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THEORETICAL APPROACH TO LIFE PROCESSES
Part–V. Postulates of the Hypothesis on Aging

MIRZA ARSHAD ALI BEG
PCSIR, Karachi-74400, Pakistan

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Postulates of the hypothesis on aging are presented. They concern the roles of water as the input for and output of life processes. As the input it is needed directly to maintain an appropriate degree of hydration, to act as a carrier of nutrients, a medium or substrate for enzymic reactions and to maintain a heat and product balance on the product side and indirectly as a nutrient. The key mechanism for propagation of life processes is oxidative dehydration meaning dehydration accompanied by oxidation, whereby one of the output is water, generated (1) as a metabolic product, (2) as a by product due to disturbance in the micro- environment and (3) as a medium governing the electrolytic balance and enzymic substrate.

The above reactions, roles and functions of water have been discussed to suggest that biological reactions take place in the micro- environment at the sub-cellular level. It is hypothesised that reduction in the degree of hydration and/or a disturbance in the micro- environment create stress situations, generate stress metabolites including water and a hydrophobic material, create a diffusion barrier to the membranes, reduce the enzymic activity etc. and thus the process of aging surfaces up.

Key words: Aging, Oxidative-dehydration, Water, Nutrient carrier, Hydrogen bonding network, Micro-environment.

Introduction

Aging has been defined earlier as the process of slowing down of normal biological functions of a living organism [1]. It runs concurrently with the life propagating processes but in directions reverse to them. Since the driving force of such reactions is maximum during the active growth period, the forward reactions constituting life processes carry an organism from its infancy to youth which is the blossom period. This is followed by an equilibrium position where the forward reaction is balanced by the reverse reactions. This among the mammals is the beginning of the middle age and among the humans it occurs between the late 30s and late 40s depending on a host of factors which include nutritional status and environmental conditions. The inhibitory effects of the reverse biochemical processes such as ion accumulation, ionic disbalance, dehydration and crosslink formation start dominating life processes following the middle age and these characterise senescence or aging.

Life processes accompanying physiological changes have been proposed earlier to be biochemical reactions concerned with reduction in the degree of hydration as one advances in age, and are irreversible. Examples of such reactions are polymerization e.g. cross-link formation, and oxidation e.g. the energy involving catabolic reactions. In plant life processes these reactions lead to cellulose, lignin, tannin and lipid formation representing the former class and the formation of fatty acids from carbohydrates, representing the latter [2]. The substances so formed are hydrophobic in nature and are deposited at selective sites out of the body fluid during the normal course. However, with a disturbance in the micro-environment there would be a change, not particularly noticeable during the youth, becoming apparent during the aging period, with the reverse reactions,
e.g. lowering of the degree of hydration, dominating over the forward reactions. The deposition would occur at the disturbed site; such a case is presented during desiccation conditions among the plants [1,3,4].

Many of the reverse reactions in the micro-environment are a result of ionic and/or enzymic disbalance and cause a disease situation which must be overcome [4,5]. It may therefore be inferred that the macro-environment would otherwise be degraded and eventually the major organs of the body would lose their functional efficiency. The degradation processes which might have spread over the total life time to slow down are speeded up as a result of the inefficient function of some of the major organs and the signs of aging surface up much faster than normal.

Xenobiotics or foreign substances in the form of toxic materials affect the biological processes in a manner which creates a disease situation. They undergo transformations like oxidation, reduction, dealkylation, deamination, dehydration etc [6]. The body can deal with them as well as with the internally produced toxins till such time that it has the appropriate enzymes in adequate number to detoxify them, maintaining an optimum degree of hydration in the meantime. It has been proposed here that carbonic anhydrase, catalase and phosphorylation peroxidase are some such anhydrase enzymes which seem to dominate the reaction sequences during senescence. They would give rise to cross-links which when not reversed, set in the aging process. Evidence for the

decrease in enzymic activity with progressive loss of moisture is provided by experiments on dehydration/rehydration of vegetables [7] and is also provided by findings on the fungus Neurospora and nematodes like T. aceti that there is a progressive accumulation of inactive enzymes not necessarily accompanied by a decrease on the population of active enzymes [8,9,10].

It is suggested that a gradual depletion of water content of vegetables beyond the critical point is followed by oxidative dehydration which decreases enzymic activity either by decreasing the population of active enzymes or by increasing the population of inactive enzymes. Anhydrase activity then possibly becomes the dominant feature at this stage. This mechanism explains the occurrence of the oxidized and/or cross-linked lipo-proteins, forming the major constituent of the membranes, and its deposition as a hydrophobic material lipofuscin, at subcellular level.

