PEROXIDASE LIKE ACTIVITY IN ABIOGENIC PARTICLES

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Peroxidase has been found to be very closely related to catalase in structure and in function. Both are present in organism which employ cytochrome system. This system derives energy from the interaction of oxygen with the products of Krebs cycle. This oxygen is reduced to hydrogen peroxide which is toxic and is destroyed by catalase.

In order to make a system which is self sustaining it must be continuously provided with energy. The stepwise oxidation of organic substances in the living system provides energy.

In the abiogenesis of biochemical substances which perhaps went to form the first living system, the source of energy utilised were probably many and the question of the relative contributions of extraneous energy sources and internal chemical transformations is an open one.

It is generally agreed that organic materials originated prior to the first living system. Organic carbon is thermodynamically at a high energy level than is inorganic carbon. The photosynthetic production of organic carbon from inorganic and the stepwise oxidation of such organic compounds to produce energy illustrates the central part played by organic carbon as the source of energy for chemical transformations within cells. The further requirement of catalysts for such a stepwise series of energy liberations provides the rationale for an investigation of the catalase like activity of abiogenic materials.

Here in this paper an attempts has been made to determine the peroxidase-like activity in the particles which are formed abiogenically in the mixture containing minerals as calcium, magnesium, potassium, sodium, chloride and sulphate ions together with high concentration of diammonium hydrogen phosphate. The mixture had formaldehyde as the source of organic carbon and molybdenumoxide as catalyst. The particles are formed on exposure to sunlight . These particles have boundary wall and internal structure[1].$ The presence of nucleic acid bases has been reported in these particles.[2] It was therefore interesting to see whether these particles had peroxidase-like activity also.

Methods and Result

The peroxidase-like activity was determined by oxidation of ascorbic acid with hydrogen peroxide as is indicated by the decrease in the absorbance of the reaction of mixture at 264 um (2,3,4). The peroxidase-like activity was determined by the oxidation of ascorbic acid in the presence of hydrogen peroxide and residual ascorbic acid was titrated against standard iodine solution. Thus all the mixtures in which peroxidase-like activity was determined contained different amount of diammonium hydrogen phosphate and small amount of minerals as Ca^{++}, Mg^{++}, K^{+}, Na^{+}, Cl^{-} and $SO_4^{=}$, ammonium molybdate, water and formaldehyde. The particles of these mixtures have appreciable peroxidase-like activity and this was destroyed by strong heat. The peroxidase-like activity of these particles was compared with the catalytic activity of these particles was compared with the catalytic activity of molybdic acid. It is very interesting to note that molybdic acid does not possess peroxidase-like activity, but particles having high content of molybdenum and molybdic acid have been reported to act as peroxidase in the oxidation of iodine.

Mineral solution was prepared by dissolving 20 mg each of magnesium sulphate. Calcium acetate, sodium chloride and potassium sulphate in 100 ml of glass distilled water. After these salts dissolved 20 mg of potassium dihydrogen phosphate were added and dissolve by shaking. Ammonium molybdate $(NH_4)_6Mo_7O_4 . 4H_2O$ solution was prepared by dissolving 4.0 gm of the salt in 100 ml of glass distilled water. Diammonium hydrogen phosphate 6 gm, 7 gm, 8 gm and 9 gm. of salt was used in different sets of experiments. 36% of formaldehyde solution was used. Four mixtures each containing 10 ml of 4% (w/v) ammonium molybdate solution, 10 ml of the mineral solution and 10 ml of distilled water were prepared in 250 ml conical flasks. The flasks were numbered from 1 to 4. To the flasks 6,7,8 and 9 gms of diammonium hydrogen phosphate were added respectively. All the four flasks were cotton plugged and sterilised in an autoclave at 15 lb pressure for 30 minutes. After cooling 40 ml of formaldehyde was added asceptically in the last. The mixtures were gently shaken to mix the contents avoiding the contact of the solution with cotton plug. All the flasks were kept in sunlight till the formation of particles.

After few days the mixture showed the formation of a sediment and become blue in colour. After about seven days the exposed mixtures were examined for sterility by petri dish technique and was found to be sterile. The mixtures were full of spherical cell like microstructures. On microscopic examination, these particles were separated from environmental medium by centrifugation, washed several times with distilled water and dried in a vaccum desiccator. These dry particles were used in the experiments.

