, phosphtidylinositol and phosphtidylcholine. Other lipid included four highly polar unknowns (A-D), an unknown (E) with a polairy less than that of diphosphatidylglycerol, and traces of lyso lipids (lysophosphatidylethanolamin, lysophosphatidylcholine), phosphatidic acid, and six unknown (E-K) with plarities between those of diphosphatidylglycerol and diphosphatidlethanolamine [table 3]. Silicic acid[27] and diethylaminoethyl cellulose[28] column chromatography, coupled with thin-layer chromatography of column fractions, gave the same proportions of neutral and polar lipid classes and the major and minor lipids as the TEAE cellulose column chromatography/thin-layer chromatography method, but the column separation of lipid types was not as good. ^{14}C-labelled neutral lipids separated by column chromatography of the total lipids and quantified by preparative, one-dimensional, silica gel H thin-layer chromatography, are shown in Table 4. sterols and their ester, and

tracyglycerols were the major lipids, as they are in trypanosomes[10]. Ergosterol has been found in L. tarentolae[7, 31] .Other sterols are present but unidentified. Ergosterol and related phytosterols have also been found in the other types of trypanosomatids studied (trypanosomes and insect flagellates) and trypanosomes taken directly from host blood.

The imbalance in distribution of 14C in the 14C-labelled neutral lipids of L. tarentolae, favoring triacylglycerols, has been observed before by Meyer and Holz[9], and also by Halevy[7] with L. tropical. ^{32}p-labelled polar lipids of L. tarentolae separated by column chromatography of the tolal lipids, and quantified by preparative, two-dimensional silca gel H thin-layer chromatography, are shown in table 5. Major lipids were phosphatidylcholine, phosphatidylethanolmine, phosphatidylinositol, diphosphatidylglycerol. The phosphatidylthanolmine of L. tarentolae reacted positively on thin-layer chromatograms to a spray reagent for aldehydes[30], suggesting the presence of plasmologens. Accordingly, the major phospholipids were degraded sequentially after Sheltaway[19]. Approximately 1/3 of the phosphatidylethanolmine was in the alkozy form, with 3/2 of that alk-l-enyl-acyl and ½ alkyl-acyl. One fifth of the phosphatidylinositol was alkozy lipid, evenly divided between alk-l-enyl-acyl and alkyl-acyl forms. Phosphatidylcholine and diphosphatidylglycerol were greater than 95% diacyl phospholipids [table 5]. The principal fatty acids of the lipid classes (total, neutral phospholipids) were 14:0, 16:0, 18:0,18:1, (n-9) 18:2 (n-6) 18:3 (n-3) 20:3 (n-3) 22:5 (n-6), and 22:6 (n-3) [table 6]. No table also were presumptive branched chain and cyclopropane fatty acids, particularly in the phospholipids fraction.

The observation of large amounts of presumptive br-S_{18} and cyclo-$_{19}$ fatty acids in the phosphatidylethanolamine of L. tarentolae, and their appearance in the total phospholipids fatty acids of other leishmanias, recommend further analysis.

Saturated, monosturated, diunsaturated and polysaturated fatty acid methyl ester fraction collected by preparative, silica gel G, argentation thin-layer chromatography contained the following major components: saturated 18:0 (38%), cyclo-19 (35%), br-C18 (13%), 16:0 (3%), 14:0 (2%), monosaturated-18:1 (97%); diunsaturated 18:2 (n-6) (93%); polyunsaturated-18:3 (n-3) (90%), [table 7]. The assignment of cyclo C_{19} was initially made on the basis of its tentative identification[10]. However, very recently, Proton magnetic resonance data appear to confirm its presence in L. tarentolae[37] [fig 5].

We have gone into an elaborate discussion of the trypanosome and the leishmania because these two constitute the most studied group among the phylum protozoa primarily because of the major roles their lipids play in their established resistance to chemotherapy. In the case of plasmalogen, preliminary evidence has been reported to the effect that the ether lipids are localized to some extent in the surface membranes provide the interface between the cell contents and the environment[3]. It is not altogether coincidental that lipids containing the ether linkage and or the carbon phosphorus bond are markedly resistant to lipolytic enzymes[3]. It is indeed quite possible that they serve as specialized protective device for a cell exposed to chemical and enzymic agents. In the

case of the cyclopropane fatty acids however, this assumption has been faulted by the discovery of cyclopropane fatty acids in E. coli and plants[31]. Also tissues of ovigerous female desert millipedes contain large amounts of cyclopropane fatty acids. All these organisms are to some extent subject to chemotherapy. But the finding of a single major cyclopropane fatty acid in trypanosomatids contracts sharply with the situation among other organisms which contain more than one molecular species. The resistance to chemotherapy of leishmania may well lie in the finding by Aneckwe[3] that the EF and the LPPG are the same structurally, and that the protein content thitherto thought of as a single protein was in fact seven different proteins more so as the protein is located on the membrane. Thus the problems encountered by chemotherapeutic agents intended to breakthrough a single protein barrier now finding themselves having to break through seven protein barriers and thereafter contend with the lipid moiety of the lipoprotein membrane, may explain the resistance of these organisms to chemotherapy.

SUMMARY

1. Lipid Biosynthesis in microbes follows the same pattern as in higher organisms. Thus, lipidosis of higher organisms e.g. mammals, can be studied by the use of microbes.
2. Microbes contain sulfated cerebrosides which information helps to understand their role in metachromatic leudkodystrophy.
3. The Excretion Factor (EF) in the leishmania is similar structurally to the Lipopeptidophosphoglycan and both contain seven isomers of a single protein, which explains why the organism is resistant to drugs.
4. The Trypanosomes contain a plasmalogen which affords it protection against chemotherapeutic agents, thus explaining why it has been difficult to cure sleeping sickness.

