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REVIEW ARTICLE

Ageing, Mitochondria and Diet

J.O. Olowookere

Biomembranes & Bioenergetics Research Laboratories, Department of Biochemistry, Faculty of Basic Medical Sciences, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, P.M.B. 2005, Ikenne-Remo, Ogun State, Nigeria

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I. INTRODUCTION

(i) Biogenesis of Mitochondria

The mitochondrion, as an organelle, is no doubt a specialized one for respiration and oxidative phosphorylation (1). The organelle also performs many other important functions. It has been documented (2) that mitochondria synthesize heme, lipids, amino acids, and nucleotides as well as mediating the intracellular homeostasis of inorganic ions. Although, respiration and ATP – synthesis are two main functions of mitochondria but, under anaerobic conditions, the two functions may just be a tip of the iceberg relative to the totality of metabolic functions of the cell which the organelles, mitochondria, mediate(3). In summary, it is now known that mitochondria are essential organelles which cannot be lost from the cell without the loss of cell viability. For eukaryotes, "No mitochondrion, no cell!" (4)

There is absolutely no doubting the fact that after the nucleus, the mitochondrion is next, considering essentiality for life, for they alone possess the self-replicating molecules, Deoxyribonucleic acids (DNA), which carry the blue prints for life.

Unlike the envisaged simplistic binary fission encountered in some free-living unicellular organisms, mitochondria biogenesis, is a *serious molecular business*. It involves the transcription and translation of proteins on both the nuclear genome as well as on the mitochondrial genome. Mitochondrial biogenesis involves the flow of molecules into and out of the mitochondria. Extensive work (5-10)have shown that there are co-ordinating signals between the mitochondrial and the nuclear genomes. The nucleus transmits

signals to the mitochondria via nuclear-encoded proteins that are imported into mitochondria to regulate the expression of mitochondrial DNA. Some of these coordinating signals may also be transmitted via nuclear encoded RNA, that is imported into mitochondria. Mitochondria do not appear to export macromolecules, but may influence the expression of nuclear genes, for mitochondrial proteins via exported mitochondrial products, such as heme and ATP.

Evolution of mitochondria probably, therefore, involved extensive transfer of genetic information from the endo-symbiotic "primitive mitochondria" to the host nucleus; hence present mitochondria, are totally dependent on their host cells. Biochemically, the fact of the matter is that the vast majority of mitochondrial proteins are *encoded by nuclear genes*, synthesized in the cytosol and then imported into the mitochondria(11-13). *Biogenesis of Mitochondria is, therefore, the "re-creation" of the organelle mitochondrion through the "cooperative assemblage" of its constituents by both the nuclear and mitochondrial genomes*.

(ii) Mitochondrial Bioenergetics

(a) Energy Exchanges In Living Cells

Mitochondrion may be defined as double membrane-bound organelle in the cytoplasm of aerobic cells which houses the respiratory enzymes systems. The *most orchestrated function* of the mitochondrion is its role in energy transduction. The synthesis of adenosine triphosphate (ATP) at the expense of electron transport to oxygen, by mitochondrion, is generally referred to as BIOENERGETICS.

Potential energy, locked up in foods, is converted to useful energy (ATP) for the cells. The efficiency of mitochondrion in this energy conversion (BIOENERGETICS) was put at 48 percent, the rest is lost as HEAT. The process of conversion of potential energy (from foods) to usable energy (ATP) plus Heat in living systems, is termed "Biological Energy Changes" OR BIOENERGETICS.

(b) Mitochondrial Coupling Membranes:

Mitochondria have double membranes. This feature, apart from the semi-autonomous genome, is another unique peculiarity of this organelle. It has also been documented that the chloroplast are morphologically related to the mitochondria by having double membranes. The fact that the chloroplast and the mitochondria are primary and secondary energy transducers point to a common ancestral line of evolution(14).

Functionally, the topology and organisation of constituent smaller molecules, e.g. vitamin A and membrane-bound enzymes in the inner mitochondrial membranes, have been explained in *a new molecular model for biological membranes* (15).

(c) Thermodynamic Aspect of Mitochondrial Energy Transduction

Of relevance to the subject of mitochondrial energy transduction is the second law of thermodynamics. This law, in its simplest form, states that *"No spontaneous transformation of energy to another is ever 100 percent efficient."* This is because, there is bound to be some loss of energy as heat, and it also means that the energy transducing capacity of mitochondria, in an isothermal medium, is not 100 percent efficient. Lehninger has shown experimentally that the efficiency of mitochondrial energy transduction is 48% in animals.

The heat dissipated is used to maintain the cellular homeostasis of the internal milieu within a narrow range of temperature change, which is 37^0C in man. The roles of numerous enzymes in the inner mitochondrial membrane ensure the success of this biological energy transformation.

(d) Chemiosmotic Theory of Energy Tranduction

The term "Chemiosmotic" is coined from two words, namely "Chemical" and "Osmotic." The most acceptable theory of energy transduction has been shown to have both Chemical and Osmotic components(16). According to Mitchell(16), who is the proposer of the chemiosmotic theory; he explains that the components of the respiratory chain are vectorially organized in the mitochondrial inner membrane.

The electron transport is accompanied by the translocation of proteins outwards across the inner membrane thus generating the electro-chemical gradient across the inner membrane. The potential difference is a measure of the energy pool which can be utilized by the energy requiring mitochondrial processes.

It is useful here to explain further that the function called "membrane potential" in electrophysiology is the same as the "electric membrane potential", which would generally be part of the "protein potential" (17). When quantitatively related, the various components of 'membrane potential' are related thus:

$$\Delta P = \Delta \psi - ZpH$$

where $\Delta \psi$ = electric membrane potential

P = Protonic membrane potential

$-ZpH$ = Chemical or thermodynamic parts of membrane potential.

From the above, it could be appreciated that "membrane potential" is greater than the "electric membrane potential" by the value equivalent to ZpH. The difference represents the chemical or thermodynamics component of the membrane potential as shown in the equation above. The Hydrogen-ions concentration gradient which gives rise to the "protonic membrane potential" represents the OSMOTIC component, and indeed, the main driving force, in the formation of ATP.

It has, therefore, been firmly established(17) by the chemiosmostic theory that OXIDATIVE as well as the PHOTOSYNTHETIC systems are bioenergetically 'coupled' by protonic derived force.

(iii) Universality of the Ageing Process

Imagine always enjoying the prime of life. Imagine vigorous health and keenness of mind that never wane. Does such a delightful prospect sound like fantasy? Then consider this curious fact: although some species of parrots can live up to a hundred years, mice rarely live more than three years. Such diverse life spans have led some biologists to reason that AGEING must have a cause and that if AGEING has a cause, it may as well, have a cure!

Living organisms are very different from machines. The most distinguishing characteristic of living organisms, in fact, may be their ability to carry out repairs after suffering an injury. The way the human body repairs itself after an injury is marvelous; but the routine it makes are, in some respects, even more remarkable.

Consider the bones, for example, seemingly inert when viewed from outside, but, indeed, bone is a living tissue that ceaselessly destroys and rebuilds itself throughout adult life. This rejuvenation virtually replaces the entire skeleton every ten years. Other parts of the human body is renewed more often.

Some cells in the skin, liver, and intestine may be replaced almost daily. Every second, the human body produces about 25 million cells.

There are more questions than answers regarding the phenomenon of AGEING. One thing that is very fundamental is that *ageing is universal*.

This universality stems from Deoxyribonucleid Acids (DNA) which is the molecule for the blue print of life.

Why does a house cat live for 20 years, but a similar-sized opossum lives for only three years? Why can a bat live for 20 or 30 years, but a mouse only for three years? Why can a giant tortoise live for 150 years, but an elephant, only for seventy years? The Yorubas of South Western Nigeria, has a proverb which runs thus: "*The size of the cat is not a function of its nutritional status; rather it is as a result of its "nature*". Thus, NURTURE and NATURE control size of most animals. The more important factor is NATURE or GENES for determination of overall size of any species.

Indeed, maximum life span for each specie is encoded in the GENES. Based on the varying life spans, encoded on the genes of each specie, we have the following:

S/N	NAME OF ANIMAL	LIFE SPAN
1	Bee	90 days
2	Mouse	3 years
3	Dog	15 years
4	Alligator	50 years
5	Elephant	70 years
6	Man	80 years
7	Parrot	100 years
8	Giant Tortoise	150 years

Universality of Ageing-Encoded Life Span (18)

(iv) Diet and Ageing

Growth is a characteristic of living things. Growth manifests itself in girth, length, and width. Growth and Ageing are like cause and effect. Whereas growth and ageing are attributes of all living things; ageing is an index of growth. Ageing is generally controlled by "Genes" which are life blue prints(19) but growth is controlled by NURTURE. Ageing is a universal phenomenon, controlled by "genes". The variable life spans of animals are encoded on their genes. The modulating effects of the physical environment and its numerous variables as well as its multiple determinants on both GROWTH and AGEING are worthy of note. Diet has a direct effect on Growth and an indirect one on ageing. In view of the fact that the effect of diet on ageing is indirect, many observers fail to see it!

Biochemists generally believe that a number of definable molecular processes in living organisms underlie the ageing process. In Gerontology, we come across many theories as there are investigators. This trend is hardly healthy and needs to be streamlined.

Physiologists believe that old age is not an illness. It is a continuation of life with decreasing capacities for adaptation. This view of ageing, in terms of progressive failure of the body's various homeostatic adaptive responses, has gained wide acceptance only recently.

There is, in fact, a strong tendency to confuse what we now recognize as distinct ageing process with those disease-conditions frequently associated with it.

Researchers, however, agree on the fact that ageing process involves a gradual wearing-off of somatic cells. These early researchers, while thinking on how the total number of cells in the body diminishes, implicated cell division by MITOSIS as a major factor in ageing process. It had been known for many years that, in the adult human, certain specialized cells – notably nerve and muscle cells lose their ability to divide. It is also noteworthy that the modification of the chemical environment of some cells in-vitro, by the addition of large quantities of VITAMIN E results in the cells dividing 120 times rather than 50 times usually observed before mitosis ceased.

Vitamin E is an anti-oxidant. The vitamin protects unsaturated membrane lipids from oxidation. It is this role of Vitamin E vis-à-vis its effects on the elongation of mitosis in-vitro, which invariably translates into the elongation of the process of AGEING. Although, the roles of free radicals in biologic reactions have long been recognized; only recently, with the development of new technology, has their full relevance in biochemical reactions as well as cellular toxicity been fully understood. It is now known that free radicals precipitate ageing in humans(19).

The "culprit" in ageing has been identified as the "free-radicals." Roles of Diet in Ageing, therefore, centres around how to make use of "free-radicals' quenching or scavenging foods to slow down the ageing process. The observed effect of anti-oxidant vitamins e.g. Vitamin E in prolonging the MITOTIC cell divisions raises a fundamental question of possible involvement of nutrients in longevity. The

documentations that free-radicals' scavengers are also potent anti-oxidants e.g. vitamins and lycopene (from fresh tomato) fully support that 'hypothesis' that certain diets enhance longevity. The two seemingly parallel lines produced by DIET and AGEING eventually converged. The earlier an individual takes note of this possible link, the longer that individual lives.

(v) Fruits and Vegetables As Anti-Ageing Foods

Mahatma Ghandi (1869-1948), once asserted that "*To maintain health, fruits and fresh vegetables must be fundamental part of our food*." This assertion is backed up by the fact that most fruits contain the anti-oxidants - vitamins and carotenes that "quench" free-radicals and scavenge for them in the cells. Their efficacy in fighting the ageing process has been documented (20). Most fruits and fresh vegetables of different countries and states have been recommended; but specifically, find listed below the best of them all.

TEN SUPER-ANTI AGEING FRUITS AND VEGETABLES

(1) **AVOCADO**

It is one of the super-guardians of cells because of its abundance of glutathione, the "master antioxidant" that, among other miracles, helps neutralize highly destructive fat in foods.
True, avocado is high in fat, but much of it is good fat – monosaturated, a type that resists oxidation. Eating avocados also lowers and improves blood cholesterol, better than low-fat diet does, according to research. The fruit is also rich in blood-vessel protective potassium.

(2) **BERRIES**

Blueberries, cranberries, strawberries, raspberries – they are all loaded with anti-oxidants caked antiocyanins more than any other food – three times more than the second richest sources; red wine and green tea. Both blueberries and cranberries help ward-off urinary tract infections. In one study, older people who ate the most strawberries had the lowest rate of all kinds of cancer. Berries are particularly rich in antioxidant vitamin C, an overall youth portion.

(3) **BROCCOLI**

It is hard to say enough for the anti-ageing properties of broccoli. It is blessed with an awesome array of antioxidants, particularly strong in one called sulforaphane, discovered by John Hopkins scientists. Fed to animals, the broccoli chemical revered up the activity of dioxification enzymes that slashed cancer rates by two thirds. Broccoli is packed with free-radical fighters, vitamin C, beta carotene, quercetin, indoles, glutathione, and loutein. Broccoli is one of the richest food sources of the trace metal chromium, a life extender and protector against the ravages of out-of-control insulin and blood sugar. In women, broccoli helps the body get rid of the harmful type of estrogen that promotes cancer.
Broccoli eaters also have less of colon and lung cancer and cardio-vascular disease. Eating broccoli has even been linked to longer survival in lung cancer patients.

(4) CABBAGE

Like broccoli, cabbage is a cruciferous vegetable with potent antioxidant activity. Men who ate cabbage once in a week compared with once in a month had only 66 percent the risk of colon cancer, one classic study found. Cabbage also seems to deter stomach and breast cancers. A specific antioxidant in cabbage, indole-3-carbinol, accelerates the disposal of a harmful form of estrogen that promotes cancer. It was discovered that about 70 percent of a large group of women who ate cabbage started burning off dangerous estrogen within five days. Effective dose is a fifth to a third of a head of cabbage. Savoy cabbage (the crinkly type) is strongest. To get the most anti-ageing benefits, eat cabbage and other cruciferous vegetables raw or lightly cooked.

(5) CARROTS

Carrots are legendary in fighting off ageing diseases. A recent Harvard study found that women who ate carrots, at least, five times a week, reduce their risk of having stroke by 68 percent!

In another research, eating a couple of carrots a day lowered blood cholesterol by 10 percent in men. Countless studies pinpoint beta carotene carrots, main antioxidant asset, as a powerhouse against ageing and disease. The beta-carotene in a daily medium carrot cuts lung cancer risk in half, even among former heavy smokers. People with low-levels of beta-carotene in their blood are more apt to have heart attacks and various cancers and to die or be disabled by strokes. Beta-carotene helps protect the eyes from sight-robbing diseases that develop with ageing. The orange pigment also boosts immune functioning. One medium carrot contains about 6 million of beta-carotenes. For a real injection of beta-carotene, try carrot juice, one cup contains 24 milligrams of beta-carotene.

(6) CITRUS FRUITS

The orange is so full of antioxidants that officials at the National Cancer Institute have called it a complete package of every class of natural anti-cancer inhibitor known including carotenoids, terpenes, flavonoids, and vitamin C. Grapefruits, too, have a unique type of Fiber, especially in the membranes and juice sacs, that reduces cholesterol dramatically and, even, may reverse the ageing disease, atherosclerosis.

(7) GRAPES

The anti-ageing secrets of grapes is simple and powerful. Grapes contain twenty known antioxidants that work together to fend off oxygen-free radical attacks that promote disease and ageing.

The antioxidants are in the skin and seeds, and the more colourful the skin, the greater the antioxidant punch. That means, red and purple grapes are more powerful. Grape antioxidants have anticlogging activity, inhibit oxidation of LDL cholesterol, and relax blood vessels. The antioxidant quercetin, also plentiful in onions and tea, appears to be one of grapes foremost anti-ageing components.

(8) ONIONS

A close kind of garlic, the onion, too, has diverse anti-ageing activity. Full of antioxidants, onions help prevent cancer, especially stomach cancers 'thin the blood' discouraging clots, and raise good-type HDL cholesterol. Red and yellow onions (not white onions) are the richest foods quercetin, a celebrated antioxidant that inactivates cancer-causing agents, inhibits enzymes that spur cancer growth, has anti-inflammatory, anti-bacterial, anti-fungal, and anti-viral activity.

Quercetin also keeps bad LDC cholesterol from turning toxic and attacking arteries. In some studies, onions have quashed the ability of fats, such as butter, to initiate formation of blood clots to clog arteries. In horses, onions have long been used to dissolve clots.

(9) SPINACH

This green leafy vegetable is packed with a variety of anti-oxidants. No wonder, spinach and its components often show up in studies, as warding off a variety of free radical-inspired diseases, including cancer, heart disease, high blood pressure, strokes, cataracts, muscular degeneration, and even psychiatric problems. One of the most striking antioxidants in spinach is lutein, thought to be as a strong anti-ageing agent as well known beta-carotene; spinach, however, is rich in both. Eating high amount of spinach cuts the risk of macular degeneration, a potential cause of blindness by 45 percent. Eating a cup of raw spinach or a half cup of cooked spinach daily may slash lung cancer odds in half, even among former heavy smokers. Spinach is very rich in folic acid, a brain and artery protector – as well as anti-cancer agent.

(10) **TOMATOES**

The familiar tomato is an unexpected anti-ageing powerhouse. Tomatoes are, by far, the richest and virtually only reliable source of a remarkable antioxidant, lycopene. Mind-boggling new research suggests that lycopene preserves mental and physical functioning among the elderly. High blood levels of lycopene

also reduce the risk of pancreatic and cervical cancer. Those who take the most raw tomatoes are also only half as likely to have cancers of the digestive tract – oral cavity, pharynx, oesophagus, stomach, prostate gland, colon or rectum as those eating the least tomatoes. Tomatoes chemicals – acid and chlorogenic acid suppress formation of cancer – causing Nitrosomonas. Only tomatoes and watermelon contain substantial amounts of lycopene.

(vi) Is Ageing A Diseased Condition?

Physiologists believe that ageing is not a diseased condition. It is a continuation of life with decreasing capacities for adaptation. More and more scientists are now supporting this line of thinking. Biochemists and Molecular Biologists agree that the various life-spans of various species of animals are essentially encoded on their genes. To the extent that 'genes' are vulnerable to various insults and assaults; their sanctity and functions can be derailed! The extent of derailment determines overall effects on the process of Ageing and longevity.

It is a fact that there are a lot of diseases that accompany the ageing process; ten of them are listed below:

- Hypertension
- Arteriosclerosis
- Diabetes
- Alzheimer's disease
- Cancer
- Congestive heart disease
- Dementia
- Parkinson's disease
- Diminishing Vision
- Diminishing hearing ability

The effects of ageing are most pronounced in Africa and developing countries being accentuated by poverty and ignorance. While a youthful attitude is desirable, no one needs to simply accept a decline in vitality ; be it wrinkles and balding, gray hair or loss of muscle mass and tone – as his or her fate, because one has reached a particular age. For every practical purpose and healthy living, we all should endeavour to AGE GRACEFULLY and regard age as just a number, whilst we try to regain our youthfulness through selected anti-ageing foods and general exercises. Indeed, anybody that does not tune inward to regulate his or her internal clock would get blown along with the tempest that accelerates the ageing process in man.

II ENERGY TRANSDUCTION IN MITOCHONDRIA

(i) The Organization and Function of the Coupling Membranes

The redox reactions which comprise respiration (or photosynthetic electron transfer) occur in a strictly defined sequence. In this respect, they resemble the multienzyme pathways of glycolysis or the tricarboxylic acid cycle. However, in contrast to these latter processes, electron transfer and the associated reactions which lead to ATP synthesis are completely membrane-bound. Thus, photosynthesic energy conservation occurs in the thylakoid membranes of plant chloroplasts and in the chromatophores or internal membranes of photosynthetic bacteria. Oxidative phosphorylation is located in the mitochondrial inner membranes of aerobic eukaryotes and in the plasma membranes of bacteria. Since these membranes normally couple their various redox systems to the synthesis of ATP, they are often referred to as coupling membranes.

