"PROBLEM OF ORIGIN OF LIFE AND INDIA'S CONTRIBUTION"

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On 19-24 August, 1957, was held the first International Symposium on, "The Origin of Life on the Earth", organised by the International Union of Biochemistry at Moscow. Future historians of this subject would consider this symposium as the beginning of a new era when scientists finally began to admit that it is respectable to have experimental interest in various problems connected with the making of life or biopoesis, a word coined by N.W.Pirie.

The work on Origin of Life is of great interest academically. May be some aspects of this prove to be of commercial interest also. The wise men of all ages were speculating about this problem because in some way it is connected with the human understanding of his surroundings as well as himself and may be it proves benificial in establishing a code of conduct for human behaviour for peaceful existence of the whole humanity.

In all the religions there is some expression of the problem of origin of life. Some associate it with the functions of God. The understanding is that he who has created the life around us is the wisest, most powerful and just and

that we must make our Code of conduct in a way as not to displease him. The oldest record about origin of life is in Rigved and Atharved where it is stated that life originated in water by the interplay of the primary elements. The modern researches in this field point in this very direction.

We have been investigating the problem of origin of life since more than last two decades and have considerable success in solving some of the intricate aspects of the problem.

We made a start in this field in 1954 when we observed that if an aqueous mixture of paraformaldehyde, a source of fixed nitrogen and inorganic catalyst as ferric chloride is sterilised and exposed to light, a number of natural aminoacids are synthesised in the mixture (1,2). The effects of different organic sources (6), ammonium salts, inorganic (3) and organic (5) catalysts and pH of the mixture (4) on the photochemical formation of amino acids have been investigated (7.8).

In 1958 we observed that if sterilised aqueous mixtures of amino acids, a sugar as a source of energy material and inorganic catalysts, are expoed to sunlight, a number of peptides of low molecular weight are synthesised in the mixture (9). The effect of different catalysts and

nature of aminoacids used have been investigated (10,11). The effect of U.V. exposure on the formation of peptides has been studied by Dr. D.N. Perti (12).

It has been observed that in abiogenic sterilised aqueous mixtures containing paraformaldehyde as source of organic carbon, ammoniacal nitrogen as source of nitrogen and inorganic catalysts as colloidal ferric or molybdic oxides, are exposed to light some natural aminoacids are formed in the mixtures, which on further exposure ~~from~~ formed small peptide molecules. In 1958 we suggested an aqueous line of molecular evolution (13) stating that in pribiological era wherever there was water as in pools and oceans, a source of organic carbon and inorganic nitrogen and minerals of the soil to act as catalyst, sunlight helped in the formation of amino acids and peptides and these acted as building materials for the subsequent life synthesis.

In 1963 we succeeded in synthesizing such particles which grow from within, multiply by budding and have metabolic activity (14). This was achieved by exposing a source of organic carbon mostly paraformaldehyde, ammonium salts, biological minerals, some inorganic catalysts and phosphate in water to sunlight under sterilised conditions (15,16,17). These particles have boundary wall and internal structures. They have many biochemicals as amino acids, nucleic acid bases, sugars, organic acids and enzyme-like materials. The particles

could be stained with biological dyes. We named these particles as JEEWANU, a Sanskrit word for the Particles of Life(14).

The work on Jeewanu was soon confirmed by Dr. M.H.Briggs of England independently in his laboratories and he presented a paper of his observations in the 4th International Conference on Photobiology held in Aug. 1964 at Oxford. He further confirmed some more of our experiments on Jeewanu and published another confirmation of his work in Spaceflight journal in 1965 (18). In the conclusion of this paper he writes, "While the definition of 'Life' and 'Living' is a difficult problem, it can be said that these microscopic objects satisfy many of the criteria of living cells. It seems entirely probable that objects similar to those observed in the present experiments were formed in abundance in the Oceans of the primitive earth and were the immediate precursors of Cellular life."