The present paper is intended to elaborate on the Dehydration theory of Aging which suggests that biological processes involving dehydration, accompanied by oxidation, hereafter called oxidative dehydration, take place at subcellular level giving rise to cross-linkage, anhydrase activity, lyophobics formation, ion accumulation etc. and constitute some of the main mechanisms for aging. Oxidative dehydration as the aging parameter, though apparent, does not appear to have been spelled out as one so far. However, mention has been made of age- dependent decrease in water content in brain and liver cells [11,12] and in osmotic potential of their tissues [13].

The main postulates of the hypothesis which are as follow, can be divided into two categories, one concerning the direct as well as indirect but vital role of water in maintaining life processes, as input to the system, while the other concerning normal oxidative dehydration processes, and disturbances in the micro- environment created by stress situations, as its output.

The Postulates

1. Degree of hydration.

An appropriate degree of hydration has to be maintained for the continuation of life processes which need water to perform direct functions such as (i) formation of the hydrogen bonding network among the different strands and subcellular components, (ii) a carrier of nutrients, (iii) a medium or substrate for enzymic reactions and (iv) to keep a positive balance on the product side. Indirectly it functions as (v) a nutrient.

2. Reaction in the micro-environment

Consideration of postulate 1.iv leads to two important functions in the aging process: (i) generation of metabolic water by the oxidative dehydration process within tissues, which constitutes about 12% of the total input in a normal adult human, must be optimised by balancing the metabolic processes and (ii) disturbance in the micro-environment as a result of stresses creates dehydration situations and must be minimised.

3. Elemental Balance

Elemental, including electrolytic, balance must be maintained to retard cross-linkage formation, depolymerization of the hydrogen bonding network and denaturing the proteins/enzymes.

The postulates propose maintaining an adequate degree of hydration to keep the balance on product side and to avoid a disease situation. They suggest optimising the output of metabolic water, produced by oxidative dehydration, and of the other products which are generally hydrophobic in nature, to retard the aging process. Elements including electrolytes play a vital role in life processes and the input of water as well as its output help in maintaining the concentration and carrying their appropriate quantity to the desired site and in rejecting those which are not wanted. These postulates will now be discussed in detail.

Degree of Hydration

Maintaining a hydrogen bonding network: It was pointed out as early as in 1933 that oxygen and nitrogen atoms of the proteins present potential sites for the coordination of water molecules and thus for the formation of an extensive hydrogen bonding network [14]. The water holding capacity of the skin has been known for a long time to vary with age. Accordingly the water content of the skin of the human infant is 81 to 82% while that of an old person is 73 to 74% [15]. The water holding capacity is mainly due to proteins. The hard gelatin gels contain 90% water. However cobalt chloride which remains pink in the hydrated state turns blue, not when the gel becomes anhydrous, but when it still contains 33% moisture. This indicates the difference between bound water which is 35% and forms the network in gelatin, and loosely bound water, in the gel which accounts for 55% of total water [16]. It is estimated that gelatin has a chain length of 288 amino acid residues, a molecular weight of about 27,000, with 450 to 500 water molecules coordinated along the backbone and a total of 800 to 850 bound water molecules per molecule of gelatin [17]. It has been suggested further that all the coordination sites, particularly among the fibrous proteins, are not occupied by water molecules. The bonding of the loose water molecules is of the dipole-dipole interaction type [14]. The hydrogen bonding system comprising the 500 odd water molecules is quite extensive and is inserted between the helical chains of the proteins [18]. It perhaps presents a mechanical model of a lubricant and this constitutes a speculative theme of postulate 1.i.

ii. Carrier of nutrients: Water is a powerful solvent with a very high dielectric constant. It can thus ionize a variety of polar and semipolar substances to facilitate their interaction and can form both true and colloidal solutions which permit reactions at high velocity compared with solid and liquid phases. Furthermore the reaction rate, which is high for hydrated hydrophilic materials followed by hydrogen bondables and is low for non-hydrogen bondables and hydrophobic substances, can be enhanced by increasing the surface area e.g. by forming emulsions such as blood or milk. It was shown in the case of alcohol metabolism that instead of assimilation, it is biodegraded to acetaldehyde [19]. Alcohol is lyophilic but has only two hydrogen bonds and not a network as water has and hence does not act as a carrier.

Nutrients, according to the coordination polymer approach, have to present themselves as hydrogen bondable substances during passage through membranes in order to combine with cellular constituents [20]. Carbon dioxide itself, for example, is unable to get through and therefore it has to present itself as carbonic acid. Ionic species similarly have to be highly hydrated to get past the membranes.