For the determination of peroxidase like activity following solutions were prepared.

Special phosphate-citrate-oxalate buffer suggested by Purr (1950) used for the determination of peroxidase-like activity, 15.65 gm of disodium hydrogen phosphate $Na_2HPO_4.2H_2O$: 7.70 gm of citric acid $1H_2O$ and 0.686 gm of oxalic acid $2H_2O$ were weighed and dissolved in glass distilled water and diluted to 250 ml pH of the buffer was measured and it was found to be 5.5. Ascorbic acid 1% was prepared in 1 vol. buffer and 9 vol. water and concentration was periodically checked by titration with standard iodine and starch. 2% of hydrogen peroxide was used. Iodine solution N/100 was prepared and standardised by standard sodium thiosulphate solution 0.058% of molybdic acid solution was made in glass distilled water. Bahadur and Ramgangyaki (1) showed that those particles contained 39.2% molybdenum on chemical analysis. Therefore for 0.1% of particles 0.058% of molybdic acid has to be used. 0.1% of particle suspension and 2N sulphuric acid were used.

To determine peroxidase-like activity 10 ml of ascorbic acid 20 ml of hydrogen peroxide and 40 ml buffer solution were taken and brought it to 25° in a thermostate and10 ml of particle suspension was added followed by water to a final volume of 100 ml. 5 ml. of this pipetted out rapidly into a second flasks containing 10 ml.

of 2N H_2SO_4. The residual ascorbic acid was enmarked from time to time by titrating it against N/100 iodine solution using starch as indicator. The spontaneous oxidation of ascorbic acid by hydrogen peroxide without adding particles suspension was also recorded simultaneously.

TABLE 1

Peroxidase-like activity in the particles of Mixture No. 1 in terms of ml of N/100 iodine solution

	0 min	5 min	10 min	15 min	20 min	25 min
Spontaneous (without particle suspension)	5.0	4.9	4.8	4.7	4.6	4.6
With particle suspension	3.5	1.5	1.3	1.0	0.7	0.7
With heated particle suspension	4.9	4.5	4.2	4.2	4.0	4.0
With molybdic acid alone	4.6	4.4	4.2	3.8	3.5	3.5
With heated molybdic acid	5.0	4.6	4.4	4.2	3.8	3.8

MIXTURE No. 2

	0 min	5 min	10 min	15 min	20 min	25 min
Spontaneous (without particle suspension)	5.0	4.9	4.8	4.7	4.6	4.6
With particle suspension	3.1	1.4	0.8	0.6	0.5	0.5
With heated particle suspension	4.3	4.2	4.2	4.0	4.0	4.0
With molybdic acid alone	4.1	4.0	3.7	3.5	3.4	3.4
With heated molybdic acid	4.4	4.2	4.0	3.8	3.8	3.6

TABLE 3

Peroxidase-like activity in particles of Mixture No. 3 in terms of nl of N/100 iodine solution..

	0 min	5 min	10 min	15 min	20 min	25 min
Spontaneous (without particle suspension)	5.0	4.9	4.8	4.7	4.6	4.6
With particles suspension	3.6	1.8	0.7	0.5	0.4	0.4
With heated particle suspension	4.4	4.1	3.8	3.8	3.8	3.8
With molybdic acid alone	4.6	4.0	3.7	3.5	3.4	3.4
With heated molybdic acid	4.5	4.2	4.0	3.8	3.7	3.6

MIXTURE No. 4

	0 min	5 min	10 min	15 min	20 min	25 min
Spontaneous (without particle suspension)	5.0	4.9	4.8	4.7	4.6	4.6
With particle suspension	3.3	1.8	1.4	1.0	0.6	0.6
With heated particle suspension	4.2	4.0	3.8	3.8	3.8	3.8
With molybdic acid alone	4.5	4.1	4.0	3.8	3.6	3.6
With heated molybdic acid	4.5	4.3	4.1	4.0	3.8	3.6