In 1968 American Scientists Mueller and Rudin again confirmed our work. On page no. 237 of the Bioenergetics Vol. 3, edited by D.Sanedi in 1970, Paul Mueller and Donald O. Rudin in their article on "Translocators in Biomolecular Lipid Memberenes" write, "..... with the advent of Miller-Urey (Miller 1954) synthesis of amino acids by electric discharge in a Primordial atmosphere, it was soon found that more complex peptides were generated and that they could also be synthesised by other energy sources, such as thermal energy under anhydrous conditions (Fox, 1965), and

even more to the point, by sunlight (Bahadur 1966).....without doubt the most interesting of these synthesis, and also the most ignored in the literature has been the photochemical synthesis of membrane bound protein coacervates of Bahadur (1966) from simple salt solutions, citrate or paraformaldehyde, modern atmosphere and sunlight. These results have been confirmed in other laboratories (Briggs 1965, Mueller and Rudin 1968 C)."

Then on page no. 239 they write, "If the successful production of lipid in modified Bahadur system can be accomplished, then new surface catalytic effects having crude template properties could come into play as well as the other attributes of lipid membrane in conjunction with sets of peptide catalysts capable of synthesising each other under the lipid as well might provide a minimal self-duplicating and energetically self-sustaining system which could probably be designated as living.

In any case it seems probable that with achievement of control over a few more parameters along the lines discussed here we can look forward to the experimental production of living protocells by spontaneous generation."

The tests for phospholipids have been done in detail (19). It has been observed that if the particles prepared photochemically after separating them from their environmental medium are dried and then extracted with a phospholipid solvent as chloroform : methanol mixture and the solvent is

then evapourated a viscous yellow liquid is obtained which gives the tests of phospholipids (19).

It has been observed that if the mixtures are first mixed with a phospholipid solvent as chloroform - methanol mixture and exposed to sunlight while being continuously shaken in a mechanical shaker, no particle formation takes place, If a control mixture without phospholipid solvent is similarly kept continuous agitated in a mechanical shaker while exposing, the particle formation takes place in the mixture. This is because in the mixtures which contain phospholipid solvent as soon as phospholipids are formed, they get dissolved in the solvent and no phospholipid is available for the formation of the membrane and the particle formation does not take place.

We suggested that the formation of particles capable of growth, multiplication and metabolic activity is possible because matter has inherent properties of duplication under suitable conditions and that a system of matter in equilibrium has inherent properties of adaptability (14, 15, 20, 21, and 22).

As the particles described above were very much similar to thepresent day cells in chemical composition an attempt was made to prepare particles which may grow from within, multiply by budding and have metabolic activity yet which

are made of materials quite absent in the present-day cells. This was done in accordance with Prof. N.W.Pirie's observations who in the introduction of the book "Life, Its Nature, Origins and Distribution", by J. Marqu and published by Oliver and Boyd London (1968) wrote, "It seems certain that none of the higher forms of terristorial life could survive in the open on any other member of solar system. Some of our bacteria could survive on some planets. It is completely fallacious to argue from this that the only possible form of extraterrestrial life will be very simple. They may be complex but different. It may be useful to consider in advance the three most obvious possible results of close biological study of samples from a planet such as Mars. No organism may be found nor any evidence that these have ever been systems there that we would wish to call living. This will mean that the precondition for biological integration are more stringent than many of us have thought. Something similar to terristrial life may be found : not necessary the 'bug-eyed monsters' beloved of the cartoonists, with metabolic systems depending on proteins, nucleic acids and other familiar types of molecules. This will be the least significant result, for it could be taken as evidence either that these molecules have a unique merit and that life is dependent absolutely on their properties, or the terristrial and extraterrestrial life come form a common source.

The most interesting result would be the discovery of organisms making use of chemical processes not used on Earth. If they are used exclusively, they will be strong if not conclusive evidence of independent origins. If the use of the novel processes is extensive but not exclusive, it will not be possible to rule out a common origin followed by prolonged independent selection. This state of affairs will however contribute enormously to our understanding of the nature of life, that is to say of the fundamental quantities, if any, that distinguish vitality from every other life of integration."