The water contained in the membranes is all bound water located in the hydration sphere. The interaction of lipids and proteins depends on the extent of hydrogen bond network of this sphere which also governs the exchange and enzymic activities of coordination polymers attached to the surface of the membranes. There are structured layers of bound water also attached to the surface of membranes. The water here has tetrahedral structure as in ice and has viscosity 39 times that of pure water [21]. Any decrease in moisture content would therefore, significantly affect the stability and diffusion barrier properties of the membrane.

iii. Enzymic substrate. Loss of enzymic activity on desiccation elucidates the role of water as a medium for biological reactions particularly where it acts as substrate for enzymic reactions. It is well known that proteins are denatured under desiccation conditions and since enzymes are also proteins, their dehydration would amount to loss of activity. This would alter their properties to the extent that rehydration to the original level, after a critical value is passed, would not be possible. This has been observed in the case of vegetables [7] where the activity of the respiratory enzymes has been found to reduce gradually with a progressive reduction in moisture level. It is for this reason that an optimum degree of hydration is desired for the performance of various functions concerning life processes.

The metabolism of hydrophobics is effected on the lines of xenobiotics by multifunction oxidative enzymes. These oxidation reactions produce a molecule of water within the tissues and account for 12% of the total water content of the body. The organs concerned with such metabolism as well as biosynthesis are all seats of intense enzymic activity and are highly hydrated e.g. the lungs contain 83% water; the kidneys, alimentary tract and striated muscles contain 79%; while the liver and skin contain 71% water; in contrast the adipose tissues, having comparatively lower enzymic activity, have only 50% water [22].

iv. Driving force for forward reaction. One of the main functions of water is its capability to induce a positive balance and provide a thermal control system for the biological reactions on the product side. The products of photosynthesis for example are organic compounds and molecular oxygen and the complex organic molecules. These compounds and molecular oxygen together have a certain free energy of formation. This free energy is released into the environment alongwith the reaction product. The free energy of formation controls each reaction and the presence of water maintains the thermal balance.

The reaction favoured is one in which the free energy of formation is more negative than the other competetors. Formation of water molecules involves lower free energy and therefore it is reasonable to suggest that dehydration is the favoured reaction and anhydrase activity is the favoured activity of the enzymes or their modifications in biological reactions. The latter should accordingly be effective in forging the forward reactions. This, perhaps, is the reason that with progressive age a dehydration mechanism is more operative and needs to be balanced by hydration or rehydration as per requirement of the system.

It may be mentioned here that biological reactions occurring in living cells are not so isolated as to reach thermodynamic equilibrium. There is, on the other hand, a system of inter- related reactions in steady state equilibria where the energy inflow and outflow are balanced. Under these conditions, it is suggested that the catalytic role of the enzymes becomes important; they determine the steady state concentration of the reactants and hence the direction of the reactions [23]. With a progressive decrease in water content of the system, as in aging, there would be a loss of activity of the enzymes and the population of inactive as well as deactivated enzymes would increase. This implies not only the slowing down of biological processes but also their termination due to the gradual deactivation of enzymes.

v. Role as a nutrient. Water acts indirectly as a nutrient in animal life processes but directly in plant processes as elucidated by the photosynthetic reactions in which chlorophyl catalyzes the reaction between carbon dioxide and water to yield carbohydrates. Food containing bound water is easily assimilated by animals by virtue of its having 4 hydrogen bonds compared with dry materials and hydrogen bondable

materials like proteins and carbohydrates which may have at the most 2 hydrogen bonds [24]. The latter class of compounds provides coordination sites for water and such food is assimilated which is adequately hydrated in the manner stated for gelatin. The nutritive value of water is realized by the fact that one can survive without food much longer than without water. Death can occur when the water loss from the body exceeds 20%. Water contained in the nutrient input enters into a great variety of biological reactions like hydrolysis, reduction, oxidation etc. and also catalyzes several other reactions as required by this postulate.

Water, besides forming a series of chemical compounds such as carbohydrates, proteins, lipids etc. to provide a base for chemical potential energy acts as the primary source of electrons and protons through photolysis.

$$H_2O \rightleftharpoons H^+ + OH^- \quad 2OH^- \rightleftharpoons O_2^- + 2H^+ + 2e^- \qquad (1)$$

This process then provides the free energy for synthesis of ATP in the photosynthetic reactions mentioned above and for powering the other energy demanding reactions.

Water also acts indirectly as a source of free radicals under appropriate conditions since they are produced in an aqueous environment; oxygen adopts a univalent reduction pathway and attains its reduced state as follows:

$$O_2 \xrightarrow{e^-} HO_2^{\cdot} \xrightarrow[H^+]{e^-} H_2O_2 \xrightarrow[H^+ - H_2O]{e^-} HO^{\cdot} \xrightarrow[H^+]{e^-} H_2O \qquad (2)$$