Clearly, one of the functions of these membranes is to organize the individual redox carriers and coupling enzymes such that they form an efficient and easily regulated energy-transducing unit. In addition, the coupling membrane probably plays an intrinsic role in the mechanism of energy transduction itself since the latter cannot occur in the absence of a membrane structure and is seriously impaired if the integrity of the membrane is damaged; the precise nature of this intrinsic role is, at present of considerable

controversy. Finally, the coupling membrane usually represents one of the major permeability barriers of the cell, strictly controlling the movement of certain solutes into and out of the compartment which it encloses. Thus, the membrane grossly controls its own activity by regulating the supply of certain substrates or cofactors and, at the same time, protects itself from the potentially deleterious effects of osmotic imbalance. These functions, although common to all coupling membranes, have been most intensively investigated in the eukaryotic mitochondrion.

(ii) Mitochondrial Structure and Function

The mitochondria are the sites of oxidative phosphorylation in all animal and plant tissues, and also in aerobically-grown yeasts and protozoa. In the former, they are usually found in the greatest abundance where the need for ATP is greatest or where the supply of substrates for oxidation is most prolific. The liver, pancreas, epithelia, brain and muscle are particularly rich in mitochondria which are able to oxidize the fatty acids supplied by the fat droplets.

Although mitochondria were first observed microscopically in the mid-nineteenth century, it was not until 1948 that Hogeboom, Schneider and Palade were successful in isolating these organelles intact. The trick was to disrupt the tissues gently in an isotonic, buffered sucrose solution which prevented the lysis of these relatively fragile structures. Fig. 1a shows mitochondrial structures as described by Lehninger (1970). The main function of mitochondria is biological energy transduction, i.e. synthesis of ATP from ADP and inorganic phosphate.

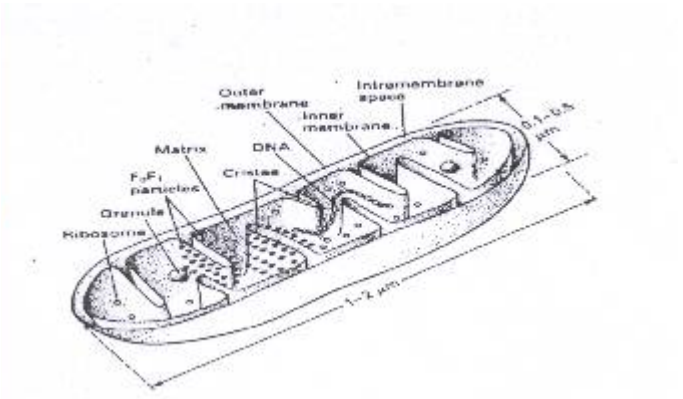


Fig. 1a. A longitudinal, three-dimensional structure of a mitochondrion.

(iii) Energy Coupling Sites in Mitocondria

Three energy-coupling sites between NADH and molecular oxygen (sites I, II and III) were located as shown in Fig. 1b. A fourth site (site O) can be demonstrated experimentally at the level of transhydrogenase ($NADPH - NAD^+$) using carefully controlled substrate concentrations. But since the E_o' of this reaction is very low, this fourth site is probably of little significance to energy conservation *in vivo*.

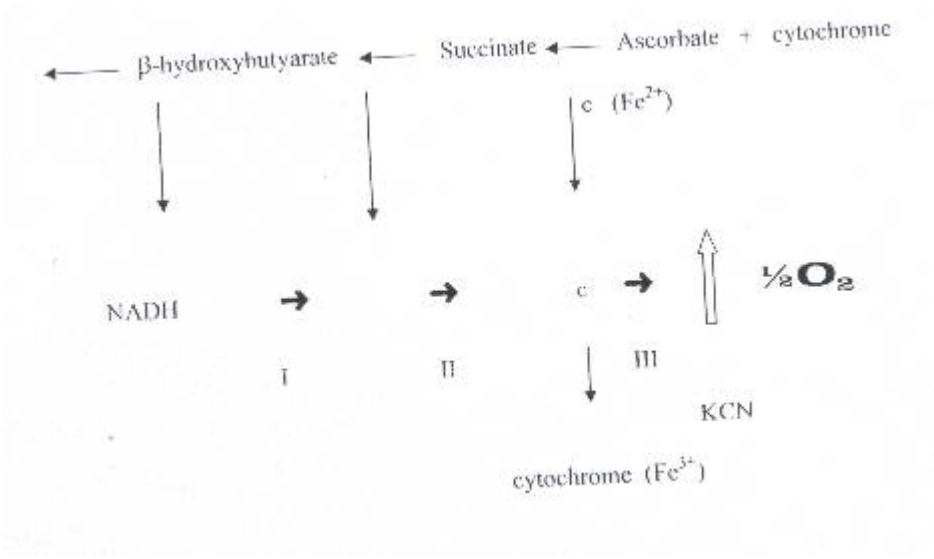


Fig. 1b: Energy Coupling Sites

Table 1: Energy Coupling Sites

Donor	Acceptor	Inhibitor	P/O (P/2e)
β-Hydroxybutyrate	O_2		3
Succinate	O_2		2
Ascorbate + c (Fe^{2+})	O_2		1
β-Hydroxybutyrate	c (Fe^{3+})	KCN	2
Succinate	c (Fe^{3+})	KCN	1

(iv) Respiratory Control in Mitochondria

In 1956, Chance and Williams reported that in the presence of excess substrates, oxygen and inorganic phosphate, the respiratory activity of intact mitochondria was effectively controlled by the availability of ADP. This process is called respiratory control.

In the absence of the phosphate acceptor (ADP), the respiratory rate is low (Fig. 2) and the mitochondria are said to be in the controlled state (or state 4). Following addition of ADP, the respiratory rate increases dramatically (active state or state 3) and remains rapid until the ADP is almost completely esterified, at which point the respiratory rate declines, again, into state 4. Successive ADP-induced cycles can be seen until the reaction mixture becomes depleted of either substrate or oxygen.

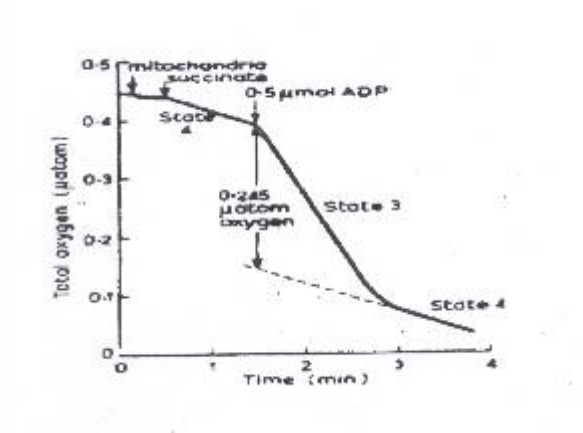


Fig. 2: Respiratory Control

This type of experiment indicates that respiration and energy conservation (ATP synthesis) are tightly coupled in intact mitochondria; the former being controlled by requirement for the latter. The consumption of substrate and oxygen is thus determined by the mitochondrial requirement for ATP such that, under normal conditions, unnecessary respiration does not occur.

It is now clear that the state 3 to state 4 transition is not a direct effect of ADP concentration, i.e. does not depend on the saturation kinetics of the phosphorylation system, but results indirectly from its effect on the phosphate potential. The latter reflects the work (G) which must be carried out by the respiratory chain in order to effect ATP synthesis. From the equation

$$G = G^{o'} + 2.303\ RT \log [ATP]/ [ADP][Pi]$$

the phosphate potential clearly varies with the [ATP]/[ADP][Pi] ratio. Slater has neatly measured the phosphate potential for rat liver mitochondria catalyzing state 4 respiration and found a value of 67.4 kJ mol^{-1}. This is almost equivalent to the theoretical maximum value of 73.0 kJ mol^{-1} (219/3) which can be developed by the respiratory chain. It follows, therefore, that respiration and phosphorylation are in thermodynamic equilibrium in state 4 and the rate of respiration is ultimately controlled by the back pressure of the phosphate potential.

An analogous type of control has also been demonstrated for a number of bacterial respiratory systems and also for photosynthetic electron transfer in chloroplasts. In the former, the primary dehydrogenases are occasionally subject to direct kinetic control by the ambient energy change. This concept was introduced by Atkinson to describe the energy status of the cell and is equal to

$$\frac{\frac{1}{2}\ \{ [ADP] + 2\,[ATP] \}}{\{ [ADP] + 2\,[ATP] \}}$$

Values may, therefore, range from 0 to 1.0 but are usually about 0.8. It appears to play a very important controlling role in metabolism via its action on key regulatory enzymes of both anabolism and catabolism, but generally has only a minor influence *per se* on respiration.

There is some evidence that the succinate dehydrogenase of the mitochondrial respiratory chain may also be subject to direct control involving activation or deactivation by various redox and energy signals.

These include succinate itself, oxaloacetate, ATP and ubiquinone. The purpose of this highly specific control system is apparently to ensure that the respiratory chain preferentially oxidizes NADH (i.e. the most

abundant and energetically potent of its two major substrates) when the cell requires maximum ATP synthesis.

Several other important parameters may also be calculated from the type of experiment described in Fig. 2. Of prime importance amongst these is the ADP/O ratio which is, of course, numerically equal to the P/O ratio or P/2e ratio. Since respiration and phosphorylation are closely coupled, then the extent of state 3 respiration is determined by the amount of ADP which is available for esterification.

From this experiment, it can be seen that the addition of 0.50 mol of ADP causes the rapid uptake of an extra 0.245 atom of oxygen (substrate, succinate):

ADP/O = 0.5/0.245 = 2.08

It is also possible to measure the respiratory control index (RCI) which is simply the ratio of the state 3 to state 4 respiratory rates. The value of RCI, which is rarely above 7 – 8 and is dependent upon both the nature of the substrate and the source of the mitochondria, gives an approximate guide as to the integrity of the coupling membrane.

(v) Uncoupling Agents of Mitochondrial Oxidative Phosphorylation

A large number of compounds are known which, when added to mitochondria, abolish respiratory control (Fig. 3) and ATP synthesis, but stimulate ATPase activity. Since energy conservation is no longer coupled to respiration, these compounds are called uncoupling agents (ΦH). Several types of uncoupling agents are known but the simplest are all lipid-soluble, weak acids, e.g. 2,4-dinitrophenol (DNP) and carbonyl cyanide metriflurophenylhydrazone (FFC, Fig. 4). These uncoupling agents appear to affect all three phosphorylation sites to a similar degree and have no requirements for either ADP or inorganic phosphate. It is likely, therefore, that a non-phosphorylated, energized state exists between respiration and the final states of ATP synthesis which is susceptible to destruction by lipophilic anions (Φ). This view is supported by the observation that the normally low ATPase activity of intact mitochondria is stimulated several-fold by the addition of an uncoupling agent.

Similar conclusions have also been drawn for photosynthetic phosphorylation and bacterial respiration. The former is uniquely uncoupled by NH_4^+ ions, a phenomenon which reflects the inverted polarity of the thylakoid membranes of the coupling membranes of intact mitochondria and bacteria.

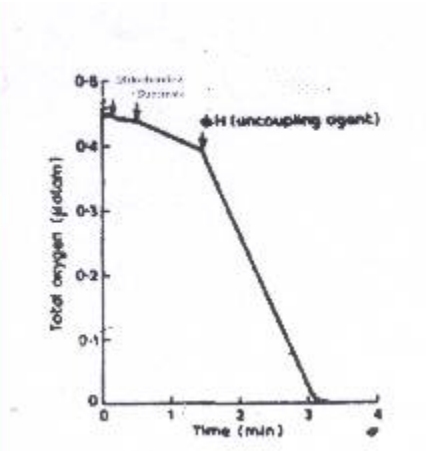


Fig. 3: Action of Uncoupling Agent (ΦH)

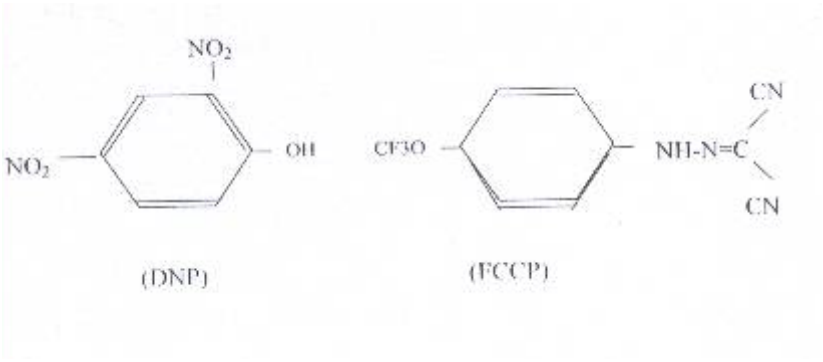


Fig. 4: Structures of DNP and FCCP

(vi) Phosphorylation Inhibitors in Mitochondria

In contrast to the action of DNP or FCCP, the antibiotics oligomycin and aurovertin inhibit ATP synthesis by their direct effect on the reversible ATPase complex. These compounds are thus classified as phosphorylation inhibitors. Since respiration and phosphorylation are tightly coupled in intact mitochondria, these compounds cause inhibition of state 3 respiration, bringing it back to state 4 rate (Fig. 5). Very interestingly, the addition of an uncoupling agent to oligomycin-inhubuted mitochondria immediately causes the relief of this inhibition and respiration increases to the normal uncoupled rate. The ability of uncoupling agents to overcome inhibition by oligomycin has important implications for the mechanism of oxidative phosphorylation, since it indicates that the former acts closer to the respiratory chain than the latter. In support of this, uncoupler-stimulated ATPase activity is strongly inhibited by the addition of oligomycin.

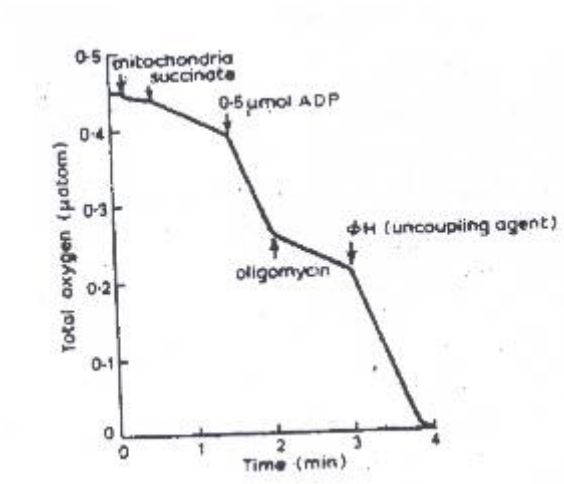


Fig. 5: Action of phosphorylation inhibitors

(vii) Reversed Electron Transfer in Mitochondria

Since respiration and phosphorylation are in thermodynamic equilibrium, it should be possible for intact mitochondria to reverse the direction of electron transfer at the expense of ATP hydrolysis. This

hypothesis was confirmed in 1960 by the separate demonstrations by Chance and Klingenberg that mitochondria catalyse the ATP-driven reduction of NAD^+ by succinate (Fig. 6). This reaction is highly efficient energetically, hydrolyzing only one molecule of ATP per molecule of NAD^+ reduced. Since this process proceeds optimally under anaerobic conditions, or when the respiratory chain is inhibited on the oxygen side of cytochrome b, it is often referred to as anaerobic, reversed electron transfer. NAD^+ reduction is abolished in the presence of uncoupling agents and/or phosphorylation inhibitors.

In contrast, it is also possible to drive the reversal of electron transfer at site I using energy which is conserved as a result of forward electron transfer at sites II or III. This process of aerobic, reversed electron transfer is also inhibited by uncoupling agents, but is usually stimulated slightly by phosphorylation inhibitors.

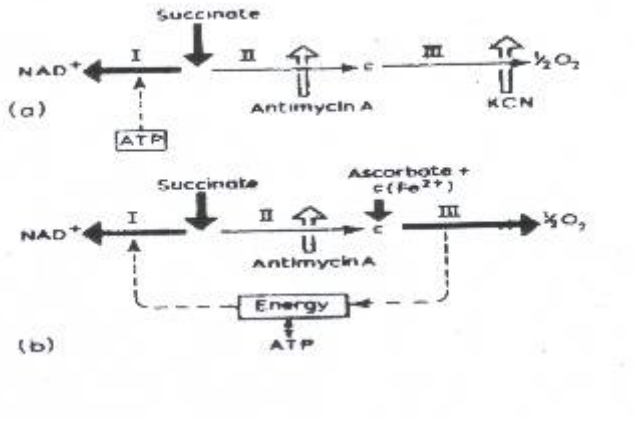


Fig. 6: Reversed electron transfer. (a) Anaerobic, (b) Aerobic.

The transhydrogenase affords a similar example of energy-dependent, reversed electron transfer during the reduction of $NADP^+$ by NADH.

Complete reversal of mitochondrial respiration (i.e. from water to NAD^+ with the liberation of molecular oxygen) has recently been successfully demonstrated using ATP at a very high phosphate potential as the source of energy.

Reversed electron transfer in mitochondria, therefore, reflects a tight, reversible coupling between oxidation and phosphorylation but it is probably of little physiological significance. In contrast, the ability of many chemolithotrophic bacteria to reduce NAD^+ at the expense of high potential, inorganic electron donors is crucial to their autotrophic mode of life.

(viii) The Concept of Energized States in Mitochondria

It is clear from the foregoing descriptions of the energy-conserving and energy-utilising properties of coupling membranes that energy transfer between the redox system and the ATP synthetase is fully reversible and must occur through a common non-phosphorylated energised state (Fig. 7). Experimental evidence suggests that the latter is destroyed or dissipated by uncoupling agents, but not by phosphorylation inhibitors which appear to act at a more distal point from the chain. The energised state probably drives a number of membrane-associated functions *in vivo* (e.g. reversed electron transfer, ion or nutrient transport) at the ultimate expense of either respiration, photosynthetic electron transfer or ATP hydrolysis.

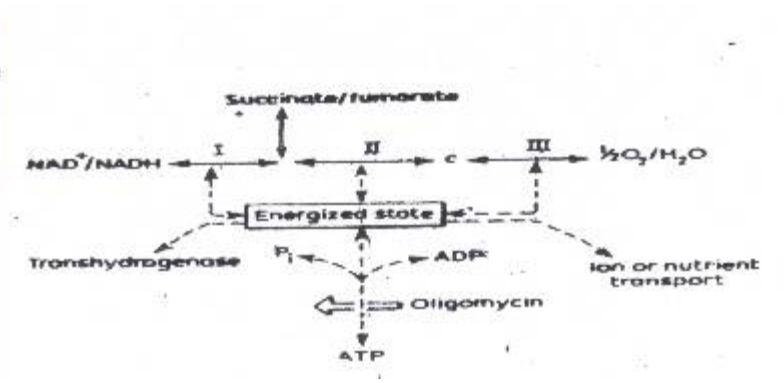


Fig. 7: The energised state.

(ix) Current Models of Membrane Structure

Danielli and Davson (1935) proposed the Unit Membrane Model. This model stated, among other things, that membranes are made of bilayers of protein-lipid. In 1959, this model was improved upon and put in better perspective by Robertson. The Danielli-Davson-Robertson unit membrane model thus became the precursor of the current models of biological membranes. This model described biomembranes as having protein-lipid and lipid-protein outlines. The core is essentially the lipid bilayer while the outer part is covered by protein. The current models of biological membranes are:

(1) Fluid Mosaic Model (Singer and Nicholson, 1972)
(2) Biphasic Molecular Model (Olowookere, 1986).

These models describe both the structural, functional and thermodynamic aspects of biological membranes such that most of the results of all biochemical, microbial and molecular experiments could be explained and reasonably discussed. They also provide the basis for the much sought after multidisciplinary applications in areas like pharmacology, pharmaceutical chemistry and other areas of medicine.