A number of such particles which had material which is not present in to-day's cell were prepared by us. One such particles have 80 per cent cuprous oxide (23). We named these particles as Cuprous Oxide Jeewanu (24,25). These showed the properties of biological order. These particles could even be trained to use an entirely different source of organic carbon as their nutrition (23). Many such particles from cerrous sulphate (26), Cobalt and nickel salts (27) were also prepared, indicating that it is not the question of any particular sets of chemicals which could under some specific condition produce the Protocells, but that several different types of material, some quite different from the material found in to-days cell, could under suitable conditions from such objects which could grow from within,

multiply by budding and have metabolic activity (15, 20). With evolution only the present protein-nucleic acid life is left (15,20).

In general it can be said that life originated in water and sunlight played an important role in this process (16,22).

The earlier experiments of photochemical abiogenesis needed several months of sunlight exposure. In 1970 the procedure of the preparation of these micro-structures and abiogenesis of biochemicals was developed which cuts down the period of exposure to a few days (28).

The particles formed on exposure of these mixtures show boundary wall and internal structure under phase contrast microscope (29). These particles can be fixed with biological fixatives and stained with a number of biological stains for RNA, proteins, carbohydrates and phospholipids (30,31) and their permanent slides prepared.

Chemical tests for nucleic acid bases have been performed in detail and the mixture is found to contain adenine, guanine, cytosine and uracil and amongst these adenine is in largest amount. Thymine is formed only on prolonged exposure. The mixture contains ribose and desoxyribose and it appears that RNA is formed first and then DNA (32, 33, 34).

These mixtures indicate the formation of considerably

large quantity of aminoacids and an attempt is being made for determining the optimum condition for the formation of amino acids and their separation from the mixture.

These particles are sensitive to antibiotics and sulphur drugs but their sensitivity is different than that of common terrestrial microorganisms. Thus penicillin and streptomycin have no appreciable effect on their formation if present in the irradiated mixtures, chloromycetin acts as activator and tetracycline is a strong inhibitor. These particles are sensitive to sulphur drugs also. Here again the sensitivity is different from that of the terrestrial microorganism. Some sulphur drugs act as activaters, some have no effect and some are inhibitors.

The concept that matter has inherent properly of duplication under suitable condition and that a system of matter in equilibrium has inherent properly of adaptability which are actually expressions of quantum mechanical resonance special stability force and Le Chatelier's Principle respectively on rethinking appear as an expression of Vedant Philosophy as well.

Human beings are intelligent. Intelligence is perhaps ability to make the right judgement. This judgement is actually based on what we have learned and experienced. If our learning and experiences are broken down to a formula and these are

fed in a computer, it will give the same judgement as we do and that too much faster. That is our intelligence is automisation and is computer like in function. Our ego forbids us to call it automisation. If taking right judgement is considered as intelligence then our subconscious mind which controls the function of our body is also intelligent and so is a unicellular organism which is able to perform allthe functions of life. If we call this as intelligence then what about an atom with its most intricate internal arrangements. That can not be without intelligence though we will like to call it automisation,but we have seen that even our conscious intelligence is automisation which our ego makes us call as something different. If all this is considered as intelligence, the intelligence is cosmic.

Consciousness is said to be ability to respond to external stimule. But any system of matter in equilibrium when subjected to a constraint, if possible, a change occurs of a type that the constraint is partially annulled. So a system in equilibrium can as well be called as conscious. If all the system of matter in equilibrium is conscious the consciousness is also cosmic. This cosmic intelligence and consciousness is called "Satchitanand", the God.

If so then it is not a question of synthesising life or

living system. Under suitable condition, the matter shows its inherent properties in the form of such system which have properties in a new dimension which we call as the properties of biological order.