Fig. 9b.: The cyclopropane fatty acid of L. tarentolae

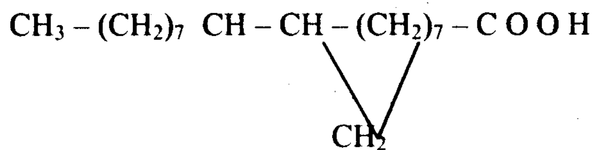


Fig. 10

Gas-liquid chromatogram of the Fatty acids of the EF and LPPG of the Leishimania

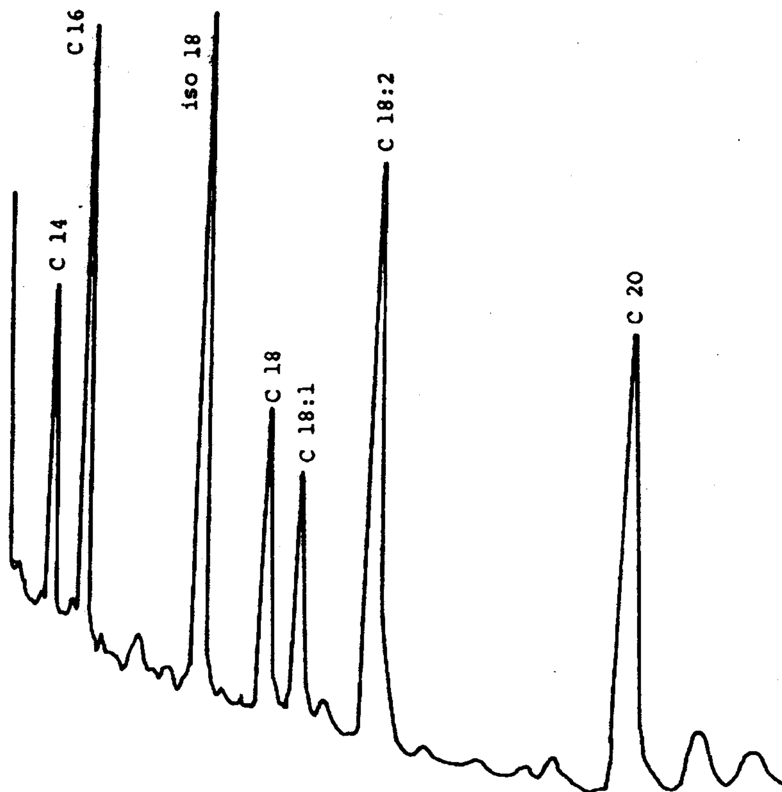


Fig. 11

Gel Electrophoresis of the single protein from the EF and LPPG of the Leishimania

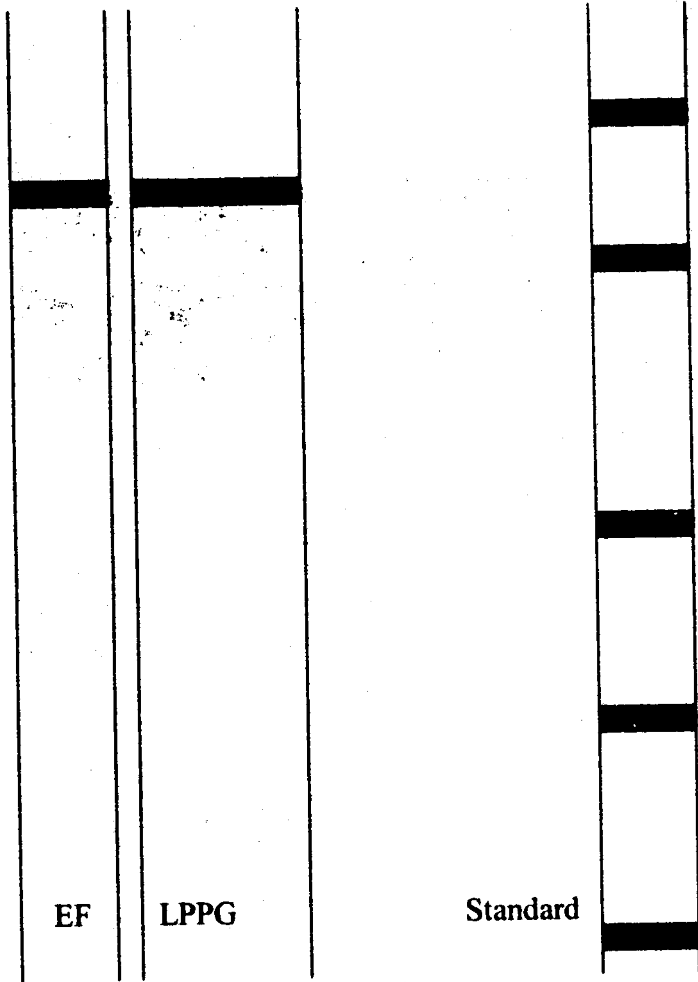
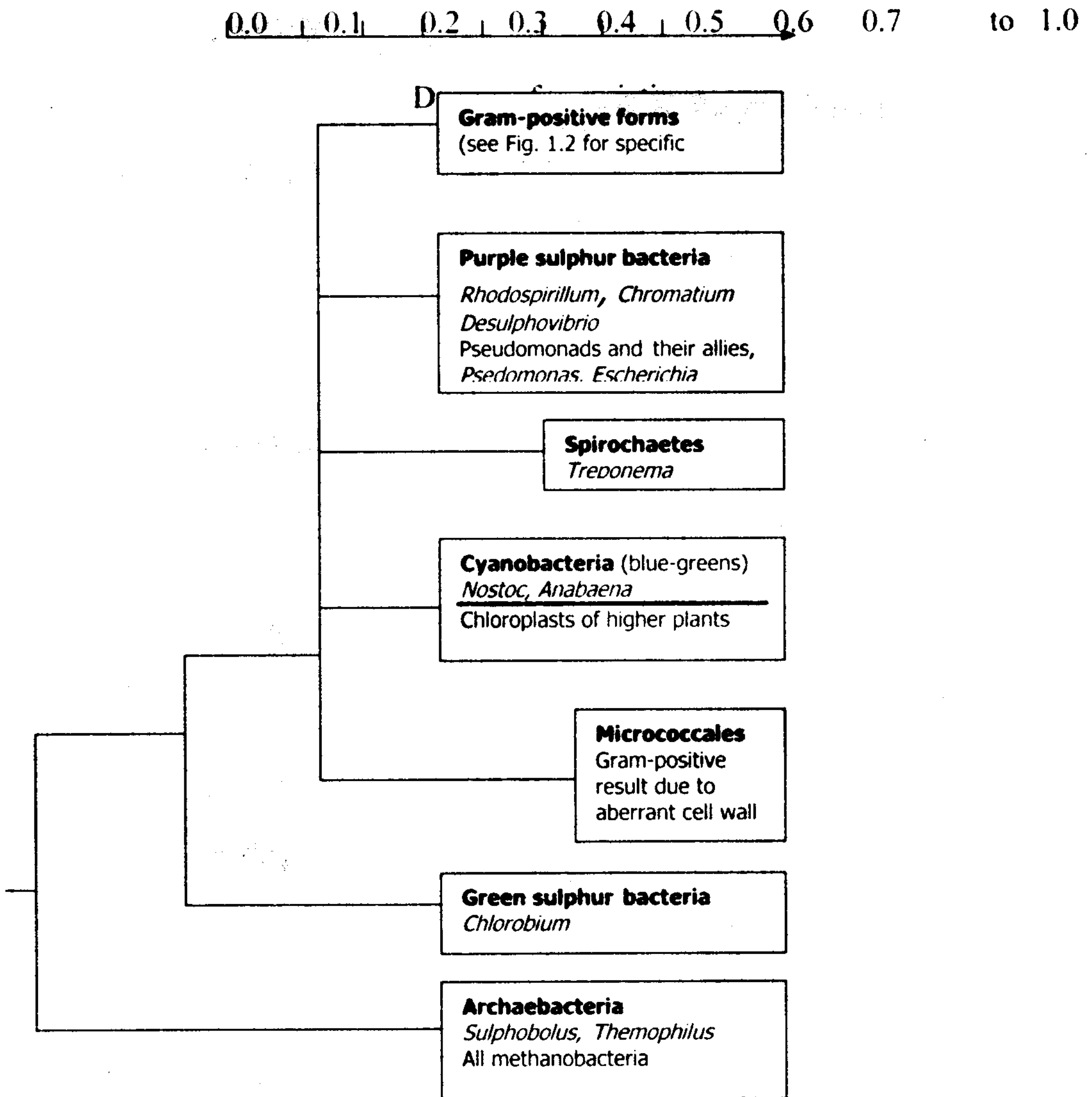