(1) The Fluid Mosaic Model of Biological Membranes

In 1972, Singer and Nicholson (21) proposed a fluid mosaic model for the gross organization of biological membranes. The essence of this model is that membranes are two-dimensional solutions of oriented globular proteins and lipids. This proposal is supported by a wide variety of experimental observations, the major features of which are:

(i) Most of the membrane phospholipids and glycolipids are in bilayer form. This lipid bilayer has a dual role of being a permeability barrier as well as a medium for suspending for integral membrane proteins.

(ii) A small proportion of membrane lipids interact specifically with particular membrane proteins and may be essential for their function.

(iii) Membrane proteins are free to diffuse laterally in the lipid matrix unless restricted by special interactions, whereas they are not free to rotate from one side of the membrane to the other.

Membranes are asymmetric

Membranes are structurally and functionally asymmetric, as exemplified by the orientations of glycophorin and the anion channel. Also, more generally, by the external localization of membrane carbohydrates. The outer and inner surfaces of all known biological membranes have different components and different enzymatic activities.

A clear-cut example is provided by the pump that regulates the concentrations of Na^+ and K^+ ions in cells. This transport system is located in the plasma membrane of nearly all cells in higher organisms. The Na^+-K^+ pump assembly is oriented in the plasma membrane so that it pumps Na^+ out of the cell and K^+ into it (Fig. 9). Furthermore, ATP must be on the inside of the cell to drive the pump. Ouabain, a specific inhibitor of the pump, is effective only if it is located on the outside.

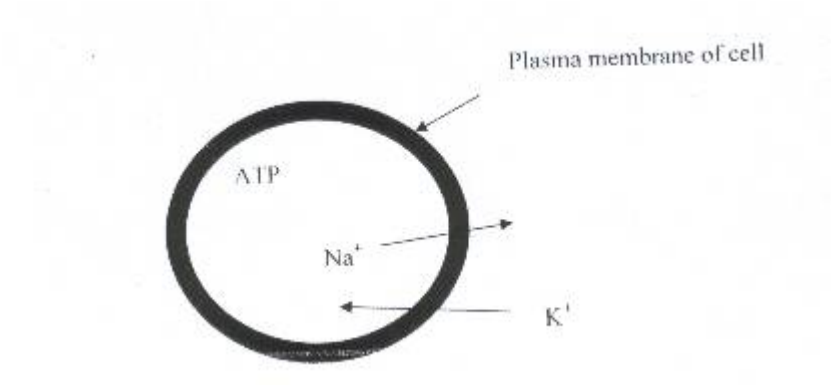


Fig. 9: The asymmetry of the Na^+-K^+ transport system in plasma membranes.

Some membrane proteins are deeply embedded in the lipid bilayer

Some membrane proteins can be released by relatively mild means, such as extraction with solutions of high ionic strength (e.g. 1M NaCl). Other membrane proteins are bound much more tightly and can be separated only by using detergents or organic solvents (Fig. 10).

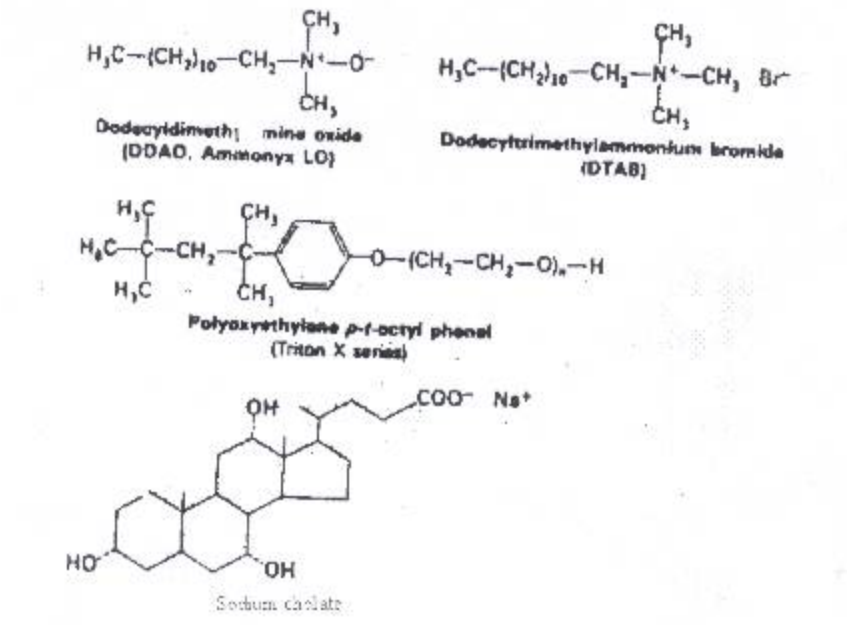


Fig. 10: The structures of some detergents used to solubilise membrane proteins.

Membrane proteins can be classified as being either peripheral or integral on the basis of this difference in ability to dissociate from membranes (Fig. 11). Integral proteins interact extensively with the hydrocarbon chains of membrane lipids and so they can be released only by agents that compete for these non-polar interactions. In contrast, peripheral proteins are bound to membranes by electrostatic and hydrogen bonding interactions. Thus, these polar interactions can be disrupted by adding salt or by manipulating the pH of the solution. It now appears that most peripheral membrane proteins are bound to the surfaces of integral proteins.

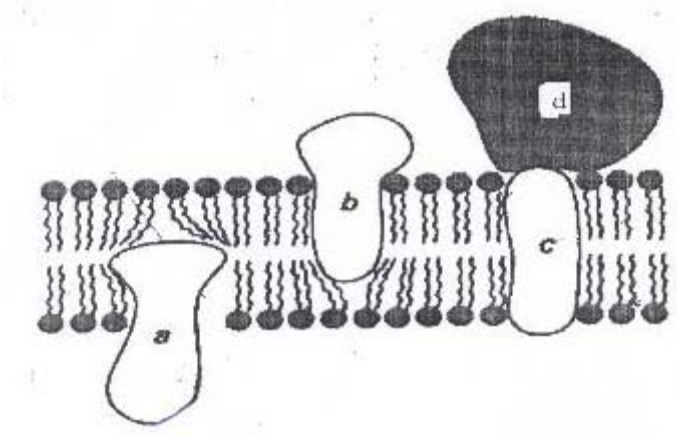


Fig. 11: Integral membrane proteins (a, b, c) interact extensively with the hydrocarbon region of the bilayer. Peripheral membrane proteins (d) bind to the surface of the integral membrane proteins.

(2) Biphasic Molecular Model of Biological Membranes

The chemical composition of a particular membrane is not necessarily constant. This is particularly true of the constituent 'mosaic' molecules than the structural lipoproteins. Change in the composition of membranes can be induced by hormones, drugs or, more conveniently, by nutritional manipulation (22). The need to effectively explain the correlation between the structure of a cell membrane and its amphipathic properties vis-à-vis its *functional* roles in health and disease led to the proposition of this model.

Description of model

Phase-contrast molecular model of biological membranes is pivoted on the anisotropic property of cell membranes. The specific location and orientation of the various molecules are very significant in the functionality of biological membranes. This model, therefore, proposes that the locations of these molecules is phase-contrasted. Whereas sugar residues, immunoglobulins, receptors and porter molecules are located on the outer phase (outside) of a single membrane, membrane-bound enzymes, anti-porters, and vitamin A may be located on the inner phase (inside) of a single membrane or span the whole structure. The model also proposes that for multimeric membrane enzymes, e.g. cytochrome oxidase, the topology is always specific as envisaged in the phase contrast phenomenon for functional effectiveness.

Any disruption of these molecules, violating the phase-contrast arrangement *in vivo* or *in vitro* will result in functional aberration. This phase-contrast molecular arrangement form the bedrock of *functional* interpretation and explanation of why and how the properties (structural and functional) of biomembranes may be affected by nurture, age and state of health.

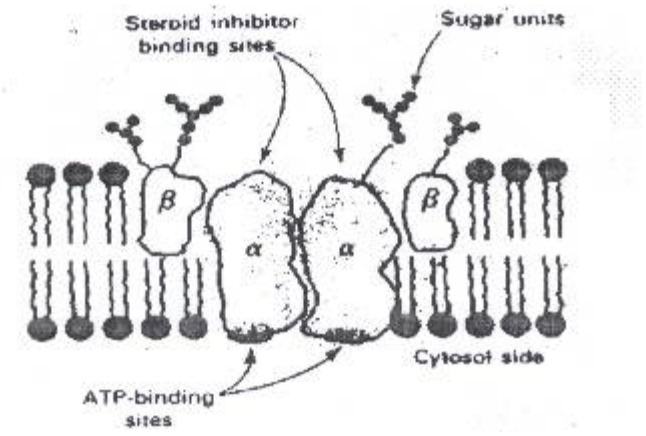


Fig. 12: Schematic diagram of the subunit structure and orientation of the Na^+-K^+ pump in plasma membranes illustrating the biphasic molecular model for biological membranes.

In a series of experiments between 1976 and 1985, using rat liver mitochondria as a model and suitable dietary manipulations, 20 litter mater, male and female weanling rats (Wistar strain) were fed a low-protein diet (LPD, 3.47% protein) for 30 days. At the end of the feeding period, the rats developed a marasmic-kwashiorkor syndrome. Ten of the rats were killed by cervical dislocation and the livers were removed into 0.25M sucrose solution. Mitochondria were isolated using the method of Schneider (24). Various biochemical experiments were carried out to study the structural and functional status of the mitochondria.

The remaining ten rats were rehabilitated for 3 weeks by feeding 21% dietary protein. The rat liver mitochondria were isolated and the same set of experiments were carried out on them. These experiments were repeated many times. Various lesions of rat liver mitochondrial membranes were observed in the animals. The deductions from these observations led to the proposal of the phase-contrast molecular (functional) model for biological membranes. These observations are itemised below:

(i) Deviation from vectorial movement, low ATP formation and high ATPase activity (see Ref. 25).

(ii) Drastic fall to 24% and a sudden jump to 90% in the activities of cytochrome oxidase directly traceable to the distortion in the specific orientation/topology of its various subunits (see Ref. 26).

(iii) Defective vitamin A utilization as membrane component, leading to functional aberration (see Ref. 27a).

The proposed model is a *functional* model. It does not subsume or replace the 'fluid mosaic' model. Rather, it complementary to it and was proposed by Olowookere in 1986 (27b).

III. THEORIES OF AGEING

The various theories of ageing as documented in (19) are as follows:

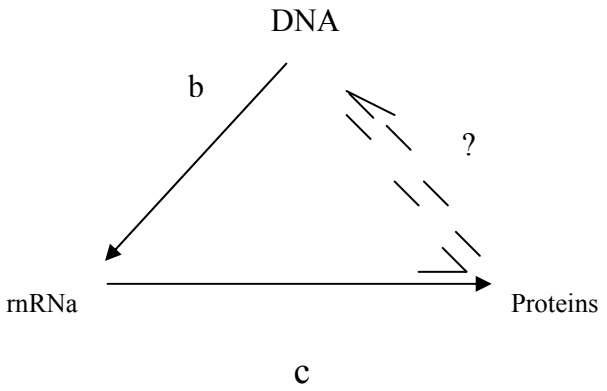
(i) Cellular-Based Theory

This is also known as "wear and tear" theory of ageing. Hence, it states that ageing process within cells are the consequence of continual exposure, accompanied by wear and tear throughout life to adverse exogenous influence. These "assaults" lead to progressive encroachment on the cells' survivability. That is, ageing involves a gradual decline or wearing off of some body cells.

Experiments performed by scientists like Moorhead (1961), Hayflick (1965) and others using "Senescent cultures", have verified, to certain extent, this theory. The control demonstrating a relationship between the termination of cellular division in-vitro and ageing in-vivo is confirmed in experiments using unicellular organisms.

(ii) Orgel's Errors' Theory

In 1968, Orgel proposed a theory of ageing that was based on the fidelity of protein synthesis. Also, the theory is known as the "ERRORS' THEORY". According to this theory, ageing results from a systematic accumulation of random errors in translation and transcription that occurs during DNA replication.



Crick's Central Dogma, 1958

Fig. 13 *{ a = Replication of DNA*
{ b = Transcription of mRNA
{ c = Translation of proteins

The theory asserted that frequent errors in DNA replication will produce imperfect mRNA molecules which may lead to faulty proteins which, in turn, leads to faulty enzymes. The frequency at which this occurs is, however, low. The theory concludes that ageing proceeds at the same rate as the accumulation of errors in the Central Dogma of Francis Crick, 1958 (Fig. 13).

(iii) Genome-Based Theory

There are some theories on ageing which are based on the Genome.

(a) **Finite doubling potential**: The Mitotic cell division has been shown to be finite in a cell. In human, fibroblast mitosis occurs at about 50 times. This was first shown by Dr. Leonard Hayflick in the 1960s. Other researchers confirmed this phenomenon independently. Introduction of aged cells into young animals have no effect on their potential to divide. Their experiments pointed to the fact that internal regulatory mechanism in the cell is the primary determinant of the ageing process and not the environmental agents or exogenous factors.

(b) **Somatic Mutation**: Mutations in the somatic cells have been implicated to be the crux of ageing process. Mutation and/or mutagens are potential agents that could facilitate the process of ageing – particularly by assaulting the DNA molecules.

(c) **Redundant Message Theory of Ageing**: The redundant message theory was proposed by Medvedare. It states that selective repetitions of some genes, astrons, operons, and other linear structures on the DNA molecule, the bulk of which are repressed, behave as "redundant messages". These are called into action when genome becomes faulty. Hew opined 1/500 genes are active and 499/500 are repressed and if mutagenic factors equally act on active and repressed astrons, the mutation rate of repressed genes must yield more mutations which then potentiate the process of ageing.

(iv) Evolution Theory of Ageing

This theory was proposed by Kirkwood. It proposed that ageing is not programmed as an active process of self-destruction by organism. This theory asserts that ageing is most plausibly explained because the acquisition of greater longevity usually involves some cost, and in view of the fact that natural selection, as a process has little to do with events of old age, most organisms that are selected must have inherent features which make them escape death at the early age. Quite similar to Genome-based theory.

(v) Hayflick Theory: Telomere, Telomerase, and Ageing

Senescence can be defined as intrinsic adverse changes during ageing of an organism. This manifests as an increasing likelihood of death as a function of time, measured from birth or from some developmental stage. Cell senescence refers to the limited proliferative capacity of cultured somatic cells.

Telomerase and Ageing

Telomeres are distinct ends of chromosomes. They can be seen under the light microscopes. They provide non-sticky ends to chromosomes, such that chromosomes cannot fuse end to end.
Length of telomere indicates age or number of divisions that cell has gone through. Replication fork advances as DNA duplex unwinds. Leading strand reaches up to telomere.

In each round of replication, telomere is progressively whittled away. In normal cell, telomere shortens with each division. Eventually, it becomes so small that cell cannot divide. TELOMERE ACTS LIKE A MOLECULAR CLOCK THAT CONTROLS AGEING. Telomeres are the protein – DNA structures at the ends of eukaryotic chromosomes. In yeast, and probably most other eukaryotes, telomeres are essential.

Functions of Telomeres

1. They allow the cell to distinguish intact from broken chromosomes.

2. They protect chromosomes from degradation.

3. They serve as substrates for novel replication mechanisms.

Telomeres are usually replicated by telomerase, a telomere – specific transcriptase. Also, telomerase – independent mechanisms of telomere maintenance exist. Telomere replication is both cell-cycle and developmentally regulated. Since telomere loss causes the kinds of chromosomal changes associated with cancer and ageing, hence its biology has medical relevance, there is definite evidence to suggest that in higher organisms, they normally play a role in controlling cell ageing.

Telomerase is an enzyme that synthesizes telomeres. It is a *unique* enzyme with RNA sequence attached to protein. Its terminal looks like DNA as it adds TTAGGG repeats at 3^1 end.

Functions of Telomerase Enzyme

1. It is an enzyme that synthesizes telomeres.
2. It serves as template for the synthesis of telomeres.
3. It balances loss portions of telomeres.
4. It is inactive in somatic cells.
5. Ageing or senescence is linked with telomerase enzyme.
6. Presence of telomerase in cells ensure that constant, regular, and continual resynthesis of telomere's repeated sequences of (TTAGGG) – which is the code for the cap that is placed at the end of telomeres that prevents their endings from unwinding.
7. Abnormal activation of telomerase may contribute to cancer development.
8. Telomerase enzyme blocks any process that limits lifetime of most of the cells.
9. Cancer cells make telomerase enzymes, this enzyme permits telomere to maintain its length hence unlimited division is possible in cancerous cells.
10. Telomerase inhibitors will be effective on cancerous cells.
11. Telomerase can be a useful diagnostic and prognostic tool in cancer.
12. Telomerase may be used to grow back the telomeres and thereby to extend life of human cells.

The telomerase enzyme has the chemical agent promoting cells' longevity and vitality. The gene for telomerase has been dubbed as the "agent of life". It has also earned itself the title as the "Ambrosia of life".

Geron is the leading Biotechnology company which has focused on human ageing. Its mission is to develop novel therapeutics for the many manifestations of ageing through breakthroughs in the basic genetic mechanisms of cell ageing and immortalization. The company's technology centers round "The genetic clock of cell ageing – the telomere and the key that is believed to rewind the clock of cell ageing – telomerase".

(vi) Calorie – Restriction Theory

There is a clock that is ticking away in every living being – for a dog for 10 years, for a turtle for 150 years, for a mouse for 3 years, and for human beings, for 70 years. This clock is probably linked to *metabolic rate* of animals, that is, the rate at which animals burn its fuel (food). The crux of the matter really is "metabolism produces free radicals; more metabolism, more free radicals – *Reduce the calories by 40% and increase the life span by 40%.*

In the Nutritional Model Theory of ageing which is also synonymous to Calorie – Restriction theory of ageing, this phenomenon is carefully explained and entrenched. This theory says that if an animal is fed 50 – 60% energy less than what it will normally obtain on its own, it will live longer and be healthier.

This model/theory actually works by itself. It is recommended by this author. Spiritualists, have long found out that moderate FASTING prolongs life, but, however, prolonged fasting occasioned by starvation, is inimical to longevity!.

(vii) Immunological Theory

This theory of ageing is also known as mutating autoimmune theory of ageing. This theory says that normal cells have normal functions and secrete normal proteins in, on or through the cell membrane. None of these normal proteins ought to cause any form of immune response. When, though, these cells mutate with time, they secrete foreign proteins in, on or through the cell membrane which DOES solicit an immune response by the body. This response shuts down the cell. Alternatively, this theory also suggests that whole cells mutate over time and cause biological errors leading to the demise of the organism.

(viii) Free Radical Theory

Oxidation reactions occur when the combustion of oxygen that keeps us alive and well produces by-products called oxygen free-radicals. When this process occurs in metals, we call it rusting. When it occurs in humans (or animals), we call it ageing, which may makes us feel rusty as well!

Free-radicals are molecules that have lost an electron. When this happens to oxygen, we call it singlet oxygen, because it has only one of its outermost electron left. This is a highly unstable condition, and to restore balance, the radical either tries to *steal* one electron away from, or donate the remaining one, to another nearby molecule. In so doing, the free-radical creates "molecular mayhem", disrupting, damaging, and destroying nearby cells. If DNA is involved, mutations occur, a favoured theory of a common cause of cancer. In time, free-radical damage accumulates resulting into the phenomenon known as AGEING.

Free-radicals are not only produced inside us, but we take them in through smoking, food, air, and water-pollution, x-rays, sun exposure, and various poisons, to name the most common. Stattman, a researcher on AGEING in National Institute for Health, USA, surmised as follows "Ageing is a disease. The human life-span simply reflects the level of free-radical oxidative damage that accumulates in cells. When enough damage accumulates, cells cannot survive properly anymore and they just give up and DIE!"