DISCUSSION

It has been observed that a sterilised mixture containing 6 gm, 7 gm, 8 gm and 9 gm diammonium hydrogen phosphate, each having 1 volume of 4% W/V ammonium molybdate, 1 volume of a specific mineral solution and 4 volume of 36% formaldehyde, when exposed to sunlight, cell like microstructure are formed; these particles increases in number with the increasing time. No particle formation takes place when diammonium hydrogen phosphate is not added. These particles after seven days are separated from the environmental medium by centifugation, washed and dry them in vacuum desicator. 0.1% of these particles are used in the experiments to determine the peroxidase-like activity by the oxidation of ascorbic acid in presence of hydrogen peroxide, the residual ascorbic acid has been titrated against standard iodine. It has been found that the particles of all the four sets shows appreciable amount of peroxidase-like activity, which is destroyed on strong heating.

Experiments with equivalent amount of molybdic acid as may be present in the particles showed that the peroxidase-like activity of this amount of molybdic acid under similar conditions is far less and this catalytic activity of molybdic acid is not destroyed by heating. The peroxidase-like activity of the particles is far greater and is mostly destroyed on heating.

The presence of peroxidase-like activity in the particles described here suggests a mechanism of oxidation of the organic substances present in the environmental medium by the oxygen enriched compounds present in the environment. Some such process might have provided the energy needed for the various chemical transformations in the earlier self-sustaining systems.

SUMMARY

Peroxidase-like activity was observed in the particles which was prepared abiogenically. This activity on strong heating was found to be destroyed. The equivalent amount of molybdic acid under similar conditions of experiments showed some peroxidase-like activity, which was far less as compared to that of particles and was not destroyed on strong heating.

The presence of peroxidase-like activity in the particles described here suggests a mechanism of the liberation of energy by the oxidation of organic material with the environmental oxygen-rich peroxides for the primordial abiogenic molecular evolution and biochemical transformations in the probable precursors of celluer life.

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STUDY OF THE RESPONSE TO ANTIBIOTICS IN JEEWANU, THE SELF-SUSTAINING COACERVATES, CAPABLE OF GROWTH, MULTIPLICATION AND METABOLIC ACTIVITY.

(Bahadur K., Ranganayaki, S., Singh, Y.P. and Kumar, S.,
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~~(Eingegangen am 20.9.1974)~~

Jeewanu, the self-sustaining coacervates (Bahadur and Ranganayaki 1970) ~~(1)~~ having amino acids (Bahadur, Verma and Singh 1974) ~~(1,2)~~, peptides, sugars, enzyme-like materials, nucleic acid bases (Ranganayaki, Bahadur, Raina 1972) ~~(1,3)~~ and phospholipids (Singh 1974) ~~(4)~~ have been prepared by the action of light on aqueous mixture containing ammonium molybdate, diammonium hydrogen phosphate, biological minerals and formaldehyde ~~(1)~~. These particles have distinct boundary walls and internal structures and after fixing them with chromic acid they can be stained with gentian Violet, eosine and sudan blue B (Bahadur & Gupta 1972). ~~when different locale acquire distinct stain (5)~~. As these particles have a composition similar to that of the present day cell, the effect of antibiotics on their formation was studied.

~~Antibiotics have been known to stop the growth of the microorganisms or Kill them if present in larger concentration and certain organisms are not affected by some antibiotics. A few such microorganisms are also known which may use some antibiotic as their source of organic carbon and they may be even dependent on some particular antibiotics for this but there is no bacteria known for which antibiotics may act as activator.~~

~~When antibiotics are given orally to farm animals in concentration between 5 to 20 parts pm increase in the growth of~~

~~the animals has been recorded. Penicillin has been found to promote growth in chicks (6,7,8,9) and pigs (10,11,12). Tyrothricin, gramicidin and neomycin also show growth response (13). Bacitracin promotes growth in pigs (14). This increase in growth is not due to the change in the pattern of their intestinal microflora (15,16,17,18) and the changes caused by feeding antibiotics in small concentration shows changes even in the metabolic processes of the animal. It shows increase in fat (19,20,21) and plasma phospholipids (22).~~

Antibiotics show interesting effects on the formation of Jeewanu, tetracycline shows inhibitory action on the formation of jeewanu, griseofulvin, and streptomycin act as activator in dilute concentration but act as inhibitor at high concentration. Chloramphenicol acts as activator and erythromycet-in acts as activator at low concentration but changes jeewanu into a different type of a very small microstructures.