The work on Origin of Life thus has many important applications for the human being. We have yet to know the life in our neighbouring plants of our solar system or in the planets of other neighbouring suns. We must know more about our life, what is its actual nature, how did it originate, what are its limits and this knowledge is essential to enable us to recognise an alien life or living systems when it comes before us.

Our work on origin of life is well advanced. In 1965 Dr. S.W.Fox of U.S.A. claimed that his microspheres are able to grow, multiply by budding and have metabolic activity (37). In 1967 Prof.A.D.Oparin of U.S.S.R.claimed that he has been able to induce the properties of growth, multiplication and metabolic activity in his coacervates and that they are able to adopt and so evolve and thus they were immediate precursors of cellular life. But both in microspheres and the coacervates, one needs to add a particular chemical to observe on of these three properties of growth, multiplication and metabolic activity, then remove the added chemical and then introduce a second chemical to observe the other property. Thus they do not

~~than~~ have all the three properties in the same system which is essential before the object can be considered a-s living.

In Jeewanu, once the experiments are set up no introduction of additional compound is needed to effect any properties. The particles are formed in the mixture, grow from within to a maturity size, multiply by budding and have metabolic activity. So one can see the properties of growth, multiplication and metabolic activity taking place in the particles as in thecase of any microorganism. In this work we are ahead of these leading groups of scientists.

It is our feeling that if this line of work can be pursued with appropriate facilities it can bring laurels to our country. Such investigations are likely to reveal some facts of great scientific value, possibly also of commercial value, and at the same time it may lead to significant change in the philosophy of life. The work done so far indicates that living systems were synthesised because of some built in properties of the matter itself which forms systems capable of self-perpetuation and that perpetuation is the urge of nature.

It is proposed to establish a separate laboratory for research work in this and allied fields.

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The Story of Origin of Life

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Since the dawn of human civilization man is interested to know as how the lifeless matter became alive for the first time. It has been one of the most baffling problems of the scientists and philosophers of all the ages. Scientists now know that multicellular organisms evolved from the small unicellular life forms and their origin is dated some 2.5 to 3 billion years ago. Most of the scientists also agree with the idea that in all probability this conversion of lifeless material into living forms took place on this earth.

With the development of microscopes and perfection of the technique of sterilization the old concept of Spontaneous Generation was discarded and a new theory known as Molecular or Chemical Evolution was introduced to explain how the first livings might have formed for the first time on the then otherwise lifeless earth. Prof. Oparin of Russia and Prof. Calvin of the States are the chief exponents of this theory.

The theory of Molecular Evolution suggests that first a series of chemical transformations were taking place on the earth resulting in the formation of certain specific molecules. These were subsequently utilised for the formation of the earliest cells. The term evolution for these chemical transformation was used with the understanding that as more and more suitable molecules for the formation of the earliest living forms were being formed from less suitable molecules, some sort of evolution may be conceived in these processes.

However, the use of the term molecular evolution created some misunderstanding in the minds of scientists in particular and public in general. Evolution as we know in its true sense can only take place when there is a system which can duplicate and if this system is slightly modified by its environment or due to the inperfect mechanism of duplication resulting in slightly different system then this new system can also duplicate. Without a clear understanding of these ideas the use of the term evolution can cause confusion and this is actually the case here.

What the scientists were actually searching on the name of the origin of earliest living forms was the process of formation of such molecular associations which were able to duplicate and adapt and by introducing the term molecular evolution it is assumed that such systems of matter were already in existence even before the first living systems were actually formed for the first time.

The idea of Molecular Evolution had one good result and that is, scientists started working on abiogensis i.e., search of the conditions in which the compounds which are present in the cell under natural conditions are synthesised. This was an important progressive step in the investigation of the problem of origin of life because before the concept of molecular evolution was introduced ~~xxxxxxxx~~ scientists did not know from where to begin the investigation of the problem of life-synthesis.