Damaging Effects of Free-Radicals

- Damage to cells occurs.
- Protein synthesis is impaired.
- Proteins become cross-linked and tangled.
- Tissues become less pliable.
- Arteries incur damages leading to arteriosclerosis.
- Genetic material (DNA/RNA) is damaged leading to cancer and impairment of natural repair mechanisms.
- Age pigments accumulate thus drowning the cells in lipofucsin and hence prevent optimal functioning.
- In general, all age-related syndromes are raised including wrinkles and Alzheimer's disease.
- Responsible for damage during cataracts due to cross-linkage of proteins.
- Damage DNA resulting in mutation hence mutant proteins and cell death.
- Degrade tissue strengthening collagen within the body's joints, skin, and organs.
- Implicated in more than 80 diseases, notable among these are: ageing,
- nervous and immune systems' disorders, cancer, heart disease,
- Parkinson's disease, Diabetes, Alzheimer's disease, and stress.

The destructive process of oxidation is the result of the work of the free-radicals in the body. Therefore, antioxidants in the body can come to the rescue.

"Pycnogenol" is extract of bark of French maritime pine. It contains powerful antioxidants – *Proanthrocyanidines* – a major ingredient in ALENOL which confers protection for the health of our circulatory system.

Professor John Phillip of Guelph University in Canada and his co-workers have found a way of postponing the natural ageing process and increasing life expectancy by up to 40 per cent. Improved version of gene SUPEROXIDE DISMUTASE (SOD) which works on free radicals has been fingered as

possible candidate for "longevity gene". The use of SOD and catalase have also been shown to increase life expectancy by 1/3 when compared with controls.

(ix) Mitochondrial Theory of Ageing

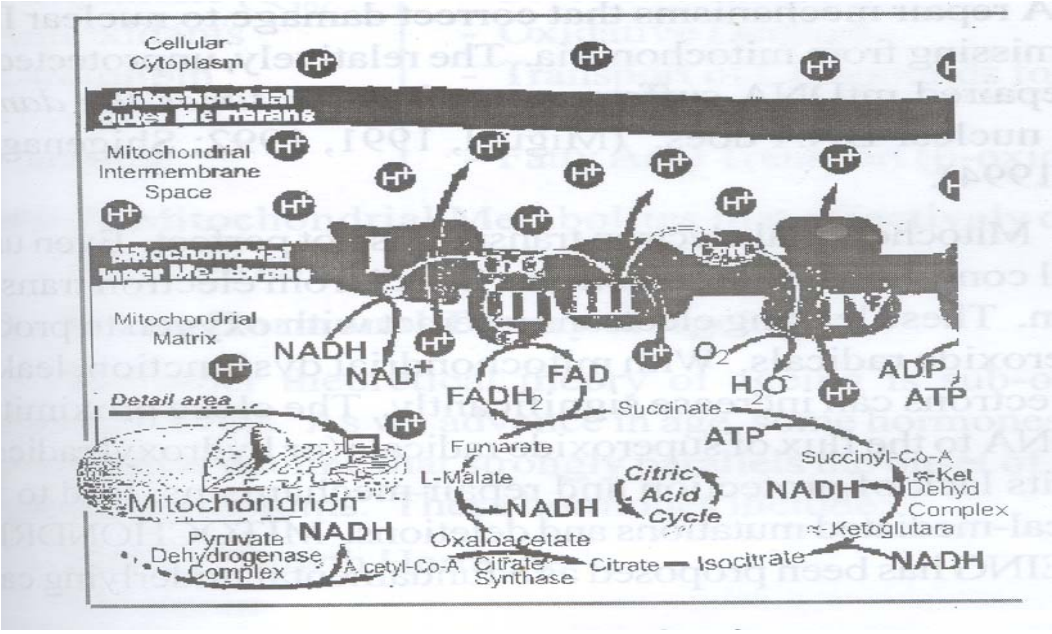


Fig. 14: Mitochondrial Energy Production

One unique property of mitochondrion (as a cellular organelle) is that it has its own DNA (deoxyribonucleic acid). DNA is the molecule of which the genes are made of. When DNA is wrapped in *histones*, a form of storage proteins, it becomes chromosomes. Mitochondrial DNA (mtDNA) is quite different from nuclear DNA is several respects.

First, it exists as a simple plasmid (a DNA loop) and, in this respect, it is more akin to bacterial DNA than the chromosomal DNA of higher organisms. Second, mtDNA is not associated with *HISTONES*. Histones are positively charged storage proteins around which nuclear DNA is wound for safe-keeping (like thread on a spool). Third, most of the complex DNA repair mechanisms that correct damage to nuclear DNA are missing from mitochondria. The relatively, unprotected and unrepaired mtDNA suffers *more than ten times the damage* that nuclear DNA does. {Miguel, (1991, 1992); Shigenaga et. al., (1994)}(19).

Mitochondrial electron transport is not perfect. Even under ideal conditions, some electrons "leak" from electron transport chain. These leaking electrons interact with oxygen to produce superoxide radicals. With mitochondrial dysfunction, leakage of electrons can increase significantly. The close proximity of mtDNA to the flux of superoxide radicals (or hydroxyl radicals), and its lack of protection and repair mechanisms, lead to free radical-mediated mutations and deletions. MITOCHONDRIAL AGEING has been proposed as a fundamental underlying cause of:

1. Free-radical stress.
2. Degenerative disease and
3. Ageing.

Evidence is accumulating that mitochondrial dysfunction underlies many common pathologies. Mitochondrial defects have been identified in Parkinson's disease, Alzheimer's disease, Heart disease,

fatigue syndromes, numerous genetic conditions, and nucleoside therapy of AIDS. It has also been shown by this author that many common nutritional deficiencies can impair mitochondrial efficiency and energy transduction processes.

Table 2: Mitochondrial metabolites that effectively decline with age.

Metabolite	**Mitochondrial Parameter Affected**
Ubiquinone (Q_{10})	Electron Transport
Antioxidants	Oxidative Damage
Cardiolipin	Transport of compounds for ATP synthesis
Carnitine	Fatty Acid Transport (□-oxidants)

(x) Sub Optimal-Hormonal Theory of Ageing: Neuro-Hormonal Theory

The other theoretical theory of ageing is sub-optimal hormonal levels. As we advance in age, some hormones begin a precipitous decline that strongly parallels the onset of ageing signs and symptoms. These hormones include:

- Human Growth Hormone
- Melatonin
- DHEA (Dehydroepiandrosterone)
- Androstenedione
- Testosterone
- Oestrogen
- Progesterone

Conversely, insulin levels tend to rise, culminating in adult onset diabetes. Also, a relative rise in cortisol, the stress hormone, is also common as age advances. Thyroid hormone does not generally decline with age. Many anti-ageing doctors insist that slow thyroid function is common, however, and when present, definitely hastens ageing and heart disease.

Human Growth Hormone (HGH) – stimulates the growth of our tissues. Our internal organs, skin, muscles, nerves, and bones are all stimulated to grow by HGH. As our levels of growth hormone shrinks, so do we!.

MELATONIN helps us to sleep and may also help to prevent cancer. One reason why people over 60 sometimes find it hard to go to sleep is declining melatonin levels.

DHEA is a building block out of which oestrogen and testosterone are made. DHEA also boosts our immune systems and brains. Testosterone, Oestrogen and Progesterone give us our sex drive, build muscle, skin, and bone, keep our minds sharp, protect our heart, and help us feel and be attractive. Thyroid Hormone helps energetic and trim. It helps to burn fat. Declining thyroid hormone leads to pot-bellies or central obesity. The declining thyroid hormone is a basic factor of the obesity. This is why dietary control of the obesity does not work!

Excess INSULIN levels are associated with diabetes, pre-diabetes and the mysterious "Syndrome X" when insulin no longer moves sugars well, known as insulin resistance, and eventually blood sugar rises. The excess blood sugar is forced into the tissues, damaging them with "advanced glycation end-products" known as "AGE" appropriately enough!

CORTISOL levels do not decline with age. Excess levels of this stress hormone are catabolic. Hence, it literally "eats you up inside" when it is excess.

(xi) Neuro-Ageing Theory

The Neuroageing theory says as we age, we undergo thalamo-hypothalamo-pituitary (THP-Axis) and neuronal degeneration. The THP-axis is the "natural pacemaker" for all cellular ageing and the concurrent effects on physiological processes.

As we age, then there are alterations in hormonal release (lowered levels as we age) and effect (reduced numbers of receptors and/or increased peripheral resistance to the hormone by its target cells). All of these lead to the decline in cell function which we call AGEING throughout the organism.

(xii) Integrated Theories of Ageing – How long should we live?

The scientific calculation of how long we could possibly live is derivable from a zoologically-based formula. In mammals, it was experimentally found out that if the time taken for the skeleton of a particular mammal to grow to maturity is multiplied by 5, the result shows the life span of that mammal.

Thus, because the dog's growing period is 3 years; it s life-span is 3 x 5 = 15 years. Human skeleton growth is complete in 25 years and so, a fair estimate of our life expectancy is 25 x 5 = 125 years. An estimate of 120 years to 125 years has been used as working figures by scientists. Anyone failing to get close to this is loosing a good number of potentially happy and productive years.

If, then, our natural ceiling is about 120 years, beyond which human life is unlikely, we should ask why so few of us come any where near that age, and why advanced state of DECREPTITUDE! Certainly, infirmity and disability are not attractive prospects, so why should the aim of a longer life be an attractive idea? Really, the proponents of "life extension" are not merely wishing to encourage various human subjects to willy-nilly trudge towards the 120 years barrier in anything other than a reasonable state of "good health" and "well-being".

Professor Leonard Hayflick of University of California, San-Francisco, is quoted as describing this "life extension" search as "THE LAST GREAT BIOLOGICAL FRONTIER" in his famous article titled "THE FOUNTAIN OF YOUTH (Newsweek, 5 March, 1990, page 34). In fact, the quest has been summed up quite neatly by another Californian, Professor Edward Schneider (University of South California), for it was he who said, "We are trying to add life to years rather than years to life". In fact, both aims are synonymous and should be equally vigorously pursued, for if quality of life, including vitality, vigour, and lack of chronic diseases could be achieved, it is almost certain that life expectancy would increase.

IV. DIET AND MITOCHONDRIA IN HEALTH AND DISEASE

(i) An Overview of Kwashiorkor

The importance of malnutrition and undernutrition as hazards of social and economic development, and as conditioning factors in a wide range of diseases have been documented by the World Health Organisation. There are more than forty dietary nutrients that the body cannot make but are essential for its normal functioning. The primary sources of energy for the body are carbohydrates, fats and proteins (during starvation). In addition to these are other requirements which include dietary fibre, minerals, vitamins, and water. Any diet in which the amounts of nutrients are inadequate or in excess leads invariably to diseased condition or may contribute to the development of condition generally referred to as malnutrition. Impaired digestion, excessive excretion of body fluids or the presence of toxic substances in food may trigger off malnutrition. *Malnutrition may be defined as a state of deficient dietary intake resulting in biochemical and functional impairment*. However, it has been shown that the maintenance of energy balance between energy intake and energy expended represents the most important nutritional problem today.

A number of methods have been used to assess the nutritional status in humans(27a). These include:

(i) Clinical investigation – these may include presence of any deficiency signs.

(ii) Anthropometric measurements. These include the measurements of parameters such as weight, height, development of various parts and accumulation of fat reserves.

(iii) Assessment of dietary intake.

(iv) Biochemical assessment – these include measurements of nutrients in the tissues, of metabolites and cofactors derived from the diet.

Protein Energy Malnutrition (PEM)

Protein energy malnutrition is not a disease that occurs explosively or on sudden onset, rather, the nutritional status of young children as regards proteins and calories may be viewed as a continuous transition from "normal" through mild and moderate degrees of malnutrition, to advanced syndromes of which the most important are kwashiorkor and nutritional marasmus(28). It has been suggested that protein energy malnutrition of early childhood should be used to cover the whole range of classifiable and unclassifiable manifestations of inadequate protein and caloric intake. Some of the features of PEM include hypothermia(29), hypoglycemia(30), low basal metabolism, and declining energy expenditure(31).

Kwashiorkor and nutritional marasmus represent the extreme end results in a chain of events extending over several months. In the poor community, cases of all stages of this transition can be seen. It is a bit difficult to decide where normality ends and where protein energy malnutrition begins (32).

Kwashiorkor

Kwashiorkor is a type of protein energy malnutrition caused by severe protein deficiency in the post weaning stages of infancy and early childhood between ages 1 – 3(30). There have been a few reports of kwashiorkor in adults. The disease affects children soon after they have been weaned off their mothers' milk onto a diet low in protein. Since, the proteins are required for the normal functioning of all metabolic processes, there are probably many sites of metabolic dysfunction in kwashiorkor(33). Kwashiorkor is prevalent in the tropical and subtropical developing parts of the world. It is a major problem among the poor strata of the population of Africa, Asia, India, Central and South America(34)

The onset of kwashiorkor is fairly rapid. The symptoms become evident 2-4 weeks with an attack of diarrhea or fever [Monacha(35)]. A similar syndrome can be produced experimentally by feeding piglets with a diet low in protein and high in carbohydrate content(36). Similarly, the same syndrome has been produced in rats weaned after 21 days of sucking (Olowookere(37, 38).

Kwashiorkor is established by a combination of the medical history of the patient and physical signs and symptoms like:

(i) **Oedema** – This is a clinical manifestation of excess of total body water which invoves extra-cellular fluid space. Oedema is more evident on the feet, legs, hands, stomach, and the face. This is the cardinal sign for kwashiorkor and the syndrome cannot be diagnosed in its absence.

(ii) **Fatty liver** – The presence of a fatty liver is thought of as the second major criterion (after oedema) for distinguishing between kwashiorkor and marasmus(39).

(i) **Low serum albumin levels** – This is a diagnostic feature for kwashiorkor(40).

(ii) **Hair changes**: There are prominent hair changes in texture, colour, strength, and plucability. Black hair may turn brown or even be discoloured(41).

(iii) **Psychological changes** – A kwashiorkor patient is usually pathetic, miserable, inert, anorexic, and usually disinterested in his surrounding environment. The patient gets irritated very easily.

(iv) **Decrease in blood essential amino acid levels** – The ratio of non-essential amino acid (NEA) to essential amino acids (EA) in the serum is useful in confirming the presence of Kwashiorkor and detecting borderline pre-kwashiokor states. The normal NEA to EA ratio varies from 1 to 2 in pre-kwashiorkor state and in kwashiorkor state, this ratio varies from 2.5 to 4 (42).

(v) **Low body weight**- Kwashiorkor children have low body weight for age – thus, their growth is stunted.

The mortality from Kwashiorkor is high in the absence of proper treatment or if the condition is too advanced. Treatment is based on administration of proteins and on correction of any existing dehydration and electrolyte imbalance. A diet rich in milk protein bas been the treatment of choice primarily because of the ease of administration and ready digestibility of milk. Prevention of Kwashiorkor lies in education towards general recognition of its dietary aetiology and encouragement of a dietary pattern which supplies more proteins of high biological quality.

Marasmus

Marasmus is a term applied to the state of chronic total undernutrition in children and it reflects a deficiency of both proteins and calories in various degrees of severity and it produces a gradual wasting away of body tissue. Marasmus is the second major syndrome of protein energy malnutrition(43). Marasmus mainly affects children in their first year of life in contrast to kwashiorkor which has its main impact on the ages 1 – 3.

When the supply of food is not enough even for limited growth, then the less essential body tissues are catabolised to maintain the more essential tissues and the clinical feature is that of marasmus (bender and Bender, 1982). A clinical picture similar to nutritional marasmus can be induced in piglets by feeding them on a diet that has low amounts of protein and carbohydrates(36, 44) .

The condition of nutritional marasmus is a characteristic by the following symptoms as described by Manocha(35).

(i) The liver is normal in appearance and size. There are no fat deposits observed in the liver as compared to kwashiorkor.

(ii) There is no oedema. This is very characteristic of marasmus to distinguish it from kwashiorkor.

(iii) There is extreme degree of wasting and the patient is grossly underweight. There is little subcutaneous fat and the body appears shrunken to the extent that the skin lays in wrinkles as in very old people especially around the buttocks and thighs.

(iv) The marasmic patient is alert and does not look as dull in appearance as a kwashiorkor case.

(v) The face is generally pinced accompanied by prominent bony points to the extent that it may look like a monkey face.

(vi) Growth failure is prominent and the patient's height is extremely subnormal.

(vii) Skin changes are not observed in marasmic condition.

(viii) Serum protein levels may be reduced but not as much as in Kwashiorkor cases.

Frequent cases of intermediate between kwashiorkor and marasmus are often encountered. These are referred to, clinically, as marasmic kwashiorkor (Bender and Bender, 1982)(27b). Complications by opportunistic bacteria precipitating infections shifts the pendulum of nutritional marasmus towards the kwashiorkor end of the spectrum (45). The age at which a child is weaned is very important in distinguishing between marasmus and kwashiorkor. Thus, early weaning leads to marasmus while late weaning leads to kwashiorkor(46). However, the overall diagnostic features for identifying each condition

is the degree of body-wasting and oedema; thus kwashiorkor is diagnosed when children are 60 – 80% of the expected weight for age and oedema is present. Whereas, marasmic-kwashiorkor is the case if children are below 60% of the expected weight for age and oedema is present, marasmus is diagnosed if children are below 60% of this weight without oedema.

(ii) An Overview of Obesity

Obesity is defined as an abnormal generalised increase in the adipose organ. It has been associated with other conditions of "overnutrition" and it makes a significant contribution to ill health via its association with hypertension, pulmonary, and renal diseases, cardiovascular disease and diabetes mellitus. Obesity is rarely recorded as a cause of death in humans but it is known to contribute significantly to the reduction in average lifespan and unquantifiable reduction in the quality of life of those who are substantially overweight. Obesity is a major problem in the developed countries.

Techniques for assessing the degree of adiposity fall into three main categories(47):

(i) Body Weight – this is the simplest possible measure of obesity.

(ii) Indices based on height and weight data, centile charts of height and weight or standard deviation of height and weight.

(iii) Direct measurement of subcutaneous fat at various sites using skinfold calipers.

There are many factors that may cause obesity and the effects of obesity are also far reaching. Thus, obesity is known to alter metabolic and endocrine balance(48).

Obesity arises often as a consequence of taking in more energy in the food than is expended in the activities of daily life. Thus, lack of physical activity will encourage the development of obesity since, in the absence of sufficient exercise, only a relatively small proportion of generously ingested food calories are converted into fat cells of the body.

Energy imbalance is regarded as an interplay between food intake, energy storage, and heat loss. This is expressed by the equation:

Energy	=	Energy	+	Energy	+	heat loss
Intake				expenditure		storage.

Obesity develops from an imbalance of this equation which could reflect either an increase in food intake or an increase in energy storage or a fall in either energy expenditure or heat loss(49). Energy expenditure by obese subjects has been shown to be higher than in control animals(50). In obesity, there is body weight gain indicating an increase in both the absolute and relative amounts of body fat. The body weight gain however is not due to fat alone but could also be due to water retention, body cell mass and body cell solids(50).

Another possible cause of obesity is an acquired hormonal disturbance (51). An excess of corticosteroid hormones induces the typical accumulation of fat on the face, neck, breasts, and other areas of the body. Other hormones like testosterone and glucocorticoids have also been shown to cause obesity when their levels are above the normal requirements(52). It has also been shown that obesity can be induced in some strains of rat by castration which leads to imbalance of hormones. Human obesity has been associated with several abnormalities of androgen and estrogen metabolism(53).

Morbidly obese men and women overproduce and over-metabolise a variety of androgens and oestrogens. Despite the elevated production rate of androgens, the plasma levels of these hormones are normal or only slightly elevated in such subjects. This inconsistence of high production rates and low plasma concentrations is explained by the high metabolic clearance rates of the androgens. Morbid obesity in both men and women has also been associated with decreased levels of sex hormone binding globulin (SHBG)(54). Such hormones exhibit higher metabolic clearance rates as well as increased concentrations of the free hormones in the blood.