Fig. 3

Major categories of prokaryotes



The degree of association is deduced by comparing a large number of characters using a computer. The scale runs from 0 to 1; 0 indicates no association, while 1 indicates that two populations are identical. This method of taxonomy is known as numerical or computer taxonomy. Seven major groups of prokaryotes are shown. The relationship of these to other groups is still uncertain.

Fig. 4

Summary of the Procedure for Isolation of the Total Lipids of T. vivax

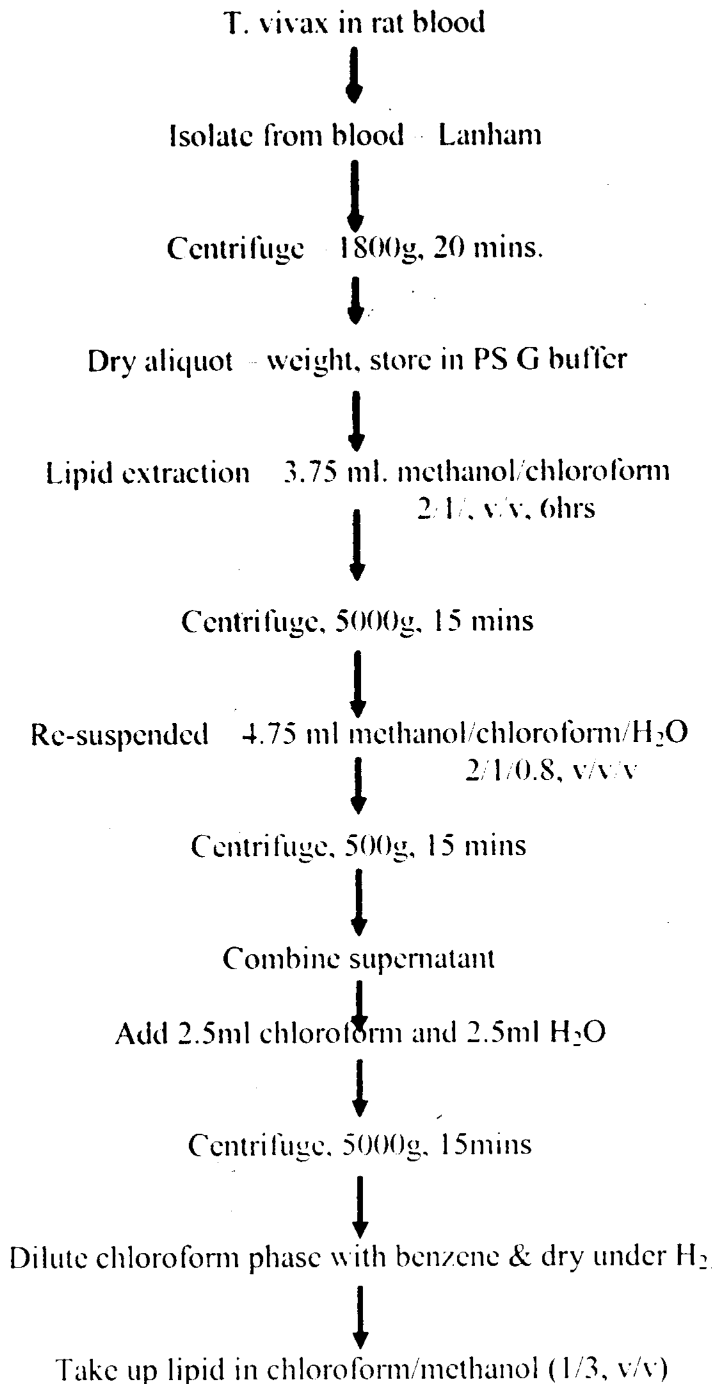


Figure 5:

Summary of the Procedure for Isolation of the Plasmalogen of T. vivax

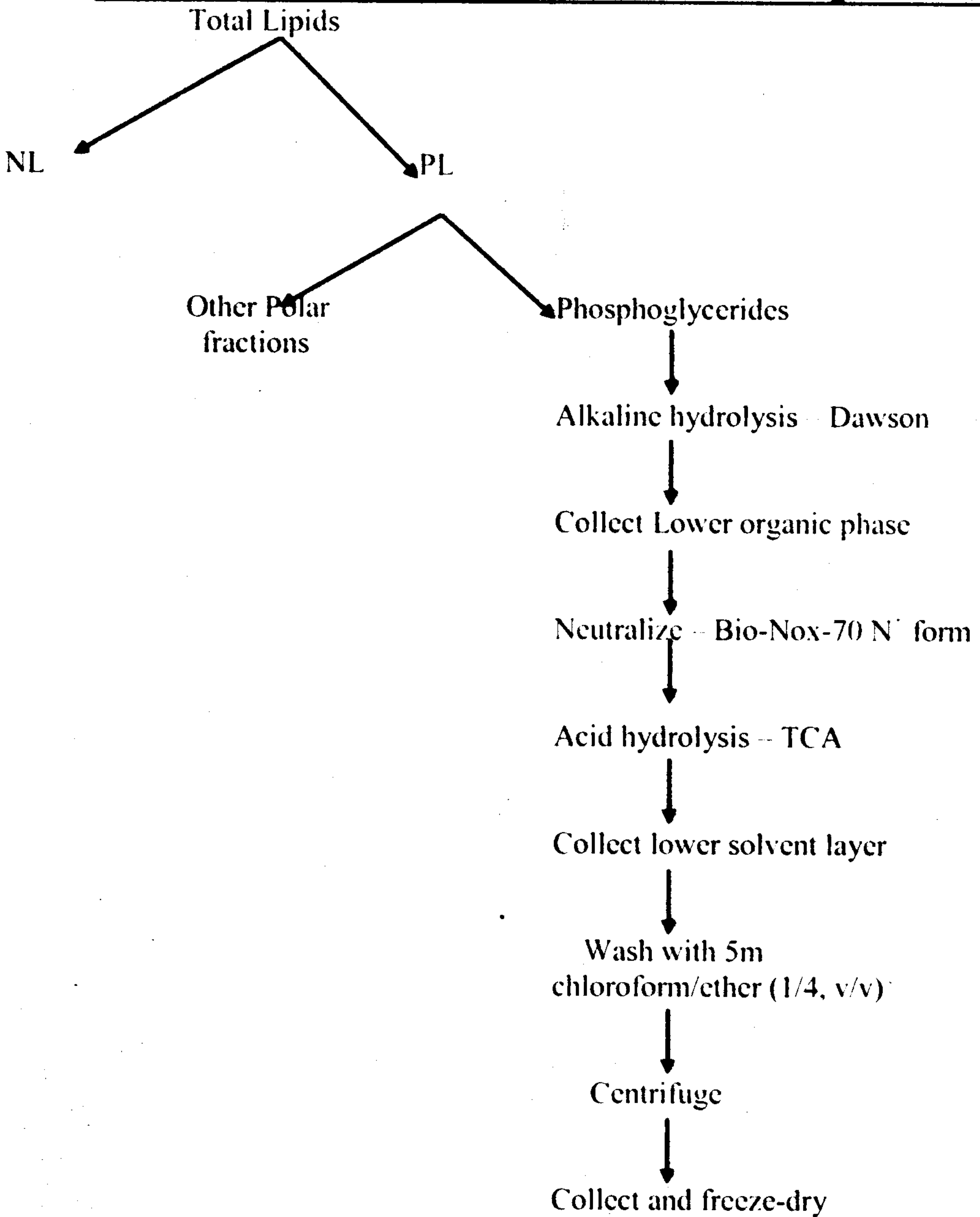


Fig. 6:

Summary of the Procedure for Paper Chromatography of the Plasmalogen of T.vivax

Dissolve extract in Solution A

Spot – Watman No. 1

Descending Chromatography

Indicator sprays

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Fig. 7

A Sulfo lipid extracted from Glomerella cingulata

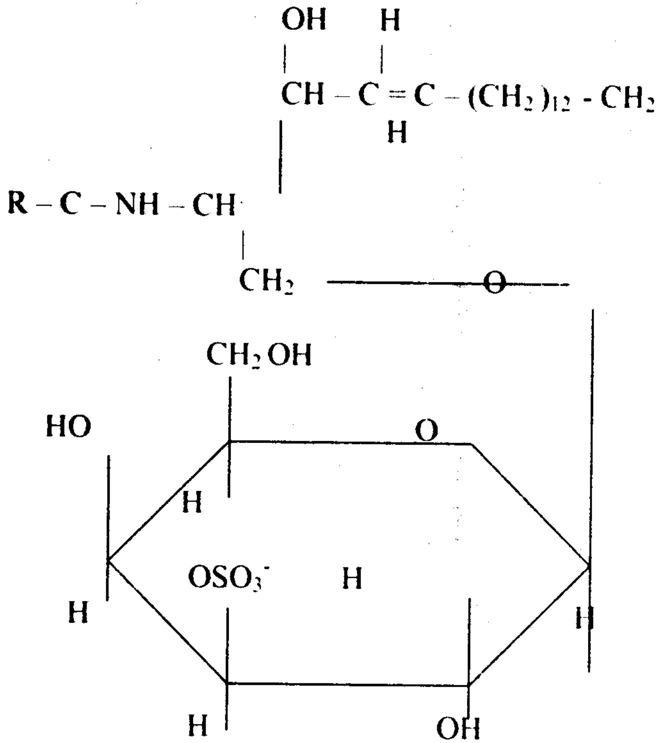

Figure 8:

Partial Structure of the Plasmalogen of T. vivax

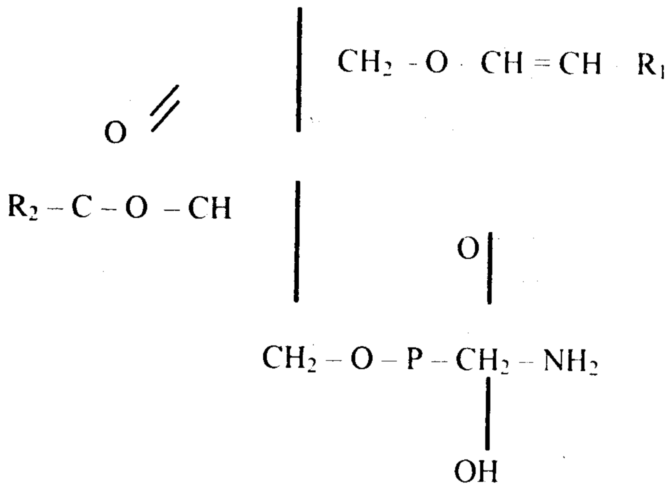

Fig. 9:
Pattern of proton resonances at the cyclopropane region of presumptive cis-9, 10-methylene octadecanoic acid of L. tarentolae