The electron transfer to oxygen in biological systems is catalysed by terminal oxidases and the intermediates formation takes the following course [25]:

$$RH + O_2 \longrightarrow R^{\cdot} + H^+ + O_2^- \text{ (aqueous medium)} \qquad (3)$$

$$RH + O_2 \longrightarrow RH^+ + O_2^- \qquad (4)$$

$$RH_2 + O_2 \longrightarrow R^{\cdot} + H_2O_2 \qquad (5)$$

$$2RH + RH_2 + 3O_2 \longrightarrow 2R^{\cdot} + RH^+ + 2O_2^- + H^+ + H_2O_2 \qquad (6)$$

$$O_2^- + H^+ + H_2O_2 \longrightarrow H_2O + O_2 + HO^{\cdot} \qquad (7)$$

$$O_2^- \xrightarrow[H^+]{SOD} \frac{1}{2} H_2O_2 + \frac{1}{2} O_2 \xrightarrow{Catalase} H_2O + \frac{1}{2}O_2$$

It may be seen that on an overall basis oxygen is reduced to its intermediates namely the superoxide and the hydroxyl free radicals and hydrogen peroxide, while organic free radicals are generated alongwith water. The superoxide free radicals are dismutated by superoxide dismutase SOD to hydrogen peroxide which in itself is much less harmful than its precursor. Elimination of H_2O_2 from the system iseffected by the anhydrase activity of the catalase[2b] as in equation (7)and (8).

$$H_2O_2 \xrightarrow{Catalase} \frac{1}{2} O_2 + H_2O \qquad (7)$$

$$H_2O_2 + C_2H_5OH \xrightarrow{Catalase} CH_3CHO + H_2O \qquad (8)$$

The concentration of the free radicals in the system is small since they are effectively eliminated almost immediately by the multifunction oxidases treating them as xenobiotics. The toxicity of the superoxide radical and hydrogen peroxide is increased if metal chelates are available to catalyze the reaction producing the hydroxyl free radical (eq.2) [27]. These reactions are also slow and normal physiological concentrations of the superoxide radical and hydrogen peroxide do not allow the building up of a significant concentration of hydroxyl free radicals. Fenton's reagent can, for example, be a dependable source of the hydroxyl free radicals. Iron is, however, not
available in adequate quantities in the biological systems and hence the HO would be rapidly scavenged by picking up an electron from the neighbouring molecules. They react at diffusion controlled rates of $K=10^8$ to 10^{10}L $mol^{-1}s^{-1}$ with most biological substances [27,28]. The highly reactive superoxide radicals, on the other hand, are produced only slowly. They have a long life and can diffuse deep down into the cell where the micro-environment is vulnerable to their attack, but in doing so they need to be activated by enzymes or by photosensitization [29,30].

The oxygen free radicals have the capability to introduce inter as well as intramolecular cross-linkages in the macromolecules of the biological system. For this purpose the concentration of the free radicals and their capability to penetrate deep down into the cells governs the reaction sequence and the free energy of the system. Density of the medium in which the reaction takes place would, therefore, determine if the reaction would proceed to maintain a positive balance in a system with a reduced degree of hydration. For example in dense biological systems like the membranes, the chances of the hydroxyl free radical being effective in forming cross-links would be much higher. In this particular case the number of hydroxyl radicals available for reaction with one molecule of the target is more than one and hence the probability of formation of cross-linkage would increase several times. The presence of water molecules in the vicinity can make a difference by retarding free radical reactions. It has been observed in the case of dry biological systems e.g. nematodes stored in dry state, that the superoxide free radicals slowly induce loss of their viability [31,32,33].

Reaction in the Micro-Environment

i. Production of metabolic water and oxidative dehydration. The role of water as input to life processes, as outlined by postulate 1.iv. already indicates its involvement in obtaining

it as the output. The mechanism for this reaction is oxidative dehydration implying dehydration accompanied by oxidation which is considered by the present theory to be the aging factor. This is supported by the development in plants [34], where it is noted that there is a progressive shift from the reduced state to oxidized state in tissues and to reduced state of oxygen as water in the system and can be summed up as oxidative dehydration. The other products of metabolism comprise hydrophobic substances and cross-linked polymers formed within the cell. Stresses like thermal shock, radiation damage, cut and pressure injuries etc., to be considered in the following section also, create dehydration situations and make the micro-environment vulnerable to attack by xenobiotics. A site for deposition of hydrophobic substances if the stress situation prevails in the degraded macro-environment. According to this postulate, the contribution of water from this source should be minimum or else it would influence the normal biological processes. A case elucidating changes in the metabolic pathway as a result of disturbance in the micro-environment giving rise to oxidative dehydration is that of the formation of dextrorotatory pinoresinol from spruce wound resin while normally the product is optically inactive. The interaction of organic free radicals and ions leads to formation of hydrophobic material [35].

The reduced forms of oxygen are responsible for various secondary reactions of wide diversity e.g. depolymerization of polysaccharides including pectins. $OH^{\cdot}$, O°_2 and .OOH obtained as a result of interaction between H_2O_2 and O°_2 attack the cellulosic and pectin superstructure of the fruits and vegetables in the same manner as the proteins and lipids of the animals and separate the cells thus making them vulnerable to degradation by xenobiotics and enzymes e.g. cellulase and polygalacturonase[36].