Recent observations in human population have confirmed that genetic factors play a significant role in their predisposition to obesity, where they may interact with dietary and environmental factors (55, 56, 57).

Heredity has also been considered in the pathogenesis of obesity explaining why obesity is more common in some families than in others. Experiments using genetically obese mice identify the adipose tissue as the main sites of extra lipogenesis.

Precisely how obese subjects regulate cellular energy reserves, or fail to, in the face of ordinary stresses and ambient temperature changes has been identified as a major problem in the pathogenesis and bioenergetics of obesity syndrome. The basic cause of dietary obesity is an intake of calories in excess of energy expenditure. The reasons for the defective energy metabolism in obesity vary widely as there are many underlying causes. Apart from dietary obesity, other forms of obesity exist. Obesity is better studied now as a syndrome with different aetiologies (58) has however shown that the various forms of obesity are directly or indirectly linked with deregulation of food intake. With the current biochemical and physiological knowledge in the aetiology of obesity, it is appropriate to distinguish among the genetic, hypothalamic, endocrine and psychological factors in obesity syndrome.

Perhaps more crucial to the issue of aetiology of obesity syndrome is the issue of oxidative energy production. It has now been shown that the process of taking in of oxygen is made more difficult in obese subjects, hence there are respiratory difficulties in obesity. Excessive adipose tissue coupled with excess load of fatty tissues carried on the chest wall have been implicated in the respiratory impairment. Obese subjects have the concomitant problem of having the whole body defectively oxygenated.

In view of respiratory difficulties in obese subjects, they usually have diminished exercise tolerance and higher resting basal metabolism. There is a growing body of evidence that a thermogenic defect plays a role in the obesity syndromes including the genetically transmitted obesity (ob/ob) (59).

Table 3: Relationship between body weight and life expectancy of a 25 year old male

Percent excess weight	Expected age at death
0	76
30 – 60	63
100	52

Jequier and Shutz have established that obese animals exhibit both hyperphagia and metabolic efficiency; yet they have low work tolerance. Beaton (1976) has shown that obesity is a condition associated with a well-established risk of morbidity and mortality. In a well-illustrated paper titled 'Multidisciplinary approach to adult obesity therapy', Blackburn and Greenberg (1983) highlighted the risk factors in obesity. These authors related obesity to other pathological conditions such as diabetes, hypertension, hyperlipidaemia, gout, lower back strain, arteriosclerosis, hernia and low work tolerance. It has been documented that although the prevalence of obesity is quite high in most industrialized countries, and as such, it is generally recognized as a public health problem, data from developing countries suggest that the prevalence of obesity is gradually increasing and may be surprisingly high among segments of the population. The extent of morbidity and mortality in obesity syndrome is shown in table 3.

It has been observed that there are inherent limitations in the use of various existing methods clinically adopted for the assessment of the energy metabolism of obese subject (Garrow, 1981). The need for better techniques, devoid of ambiguous interpretations, during rehabilitation and management of obesity has been advocated (Webb, 1983). The metabolic dependence on mitochondrial function has been well established. The biochemical properties of the mitochondrion as the power plant of the living cell coupled with its genetic peculiarity being semi-autonomous, even as a sub-cellular organelle, has made it a variable marker in the elucidation of the variability in energy metabolism and thermogenesis during malnutrition.

This assertion has been put to test, and findings of experiments from various laboratories have proved very informative (63).

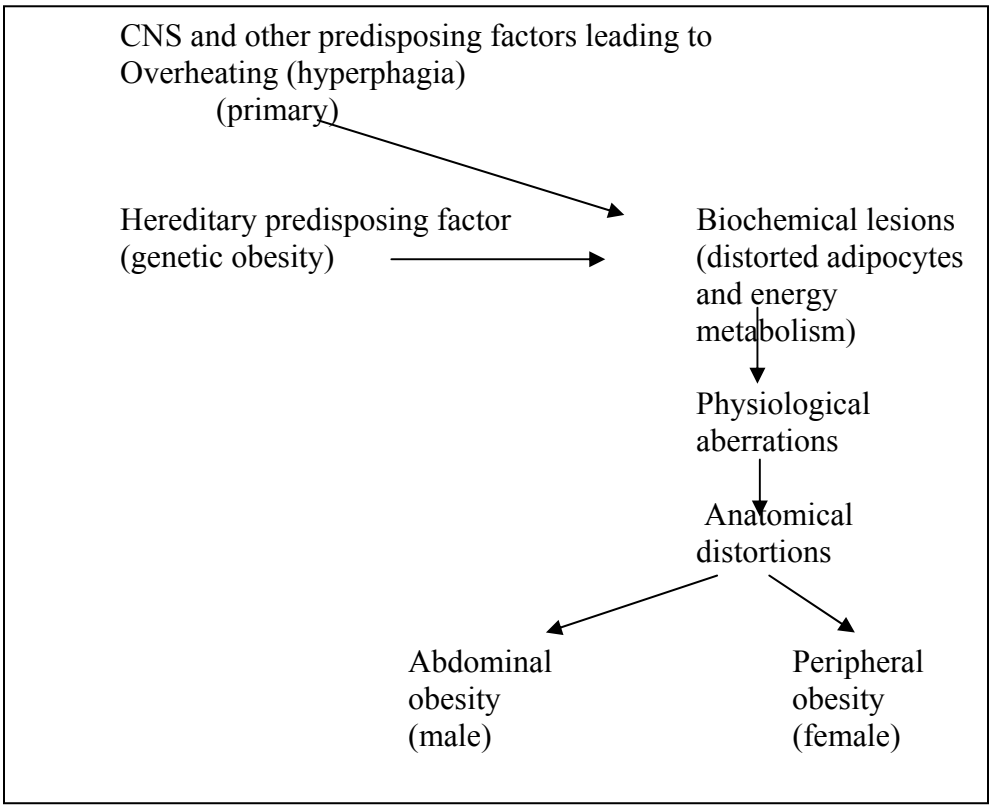


Fig. 15: A generalized scheme showing the pathogenesis of obesity syndrome in man (64).

In view of the variations in the aetiology of obesity, the thermogenic defects implicated in its pathogenesis, the difficulties in respiration during obesity, the low energy expenditure in relation to size, the observation of hyperglycemia and low glucose uptake, the higher basal metabolic rate (BMR) in obesity and the diet-induced thermogenesis (DIT); it is pertinent to describe the current state of the art on the bioenergetics of obesity and kwashiorkor syndrome. The above on the overview of kwashiorkor and obesity vis-à-vis deranged bioenergetics is the main objective of writing this book on "Bioenergetics of kwashiorkor and obesity".

(iii) Energy-linked functions of Mitochondria isolated from PEM Animals

Using an experimental procedural prototype(65) induced marasmic kwashiorkor syndrome in wistar rats. The use of Cassava farina (3.4%) as the source of energy, rather than sucrose, in the induction of marasmic-kwashiorkor syndrome was reported by the author and his collaborators(66, 67). However, a rapid method of induction of marasmic-kwashiorkor syndrome in laboratory animals was developed by Adelusi and Olowookere(68). Hence, the investigation into the molecular mechanism of PEM syndrome vis-à-vis the energy-linked functions became greatly intensified. These efforts have been fruitful as can be seen in the following paragraphs.

(a) ***Osmotic behaviour of Mitochondria during PEM***

It has been established(80) that monkeys fed a low-protein diet have polymorphic and mega-mitochondria. This, invariably, showed that the pathological condition affected the 'osmometric property' of mitochondria on which the functionality hinges, namely: chemiosmotic theory.

Olowookere *et al*. (68) showed that there is a definite correlation between the vitamin A content of the liver of hepatic mitochondria in rats and the dietary protein status of such animals.

The same authors observed that there is a 300% increase in the passive swelling of hepatic mitochondria of rats fed a low protein-high-carbohydrate diet in three different isotonic solutions. The observed changes in absorbance (at 5.20nm) or light scattering of the mitochondria was found to be inversely related to the vitamin A content of the liver.

The observations of reduced vitamin A content in the livers of PEM-diseased rats has been shown to stem from the reduction in the RBP (Shetty *et al*.)(70) which is the vitamin A-transporting complex found in the serum (71), in spite of adequate vitamin A supplement in the regimen used to induce the marasmic-kwashiorkor syndrome.

Although, vitamin A deficiency has been shown to have a profound effect on virtually every organ, it has been reiterated that the precise metabolic function of vitamin A on body organs has not been elucidated(72). From our studies(65, 66, 67, 68), it has been demonstrated that low vitamin A content (due to defective RBP synthesis) may potentiate the fragile membrane structure, thus leading to osmometry in mitochondria.

Mitchell(73), has shown that rats fed a vitamin A-deficient diet have reduced mitochondrial lipids, and lipids are an integral part of the proteins in biomembranes, which is the limiting surface of all organs.

Extensive mitochondrial swelling, as observed in the malnourished rat, has been shown to be a result of: (i) failure of oxidation phosphorylation; (ii) membrane permeability changes, and (iii) inability of mitochondria to concentrate Ca^{2+} ions(74). The conversion of 11-cis-retinol to all-trans-retinol (vitamin A isomers) has been shown to lead to the alteration in permeability of vesicle membrane, across which there is normally a potential difference (which is related to the membrane potential of Mitchell's chemiosmotic theory; hence, the cellular bioenergetics). It is, therefore, the surmise of the author that defective bioavailability of vitamin A may be a complication in PEM.

(b) ***Deviation from Vectorial Metabolism/Topological Disorganisation of Electron Transfer Complexes during PEM***

Mitchell (1977), in his 'Vectorial chemiosmosis' clearly demonstrated that the direction and magnitude of flow of electrons in normal mitochondria followed a simple vector(68), Olowookere and Olorunsogo(108), demonstrated that there is a deviation from vectorial metabolism in the hepatic mitochondria of PEM rats. The same authors showed that whereas succinate-cytochrome C reductase activity was reduced to about 48% and that of NADH-cytochrome C reductase was reduced to about 45%; cytochrome oxidase activity was drastically reduced to about 23% of the values obtained for the control. Since each of the electron complexes specifically function for each of the three sites of phosphorylation in a mechanistic manner that is vectorial, it follows that there is an attendant alteration in the topology of the electron transfer complexes during PEM(65, 66, 67).

(c) ***Cytochrome Oxidase Status and Basal Metabolism during PEM***

It has been shown(77) that the third site of phosphorylation which is mediated by cytochrome oxidase is affected most by protein depletion. The topology of the multi-unit enzyme is distorted(77). There is also an attendant loss of activity as a result of hypocupremia which normally accompanies PEM(78). Copper acts as the co-ordinating molecule for the enzyme.

It has been shown [Olowookere, (1976)] that whereas the activity of mitochondrial cytochrome oxidase was raised during rehabilitation from 23 to 91% of the values obtained for control rats, the activities of both succintae dehydrogenase and NAD-isocitrate dehydrogenase were raised from 75 to 83% and 73 to 88%, respectively; apparently in line with the general rate of recovery of the diseased rats as reflected by the changes in the body weights of kwashiorkor-repleted rats. This may be due to the multi-unit nature of cytochrome oxidase and/or the distortion of the obligatory vectorial orientation of the constituent subunit of the enzyme.

The status of cytochrome oxidase during PEM is in consonance with the clinically observed basal metabolism in PEM patients. DeMaeyer (79), observed that the basal metabolic rate falls during PEM; but rose sharply during the first week of rehabilitation – in a similar manner to the cytochrome oxidase status (Olowookere and Olorunsogo(108); Olowookere (26). It has also been shown [Olowookere (69)] that the reduction in BMR is traceable to: (i) loss of osmotic property by mitochondria of PEM animals, and (ii) distortion in the topology of electron transfer complexes – which invariably explains the clinically observed decline in energy expenditure by children suffering from PEM (Ablett and Mc-Cance, 1971), hypothermia (Brenton, 1976) and generally low resistance and apathy(30).

(d) ATP Synthesis and ATPase Activity during PEM

It has been established (65, 66, 67) that the respiratory control ratio or acceptor control ratio, which is an index of the ability of the mitochondria to make ATP is reduced by about 50% in rats suffering from PEM syndrome using either succinate + rotenone or β-hydroxybutyrate, respectively. The lesioned mitochondria that had been observed to swell in vivo(80) and in-vitro (65, 66, 67) have lost the ability to maximally synthesise ATP.

Bababunmi *et al.* (81) also reported the reduction in the respiratory control ratio of liver mitochondria of rats which had shown signs of suffering from marasmic-kwashiorkor syndrome.

It has been established (Olowookere *et al*) (66), Olowookere(67) that there was an enhancement in the activity of hepatic mitochondrial ATPase activity during PEM. It has been shown that there is a seeming correlation between ATPase activity and mitochondrial supernatural protein.

Other workers, Pimpalikar and Kaplay(83) have also reported elevated Na^+-K^+-ATPase activity in the erythrocyte cell membrane of children suffering from kwashiorkor. Studies on kidney microsomal Na^+-K^+-ATPase showed a similar elevation of this activity in experimental PEM. These workers s [Kaplay, 1978; Pampalikar and Kaplay (1981)](83) have shown that the elevation in the activity of the enzyme is a reflection of active Na^+ reabsorption and that Na^+-K^+-ATPase may have an important role in the fluid accumulation in PEM. It has been shown (Olowookere, 1980b)(77) that all these findings on the latent ATPasae and/or the Na^+-K^+-ATPase are suggestive of impairment in both the mitochondrial and erythrocyte membranes during PEM.

(e) Xenobiotic Metabolism during PEM

The breakdown of foreign compounds has been shown to involve the use of energy and that oxidation and/or reduction processes of the biomolecules are pre-requisites to biodegradation(84).

It has been established (Boyd and Decastro, 1968a, b) that the toxicity of dicophane during dietary protein deficiency is more pronounced. Bababunmi *et al*, (1981)(81) also showed that there exists a defect in xenobiotic activation prior to glycine conjugation in kwashiorkor disease. Thabrew *et al*.(87) clearly showed that the defective xenobiochemistry in animals suffering from PEM is directly related to the bioenergetics of the cell, i.e. the impaired mitochondrial oxidative phosphorylation of the animals.

Maduagwu *et al*(88) investigated nitrosamine and nitromorpholine elimination from the blood after a single intravenous dose in kwashiokor animals raised according to the method of this author. It was found that the N-demethylase activity in the liver microsomers of the PEM animals was not significantly different from that of the control animals – although, it was revealed that the activity of the enzyme was reduced per gram wet liver tissue. On the other hand, Maduagwu *et al*. (88), found that the glutathione content in the liver cytosol was much higher than that of the controls. These workers concluded that the elimination of nitroso-dimethylamine and nitrosomorpholine from the blood of PEM animals over 8 h following intravenous administration of the carcinogens showed that the clearance rate of each nitrosamein was significantly reduced in the kwashiorkor animals. The vulnerability of the PEM-diseased rats may be due to their reduced ability to synthesise ATP as shown by Thabrew *et al*(87).

(f) *Consequences of Retinol Transportation on the Cellular Bioenergetics of PEM Animals*

It has been established (65, 66, 67) that the hepatic mitochondrial of rats fed a protein-depleted diet showed excessive passive swelling (about 3-fold of controls) in isotonic solutions. It was further established that whereas an inverse relationship existed between the vitamin A content of the liver and the osmotic behaviour of hepatic mitochondria of rats fed a protein-depleted diet, there is a direct relationship

between their hepatic mitochondrial vitamin-A and the respiratory control ratio. The same author(77) concluded that the defective transportation of vitamin A invariably affected the concentration of the mitochondrial vitamin A, hence resulting in the impairment of the integrity of the mitochondria. This culminated in the leaky nature and aberration in the functions of the mitochondrial membrane, and ipso facto, a derangement in cellular bioenergetics of PEM animals.

(g) ***Evidence for Heavy Leaning on Glvcolytic Pathway Resulting from Lesioned Mitochondria of PEM Animals***

Many of the clinical features of severe PEM such as physical inactivity, unresponsive emotional tone, bradycardia, and decreased body temperature, are consistent with a decrease metabolic rate(30). The reduced BMR of PEM subjects had been proved to stem from lesioned mitochondria.

It has been shown that the ability of the malnourished child to survive a continuing shortage of dietary energy and protein, often in the face of increased energy requirements, is dependent on successful adaptations in various metabolic pathways. Kerr *et al*. (89), showed that most Jamaican malnourished children were able to maintain blood glucose homeostasis similar to that achieved in recovered children as a result of their ability to produce glucose by gluconeogenesis. These authors concluded that this remarkable adaptation to fasting in the malnourished children took place in the production of glucose, rather than in utilization of energy. Kerr *et al*, (89) showed conclusively that there is an efficient recycling of products of glycosis in malnourished children. This heavy leaning on the glycolytic pathway by eukaryotic cells is consistent with the finding that mitochondrial oxidative phosphorylation is definitely impaired as shown by various reports (Olowookere *et al*(65) Olowookere and Olorunsogo. 1985: Olowookere, (66, 67).

(iv) Roles of Defective Energy Metabolism in the Aetiology of Obesity

(a) *Defective Thermogenesis in Obesity*

It has been documented by Jequier and Shutz(97) that a thermogenic defect plays a role in the development of a number of genetically transmitted obesities. These subjects exhibit both hyperphagia and increased metabolic efficiency. It has been experimentally shown that during pair-feeding studies with lean controls, ob/ob mice became obese. These authors inferred that the reduced thermogenesis alone can be responsible for obesity in these mutants. In addition, hypothalamic obesity, induced in the rat by lesions in the ventromedial hypothalamus (VHM), also results into hyperphagia and an increase in metabolic efficiency(97).

The concept of thermogenic defect in obese subjects has gained credence as a result of studies showing a reduced postprandial response in obese individuals after glucose(98).

The obese subjects, in whom a thermogenic defect was observed, were usually selected on the basis of family history of obesity. However, conflicting reports showing an unaltered thermogenic response to a meal have also been documented(90). Dietary obese subjects not selected on the basis of family history, have also been found to demonstrate thermogenic defects.

The reasons for these contradictory results are: (a) the multi-aetiology of obesity syndrome as well as the extent of adiposity, peripherally or abdominally, and (b) the methodological problems, which vary from one investigator to another.

Techniques better than those currently being used might have to be employed to unequivocally resolve the contradiction. The various techniques being used include (a) the use of respiratory chamber; (b) extrapolation from basal metabolic rate; (c) diet-induced thermogenesis (DIT); (d) extrapolation from the energy expenditure due to physical activity, and (e) the use of direct and indirect calorimetry.

The inherent limitations of the various methods vis-à-vis thermogenesis can be a factor in the contradictory results from various laboratories. Since, there is a consensus that there exists a *thermic* effect of food, the relevant issue, therefore, to clarify is the significance of the reduced thermogenic response to food ingestion, as a factor, favouring the aetiology of obesity. The use of a single common technique for all forms of obesity, i.e. bioenergetic techniques, to ascertain the status of the energy transduction in mitochondria of obese animals is advocated and used. The results obtained from the laboratory of this author and other laboratories are hereby reported to provide an 'energy baseline' in obesity.

(b) Changes in Resting Basal Metabolism in Dietary Obesity

In a series of experiments carried out by the author and his collaborators in his laboratory as well as in the Department of Biochemistry, University of Nairobi, Kenya, it was experimentally demonstrated that there were notable changes in the resting basal metabolism in dietary obese rats.

The findings above confirmed the earlier observation that diet induced obese subjects have higher BMR (expressed in absolute terms) and a greater overall resting energy expenditure despite their reduced thermogenic response to food ingestion.

It has been reported that obesity is usually accompanied by an increased energy expenditure and a reduction in DIT(91) while kwashiorkor has been associated with hypothermia and defective ATP formation.