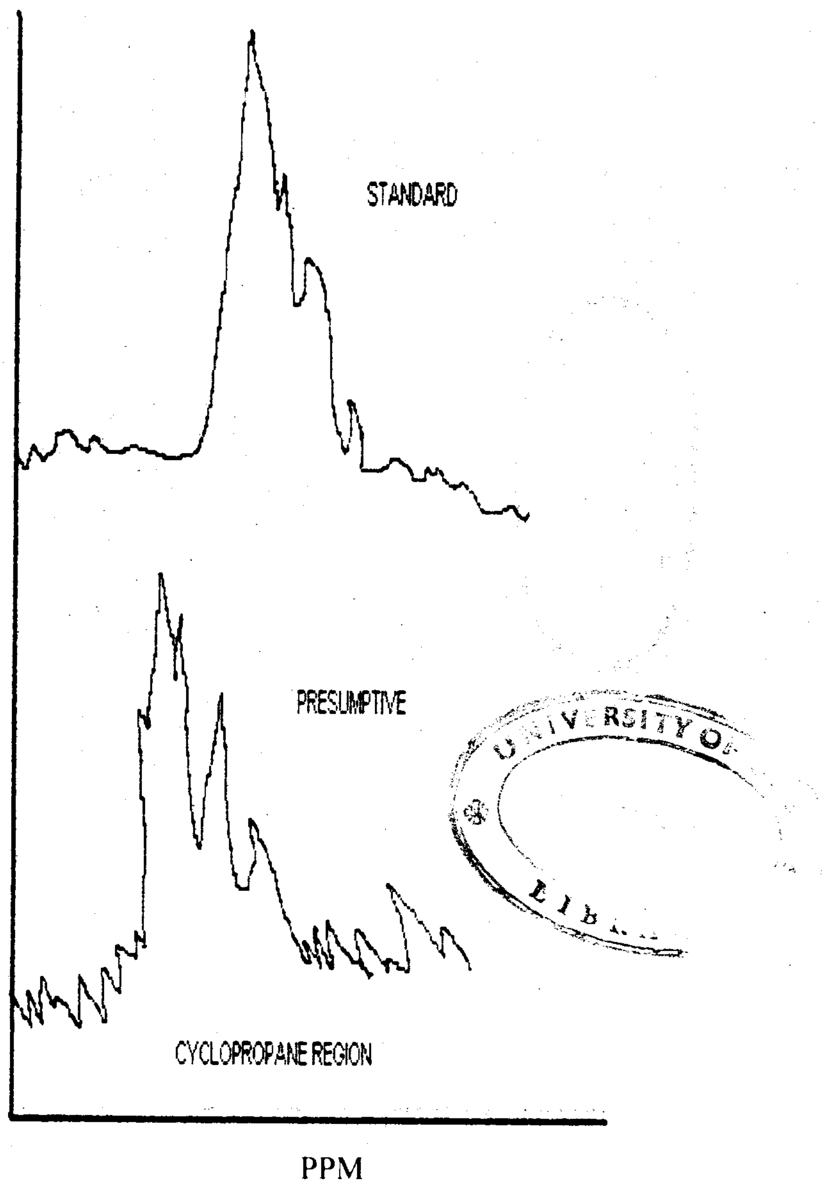

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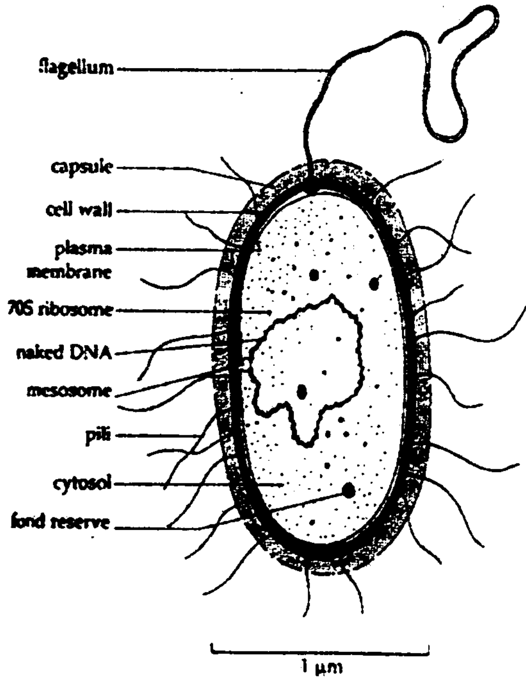
Fig. 1

Cell structure:
a) generalized prokaryotic cell; b) generalized eukaryotic cell



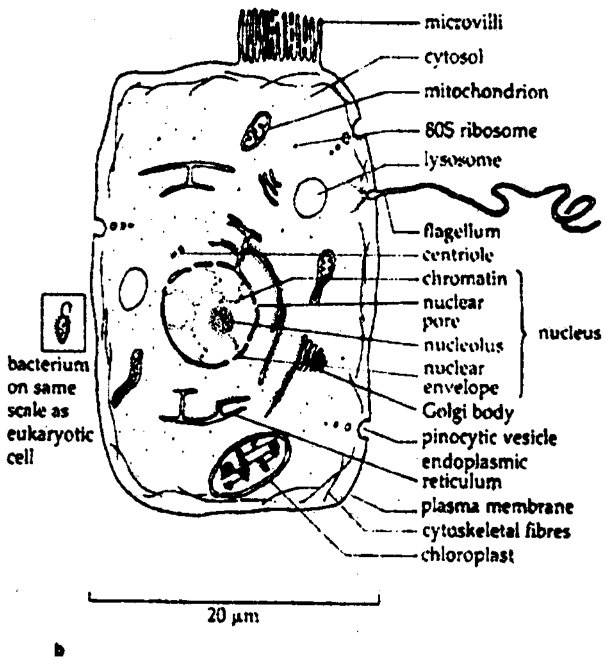


Fig. 2
The primary kingdoms (after Woese, 1981.)
Woese and Fox have given the name ukaryote to the (postulated) ancestral kingdom. E. coli (right) is a common saprophyte in the human gut. It is referred to several times in the text.