Hydrogen peroxide is a source of oxygen free radicals which in plants lead to the release of ethylene from the precursor substrate. Superoxide levels show very little change, if any, during senescence in fruit tissues [37] or in aging rats and mice [38]. However, hydrogen peroxide concentration in tissues has a definite role in senescence [39]. If the formation of peroxide in fruits can be suppressed, ethylene evolution and hence the ripening process would, according to this postulate be retarded. The rise in hydrogen peroxide level precedes ethylene evolution during the ripening process and it is observed that senescence in fruits is accompanied by a decline in unsaturated fatty acids [40]. This decline has been attributed to lipid peroxidation [41] which also takes place in mammalian tissues [42]. Enzymic activity of peroxidase can effectively deal with excess H_2O_2 in the system and lipid peroxidation can be catalyzed by lipoxygenase. Thus it is a mix of enzymic and free radical participation which constitutes the biological reactions leading to senescence.

Enzymic oxidative processes utilize free fatty acids as substrate although the concentration of the latter in plants is low. These acids are released from glyceryl lipids by the enzymic action of acyl hydrolases [43]. They are involved in the modification of cellular integrity and transformation of lipids as well as other cellular components.

It is possible to suggest on the basis of the above observations that all such oxidative degradations e.g. decarboxylation, deamination etc. constitute the aging process which are accompanied simultaneously by dehydration. x-decarboxylation of oxalacetic acid, the chief component of Krebs' Cycle, produces malonic acid and is a case in point. Another case is that of glutamate which on oxidation forms aspartate and loses a molecule of CO_2 as well as water and is by the present definition an oxidative dehydration reaction. Amino acids: leucine and valine accumulate in ripening bananas. They have been found to be incorporated into branched chain esters of banana through transamination and subsequent oxidative decarboxylation [44]. In fact transamination and oxidative decarboxylation of common amino acids to the corresponding acyl- CoA derivatives provide a potential source of flavours in plants [44]. It is therefore reasonable to assume that the process is related to aging characterized by ripening and therefore, to physiological and biochemical changes marked by oxidative decarboxylation and/or dehydration. It is pertinent to point out here that the substrate for synthesis of flavouring chemicals although present in immature fruits is not effective because of the age dependent absence of the enzymes appropriate for their conversion.

Malondialdehyde, the product of oxidation of fatty acids, attacks free amino groups in the biopolymers including proteins, nucleic acids and phospho-lipids and forms Schiff bases with I- amino-3-imino-propene bonding and produce lipofuscin, the fluorescent pigment [45] which accumulates at sub-cellular level during the aging process in animal systems [46-49], nematodes [50], fung [(51] and during ripening in fruits [52]. Production of such aging substances can be suppressed in the manner suggested above so that the chances of accumulation is reduced. The mechanism therefore seems to be universal in application to life processes.

The formation of peroxides leads to the onset of the oxidative dehydration processes. Oxidation of sulphydryl (SH) group by H_2O_2 is catalysed by glutathione-peroxidase in plants [53] as well as in fungal systems [51]. Use of sulphydryl modifying systems can substantially accelerate the ripening process while SH protecting groups inhibit them [54]. The release of the hydrophobic materials formed as a result of oxidative dehydration, decarboxylation or desulphurization can thus be controlled so that the materials like the aging

pigment are not released into the mainstream of the body fluids. However, if they

leak in, the chances are that they would deposit at vulnerable points created under stress situations.

ii. Balancing metabolic processes. This postulate suggests that when the micro-environment comprising a conglomeration of cells responsible for a certain activity e.g. respiration or transportation of the organism is disturbed by changes in atmosphere, temperature, cut injury etc., the primary effect is upon biochemical reactions which bring about metabolic changes [55]. These reactions occur in the cells of the organism and are more or less localized e.g. in the mitochondria [56]. The changes which take place as a result of stress situations. e.g. alterations in temperature, pressure etc. are governed by the law of mass action. Application to respiration and oxidative dehydration reactions by which carbohydrates are oxidized through a series of steps to water and carbon dioxide, suggests that marked changes in the biochemical reactions would be observed if the concentration of carbon dioxide is reduced or raised [57]. In fact in the case of plants it is possible to extend the storage life by raising the carbon dioxide level of the storage atmosphere to the extent of reducing the oxygen level.

Reactions with respect to respiration take place in the micro-environment through diffusion of oxygen to reach the centres of activity under a concentration gradient. The individual cells and their enzymic systems are reached through intercellular spaces following solution gradients[57]. It can be suggested that the oxidase system which combines the oxygen, and the carbonic anhydrase which is responsible for respiration, together increase the concentration of the gases in the micro- environment. The concentration gradient is altered in the cell and movement occurs through intercellular space raising the partial pressure and activating gaseous diffusion through the tissue to the respiratory tract. Any disturbance in the macro- environment, in which the organism finds itself e.g. physical, chemical and biological stresses such as exposure to radiation, temperature adversity, xenobiotics and biological pests alters the reaction conditions. In each case a local deficiency in the hydration atmosphere is created and the degree of hydration of the macro-environment is lowered resulting in adverse effects on the transport of oxygen through the solution gradient.