During the last two decades considerable progress has been made in searching out the conditions which help the synthesis of compounds of biological interest in nature. Starting from a few organic and inorganic molecules and those sources of energies which were available in the prebiological era of the earth, scientists

succeeded in synthesising amino acids, peptides, nucleic acid bases and pentoses. The sources of energies used in these processes are solar radiations and sunlight, radioactivity, electric discharges and volcanic heat. Amongst these the former two must have been generally available through out the surface of the earth and the latter two were most probably of phenomenal type and were available only in certain zones and at certain time.

Bahadur synthesised amino acids and peptides in sterilised aqueous mixtures containing organic substances and inorganic catalysts by exposing the mixtures to sunlight. Miller emphasised the role of electric discharge and Fox laid stress on the volcanic heat for the synthesis of amino acids and amino acids and peptides respectively. Oro and Ponnamperuma made notable contributions in abiogenesis of nucleic acid bases and nucleotides.

Though abiogensis thus made considerable progress during last two decades the solution of the problem of Life synthesis was still far off and no body knew as how and under what conditions these molecules formed the first ~~Right~~ living system. This abiogenic approach itself had a few dead ends. All we know at present about the living forms is through the cells which themselves have undergone evolution for at least 2.5 to 3 billion years. During this long span of time there must have been changes not only in its cytological details but also in its chemical composition. The basic consideration in the abiogenic approach is that the earliest cells were made of the same materials which are present even in the present-day cell and this itself is not a very reasonable consideration and it is equally possible that the earliest cells might have been formed from entirely different compounds and the chemical constituents of

present-day cells were formed inside the cell itself during the course of its evolution.

Since last two decades great stress is also laid on the study of inter molecular forces and other factors which can contribute towards keeping a group of molecules held together in the form of a molecular association, for at least it is certain that life must have originated in some such molecular association. The reason for these molecular coming together in some specific pattern to show the properties of biological order, thus becomes the most important aspect of the problem of Origin of Life.

Oparin has been investigating coacervate particles since last three decades and he is of the opinion that life originated as coacervate particles. Coacervates are particles in between colloidal and precipitate stage. These are small aggregates of molecules of irregular shape having a partial electric charge. These particles thus retain their individuality for sometimes. One of the methods of preparing coacervate particles is to boil gum arabic and gelatine with a little water and subsequently pouring the mixture in excess of water. Oparin observed that under specific conditions coacervate particles show growth and multiplication. He is investigating the changes in the properties of the substances when made into coacervate particles or incorporated in other coacervate particles. Oparin is trying to induce the properties of biological order in these particles by incorporating different chemicals.

Fox of U.S.A. is working on a type of particles called microspheres which are produced by boiling thermal peptides in water for one minute and cooling the mixture. Small spherical particles separate out which have a boundary wall. These particles are brittle in nature swell in certain salt solutions and dissolve

out in others.

In May 1963 Bahadur synthesised particles called Jeewanu thereby meaning particles of life, under specific chemical environment. These particles are capable of growth, reproduction by budding and have metabolic activity. Jeewanu have been prepared from a variety of materials including peptides photochemically synthesised in aqueous mixtures and thermal peptides - ammonium molybdate complex and even with non-proteinous materials. These if prepared from peptides photochemically synthesised, have a number of natural amino acids present as peptides, have sugars as ribose, desoxy ribose, glucose and fructose and purines as adenine and guanine together with a number of organic acids. The body material of these Jeewanu have phosphatase and esterase activities. These particles have complicated internal structure and these can be stained with biological dyes. Moreover, the phenomena of growth, multiplication and metabolic activity are observed in the same environmental medium without any need of using any specific chemical to effect these functions and Jeewanu can be sub-cultured in specific media, like any micro-organism. Bahadur suggested that life originated as Jeewanu particles which on subsequent evolution produced the present cellular life.