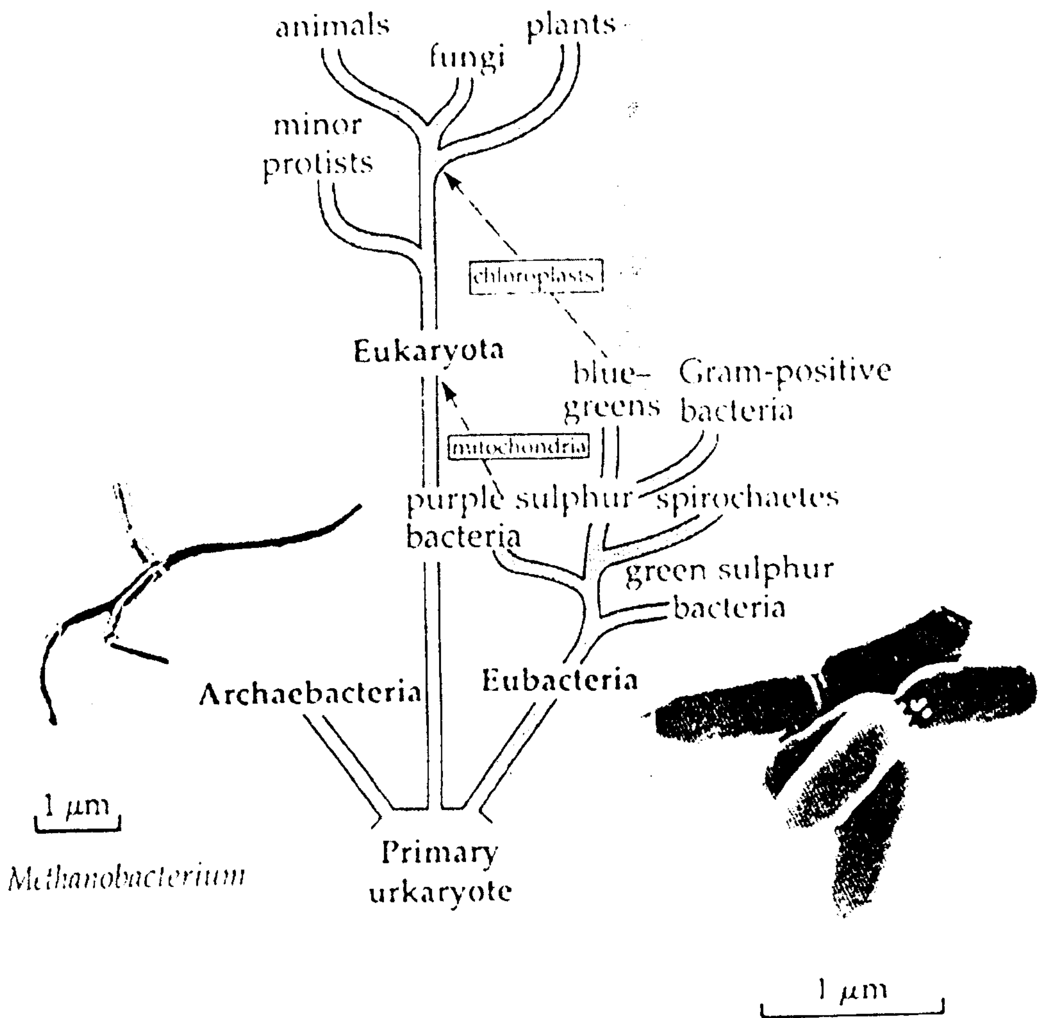


Fig. 12

Isoelectric focusing of the single protein from the EF and LPPG of the Leishimania

Table 3: Thin-layer Chromatographic (TLC) Identification of the Lipids of L. tarentolae Cultured in Neopeptone-Yeast Extract Medium With 3mCi H_332PO_4 at about 25^0C for 72hrs.

TEAE Cellulose Column Fractions	Solvents	% total of lipids by wt.	dmp 32p	Identity by TCL
1	Chloroform	34.7	0.03	Neutral Lipids
2	Chloroform/Methanol 9/1	28.8	58.8	Phosphatidyl-choline+ Trlysophosphatidyl-choline
3	Chloroform/methanol 2/1	0.4	0.3	Tr Phosphatidyl-choline tr lysophosphatidyl-choline
4	Chloroform/methanol 2/1 + 1% acetic acid	18.9	20.3	tr lysophosphatidy-lethanolamine tr N, N-dimethylphosphatidy-lethanolamine
5	Glacial acetic acid	6.7	3.3	tr Phosphatidyl ethanolamine Unknown A
6	Methanol	1.6	1.2	Tr Unknown A Unknown B Unknown C Unknown D
7	0.1H Potssium acetate in chloroform/methanol 4/1 Containing 20ml 28% aqueous ammonial/liter	9.0	16.0	Unknown E Diphosphatidyl-glycerol tr Phosphatidic acid tr Unknown F-K Phosphatidylinositorl

Table 4: Neutral Lipids of L. tarentolae cultured in Brain Heart Infusion (1 liter) with 0.25 mCi sodium (1-^{14}C) acetate, at about 25)^{0}C for 70 hrs.