Between 1976 and 1990, this author systematically assessed the bioenergetics in malnutritional states. The summary of results show that there is an aberration in energy metabolism in both undernutrition culminating in kwashiorkor and overnutrition resulting in obesity(92). This author also opines that these bioenergetic problems in obesity and kwashiorkor are basically secondary factors rather than primary. They are better described as one of the 'immediate consequences' of overnutrition and malnutrition in general. In spite of the divergent aetiology of obesity and kwarshiorkor from the dietary viewpoint, both share in common some bioenergetic aberrations. This common denominator, reported in the next section, provides an experimental basis for the generalization(93) that 'nutritionally unbalanced diets invariably lead to defective energy metabolism'. It can be surmised that whilst these bioenergetic parameters are mostly of molecular and enzymatic origin, the only apparently superficial indicator, i.e. BMR or RBMR could be measured using the method of Kleiber(94). This, no doubt, can be of clinical application in the prognosis of obesity syndrome(28).

(c) Changes in Oligomycin-Sensitive ATPase in Dietary Obseity

Oligomycin is a typical inhibitor of oxidative phosphorylation(95). This compound becomes a useful tool of investigating energy metabolism, as it confers preferential sensitivity on one of the component proteins that form the basal part of the ATPase complex. The oligomycin sensitivity-conferring protein thus, gives an indicator of mitochondrial functionality, hence the index of the formation and hydrolysis of ATP to form both useful biological energy as well as heat. It was noted that ATPase activity in mitochondria from obese rats was less oligomycin-sensitive (oligomycin-sensitivity was 60-80%) than in the control animals where oligomycin-sensitivity was between 97% and 99%(96). This observation points to yet another inherent lesion at the mitochondrial level in dietary obese rats. It may also assist in understanding the rather complex bioenergetics of obese subjects.

(d) Na^+-K^+-ATPase and Basal ATPase Status in Obesity Syndrome

The multi-subunit enzyme (ATPase) becomes very significant in consideration of formation/hydrolysis of ATP because of the importance of the equation:

$$\text{ATP} \underset{\text{ATPase}}{\overset{\text{ATPase}}{\rightleftharpoons}} \text{ADP + Pi + Energy useful + Heat}$$

Studies on the mitochondrial ATPase measured in the direction of ATP hydrolysis indicate that both the basal and carbonylcyanide-m-chlorophenylhydrazone induced activity in obese rats was about 40% lower than in control. This observation suggests that in obese rats, the enzyme has lower capacity to hydrolyse ATP than in control rats, thus suggesting a lower rate of energy generation through ATP hydrolysis(96). This may further explain the reduced thermogenesis observed in obesity(97, 98). Table 5 gives a summary of results in mitochondria isolated from obese and control rats.

Table 4. ATPase activity in mitochondria isolated from obese and control rats.

Mitochondria source	ATPase activity, µmol/min/mg protein			
	Basal		CCCP-induced	
	– oligomycin	+ oligomycin	– oligomycin	+ oligomycin
Obese rats	215.00	78.00	480.00	96.00
Control rats	370.00	10.00	720.00	8.00

CCCP = carbonylcyanide-*m*-chlorophenylhydrazone

The results from our laboratory in our studies on the status of both the basal and Na^+-K^+-ATPase are in agreement with those of Izpisua *et al.* (115). Olowookere *et al*(96) showed that the mitochondrial membranes in dietary obese rats show greater fluidity as well as significant ($p > 0.001$) decreases in Na^+-K^+-ATPase which is a membrane-bound enzyme just like most other enzymes of the inner mitochondrial membranes.

A possible conclusion from these observations are: (a) changes in the fluidity of obese rat liver mitochondria membranes ; (b) decreases in basal ATPase measured along the direction of ATP hydrolysis; (c) decreases in the Na^+-K^+-ATPase, and (d) a possible 'partial uncoupling' of the mitochondrial membranes which consequently may disrupt the topology of the ATPase-enzyme complex. The multi-subunit nature of ATPase (basal and Na^+-K^+) makes it rather vulnerable to fluidity and hence deviation from vectorial chemiosmosis(99). The changes in the cholesterol and phosphor-lipid constituents of the obese rat mitochondrial membranes may contribute to the alteration in the membrane texture, membrane fluidity and, consequently, depresses the activity of the basal ATPase as well as Na^+-K^+-ATPase in dietary obesity.

(e) The Energy Implications of Cyclical Obesity in Hibernators

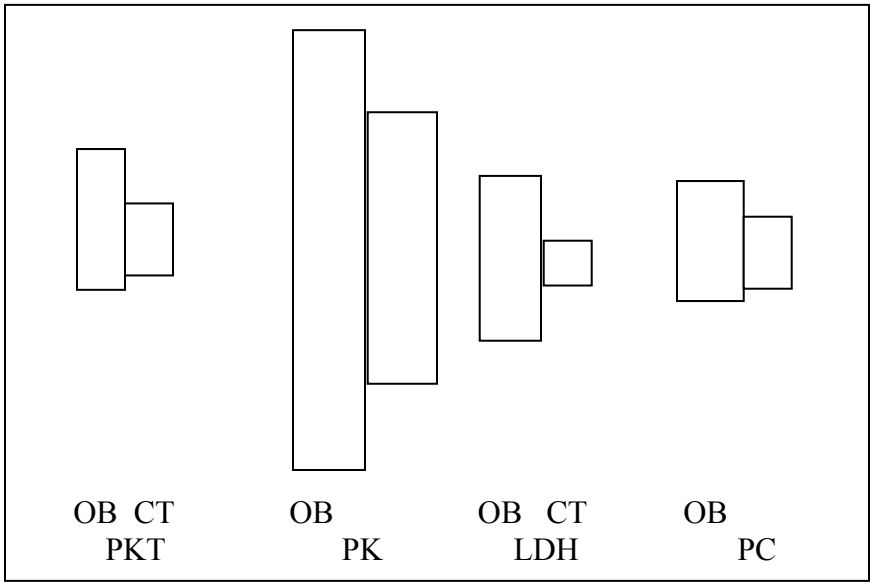
The fall fattening and winter weight loss of mammalian hibernators has been known since antiquity(100). There is now ample evidence to support the view that in hibernators, just as in nonhibernators, ablation of the VMH and lateral hypothalamus (LH) resulted respectively in obesity and weight loss(100). The golden-mantled ground squirrel (*Spermatophilus lateralis)* has been used as a model to study cyclical obesity in mammalian hibernators. The evolution of thermoregulatory and endocrine cycles in hibernators has not been seriously and rigorously investigated. However, modern studies of regulatory physiology have made several unsuccessful efforts to identify the adjustable regulator for food intakes, as well as weight gains vis-à-vis the energy implications in hibernators. The number of mitochondrial per cell during hibernations is reduced. This occurs as part of preparation towards hibernation.

The energy requirements of hibernators being necessarily lower than nonhibernators, the number of mitochondria per cell is correspondingly reduced. The production of mitochondria as cellular organelles follows mitotic pattern; this means that it is controlled by the genome. The cyclic obesity in hibernators is probably under the control of the genome which, in turn, is traceable to evolution. The 'intractable' regulator of obesity in hibernators may be a result of the complexity of mammalian genome. The answer may well lie in the modern techniques of genetic engineering. This author agrees with Mrosovsky(116) that whilst there were justifiable reasons, 20 years ago, to focus on the hypothalamus on which experiments on weight cycles revolve despite VMH and LH lesions, it is now timely to turn our attention elsewhere in the search for the adjustable regulator in cyclical obesity in hibernators. So far, no treatments are known to abolish weight cycles in hibernators. These cycles persist despite removal of the thyroids, gonads, pineal, olfactory bulb and a variety of hypothalamic and midbrain lesions. There is a hint that certain midbrain lesions attenuate or disrupt cyclicity of obesity in hibernators, but the present data are inconclusive(101).

Perhaps, the search for the adjustable regulator of weights in hibernators should be a multidisciplinary one focusing on a *biochemical molecule* (e.g. a section of DNA, a protein or enzyme or an endogenous

pharmacological determinant of behavioural and physiological changes). Using the dormouse (another mammalian hibernator model), it was also found that there was notable insulin seasonal fluctuations(102). It was also found that adipocytes had lower glucose-6-phosphate dehydrogenase activity and oxidized less glucose in response to insulin in the summer, but these indices changed in the fall and winter when the animals are in lipogenic state.

The above-named poor oxidation of glucose during the summer relative to the winter seasons, provided the first comprehensive profile of carbohydrate metabolism in hibernators and demonstrated marked seasonal changes. It also provided a direct evidence of the lesions in mitochondria isolated from obese animals. More importantly, however, the obese (OB) rats findings provide a solid scientific basis to focus on the status of glycolytic energy production which is an obligatory precursor of mitochondrial phase of energy production in eukaryotic cells.



PFK = Phosphofructokinase; PK = Pyruvate kinase; LDH = Lactate dehydrogenase; PC = Pyruvate carboxylase.

Fig. 16: Activity profiles of four glycolytic enzymes in control (CT) and Dietary obese (OB) rats

(f) Changes in Key Glycolytic Enzymes in Dietary Obesity

Glycolysis, otherwise known as the Embden-Meyerhof pathway of sugars, yields pyruvic acid and lactic acid. Glycolysis in tissues consists of the breakdown of glycogen, glucose, or other sugars to pyruvic and lactic acids. It is a process of carbohydrate metabolism generally characteristic of animal cells. Although, one stage of glycolysis requires oxidation by dehydrogenation, this is often accomplished without oxygen. Essentially, the process of glycolysis is anaerobic. The glycolytic process is necessary for most phases of carbohydrate metabolism except the interconversion of sugars to glycogen. It is an obligatory pathway of carbohydrate oxidation since the pyruvic acid formed is oxidative decarboxylated to form acetyl fragments (C_2). The cyclic processes represented in the tricarboxylic acid (TCA) cycle takes on this two-carbon

fragment via coenzyme A (i.e. acetyl-CoA) to form ATP, CO_2 and H_20. The TCA processes are localized in the mitochondria.

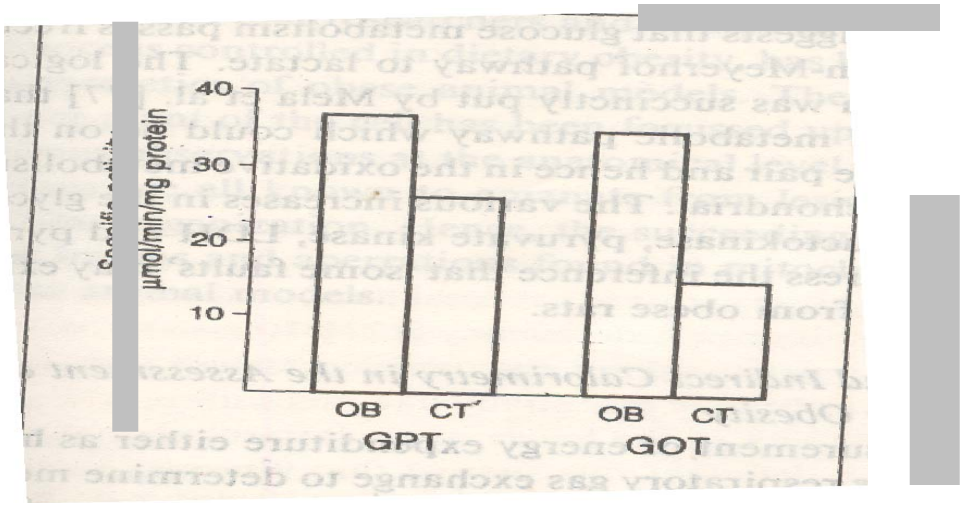


Fig. 17 Activity profiles (x 10^3) for glutamate pyruvate transminase (GTP) and glutamate oxaloacetate transaminase (GTP) in control (CT) and dietary obese (OB) rats.

Kerr *et al.* (103) have shown that there is a rapid recycling of glycolytic intermediates in protein-energy malnutrition. Mela *et al*(104) have established that whenever there is a fault in the mitochondrial energy transduction, there may likely be a corresponding compensatory faster recycling of glycolysis. It is with this background and with a view to clearly unravel the bioenergetics of obese subjects that prompted the essay of key glycolytic enzymes from the liver homogenate of animals suffering from dietary obesity.

The activity profiles of four key glycolytic enzymes assayed from the liver homogenate of animals suffering from dietary obesity were investigated in our laboratory(92). These enzymes are phosphofructokinase, pyruvate kinase, LDH, and pyruvate carboxylase.

Figure 16 shows the activity profiles of four glycolytic enzymes in control and dietary obese rats. In addition, the activities of the cytosolic glutamate pyruvate transaminase and glutamate oxaloacetate transaminase were determined (Fig. 17) (92).

It has been documented that there is an increase in LDH in undernourished (kwashiorkor) patients(105). Elevation of lactate, the end-product of anaerobic respiration, suggests that glucose metabolism passes freely through the glycolytic Embden-Meyerhorf pathway to lactate. The logical inference from this aberration was succinctly put by Mela *et al*. (104) that 'there exists a fault along the metabolic pathway which could be on the oxygen side of pyruvate-lactate pair and hence in the oxidative metabolism which takes place in the mitochondria'. The various increases in the glycolytic enzymes, i.e. phosphofructokinase, pyruvate kinase, LDH and pyruvate carboxylase, further buttress the inference that 'some faults' may exist in the mitochondria isolated from obese rats.

(g) Limitations of Direct and Indirect Calorimetry in the Assessment of Bioenergetics in Dietary Obesity

Calorimetry is the measurement of energy expenditure either as heat loss (direct calorimetry) or as respiratory gas exchange to determine metabolic rate (indirect calorimetry). Calorimetric measurements are often made in the context of dietary control for some extended period. Usually, it is accompanied with measures of body composition to assess energy stores. Essentially, calorimetry determines the 'significant features' in energy balance(106).

Webb (106) documented that obese and overweight people do not differ from others in obvious ways. He asserted that fat people are not more efficient in handling food nor are lean people extravagant

'spenders' or energy, at least, under controlled conditions. While Webb(106) agreed that there is an increased thermogenesis during overeating and decreased thermogenesis during undereating, he concluded by stating that differences in their responses to dietary intake are not clear from experimental data *et al*(107) proposed that the mechanism of the increased thermogenesis may be related to response to catecholamines. These workers infuse noradrenalin into lean and obese subjects, which caused a sizeable increase metabolism in the lean subjects but only half that response in the obese subjects.

Webb (105), having observed some of the inherent limitations in the use of direct and indirect calorimetry, suggested that future research could employ better techniques for monitoring activity vis-à-vis food intake. The same author opined that such new techniques should make it compulsory to learn how people regulate energy stores, or fail to, in the face of ordinary stresses such as anxiety, temperature changes, and variations in food intake. He concluded that such futuristic techniques may make researchers in this field to understand how energy balance is controlled in the obesity syndrome.

An attempt to pave the way to the evolution of better clinical techniques, such that researchers and clinicians could understand how energy balance is controlled in dietary obesity, has led to a series of studies bioenergetics of obese animal models. The mitochondrion which is the power plant of the cell has been focused upon and used as a model. The various observations at the anatomical level resulting in pathological conditions are all known to emanate from lesions at the molecular level of cellular organization. Hence, the succeeding section summarises the various lesions and aberrations found in mitochondria isolated from dietary obese animal models.

(v) Mitochondrial Status and Related Energy-Linked Functions of Mitochondria Isolated From Dietary Obese Animals

(a) Changes in Phospholipids, Cholesterol and Total Lipids in Undernourished and Overnourished (Obese) Animals

It has been shown that in mitochondria isolated from undernourished (kwashiorkor) rats, significant decreases occurred in the concentration of total lipids and phospholipids, while significant increases occurred in the total cholesterol and triglycerides in control pair-fed rats. In obese rats, the values of total lipids and cholesterol exceeds that of the values in control (normal) rats. The values for total lipids and cholesterol are of the order kwashiorkor **<** control **<** obese.

The phospholipid values in the deficient (kwashiorkor) group mitochondria were 4.8-5.1 mg phosphorus/g protein, and 8.6-10.7 mg/g protein in obese rats in comparison with 5.7-6.6 mg/g protein in the controls. Cholesterol levels were 6.35 mg/g protein in deficient rats and 4.6 mg/g protein in controls. However, in obese animals, the value of cholesterol was 9.2 mg/g protein.

The increase in total cholesterol in mitochondria from the deficient rats is consistent with previous observations that total liver cholesterol was increased in rats suffering from vitamin A deficiency(121).

It has also been shown that rats suffering from protein-energy malnutrition lacked the ability to utilize vitamin A and hence behave essentially like rats suffering from vitamin A deficiency (122). Changes in the levels of cholesterol and phospholipids in mitochondria from deficient rats suggest that vitamin A may be an integral structural component of the mitochondrial membrane(122). However, the increases in the mitochondria total lipid and cholesterol may alter the critical lipid/protein ratio of mitochondrial membrane.

Changes in mitochondrial membrane texture occur when there are changes in the constituent lipids, proteins, and other smaller molecules like vitamin A and manosyl fragments(123, 124). Izpisua *et al*(115) reported that mitochondrial membranes isolated from dietary obese rats exhibited greater fluidity than normal rat liver mitochondrial membrane. It is, therefore, logical to speculate, at this state, that mitochondria from dietary obese rats may also not function optimally.

(b) ***Deviation from Vectorial Chemiosmosis and Changes in Oxidative Phosphorylation Characteristics of Obese Animals***

Mitchell (72), in his 'vectorial chemiosmosis' clearly demonstrated that the direction and magnitude of flow of electrons in normal mitochondria followed a simple vector. This theory, i.e. chemiosmotic theory, was experimentally demonstrated by the same author in 1979(125). Olowookere(62, 124), demonstrated that there is a deviation from vectorial metabolism in the hepatic mitochondria of PEM rats.

Recently, Izpisua *et al.* (115), demonstrated that mitochondrial membranes isolated from dietary obese rats show greater fluidity – a situation that points to possible deviation in vectorial metabolism of obese rat liver mitochondria. Olowookere *et al*(15) experimentally showed that there are significant alterations in the oxidative phosphorylation characteristics of dietary obese rat liver mitochondria. These authors(15) showed that the resting metabolic rate in obese animals was 23% higher than the theoretically predicted value. Although, the mitochondrial oxygen consumption pattern, using malate plus glutamate or succinate as respiratory substrate, revealed that the resting respiration (state 4) was 29.1% higher in obese rats, the active state 3 (ADp-dependent) respiration was 43.3% lower in obese rats compared to controls. The respiratory control ratio (RCR), which is the biochemical index for ATP synthesis, was 43.3% in obese rat liver mitochondria relative to control. From these studies, it was concluded: (a) that dietary obese animals' mitochondria have reduced RCR, and (b) dietary obesity interferes with mitochondrial oxygen utilization at the level of state 3 (ADP- dependent) mitochondrial respiration. In summary, there was an adjustment upwards in state 4 mitochondrial respiration while there was a drastic reduction in the state 3 (ADP-dependent) active respiration of mitochondria isolated from dietary obese rats.

Since each of the electron complexes specifically functions for each of the three sites of the mitochondrial oxidative phosphorylation in a mechanistic manner that is vectorial, it follows that there is an attendant alteration in the electron transfer complexes during obesity. This view is consistent with the abnormal fluidity observed by Izpisua *et al*.[15] and the reduced RCR and ADP/O ratio reported by Olowookere *et al* [15].

(c) *ATPase Status and Brown Adipose Tissue (BAT) Mitochondria Isolated from Obese Animals*

There are a number of energy-requiring metabolic cylces in the body which participate in the expenditure of energy under basal as well as diet-induced conditions. These include the hydrolysis of ATP to ADP + Pi by Na^+-K^+-ATPase(89, 126) as well as the so-called 'futile cycles' involved in the biogenetics of obese subjects. Izpisua *et al*(115) have shown that there is a decrease in Na^+-K^+-ATPase of mitochondria isolated from dietary obese rats.