Experimental

4% $\left(\frac{W}{V}\right)$ ammonium molybdate solution and 3% $\left(\frac{W}{V}\right)$ diammonium hydrogen phosphate solution were prepared in distilled water. Mineral solution was prepared by dissolving 20 mg of each of sodium chloride, potassium sulphate, calcium acetate, magnesium sulphate and potassium dihydrogen phosphate in 100 ml of distilled water. 36% formaldehyde was used in the experiment. The antibiotic solution was prepared by dissolving 100 mg of the antibiotic in 10 ml of distilled water. The mixture is heated to dissolve as much of the antibiotic as possible and filtered. The filtrate is used as antibiotic solution.

1 ml of the ammonium molybdate solution, 2 ml of the diammonium hydrogen phosphate solution and 1 ml of the mineral solution were taken in each test-tube. The test-tubes were cotton plugged and sterilised at 10 lb pressure for 15 min. After cooling the test-tubes were divided in sets of two each. One set of two test-tubes was kept as control adding in one 0.5 ml and in the other 1 ml of distilled water. To another set of two test-tubes in one 0.5 ml and in the other 1.0 ml of the penicillin solution was added. Similarly in other set of two test tube 0.5 ml and 1.0 ml of tetracycline were added. And like this sets of chloramphenicol, griseofulvin, erythromycin and streptomycin were prepared in which one test-tube had 0.5 ml and another 1.0 ml of the antibiotic solutions.

One ml of 36% formaldehyde was added ~~in~~ to the mixture of each test-tube aseptically, the test-tubes cotton plugged, mixture shaken gently and exposed to bright sun light for 4 hours. After the exposure the mixture is allowed to stand over night in the laboratory. Next day the mixtures are shaken gently and a few drops of the mixture were taken out aseptically and examined under a microscope in oil-immersion at 1500 magnification.

~~Observation :~~ Results (Table 1)

~~Control :~~ In the control experiment without antibiotica ~~B~~blue spherical particles are observed. A few of these particles show budding. The particles are from 1 to 3 µ in diameter and there is not much difference between the slides of the mixtures containing 0.5 to 1.0 ml of distilled water.

Table No. 1. Effect of antibiotics on the formation of Jeewanu

Antibiotic used	ml of antibiotic solution added ~~in~~ to the mixture	
	0.5 ml	1.0 ml
Penicillin	Same particles as in the control in number or size	No difference
Tetracycline	Fewer particles than in the control	Number of the particles still futher decreases
	Size of the particles the same as in the control	
Chloramphenicol	The number of particles is larger than in control. Tendency to form hexagonal crystalline structures is observed and some particles are spherical as in the control ~~mixture~~ and ~~are~~ about 2 to 3 µ in diameter. A number of clusters of light blue to colourless spherical particles are also observed and many of these show budding.	The number of particles is still higher. A large number of clusters of several particles from light blue to colourless shades are observed showing budding. The diameter of the particles range from 0.5 to 1 µ. Lesser tendency of crystallisation. No hexagonal structure observed.

~~Contd.~~..

Erythromycin	The number of particles increases considerably. A few crystalline blue, rectangular structures observed. The number of particles is about four times of the control. The diameter of the particles range from 0.25 to 1.5 μ and clusters of light blue and colourless particles showing budding are observed.	Only a very few blue spherical particles of about 1.5 μ diameter are observed and quite a few of these show characteristic motion of granules within them. The whole view is full of a new type of very small particles of about 0.1 μ in diameter which move very fast and appear like dot shape bacteria. These small structure appear coming out from the big typical particles.
Griseofulvin	Particle formed are spherical and large in number. A number of clusters of light blue and colourless particles showing budding are observed and their size ranges from 0.5 to 2 μ. A few dark blue coloured crystalline structures are also seen.	The number of particle is considerably less, there being only about one tenth in the mixture in which 0.5 ml of griseofulvin solution is added. A few blue particles of the diameter from 0.5 to 2 μ are also observed.
Streptomycin	A large number of colourless spherical particles are observed. A few dark blue particles of about 0.5 to 2.0 μ diameter are also seen. The number of particles is about 10 times of the control and clusters of colourless particles are more predominant. There is a tendency of dissolution of the wall joining the particles and this results in irregular structures.	A large number of white spherical particles are observed in which the joining wall has dissolved. The number of the particles is one-fourth compared to ~~of that of~~ the mixture ~~which had~~ with 0.5 ml of the streptomycin solution. A very few typical spherical blue particles are observed.