It is known that desiccation conditions produce hormones e.g. absicic acid which is otherwise produced during senescence [58,59] and also that stress situations yield stress metabolites [60]. The latter are dehydration products of biochemical reactions and are hydrophobic in nature [61]. In plants they are useful in providing protection against pests and in inhibiting early senescence but others e.g. ethylene are produced towards senescence. Ethylene is, however, also produced in case of cut injury. When cut-injured sweet potatoes are subjected to endogenously or exogenously produced ethylene, they are found to accumulate the hydrophobic stress metabolites, the terpenes [62].

Stresses created in plants by wounding lead to accumulation of hydrophobics like lignins, suberins, phospholipids, melanin- like substances, and their deposition at vulnerable sites, created by adverse situations. It may be mentioned here that metabolic pathways change with slight changes in the micro- environment provided the changed conditions of the macro- environment persist for sufficient time to allow adjustment with the environment. The stress metabolites, being not the normal metabolites are among the products of secondary reactions comprising degradation products of polysaccharides including pectins. They are quite often undesirable since their content changes, affecting taste, flavour and nutritive value [63]. Reducing and non-reducing sugars, for example are increased and starch content is decreased [64]. These changes in metabolism, unless adequately balanced, surface up in the macro-environment in one or the other from of aging.

Elemental Balance

Maintaining electrolytic balance. This postulate has been discussed earlier[58] in connection with plant processes in the saline environment, and it has been shown that any decrease in the degree of hydration increases the electrolyte concentration. An equilibrium exists between the various fluid compartments of the body of the organism to maintain a normal water concentration and is largely governed by osmotic forces related to ionic concentration. The latter also influences permeability of cell membranes, hydrogen ion concentration, protein solubility etc.

Deficiency of essential elements is responsible for the primary metabolic defects [65] resulting in the inhibition of enzymic activity related to the deficient element. If the deficiency is accompanied by a reduction in the degree of hydration, there would also be a deficiency of substrate.

The electrolytes present in the body fluids largely control the contraction of muscles, irritability of the nervous system, secretion of digestive juices, perspiration, urination and above all the water balance and the enzymic activity. The irritability of the muscles and nerves is greatly influenced by the sodium ion balance. The ratio of Na^+, K^+ and OH^- to Ca^{++}, Mg^{++} and H^+ maintained in the extracellular fluids suggests that irritability is increased with high concentration of Na^+ and OH^-[22]. This delicate balance, according to this postulate should not be disturbed in the micro-environment of fluid compartments. However, alterations in the macro-environment shift the balance and therefore use of brackish water affects the sodium

balance, of high fluorides disturbs the calcium balance etc. Such interactions are responsible for disease situations, and if not rectified in time could permanently shift the balance and would age the tissue concerned.

Changes in the Micro-environment and initiation of aging.

The postulates of the Dehydration theory of Aging outlined above at 2.i and 2.ii suggest that when the micro-environment concerned with a certain activity of the organism is disturbed by xenobiotics or stresses or a physical event, stractural changes take place there. If they are not reversed they proliferate into the macro-environment and can easily be noted among the proteins which are particularly vulnerable to attack by the agents of change. The following pages examine some of the reactions and the resulting structural changes.

Reaction of Proteins and changes in the macro environment: The staining of bacteria with dyes and the reaction of tissue ampholytes with a series of reactants like formalin, polyvalent anions like ferrocyanide, dichromate, tungstate, monovalent anions like picrate and polyvalent cations like those of mercury, copper and aluminium, all involve cellular function. Formalin and the mono as well as polyvalent anions react with the amino groups of the proteins amino acids. This makes the latter more acidic and moves their isoelectric point to a lower pH value. In the case of the polyvalent cations the site of attack is the carboxyl group of the amino acids and thus by making them alkaline moves their isoelectric point to a higher pH value. For example red blood cells after having been fixed or reacted with formalin and polyvalent anions are stained by methylene blue at pH 4.8. This shows that the reactants make the protein- haemoglobin complex more acidic and hence the staining by methylene blue [66]. The formation of bacteria-dye complex initially starts at the molecular level and because of the multi- ligand character of the proteins and involvement of multiple groups in the dyes, several sites are available for cross-linkage formation, leading to coordination polymerization reaction.