BAT mitochondria contains yet another pathway for the release of energy as heat. This proton conductance pathway allows for the leakage of protons into the mitochondria when nucleotides are loosely bound to a 32,000 molecular weight protein on the inner mitochondrial membrane (127). Under these conditions, free fatty acids released during intracellular lipolysis are oxidized within mitochondria with the subsequent release of heat, rather than formation of ATP or any of the high energy phosphate bonds (89). As such, BAT represents a major source of nonshivering thermogenesis in the rat(128) and may as well be a contributory factor in DIT .

Regardless of the primary source of thermogenesis, the sympathetic nervous system appears to be the most important effector of this process in mammals(129).

(d) Respiratory Impairments and Low Work Capacity in Obese Subjects

The respiratory impairment and defects in mitochondrial respiration reported by Olowookere *et al*(15) brought to focus, eight bioenergetic problems, namely: (a) defective ATP synthesis; (b) defective ATP hydrolysis; (c) poor utilization of molecular oxygen; (d) deviation from vectorial metabolism resulting from abnormal fluidity of mitochondrial membranes; (e) reduced RCR; (f) reduced ADP/O ratio, and (g) abnormal mitochondrial guanosine diphosphate binding(103). The resultant effects of all these biochemical lesions culminate in the observed low work capacity of obese subjects.

(e) Does a Dietary Obese Animal Rely More on Glycolysis?

Compared to normal animals and the data presented in this review on the activity profiles of four key glycolytic enzymes (see section 'Changes in Key Glycolytic Enzymes in Dietary Obesity'), there is evidence to show that there may be greater reliance on glycolysis by obese animals than normal.

The various mitochondrial lesions listed in the above section buttress this conclusion since the mitochondria isolated from the obese animals do not function optimally. The reliance on glycolytic energy

production might be an adaptation or a compensatory mechanism, bioenergetically. This inference is consistent with the view of Kerr *et al*(84) who observed a rapid recycling of glycolysis and an increase in gluconeogenesis in malnourished children.

V. NETWORK THEORY OF AGEING AND MITOCHONDRIA

(i) Mitochondria as a Biomarker of the Ageing Process

Evolution theory indicates that ageing is caused by progressive accumulation of defects, since the evolutionary optimal level of maintenance is always below the minimum required for indefinite survival. Most of the theories of ageing also suggest that multiple processes are operating in parallel, but unfortunately they make no predictions about specific mechanisms. To understand and evaluate the many different mechanistic theories of ageing which have been proposed, it is important to understand and study the network of maintenance processes which control cellular homeostasis.

A network theory of ageing highlights the interactions of defective mitochondria, aberrant proteins, free radicals, and anti-oxidant scavengers in the ageing process.

This networking includes the protective effects of anti-oxidant enzymes and proteolytic scavengers. The model simulations not only confirm and explain many experimental, age-related findings like an increase in the fraction of inactive proteins, a significant rise in protein half-life, an increase in the amount of damaged mitochondria, and a drop in the energy generation per mitochondrium. Indeed, network theory pointedly showed that MITOCHONDRIA received the greatest "bashings" from ageing processes. Some of these are:

(i) Increase in the amount of damaged mitochondria
(ii) Mitochondrial energy generation badly affected.
(iii) Increase in the fraction of inactive proteins.

The above is now coupled with

(iv) Longevity gene located on mitochondria
(v) Derived from maternal source since it is sex-linked (Attardi, 2004)

Hence, this reviewer, has come to the irresistible conclusion that MITOCHONDRION is the BIOMARKER for ageing process. In fact, the mechanism of the final breakdown (from ageing effects) seems to be a consequence of the cooperation of mitochondria and cytoplasmic reactions, the mitochondria being responsible for a long term, gradual change which eventually triggers a short-lived cytoplasmic error loop.

(ii) Mitochondria and Ageing

One unique property of mitochondrion is that it has its own DNA (deoxyribonucleic acid). DNA is the molecule of which the genes are made of. When DNA is wrapped in histones, a form of storage proteins, it becomes chromosomes. Mitochondrial (mtDNA) is quite different from nuclear DNA in several respects.

(i) Mitochondrial DNA exists as a simple plasmid (a DNA loop) and, in this respect, it is more like the bacterial DNA than the chromosomal DNA of higher organisms.

(ii) mtDNA is not associated with HISTONES. Histones are positively charged storage proteins around which the nuclear DNA is wound for safe keeping (like thread on a spool).

(iii) Most of the complex DNA – repair mechanisms that correct damages to nuclear DNA are missing from mitochondria.

The relatively unprotected and unrepaired mtDNA suffers more than ten times the damages that the nuclear genome usually suffers (109, 110).

Mitochondrial electron transport is not perfect. Even under ideal conditions, some electrons still leak from the electron transport chain. These "leaking" electrons interact with oxygen to produce *superoxide radicals*. With mitochondrial dysfunction, leakage of electrons can increase significantly. *The close proximity of mt. DNA to the flux of superoxide radicals (or hydroxyl radicals*), coupled with its lack of protective histones and repair mechanisms precipitate the free-radical mediated mutations and deletions. MITOCHONDRIAL AGEING has been proposed as a fundamental underlying cause of

(1) Free-radical stress
(2) Degenerataive Diseases and
(3) Ageing (111).

Evidence is accumulating that mitochondria dysfunction underlies many common pathologies. Mitochondrial defects have been identified in Parkinson's disease and Alzheimer's disease(112) . Numerous genetic diseased-conditions and nucleoside therapy of AIDS have been directly or indirectly traced to MITOCHONDRIAL AGEING. It has also been shown by this author (113, 114, and 115) that many common nutritional deficiencies can impair mitochondrial efficiency and energy transduction processes.

Table 5: Mitochondrial Metabolites That Decline With Age.

Mitochondrial Metabolite	**Parameter affected by Age**
- Ubiquinone (Q_{10})	- Electron Transport
- Antioxidants	- Oxidative Damage
- Cardiolipin	- Transport of Compounds for ATP synthesis
- Carnitine	- Fatty acid Transporta (β-oxidation)

(iii) Longevity Gene is Sex-linked and Located in Mitochondria

Mice genes provide key to Long life

Dieters got a bit of hope recently from a study that shows a change in a single GENE in mice allows them to eat as much as they want while staying thin and living longer in the bargain. Many studies have shown that animals live longer when they eat, on average, about 30 percent less than normal. The findings have led scientists to speculate that people, too, can extend their lives by dieting. Calorie Restriction theory of ageing has been pivoted on "Free – Radicals Damage". The theory has been that:

> "If an animal eats less, the body produces
> fewer cell-damaging 'free-radicals' which
> result as natural by-product of metabolism
> of food, culminating in reduced mitochondrial respiration"(115).

Scientists identify genetic marker for longevity

Scientists, for the first time, have identified a common genetic mutation in people over 100 years old, a finding, they said, could be a key to discovering a way to avoid the ravages of AGEING. In a study conducted at the California Institute of Technology, in Pasadena, California, researchers found out that

centenaries were five times more likely than others to have the same MUTATION in their mitochondrial DNA.

Mitochondrial –DNA is passed from the mother to offspring

It has been shown (116) that mitochondrial-DNA, passes only from the mother to offspring. It is possible that during the process of replication, mitochondrial metabolites are less damaged by oxidation. The key mutation shifts the site at which mitochondrial DNA starts to replicate, and perhaps, that may accelerate its replication, allowing the individual to replace damaged molecules faster.

Longevity gene is "C150T" and can be found located in mtDNA

In a study of a group of 52 Italian centenarians, researchers found a common mutation in the same control region while looking at mitochondrial DNA in white blood cells. They found out that 17 percent of the 52 has a specific mutation called "C150T", compared with only 3.4 percent of 17 people under the age of 99 years. The above results were published in the proceedings of National Academy of Sciences of United States of America.

The team further investigated skin cells collected from same individuals between 9 and 19 years apart. In some, both samples showed that the mutation already existed; while, in others, it either appeared or became more abundant during the intervening years. These results suggest that some people inherit the longevity gene from their mother, while others acquire it during their life time.

The selection of "C150T" mutation in centenarians suggests that it may promote longevity in humans, or, put more succinctly, it is, in fact, the long sought for "longevity Gene"! The trait could only be inherited from mothers, as it is sex-linked.

(iv) How Free-Radicals From Mitochondria Damage Cells (In-Vivo) and Precipitate Ageing

The free-radicals (damage) theory was first adumbrated around 1949 by Gershman. However, this same theory was expanded and championed by Denham Harman, a biochemist from the University of Netraska, as a key theory to AGEING.

Free radicals are molecules with unpaired electrons in their outer shell. Such molecules can wreak havoc on normal cells by the attempting to steal an electron from another molecule in order to restore their balance. In doing so, they can initiate a destructive cycle that may multiply and quickly spread, destroying healthy cells in the process. This rather infectious pattern can spread indefinitely.

Free radicals are powerful oxidizing agents that cannot be avoided altogether in that a majority of them occur naturally from many normal reactions in the body involving cellular respiration. Whilst it is required for life and responsible for as much as 95 percent of biological energy need, Oxygen, (ironically) itself is, in fact, the most prevalent free radicals found in the body. Unfortunately, when not kept in check by an adequate supply of anti-oxidants, which attack them directly and prevent new ones from forming, free radicals can lead to all sorts of degenerative diseases. These include arteriosclerosis, cancer, Alzheimer's disease, cataracts, osteoarthritis, neurological disorder and immune deficiency.

The free radicals most critical to ageing process include super-oxide anion, hydrogen radicals, singlet oxygen, hydrogen peroxide and hypochlorous acid. At the molecular level, they can launch attacks on several fronts simultaneously, such as damaging immune cells (white blood cells), damaging lysosomes (which house digestive enzymes), attacking unsaturated fatty acids (lipid peroxidation), destroying DNA and causing DNA mutations, hardening cells and nuclear membranes, breaking-off cell membrane extrinsic proteins thus disrupting the integrity of cell membranes.

Site of Production of Free-Radicals

The specific site of production (in-vivo) of free-radicals is a key factor in determining their degree of danger. Most can be found in the mitochondria where they cause the least harm. The inner membranes of mitochondria are location sites for these free-radicals. Lipofucsin, a yellow pigment which increases with age is referred to as age spots, is derived from oxidized fat molecules. The built-up of this molecular trash

initiated by free-radicals eventually becomes a burden that damages the cell itself and causes it to stop functioning properly.

Free radicals, perhaps, do their greatest damage through a process of fusing DNA and protein molecules referred to as cross-linking.

Other Sources of Free-Radicals

Free radicals can also be found in our environment in form of:

(i) Toxic waste
(ii) Chemicals
(iii) Pesticides
(iv) Sunlight
(v) Radiation
(vi) Cigarette smoking
(vii) Other dietary sources such as coffee, alcohol, fried, and barbecued foods.

Oxidation reactions occur when the combustion of oxygen that keeps us alive and well, produces by-products called oxygen free-radicals. When this process occurs in metals, we call it rusting. When it occurs in humans (or animals) we call it ageing, which makes us rusty as the metals!

Free-radicals are molecules that have lost an electron. When this occurs to oxygen, we call it singlet oxygen, because it has one of its two outer electrons left. This is a highly unstable condition, and to restore balance, the radical either tries to steal one electron from another nearby molecule, or donate its lone electron. By doing any of the above, free-radicals create "molecular mayhem" disrupting, damaging, and destroying nearby cells. If DNA is involved, mutations occur, a favoured theory of a common cause of cancer. With time, free-radicals' damage accumulate resulting into AGEING. Free-radicals are not only taken in through smoking, food, air and water pollution, x – rays, but they are also produced within us and by us by the organelle MITOCHONDRION during ELECTRON TRANSFER PROCESS.

In-vivo production of free-radicals by mitochondrion constitutes a "self destructive" process which is being systematically unraveled by gerontologists. The human life span simply reflects the level of free-radical oxidative damage that accumulate in cells. When more than enough damage accumulates, cells can no longer survive hence, the resultant cell-death.

The food we take naturally becomes the raw-materials on which mitochondrial energy transduction processes are anchored. The complete metabolism of these food substances leads to oxido-reduction reactions during electron transfer reactions. More food taken leads to more free-radicals produced, hence, ageing results more quickly in big eaters.

Hence obesity reduces life span. Less food taken in as in starvation or "fasting" leads to low-level of "free-radicals" generation by mitochondrion in-vivo hence, people who "fast regularly" live longer than those who could not afford to miss a meal.

How Free Radicals Precipitate Ageing

1. By attacking DNA, the genetic materials, thus causing mutation. These mutations result into cancer and other diseases.

2. Mitochondrial genome is much more vulnerable to free-radicals' attack because they are close to the source of its in-vivo production.

3. Protein synthesis is impaired by free-radicals.

4. Proteins became cross-linked and tangled, thus losing their natural ability to function as enzymes and structural materials.

5. Free radicals make tissues less pliable.

6. Free radicals' assault on arteries leads to arteriosclerosis.

7. Age pigments accumulate, thus drowning the cells in lipofucsin thus preventing optimal functioning.

8. Free radicals precipitate cataracts of the eye due to cross-linkage of proteins.

9. Free-radicals have been implicated in more than 80 diseases of ageing which accelerate the processes of both ageing and death.

(v) Major Anti-Ageing Remedies

Ageing is, perhaps, an evocative term, relating, as it does, to a human process many consider natural. It is a continuation of life with decreasing capacities for adaptation.
This view of ageing, in terms of progressive failure of the body's various adaptive responses currently gaining acceptance. *To be aged is not a disease*.
Ageing, however, makes the body to be prone to being afflicted with myriads of possible ailments and disorders (117). The assaults by free radicals over the years, the bad food habits as well as series of unwholesome life styles that promote ageing need to be redressed for optimal longevity.

Anti-Ageing/Longevity Remedies

(i) Eating fresh fruits which supply the body with vitamin C (ascorbic acid) which is an antioxidant or free-radicals scavenger.

(ii) Drinking clean, pure water. This will prevent dehydration of cells and wrinkling.

(iii) Avoid heavy metal in your food as they can be very toxic to the human system.

(iv) Avoid foods rich in sodium as this aggravates hypertension.

(v) Avoid pollutions and pollutants, as they can be the sources of mutagen or carcinogens.

(vi) Avoid the underlisted toxic substances. Do not let them contaminate your food and drinks:

(a) Fluorides
(b) Aluminium
(c) Pesticides
(d) Herbicides
(e) Insecticides

(vii) Exercises

One of the very important anti-ageing remedies is regular exercises. Sedentary life is an invitation to premature ageing. The more you exercise the body, the healthier it becomes. Old people's exercises need not be vigorous or rigorous but should be regular. Any of the underlisted exercises could be considered and practised:

- Road walk
- Table Tennis
- Squash
- Cycling
- Golf (Non-competitive)
- Jogging
- Swimming (for about 15 minutes daily)

(viii) Karma Message/Relaxation

Individual should endeavour to practise karma message. Individual should relax thoroughly and fret over nothing.

(ix) Nutrition

Thomas Edison once said "The doctor of the future will give no medicine, but will interest his patients in the care of the human frame, in diet, and in the cause and prevention of diseases".

People who aspire to live long and live healthy should, by deliberate action, opt for taking BALANCED DIETS. They should take multi-vitamins and minerals supplements to ensure that the nutritional needs of the body are being met, especially, as these needs increase with age. Bio-inorganic chemists have shown (118) that the metals in the first series of transition metals in the periodic table serve as co-factors in many metalloenzymes. For instance, Copper (cuprous) forms part of the enzyme cytochrome oxidase in the mitochondria.

(x) Anti-Oxidants

These are the body scavengers of anti-ageing agents. Most people are familiar with, at least, some of the benefits attributed to such popular anti-oxidants as Vitamin C, Vitamin E, and Beta-carotene. The first line of defence against an excessive build-up of singlet or reactive oxygen at the molecular level consists of three protective enzymes naturally found in the body viz: superoxide dismutase (SOD), catalase and glutathione peroxidase.

Free radicals are defeated essentially by being trapped, or isolated and not allowed to seek out electrons from neighbouring cells by these anti-oxidants. Free radicals can then be metabolized and turned into harmless paired oxygen molecules or simply water. Dietary sources of anti-oxidants (non-enzymatic compounds) can produce similar effects.

While dietary anti-oxidants such as Vitamin C are useful in the fight against free radicals, this is not the only place they are needed in the body. The more antioxidants involved in this fight, the less are available overall. If neither the protective enzymes nor dietary sources of antioxidants can stem the rising tide of free radicals, the cells attempt to destroy them, themselves. This burns up vital energy which the cells require for more useful and more important activities.

As free radicals destroy healthy cells, those cells that are not being destroyed are expending more and more resources both in attempts to keep the free radicals onslaught at bay and to clean up and repair the damage that already has been done. Eventually, some price has to be paid. This price that is being constantly paid gradually moves from the molecular level outward, afflicting cells, tissues, and organs culminating in heart disease, cancer, diabetes, arthritis, senility dementia, Alzheimer's disease, a weakened immune system, and numerous other afflictions.

Key to understand why free radicals are central to the precipitation of "ageing" revolves around the issue of maintaining oxygen balance within the body. Techniques designed to successfully maintain this delicate balance are at the heart of most natural anti-ageing protocols today.

(xi) Chelation

Everybody should avoid heavy metals in food or drinking water, as they constitute sources of toxicants. If they inadvertently get into our systems, they should be removed by Chelation.

(xii) Boosting the natural immune system

Through the foods we take and the exercises, we are directly or indirectly boosting the natural immune system.

Part of "ageing" process is the gradual erosion of immune system. This is occasioned by devastation and assault of free radicals. However, by deliberate planning and choice of activities, foods, etc., the

immune system could be enhanced and supported to be responsive to attack by bacteria and viruses. Occasionally, use natural antibiotics to eliminate the more persistent bacteria and viruses. These are very important to ensure that serious ageing disorders and diseases do not appear. One of such boosters is Aloe Vera**.**

ALOE VERA.

Aloe vera has been considered a miracle plant by cultures, the world over, for thousands of years. It is approximately 96 percent water, with the balance of its active contents consisting of essential oil, amino acids, vitamins, minerals, and enzymes.

Aloe vera's primary strength as a healing agent lies in the fact of it being able to regenerate damaged tissues. Such actions have long made it a powerful therapy for wounds such as burns, cuts, and bruises. But this is just the tip of the iceberg.

Aloe vera is a traditional remedy for diabetes in the Arabian peninsular. Research shows that the intake of half a teaspoon of aloe vera daily for four to fourteen weeks significantly reduced the fasting serum glucose level in all patients. Another study found that five non-insulin dependent diabetics experienced a mean reduction in fasting blood sugar of 273 mg/dl to 151 mg/dl following fourteen weeks of taking a half spoon 4 times daily of aloe vera.

Animal experiments support such findings with respect to the potential of aloe vera as a valuable therapy for diabetes, and have demonstrated its anti-cancer properties and abilities to inhibit arthritis in rats. A host of studies indicate aloe vera can produce strong anti-inflammatory, antibacterial, and wound-healing effects. Aloe vera has also been used successfully as a laxative, may be effective for ulcers, and can protect against skin damage when used topically.

Current research points to aloe vera's potential as an effective immune system booster and possible candidate for the treatment against AIDS.

(xiii) Nootropics

The regular use of smart drugs and nutrients has been advocated to cushion the working of the body systems at an optimal level and to ensure the prevention of rapid oxidation that could lead to senility.

(xiv) Hormone Maintenance

The use of hormones and/or their precursors to ensure that the body remain at the approximate levels of healthy 25 year-old person. The above, in turn, will continually ensure improved mental and physical capabilities.