Discussion

Penicillin does not affects the formation of jeewanu. ~~in mixture~~ With 0.5 ~~.5~~ to 10 mg of chloramphenicol per ml ~~of the mixture helps in the formation of~~ a larger number of microstructures. is formed, and ~~in~~ Big clusters of the particles showing budding are observed; ~~and~~ most of them are either light blue or colourless. Tetracycline ~~when present in the mixture~~ decreases the formation of ~~a~~ the jeewanu ~~in mixture~~ and decrease of ~~in~~ the number of ~~the~~ particles ~~in the mixture~~ is proportional to the concentration ~~quantity~~ of tetracycline ~~in it~~. With 1 mg/ml ~~When the concentration~~ of griseofulvin or streptomycin ~~in the mixture is 1 mg/ml~~, the formation of jeewanu ~~in the mixtures~~ is considerably increased but on doubling the concentration of the antibiotics the formation of the particles is significantly decreased.

Erythromycin ~~when present~~ (1 mg/1 ml) ~~in the mixture~~ acts as ~~the~~ activator for the formation of jeewanu ~~but on~~ On doubling the ~~this~~ concentration of the antibiotic jeewanu change qualitatively and a new type of microstructures of the range between 0.1 to .2 µ in diameter come out of the jeewanu ~~and in a few jeewanu,~~ This process ~~taking place~~ can be observed under the microscope. The fine granules show rapid motion.

Summary (Abstract)

An aqueous mixture containing ammonium molybdate, diammonium hydrogen phosphate, ~~biological~~ biological minerals ~~and~~ ~~compounds~~ and ~~Na acetate and~~ formaldehyde on exposure to light shows the formation of

cell-like microstructures ~~calle~~d (Jeewanu) which are self-sustaining coacervates. Penicillin has no effect on their formation. Chloramphenicol acts as an activator for the formation of these particles and increases their growth and multiplication. Griseofulvin, and streptomycin ~~when present in the mixture~~ in smaller quantity activate s/ the formation of these particles but ~~when~~ in larger quantity of these antibiotics ~~present in the mixture~~ the formation of the particles is hindered.

Erythromycin enhances the formation of jeewanu when present in smaller quantity ~~in the mixture~~ but a large quantity of erythromycin in the mixture destroys the jeewanu formed and the mixture is full of a large number of dot-like colourless particles showing great movability.

Tetracyclin inhibits the formation of the particles and this inhibition is proportional to the amount of tetracycline in the irradiated mixture.

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PHOTOCHEMICAL FORMATION OF SELF SUSTAINING COACERVATES*

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~~How lifeless matter acquired the properties of biological order has been of interest to humanity since the dawn of human civilization. In olden days when science was in elementary form, many were the misconceptions associated with this and not a few scientists believed that living forms, e.g., insects, mice, duck etc., sprang up from decaying organic matter. The idea of spontaneous generation of life held the field till the end of the last century. After the discovery of microscope and technic of sterilisation the old concept of organic materials getting converted into well organised living forms was discarded. Seeing the apparent improbability of life synthesis under natural conditions of the Earth many scientists as Arrhenius (1) and Ch. Lipman (2) suggested the possibility of our life coming on the Earth from other planets as infection. This approach, even if true, does not explain how lifeless matter got converted into living systems for the first time. It simply shifts the zone of life synthesis from our Earth to some other far distant planet.~~

~~The expressions of Mitcherlish, Engel, Huxley and others led to~~ The present approach to the problem of origin of life ~~finally taking definite shape~~ started by the postulate of Haldane & Oparin commonly known as Molecular or Chemical Evolution theory ~~(3,4)~~ (Haldane 1929, Oparin 1938). According to them first molecules constituting the earliest cells were synthesised under natural conditions by a slow process of molecular evolution and these molecules then organised into the first molecular system with properties of biological order.