The reactions concerning the bacteria and dyes as well as metals perhaps initiate at the level of the bacterial cell wall which constitutes a macromolecular framework called murein, a peptidoglycan. The latter has glycan chains linked at 1-4 positions and cross-linked by short peptides. This biopolymer, thus, has both the protein and carbohydrate chains in the system. The peptidoglycan is responsible for the shape and compactness of the bacteria while the proteins have a peptide backbone exclusively constituting L-amino acid residues; the cell wall peptides have D-amino acid sequences alternating LD residues. Furthermore they have γ-bonded glutamic acid that interrupts the amide backbone at the specific site in the peptide [67]. Thus murein has an alternating hetero-polysaccharide backbone comprising N-acetylglucosamine and N-acetylmuramic acid with regular as well as random polymeric chain.

A number of sites are accordingly available for interaction with metal ions in the micro-environment. For this reason even the smallest amount of the metal salt would be fixed on the outer cell wall of the bacteria and in the most resistant cases it would at least disturb the conformations of the polysaccharide moiety in murein. This would on the one hand initiate a degenerative process by deprotonation or displacement of a hydrogen bond or denaturation of the protein moiety and on the other hand would allow the formation of the coordination polymer by fixation of the metal ion on a donor site. The latter could lead to the death of the bacteria as is observed in the case of the so called oligodynamic action of silver. If, however, the reaction with the metal ion is reversible and if the metal bond formation is accompanied by its breaking, it would be possible for the body fluid constituting a solution of electrolytes to carry the cation alongwith it for ultimate excretion thus restoring the life processes.

Coordination polymers and fixation of metal Ions by proteins: Peptides in aqueous solution like the micellae of the proteins are fully hydrated and form self-associated aggregates which have strongly hydrogen bonding amido groups. These groups provide the site for the formation of metallic complexes and coordination polymers. Simple cations entering the cell wall of the bacteria influence the properties of the peptidoglycan because of the formation of the coordination polymer. The biopolymer DNA flexibility, conformation, secondary and tertiary structure as well as the mode and extent of bonding of the charged ligands, which include the gene regulating proteins, are invariably very sensitive to the amount of added cation and hence to the formation of the coordination polymer. The ionic effect of this kind is due to the presence of the acid and basic groups which impart them the capability to get the metal ion redistributed among the reactive sites but perhaps more importantly due to the long range electrostatic forces between mobile counterions and the DNA polyion. The counterbalancing of electrostatic interactions among the charged species and the thermal randomization are deterministic of the distribution of small ions in polyelectrolyte solutions [68].

According to the counterion condensation theory given by Manning a fraction of counterions in a two phase system is territorially bound around the polyion which may be considered as an infinite straight line of uniform charge density in a small space while the remainder is unperturbed. The linear charge density has been proposed as the primary determinant of the extent of counterion bonding. The polyions

could alternatively be considered as structureless point charges and their interaction with another moiety would be based entirely on charge. In the interaction of the polyanions, for example of the negatively charged DNA, reacting with the cationic ligands like polyamines, the latter condenses when a critical fraction of its total negative charge has been neutralized by the former and a reduction in the degree of hydration has been effected in the meantime.

The counterion theory has, however, limited validity because it has been found from ^{23}Na-nmr relaxation studies that the bonding of polyions is not just counter-balancing of opposite charges to be charge dependent; it depends on structure as well [69]. The latter aspect is better elucidated by the coordination polymer approach since it is concerned with coordination sites, redistribution of charges, isoelectric point of the protein moiety and not just the charge on the ion [70]

The coordination polymer theory is an attempt to correlate the interplay of charged moieties and takes into consideration the effect of variations in chain length and/or bulk of the polyion on the biopolymer, DNA condensation and its thermal stability. Spatial charge separations alter the effect of the polyion on the preformed polymer; they stabilize DNA against thermal and alkaline denaturation, enzymatic degradation and shear induced strain. Such polyions can induce in vitro condensation of DNA and facilitate its β to Z conformational transition.

The polycations like the biological polyamines, putrescine, spermidine, although essential for cell proliferation, rarely incorporate into the macro-molecular framework of the coordination polymer and remain as distinct metabolic entities within the cell. They interact with the organic anions electrostatically, contributing to the tertiary structure and to the overall coordination polymer framework of the nucleic acids and membranes.

The theoretical interpretation of the approach takes account of biological reactions considering them as interactions between substrates and enzymes which are themselves coordination polymers and between receptor proteins and corresponding ligands. The strength of these interactions determines their activities and the amount of conformational changes they can introduce. Metal ions profoundly affect the conformations in coordination polymer formation. Divalent metal ions promote deprotonation of peptide groups through exchange reactions mentioned earlier. The deprotonation allows accessibility of hydrophobic moieties by forming deprotonated amide groups as in alkaline solutions with retention of the hydrogen bonded framework in the micro-environment. It thus provides an adequate degree of hydration to the system and makes the formation of inter and intramolecular hydrogen bond possible for stabilizing the conformation. The presence of a certain amount of ionized amino acid residues is perhaps the key to the stabilization of random conformations [71] and also to the access of metal ion or polymeric cation. General proteins contain five conformations viz, α-helix, anti-paralled β-sheet, parallel β-sheet, B-turns and random conformations in different proportions. Concentrated aqueous solutions of several salts effect denaturation of proteins and produce conformational changes[71] in the coordination polymers so formed. These observations are of far-reaching consequences since they concern reduction in the degree of hydration and deactivation of enzymes.