(xv) Avoid excess age-increasing hormones and enzymes

Keeping age-increasing hormones such as cortisol and prolactin low. Also, ensure that age-increasing enzymes such as (MAO) Monoamine Oxidase do not interfere with neuro-transmitters.

(xvi) Energy

Provides protection and support for the energy producing process in the body, especially for the MITOCHONDRIA, the organelles that produce the universal energy currency through a molecule called ATP.

(xvii) Specifics

There should be nutritional and medical assistance for individual areas of concern including specific treatment for hair loss (through the use of the sulphur containing amino acid called CYSTEINE).

This, of course, is to be done when the subject is not diabetic as cysteine has side-effect by inhibiting insulin. Also, impotence or loss of libido could be specially attended to through appropriate specific programme.

(xviii) Others

Readers should, in fact, keep an open mind and utilizing any other proven sources of "Life Extension" formulae e.g. the new emerging science of Electromagnetic Medicine.

(xix) Ultimately

Pure anti-ageing remedy will lie in the ability of scientists to further decipher DNA and use of those results to manipulate DNA for our own needs. The newly identified "longevity gene" should now be further studied and explored to produce the required essential ingredients as the "new keys" to longevity in man.

VI. CONCLUSION AND FUTURE DIRECTIONS

(i) Effect of Calorie Restriction on Ageing

There is a clock that is ticking away in every living being. For a dog, it is 10 years, for a turtle, it is 150 years. It is 3 years for a mouse. It is practically 70 – 80 years for human beings, as against 120 years of theoretical possibility. This clock is closely linked to *metabolic rate* of animals, that is, the rate at which animals burn its fuel (food). The crux of the matter really is "metabolism produces free radicals". Reduce the calories by 40% and increase the life by 40%.

In the Nutritional Model Theory of Ageing, which is also synonymous to Calorie Restriction theory of ageing, this theory is predicated on the fact that if an animal is fed 50 – 60% energy less than what it will normally feed on, that animal will live longer and be healthier than control animals. This model/theory actually works by itself. It is auto-regulatory. So, food can be an obsession! No wonder, people say, gormandizers have heavy brains, hence are belly worshippers! They invariably, do not live as long as they are poorly fed litter –mates.

Spiritualists, have long found out the efficacy of "Caloric Restriction on Health"; hence they FAST. FASTING prolongs life but; prolonged fasting occasioned by starvation, is inimical to longevity.

Indeed, less food, less free-radicals from within the mitochondria. Less free-radicals, slow ageing; more free-radicals from big food, hence rapid ageing. For any ailment of the aged; first, half his or her food, you will be amazed the way he/she will bounce back to health. Caloric restriction, a veritable tool to combat ageing.

(ii) Effect of Obesity on Ageing

Obesity

There is a desired body weight for age for individuals. The Body Mass Index (BMI), as referred to in medical parlance, is a very important parameter to stay healthy and physically fit.

Obesity is defined as an abnormal generalized increase in the adipose tissue (Garrow, 1978). It has been associated with other conditions of "overnutrition" and it makes a significant contribution to ill health via its association with hypertension, pulmonary and renal diseases, cardio-vascular disease and diabetes mellitus. Obesity is rarely recorded as a cause of death in humans; but it is known to contribute significantly to the reduction in average lifespan and an unquantifiable reduction in the quality of life of those who are substantially overweight. Obesity is a major health problem in the developed countries.
In developing countries, however, it is only noticeable in some segments of the population – particularly the rich and business executives with little or no time for recreation and exercises.

There are many factors that may cause obesity and the effects of obesity are also far reaching. Obesity is known to alter metabolic and endocrine balances.

Obesity arises often as a consequence of taking in more energy in the food than is expended in the activities of daily life.

Thus, a lack of physical activity will encourage the development of obesity since, in the absence of sufficient exercise, only a relatively small proportion of generously ingested food calories is used up to provide energy for physical activity. The unused calories are biochemically converted into fat cells of the body. Energy imbalance is regarded as an irreversible interplay between food intake, energy storage, and heat loss. This is expressed by the equation:

Energy intake → Energy Expenditure + Energy Storage + Heat Loss

Obesity develops from an imbalance in this equation, which could reflect either as an increase in food intake or an increase in energy storage or a fall in either energy expenditure or heat loss. Energy expenditure (Biochemically) by obese subjects has been shown to be higher than in normal subjects. In obesity, there is body weight gain indicating an increase in both the absolute and relative amounts of body fat. The body weight gain, however, is not due to fat alone, but could also be due to water retention, body cells' mass and body cells' solids.

Another possible cause of obesity is an acquired hormonal disturbance. An excess of cortico-steroid hormone induces the typical accumulation of fat on the face, neck, breasts, and other areas of the body. Other hormones like testosterone and glucocorticoids have also been shown to cause obesity when their levels are above the normal requirements. Scientists have demonstrated in the laboratories that obesity can be induced in some strains of rat by CASTRATION which precipitates imbalance in hormonal levels. Human obesity has been associated with several abnormalities of androgen and oestrogen metabolism. Morbidly obese men and women overproduce and over-metabolise a variety of androgens and oestrogens. Despite the elevated production rate of androgens, the plasma levels of these hormones are normal or only slightly elevated in such subjects; quite inconsistent with the exaggerated production rate.

This inconsistency of high production rates and low plasma concentrations is explained by the high metabolic clearance rates of the androgens. Morbid obesity in both men and women has also been associated with decreased levels of sex hormone binding globulin (SHBG). Such hormones exhibit higher metabolic clearance rates as well as increased concentrations of the free hormones in the blood.

Recent observations in human population have confirmed that genetic factors play a significant role in the predisposition to obesity. With the current biochemical and physiological knowledge in the aetiology of obesity, it is now appropriate to discuss obesity under the various contemporary captions:

1. Dietary Obesity
2. Genetic Obesity
3. Hypothalamic/Endocrine Obesity

A generalized scheme showing the pathogenesis of obesity syndrome was put forward by the author (that is, Olowookere) (90) as shown in Fig. 18 below:

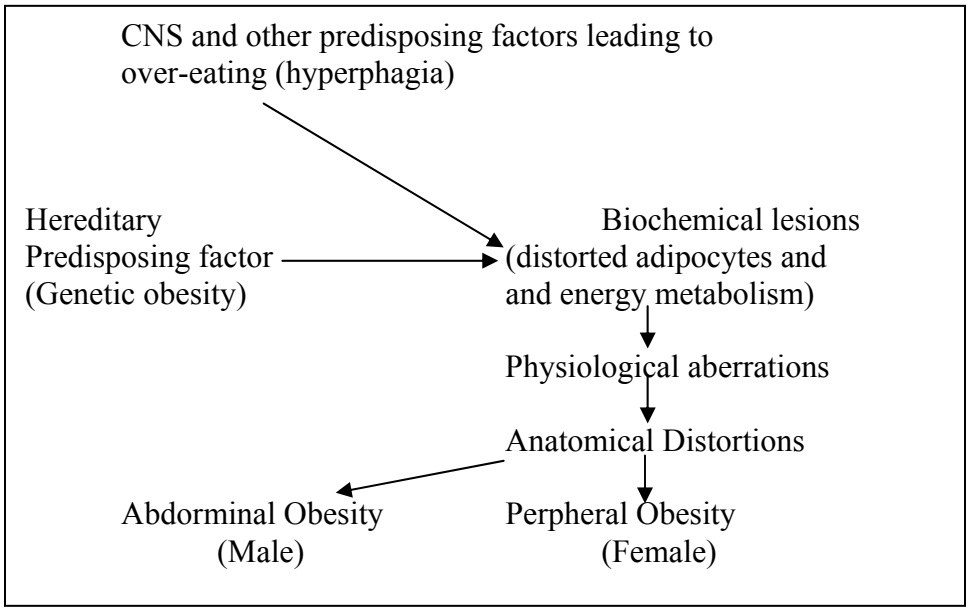


Fig. 18: A generalized scheme showing the pathogenesis of obesity syndrome in man (Olowookere) (90).

OBESITY REDUCES LIFE EXPECTANCY IN MAN

Perhaps more crucial to the issue of obesity is the fact that it reduces life expectancy. Table 3 shows the relationship between body weight and life expectancy in a 25-yeart old male.

In view of the fact that obesity is related to other pathological conditions such as diabetes, hypertension, hyperlipidaemia, gout, lower back pain, arteriosclerosis, hernia and low work tolerance, it is logical to conclude that obesity reduces life expectancy in line with data shown on the relationship between body weight and life expectancy in a 25-years old male referred to above.

Old people should therefore:

(i) Avoid over eating
(ii) Avoid fatty foods
(iii) Avoid over-weight
(iv) Avoid junk foods/soft drinks
(v) Reduce intake of calories
(vi) Fast at least once a week (as routine and not a spiritual observance)
(vii) Exercise their bodies always
(viii) Should not take alcohol
(ix) Eat vegetables always (vegetarians are known to live longer)
(x) Eat fresh fruits and natural foods
(xi) Avoid synthetic foods
(xii) Avoid artificial colouring agents in foods (as some of them are carcinogenic.

Watch Your Weight and What You Eat to Avoid Obesity and Its Co-Morbidities

"Overweight is bad and inimical to good health;
Over-nutrition leads to obesity,
Obesity reduces life-span/longevity,
Slimness potentiates good health,
Slim stature leads to good physique and physical fitness,
reduce your food intake,
Reduce patronizing fatty foods,
Live well; eat less
Eat less, live long;
Watch your weight as well as what you eat
Avoid compulsive eating".

(iii) Key Diseases Associated with Ageing

There are various diseases that are normally associated with the process of Ageing. These diseases are referred to as Age-Related Diseases. These are diseases that are simply more prevalent in the advanced years of life (119). They include the underlisted:

- Systematic arteriosclerosis
- Acute myocardiac infarction
- Cerebra – Vascular disease
- Hypertension
- Type II Diabetes mellitus
- Glaucoma
- All neurological degeneration diseases
- Dementia
- Muscle Weakness

- Alzheimer's Disease
- Parkinson's Disease
- Cataract
- Hearing impairment
- Osteoporosis
- Osteoarthritis
- Epiphora (a disease of the eye)
- Obstruction of nasolacrimal duct
- Presbyopia (eye disease/occasioned by reduced focusing power)
- Atrial fibrillation (Commonest hearts dysfunction of the aged)

- Sinoatrial block } Valvular diseases of the
- Atrio-ventricular block } heart common with the aged

- Cancer
- Gout
- Senility

Mitochondrial Dysfuction Underlies Most Ageing Pathologies

Another mitochondrial correlate of the ageing phenomenon is the various pathologies that are associated with Ageing. Two previous correlates have been identified thus:

(a) Production of free-radicals in-vivo (by mitochondria) which invariably precipitate ageing.

(b) The discovery of the longevity gene "C150T" on the organelle mitochondrion.

The third mitochondrial correlate of ageing is that the aetiology of most diseases of the aged has been traced directly to mitochondrial dysfunction as detailed below.

Mitochondrial electron transport is not perfect. Even under ideal conditions, some electrons still "leak" from the electron transport chain (120). These leaking electrons interact with oxygen to produce "superoxide radicals". With mitochondrial dysfunction, leakage of electrons can increase significantly. The close proximity of mtDNA to the flux of superoxide radicals (or hydroxyl radicals) coupled with the lack of protection of mtDNA vis-à-vis its defective repair mechanisms, lead to free radical-mediated mutations and detections. Mitochondrial dysfunction has been directly implicated as a fundamental underlying cause of:

1. Free-Radical Stress
2. Degenerative Diseases
3. Ageing

Evidence is accumulating in support of the fact that most of the common pathologies of the aged directly stem from mitochondrial ageing cum dysfunction. In fact, mitochondrial defects have now been identified in Parkinson's disease, Alzheimer's disease, Heart Disease, Fatigue syndromes and other numerous genetic and pathological conditions including nucleoside therapy of AIDS.

Indeed, apart from the genetic molecule (DNA) which is universally distributed in all cells, the most implicated organelle in ageing is MITOCHONDRION, hence could possibly serve as the much needed BIOMARKER for monitoring ageing as well as a veritable research tool for the development of management strategies and possible geriatrics medication derivable from the results of investigations carried out on the "longevity gene."

(iv) Sociology of the Aged: Need for a Governmental Policy on Old People Recreation Centres With Full Complement of Well-Trained Staff

This section focuses on the sociology of ageing. The section deals with the social divisions in a society and focuses on the old people as a distinct stratum. This section therefore, succinctly explores, "age" as one factor that can be used to classify people into social divisions – largely because of the various experiences and needs of different age groups. Other things such as, sex, ethnic group, religious beliefs and levels of disability are also useful in social divisions.

All these divisions and the many more which exist, lead us to being treated by others in very different ways, and consequently, allow a wide range of different activities and opportunities. In this write-up, we will take one of the many divisions which exist – that of 'age' and explore the way it affects an individual's life. We should be aware that the purpose of this discourse, in this book, is to cover every aspect of age as a social division exhaustively but merely examine the issue of old age and highlight its significance vis-à-vis adaptability and optimization of natural resources and historical facts. Academically, I believe that sociology can best be described as the study of 'cohesion, division, and change in society, and that virtually every issue can be looked at through these three elements. What holds people together, what pulls them apart, and what relationship do these have to social change over time are the relevant questions. It seems to me that old age, although unique; do share the common sociological fact that is really an example of divisions in a given society.

Significance of Age in Social Divisions

One of the most significant divisions in all our lives is that of age, for most people, this is both obvious and surprising. Obvious, because we think of ageing as a natural process during which our bodies change and our behaviour alters. Older people, as throughout history, bemoan the fact that young people are less thoughtful, less caring, and less polite to their elders; young people complain about the way older people moan.

Sociologists see age in different forms. They notice the way that behaviour is culturally influenced, that is, there is no direct relationship between age and a particular form of behaviour. Historically, children worked in some periods and were cosseted in others, adults played games and engaged in wars, and older people either had positions of power, or were regarded as burdens to be eliminated. It is expedient to whittle down all forms of prejudices hence, demonstrate how powerful age is as a source of social division in its own right, and separately how it interlinks with social class, race and gender.

In 1850, less than 5 percent of the population was aged over 65, but this group now forms almost 15 percent, and it is estimated that in 2020, one in five people in developed nations like USA and Britain will be over 65 (121, 122). There is a noticeable gender imbalance in that women have longer expectations of life than men, and by the age of 85, they outnumber men by 4 to 1. This growth in the numbers of older people is explained by the increasing expectation of life, which results from higher living standards, better nutrition as advocated in this book and improved housing. Reverse is the case in the developing countries like Nigeria in numbers of older people.

Old age is regarded as having very few positive characteristics, and according to Harris(122), the aged are perceived as ill, tired, slow, and inefficient in their thinking, and are regarded as having no sexual interest, unless they are odd or perverted. Possibly, because of the stigma of old age in our society, the majority of older people rejects the label of being called 'old' and perceive a difference between physical strength decline, giving an image of an old person which others see, and their self-perception that 'inside' they are still young. As people get older, they are likely to have increasingly negative opinions about themselves.

Position of Elderly People In Nigeria

It is generally believed that old age should be a time every hard working person looks forward to. It is a time to withdraw from the usual hustling and bustling associated with daily living and watch others struggle to go through the path they had once trodden. It is a time to enjoy the fruit of early years of hard work. It is a time to have everything at one's beckon and call.

Of course, that is not usually the story of most of our senior citizens. Old age, to many of them, is an endless drama of disappointments. A lot of the old people around want to go back to their youthful years

and re-enact the feat that gave them joy and fulfillment. But that only remains a wishful thinking. To a lot of them, old age is characterized by a catalogue of unfulfilled dreams.

Rather than be an enjoyable experience, old age, to a lot of the elderly, seems to be a never-ending nightmare. It is a time when there is a decline in organ and tissue function. The eyes become weak, the bones get old and creaky, a lot of the cells die which results in the weakening of the body. Many of them become senile and lose the use of various parts of their body. In spite of the contributions of the elderly to the Socio-economic growth of the nation, they are neglected in their old age. Many of them die while queuing up for their pension which is normally paid in arrears!

The old people would probably not consider their plight lamentable if there were people around to make life comfortable for them. But this is the time that a lot of children abandon their parents. The effect of this is the evolution of a strange culture in our society, which is, old people taking to the streets to beg for alms!

Universal Expectations of the Aged

The "Universal interests" of the aged can broadly be divided into five. They are:

1. To live as long as possible, at least, until life's satisfaction no longer compensates for its privations, or until the advantage of death seems to outweigh the burdens of life.

2. To get more rest, release from the necessity of wearisome exertion at humdrum tasks and the protection from too great exposure to physical hazards – opportunities, in order words, to safeguard and preserve the waning energies of physical existence.

3. To remain active participants in personal and group affairs in either operational or supervisory roles – any participation, in fact being preferable to complete idleness and indifference.

4. To safeguard and even strengthen any prerogatives acquired in a long life (e.g. skills, possessions, rights, authority and prestige); and

5. To meet death, when necessity requires it, as honourably as possible, without too much suffering and with maximal prospects for an attractive hereafter.

The above are broad desires of the aged. The specificities of which have to be determined within the context of the peculiarities of each society. In fact, the specific determination of each of the five desires and how they can be fulfilled constitute important research areas for social gerontologists; and, in fact, form the pivot of my recommendations in this review on "Aging , Mitochondria, and Diet."

Recommendations

It is recommended that there should be a change of attitude to the elderly so that the elderly would see the younger generation and even the nation as being appreciative. To witness that the people who had contributed their quota to the development of the country were given the opportunity to enjoy in their old age.

It runs contrary to our culture in Africa, the idea of taking old people into old people's homes. And this foreign idea is both strange and wicked. What old people need is not an old people's home but an old people's centre, "a place where they can go for recreation and fellowship with others like themselves." The centre should have a full complement of well-trained staff and Nurses to attend to the old people.

As a matter of fact, the predicament of the elderly could be traced to the downturn in the economy, which led to the breakdown in the family system and the younger generation should be called to remember that they too would, one day, become old and urged them to sow what they wish to reap.

(v) Future Directions on Ageing Research

The last major frontier of Biological Research is Ageing. It is however multi-pronged and both basic cum applied. Many centres of gerontological research across the globe have made many startling discoveries on the phenomenon of ageing. The laboratories of GERON INTERNATIONAL which is a drug-manufacturing outfit is collaborating with many other laboratories in various continents to further unravel the mysteries shrouding the phenomenon of ageing. The recent advances in MOLECULAR BIOLOGY RESEARCH have further illuminated this rather vast area of research.

Mitochondrial involvement and roles in ageing are becoming clearer. Further elucidation of the roles of this ORGANELLE in AGEING is definitely required to fully understand the molecular biology of ageing. Indeed, the biogenesis of the organelle, mitochondria, was fully understood from Molecular Biology perspectives. Contrary to the classic endo-symbiotic theory for the origin of mitochondria vis-à-vis the universality of their functions (from yeast to man)(123), Molecular Biology argues against the gradual integration of mitochondrial genome into the nucleus. These molecular studies gave birth to the new, an accepted independent mitochondrial genome. Further characterization of this mitochondrial genome and its full involvement in the ageing process, would, undoubtedly, further garnish our present knowledge and hence, our management strategies and hence geriatrics medication/formulations for the aged.

In conclusion, mitochondria are complex organelles, whose formation necessitates the intricate cooperation of nuclear and mitochondrial genes and their products. The mitochondrial genome, having both transcription and translation in the same mitochondrial compartment, though fully characterized (124, 125), still remains an enigma vis-à-vis ageing phenomenon! The new "MITOCHONDRIAL AGEING THEORY(126) captures the whole essence of the molecular basis of degenerative pathologies during ageing. Full x-ray of the molecular genetics of mitochondria vis-à-vis ageing should be further investigated. It is a core Basic Research needed before further Applied Researches in Gerontology. This, to my mind, is a classical example of a basic research agenda, which dovetails into an applied research solution by unraveling the process of AGEING.

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