~~Here~~ The term molecular evolution has been used in a restricted sense. It only means the ultimate formation of molecules which formed the earliest cells by chemical transformation which were taking place on earth ~~(5)~~ (Bahadur 1969). The term evolution has not been used in the strict biological sense where it is believed that evolution can follow only when we have a replicating system which even on modification by natural reasons can duplicate in this modified form. ~~Actually the main~~ The purpose of the investigation on origin of life is ~~just~~ to search the natural conditions which formed such replicating self-sustaining systems to begin with ~~(6,7)~~. (Blum 1961, Bahadur and Ranganayaki 1966)

The second reservation in the theory of molecular evolution is that it is taken for granted that earliest cells were made of the same materials which are found in the present day cells. It is a big assumption for it is equally probable that the chemicals which are present in today's cell might not have been there to begin with and were formed as the product of some evolutionary metabolism operating in the cells ~~(8)~~ (Bahadur 1967). At least so far as the size of molecules are concerned it may be quite probable. ~~Today's cell has high molecular weight substances, as proteins and nucleic acids in them, as their,~~

~~*Text of the lecture delivered at Photobiology Symposium on Photochemical and Photophysical Processes in Biological Systems sponsored by University Grant Commission and Indian National Science Academy and organised by Jadavpur University, Jawaharlal Nehru University, Indian Agricultural Research Institute and Indian Photobiology Group at Indian Agricultural Research Institute, New Delhi on 27th Jan. 1972.~~

vital components and it is quite possible there were other molecules in theearliest cells of smaller molecular weights yet in some way performing the functions which are being carried out by these high polymers of today's cells (8).

~~As we know only one form of life on our earth and that~~ is ~~protein life,~~ ~~it was considered that~~ it is quite probable that their precursors were made of at least amino acids and abiogenesis of natural amino acids in nature was greatly sought. With the same process of logic the abiogensis of almost all the chemicals present in the present day cells was searched out and this yielded some very encouraging results during the last two decades.

Loeb was the first to use electricity for the abiogenesis of amino acids (9). He observed that by passing silent electric discharge in a solution of formamide in water a number of amino acids are synthesised in the mixture. In 1953 electricity was again used for the synthesis of amino acids and this time electric discharges were passed in a mixture of methane, NH_3, H_2 and H_2O (10). Photo-chemical formation of amino acids in sterilised mixtures containing formaldehyde, ammoniacal or nitric nitrogen and iron or molybdenum as inorganic catalysts has been observed by Bahadur in 1954 (11,12). Pavlovskaya and Pasynskii ~~(13)~~ obtained amino acids by exposing similar mixtures to Ultra violet rays. Many other sources of energy have been used for amino acid synthesis ~~(14,15, 16,17,18,19,20,21,22)~~.

Bahadur 1954, Bahadur and Ranganayaki 1955, Bahad et al. 1958

9,10,

→ (Pavlovskaya & Pasynskii, 1957)

The formation of peptide bonds under natural conditions was another important aspect of abiogenesis. ~~Fox (23) synthesised high molecular weight peptides by heating a mixture of amino acids in the absence of air at 160°C and subsequently dialysing the product. The temperature of 160°C was not attained on the surface of the earth after the appearance of water on the earth and may be available in small packets near volcanoes.~~

In 1957 Bahadur observed that peptides of low mol. wt. are formed in sterilised aqueous mixture containing amino acids, sugar as energy source and iron or molybdenum as inorganic catalysts on exposure to sun light ~~(24,25,26,27,28)~~. Formation of peptides in aqueous medium has also been studied by Calvin and Steinman using dicyanamide as condensing agent ~~(29, 30, 31)~~. (Steinman and Lemon 1964, 1965, Steinman and Kenyon 1965)

(Bahadur, 1958, 1961, 1961, 1962, 1965)

Abiogenesis of other compounds of biological interest has been worked out and conditions leading to the formation of nucleic acid bases and sugars were discovered. Several detailed reviews of ~~of this~~ abiogenesis are now available ~~(32,33)~~. (Steinman 1969, Oparin 1969)

The important point in the origin of life is to investigate as how these molecules formed the earliest cells. If such systems are synthesised using the chemicals, whose abiogenesis is sought, only then the work on abiogenesis becomes pertinent in solving the problem of life synthesis.