Neutral Lipids	% of Neutral Lipids	
	by wt.	dpm 14C
Monoacylglycerols	1.2	0.4
Sterols	43.3	6.6
Diacylglycerols	1.5	1.8
Unesterified fatty acids	0.4	0.7
Triacylglycerols	43.0	74.4
Diacyl-alkoxy lipids	1.7	5.1
Sterol esters	9.1	11.0

Table 5:
Phospholipids of L. tarentolae cultured in Neopeptone-Yeast extract medium

Phospholipid	% of Phospholipid		
	Weight	Phosphorus	dpm^{32}p
Unknown E	5.7	1.8	tr
Diphosphatidylglycerol	5.7	4.2	3.5
Unknown F-K		tr	tr
Phosphaditic acid	tr	tr	tr
N, K-dimethylphosphatidyl-ethanol amine	tr	tr	tr
Phosphatidylethanolamine	30.0	29.5	28.2
Phosphatidylinositol	9.5	13.7	11.1
Phosphatidylcholine	51.8	43.9	52.0
Lysop hosphatidylcholine	tr	tr	tr
Lysophosphatidylethanolamine	tr	0.5	0.5
Unknown A		2.2	0.5
Unknown B	2.6	1.8	1.8
Unknown C		2.9	2.3

Table 6: **% Total Fatty Acid Methyl Esters (FAME) of L. tarentolae Grown In Brain Heart Infusion**

FAME	TOTAL LIPIDS	NEUTRAL LIPIDS	PHOSPHOLIPIDS
	1	1	-
br-C_{14}	-	-	-
14:0	4	6	2
br-C_{16}	1	tr	1
16:1	tr	1	Tr
16:0	2	1	1
17:0	-	-	-
br-C_{18}	5	1	5
18:0	12	13	11
18:1 (n-9)	28	30	27
18:3 (n-6)	8	6	8
Cyclo-C_{19}	tr	tr	1
18:3 (n-3)	28	32	30
18:3 (n-)	-	tr	-
20:2 (n-6)	tr	tr	Tr
20:3 (n-6)	-	tr	-
20:3 (n-3)	1	1	1
20:4 (n-3)	1	1	1
20:5 (n-3)	tr	1	-
22:5 (n-6)	1	tr	2
22:6 (n-3)	8	2	10

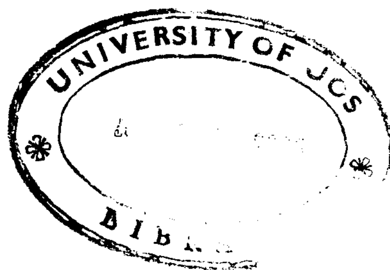

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Table 7: % total Fatty Acid Methyl Esters of Individual Lipid Fraction of L. tarentolae Grown in Neopeptone-Yeast Extract Medium

FAME	Triacyl-glycerols	Sterol esters	Diphos-phatidyl-glyceramine	Phospha-phatidyl-ethanol	Phospha tidyl-etamine	Phospha-tidyl-choline
14.0	8	3	1	1	1	1
br-C_{16}	-	1	-	2	1	-
16:0	1	1	2	1	2	1
16.1	tr	1	1	1	1	1
br-C_{18}	1	1	4	22	1	1
18.0	10	6	2	5	27	6
18:1 (n-9)	25	39	13	41	56	18
18:2 (n-6)	7	7	9	9	3	8
Cyclo-C_{19}	-	1	tr	8	-	1
18:3 (n-6)	tr	1	-	-	-	-
18:3 (n-3)	37	30	22	7	3	47
20:2 (n-6)	tr	tr	1	-	-	1
20:3 (n-6)	tr	tr	1	-	-	1
20:3 (n-3)	2	tr	1	-	-	Tr
20:4 (n-3)	1	1	1	-	-	1
20:5 (n-6)	1	1	-	-	1	Tr
22:4 (n-6)	1	1	tr	1	1	-
22:5 (n-6)	tr	-	6	-	-	1
22:5 (n-3)	1	-	1	-	3	1
22.6 (n-3)	4	4	31	1	1	12

Table 8: Chemical Composition of L. Donovani LPPG

Chemical species	Species/quantity	Condition/reagent
Proteins	One – 4.5 x 10^3m wt.	One-dimensional SDS-Polycrylamide
	Seven bands	Isoelectric focusing
	One band	Two-dimensional Gel-electrophoresis
Lipids	14:0, 1.4% 16.0, 0.20% iso 18.4, 93% 18:0, 2.00% 18:1, 1.90% 20:0(n-9), 6.46%	BF-methanol Diazomethane-Methanolysis Acid-hydrolysis Methylation
Carbohydrates	10µg sugar/ml LPPG PASS negative	Anthrone
Phosphorus	1.1µgP/ml LPPG	Fiske-Subbarrow
Amount analyzed	1g	

Table 9: Characteristics of prokaryotes and eukaryotes. The blue-green 'algae' conform entirely to the definition of prokaryotic. This text therefore adopts the more modern approach of treating them as true bacteria (Cyanobacteria) and identifies them as a group by the term 'blue-greens'.

	Eukaryotes (animal-plant cells) (eu meaning true, and karyon meaning a nucleus)	Prokaryotes (bacteria) (Pro meaning without, and karyon meaning a nucleus)
1 Nucleus	Present	Absent
2 Nucleolus	Present	Absent
3 DNA	(i) Mostly in linear chromosomes in nucleus (ii) Some in mitochondria, chloroplasts	(i) Mostly confined to single loop (chromoneme) attached to plasma membrane. (ii) Some in plasmids
4 DNA packaging	Histones package DNA in nucleosomes	No histones
5 Cell walls	(Plants only) Mostly cellulose, hemicellulose and pectate	Mostly peptidoclycan
6 Plasma membrane	Phospholipids, sterols, proteins. May form pinocytic-phgocytic vesicles or finger-like microvilli (animals only)	Phospholipids, and proteins; no sterols. No pinocytosis, phogocytosis, microvilli

12	Organization	Cells are mostly components of highly differentiated complex multicellular organism	Unicells, or short chain of similar cells
13	Size	Large (10^4-$10^5 \mu m^3$)	Small (1-10 μm^3)
14	Other	None	Gas vacuoles common in planktonic forms

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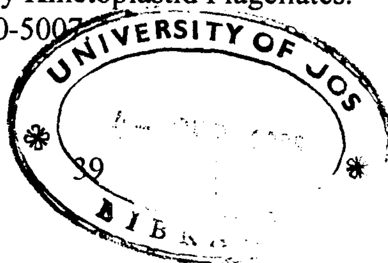

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ABOUT THE AUTHOR

Born November 17, 1940 at Ukpor in Anambra State, Professor Gregory Ejinkonye Anekwe got his primary education at St. John's Primary School Onitsha under the tutelage of his father Mr. Paul Anekwe. From 1955 to 1959 he attended the famed College of the Immaculate Conception, Enugu, from where he obtained his Cambridge School Certificate in Grade One. In 1960, he entered the prestigious Nigerian College of Arts, Science and Technology, Enugu, for his Advanced Level Studies with a Federal Government Scholarship. In 1962, while still at the Nigerian College, he won an International Cooperative Administration Scholarship from the United States Government and became one of the 10 Africans selected to study at Cuttington University, one of Africa's oldest universities (founded in 1889). He obtained his B.Sc. degree magna cum laude in 1964, thereby completing a four-year program in three years.