Confirming the observations of Bahadur on his work on Jeewanu in 1965 an eminent English exobiologist, Dr. M.H.Briggs wrote in the July issue of the journal Space flight, that particles like Jeewanu were formed in large number in the oceans of the primitive earth when they got irradiated by sunlight and is entirely probable that Jeewanu were the immediate precursor of the cellular life.

In 1965 Fox also reported the phenomenon of growth and multiplication by budding in his microspheres in the report 05 of the Institute of Molecular Evolution of Miami University.

The forces and factors affecting the synthesis of molecular association with the properties of biological order also started becoming clear though a lot of work has still to be done in this direction. According to Schroedinger atom is the germ of solid. There are two ways in which atoms and molecules of a solid can be arranged. One is the periodic arrangement forming the solids known as crystalline material. The other is the unperiodic arrangement resulting in amorphous state. However there can be a third stage of arrangement of atoms in a solid in between these two states, which may be called as semiperiodic arrangement. If the atoms are arranged in this semiperiodic fashion, it will result in faulty or irregular structures which though tries to achieve the form of a crystal yet fails to do so. The earliest living systems were most probably formed as some such semiperiodic structure or defective irregular crystals from molecules which were of materials of viscous consistency and were loaded with large number of groups and associated molecules resulting in morphological looking objects. The guiding force for the formation of such structures would have been the effort of this group of molecules achieving a spacio-energetic pattern representing the state of minimum energy. In these structures several molecules were held together by various intermolecular forces and this complicated molecular mesh started showing the properties of biological order when certain conditions were satisfied. The necessary conditions have been discuss ed in detail by Bahadur.

This poses an interesting problem. Can every piece of m under the specific conditions show the properties of biological order or are life and living forms the monopoly of protein like materials only. Bahadur and Ranganayaki hold that matter has inherent properties of multiplication and adaptability and because of these properties of matter, living systems were synthesised. Le Chatelier's Principle and the quantum mechanical resonance special stability force are the expressions of these properties of adaptability and duplication of the matter respectively. According to them, if a system of matter in equilibrium is subjected to a constraint a change occurs if possible of a type that the constraint is partially annulled and the synthesis of a molecule under appropriate conditions resultant stable synthesised structures of those forms which are already in existence will be more than the other synthesisable probable structures at the same thermodynamic level and needing same amount of energy of activation.

This idea demands the synthesis of such molecular structures which should show the properties of biological order yet may be made of materials quite different from what is present in the present-day cell. One such molecular association known as cuprous oxide Jeewanu has been synthesised by Bahadur and co-workers. These particles have about 48 per cent copper, 4 per cent carbon and about 0.3 percent nitrogen. Other than these the particles contain biological minerals and molybdate. They have boundary wall and complicated internal structure and the material constituting these show catalase activity which is destroyed on heating. These particles are devoid of amino acids, peptides and proteins.

Recently morphological looking objects have been reported in carbonaceous chondrites. Urey and Nagy suggest that thes are forms of extra-terrestrial livings. Some consider these as mineral granules or terrestrial infection. The particles are called organised elements. The important point is that these have high concentration of iron, nickel and chlorine and are devoid of protein. These particles have morphological appearance.

In Oct. 1963 Russian scientist Kalendrkov reported the synthesi of cells which have morphological look and show the properties of growth and multiplication. However these two do not contain the usual constituents of the present-day cell. It has been a matter of general observation that many minerals of the earth show presence of morphological looking objects and these are considered as mineral granules and no importance is attached to them from the point of view of origin of life.

In the above back ground it appears that under certain specific conditions morphological looking objects or molecular associations result from a variety of minerals and organic substances. Under suitable conditions, which now can be conceived, life originated in some such molecular association. In these the inherent properties of matter viz-. duplication and adaptability operated and resulted in objects capable of growth, multiplication and metabolic activity. If the necessary conditions remained favourable for a long time and changed only slowly, these objects could under go evolution resulting in more and more complicated forms of life. The synthesis of such molecular associations have been achieved and these are the Jeewans.