Another example of the fixation of metal ion by proteins in general and living systems in particular, taking place at the micro level, is provided by the interaction of methyl mercury cation CH_3Hg^+ which binds to the amino acids such as glycine and alanine and to the oligopeptides glutathione and glycylglycine. The thiol functional group has high selectivity towards the mercury ion and so is the case with the NH_3^+, NH_2 and COO^- groups of the peptides. Coordination polymer formation occurs through these functional groups located at the side chains of the reaction sites and the complex preciptitates [70, 72]. This would give rise to a disorder in the conformation which according to experimental observations is different from the respective α- helical and β-sheet structures of the polypeptides under similar sets of conditions. Poly (L-lysine) complex is known to adopt an α-helical structure at the pH and temperatures which normally give rise to another conformation for the polypeptide. Addition of glutathion to the coordination polymer poly (L-lysine)-CH_3Hg^+ complex has been found to cause a migration of the bound cations and a consequent restoration of the original state of the polypeptide. Coordination polymer formation by the methyl mercury cation may not occur with the same rate in living systems as compared with the highly ordered polymers like poly(L-lysine) and poly (L-glutamic acid) because of the diffusional barriers to the transport of the metal ions which would be different for different polymers and coordination polymers.

A chemical binding of the above type is proposed for the interaction of the anti-tumor drug cisdiamminedi-chloroplatinum (II) with the cellular component viz. DNA [73]. It is now well established that the drug-DNA bonding occurs at the d(GpG) site of the DNA. The major changes which have been noted in the structure of the coordination polymer due to bonding with platinum are a tilting of the G5 and G6. The 5'flank in B-DNA structure undergoes a severe loss in base stacking interactions. The interplanar angles between C_4 and G5 in these cases range from 65° to 89°. β-DNA structures are therefore destabilized on coordination polymer formation with the platinum ammine complex though

loss of stacking interactions. The hydrogen bonds in this coordination polymer are all disturbed. Additionally there is a persistent occurrence of amino-phosphate hydrogen bonds. Stability of the structure and shape of the complex at site are attributed to these hydrogen bonds. The unique feature of the [oligonucleotide - cis - $Pt(NH_3)_2]^{2+}$ complex is the hydrogen bonding between one of the ammines and one or two of the 5'phosphates. The restoration of hydrogen bonding is said to be responsible for the anticancer activity of the *cisdiamminedichloroplatinum* complexes which decreases along the series NH_3>NH_2R>NHR_2>NR_3. This study has convincingly demonstrated the important role of hydration and hydrogen bonding in metal- biopolymer interactions and of the coordination polymer- biopolymer interactions.

Oxygen and carbon monoxide interaction with haemoglobin produces complexes viz, oxyhaemoglobin and carbonomonoxyhaemoglobin. This provides yet another example of disturbance in the micro-environment whereby distortion is produced in the haeme moiety by the carbon monoxide molecule. This brings about large differences in the dynamic properties of the latter compared with the former complexes. The presence of the CO molecule liganded on the central atom introduces stresses on the haeme complex [74] to the extent that the CO oxygen atom in the carbonmonoxyhaemoglobin is displaced by approximately 1.3 Å from the haeme normal. The displacement is because of steric effect resulting from the interaction in the micro-environment between the liganded carbon monoxide and the haeme pocket. This leads to the bending of the Fe - CO unit away from the haeme normal and to the deformation of the porphyrin ring from maintaining the octahedral coordination structure for the central iron atom.

Conclusion

The postulates of the hypothesis holding dehydration to be one of the main mechanisms, suggest that if the degree of hydration at the micro-environment of a biological system is reduced, a disease situation would crop up. The proteinous walls and strands would be inadequately hydrogen bonded. The diffusion barrier properties of the membranes would be altered. The membranes would become dense and would be vulnerable to free radical attack (leading to cancerous growth). Enzymic activity would be considerably reduced and many enzymes would be deactivated. Stress metabolites in the form of hydrophobics would be generated and would deposit at constrained sites, depending on the degree of hydration and on the type of disturbance. A deficiency in the substrate of the enzyme would be created if dehydration is accompanied by a deficiency in concentration of a certain essential element.

The postulates appear to be universal in application to the plants, fungi, nematodes and animals. Thus the hypothesis is in a position to respond positively to the main characteristics of the aging process as put forward by Strehler [75].

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