In 1965, he was awarded the Carver Research Foundation Fellowship from the United States of America and left for his Masters degree program at Tuskegee University. In 1967, he obtained his M.Sc. in Molecular Biology and was promptly inducted into the Beta Kappa Chi Honors Society as the student with the highest grade point average in his class.

That same year, he obtained a teaching assistantship from New York's St. John's University for his Ph.D studies in Biochemistry. At the age of 29, he obtained the Ph.D garnering simultaneously the accolade of a Dissertation Award recipient.

In the same year (1970), he won a summer post-doctoral fellowship from the U.S Department of Energy to study at Oak Ridge, Tennessee. In September of the same year, he was appointed Assistant Professor of Biochemistry at his alma matter, Tuskegee University.

From 1971 to 1972, he was a post-doctoral trainee of the U.S National Institute for Neurological Diseases and Stroke at the Marine Biological Laboratories, Woods Hole, Massachusetts. From 1973 to 1974, he was post-doctoral fellow of the United States National Institute of Health, Heart and Lung Institute at the College of Medicine, Upstate Medical University Syracuse, New York, were he specialized in tropical diseases –specifically Trypanosomiasis and Leishimaniasis. He returned to Upstate Medical in 1982 as a Senior post-doctoral.

In 1977, he won the Senior African Scholar's Award for Research from the U.S. Department of State and moved to Harvard University Cambridge Massachusetts where he was a Research Associate of the Nobel laureate, Konrad Emil Bloch.

From 1970 to 1975, Professor Anekwe was Assistant to Associate Professor at Tuskegee. In 1976, Senior Lecturer, Consultant biochemist, and Acting Head of Biochemistry at College of Medicine, University of Lagos; in 1981, Visiting Professor of Biochemistry, University of Nairobi, Kenya, as a Commonwealth Academic Exchange Fellow. In 1984, he was appointed Professor and Head of Biochemistry, University of Jos, making him the first Nigerian to be so appointed. In 1988, he was appointed Senior

Research Fellow, London School of Hygiene and Tropical Medicine, London University. Between 1988-89, he was Professor, Head of Chemistry and Dean of Science Cuttington University, and from 1996 to 1998, Visiting Professor of Biochemistry, University of Zambia. He has also been an Invited Lecturer at Morehouse College, Atlanta, Upstate Medical University, Syracuse, New York, and Harvard University Cambridge Massachusetts. He has also lectured at Goteborg, Sweden as a H.H. Powers Foundation Fellow, Sussex, England, Toronto and Montreal Canada. In 1973, he was invited to Italy as a protagonist for the third Inter Petroleum Congress in Rome.

Author of 62 scientific articles, 43 or 73% of which appeared in highly reputable journals in Europe and America, Professor Anekwe is the proponent of the Combined Law of Thermodynamics, and author of three textbooks of Biochemistry. He has supervised single-handedly a total of 66 projects leading to 66 theses and dissertations from undergraduate to doctoral and postdoctoral levels in Africa and the United States of America. His first female M.Sc. student in America wrote the formula for Joy Soap. He was instrumental to the introduction of the B.Sc. program in Biochemistry at the University of Lagos in 1976 and postgraduate program in Biochemistry in the same University in 1978. He produced the first two M.Scs at Lagos University in Biochemistry. He also introduced the postgraduate program in Biochemistry at the University of Jos. He has served in numerous capacities at the University of Jos in particular and Nigeria in general; he was the first Senate elected Chairman of the Examination Misconduct Committee at the University of Jos, Chairman, Committee on Criteria for Promotion of Academic Staff, Faculty of Medical Sciences (1990), Chairman, Panel of Investigation Department of Chemistry, Faculty of Natural Sciences (1990), Chairman, Panel of Investigation Faculty of Education (1992), Vice-chairman, Nigerian Association of Medical Scientists, Jos branch (1994), Sub-Dean, Pre-clinicals, Faculty of Medical Sciences (1994), Chairman Faculty Research Grants Committee, Faculty of Medical Sciences, (1994-1996), Chairman, Standard Organization of Nigeria, Committee for Standards for Soya Beans and Soya milk (1995), Chairman, Standards Organization of Nigeria, Committee on Standards for Rough rice, Brown rice, Milled rice and Ground rice (1995), Senate Representative Council Tenders Board, University of Jos (1995), and Chairman, Administrative Committee on Investigation of the Collection of Illegal Fees from students (2004).

Declared by the International Biographical Center, Cambridge England as one of the most biographied individuals in the world, Professor Anekwe is listed in Personalities of the South by the American Biographical Institute, Who's Who in Nigeria, Who's Who in Africa, Who's Who in the World (twice), 2000 Outstanding Scientists of the 21st Century, Living Legends, 2000 Intellectuals of the 21st Century Man of the Year (twice by two different Biographers) an Inaugural Member, Leading Scientists of the World, 2005, and MEMBER OF THE ORDER OF EXCELLENCE. He was a member of the Board of Governors, Nigerian Institute for Trypanosomiasis Research Kaduna, and

member, Special Research Project, National Institute for Policy and Strategic Studies, Kuru.

He is a member of the New York Academy of Science, member, American Association for the Advancement of Science, member, American Institute of Chemists, member, American Oil Chemists Society, member, International Society for Fat Research, member Nigerian Society for Biochemistry and Molecular Biology, member, Zambian Society for Biochemistry and Molecular Biology, and a Professional Chemist Accredited.

He is a member of the International Biographical Center (IBC), Research Council, Member Advisory Council International Biographical Center Cambridge, England, Winner, of the 21st Century Award for Achievement and 2003 Winner International Scientist of the Year Award.

He is married to Lucy Ifeoma, a nurse, herself a product of Lagos University Teaching Hospital, University of Leeds, England, and University of London. They have three Children—two boys and one girl.

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