~~It is because of the apparent improbability of solving this aspect that most of the scientists lose their interest in biopoesis, and though many scientists have taken part-time interest in the field of origin of life during the last two decades there are hardly a few in the whole world who are investigating the problem of origin of life as their whole time work.~~

~~The problem of origin of life needs specific knowledge of many disciplines of science, some of which arequite far off from biology and not being able to see through all the problems involved many~~

~~scientists become reluctant in pursuing the investigations on life synthesis. Pasteur's work that life can be synthesised with living things only and some religious beliefs that formation of living from non-living materials is the function of God, contributed a good deal in creating such a back ground in the mind of the people that the problem of origin of life cannot be solved.~~

~~Let us take the case of a normal biologist. He thinks that talking about the origin of life is talking about the natural synthesis of protoplasm or cells and with time as the knowledge of these 19 increasing he is getting certain tha t complex material like protoplasm and extremely intricate material like cell cannot be synthesised under natural conditions and so he almost ceases to take active interest in the problem and his apparent interest in the problem of origin of life is more to justify his being a biologist than a serious interest to unveil this mystery. He is correct so far he thinks that protoplasm or cell cannot be synthesised under natural conditions and the problem of origin of life has not much to do with the synthesis of the present protoplasm or the cell. It only deals with the study and probable factors which lead to the synthesis of those precursors which on evolution of about 3 billions of years, when in all probability they were synthesised for the first time, resulted in the present cellular life and the protoplasm contained therein.~~

~~A biologist extends the evolution backward and finds it easy to explain the origin of complex forms of living systems from earlier simple structures but when it comes to the problem of life synthesis he expects the synthesis of a cell which is an evolutionary product of 3 billion years. To begin with not the present cells, but their precursors would have been synthesised.~~

Backward exploitation of the diversity of the phylology in abiogenic time suggests that to begin with there must have been few species ~~(34)~~ (Pirie 1954). The work of Dillon ~~(35)~~ (Dillon 1962) shows that the problem of origin of organelles of a cell is not the problem of origin of life but that of evolutionary cytology and the earliest cells must have had very simple internal structures. Lwoff's ~~(36)~~ (Lwoff 1943) work on the loss or gain of the properties of individual cells during evolution with time suggests that the evolution is loss and not gain in the properties of the individual cell. ~~With evolution the cell becomes more sophisticated and different tissues become capable of performing some specific function more efficiently, but individually the cell loses its properties. Thus though man is far ahead in evolution than unicellular organism, no cell of his body has that many properties which an unicellular organism has.~~

Putting the work of Dillon and Lwoff together it appears that to begin with there were fewer species and the earliest cellular living systems were very simple in structure and were full of properties of biological order ~~(37)~~ (Bahadur 1964) and these were the precursors of cellular life. The study of origin of life is the investigation of the natural formation of these precursors of cellular life. ~~Only these on evolution resulted in the present form of cellular life and the protoplasm contained there in.~~

~~The main problem of life synthesis is investigation of how much a system was synthesised. This system certainly had one property and that is adaptability, otherwise no matter what properties this system would have it could not have been able to adapt and even if they would have got synthesised in the nature we would have only those earliest synthesised forms on our earth and not higher forms of life.~~

According to Bahadur matter has inherent property of duplication under suitable condition and a system of matter in equilibrium has inherent property of adaptibility (~~38, 5, 39~~) and Le Chatelier's Principle and quantum mechanical resonance interaction special satability force are expression of these properties (~~40~~, 41, 42, ~~43~~) hhh

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~~If a mild constraint is caused on a system and if there is a change in the system due to this constraint of a type that the constraint is partially annulled, then it is said that the system is capable of adaptability. This property of a change taking place in a system, if possible, in response to a constraint caused on the system is the inherent property of a system of matter in equilibrium according to Le Chatelier's Principle (38, 37, 5)~~ which holds that if a system of matter in equilibrium is subjected to a constraint if possible, a change occurs within the system of a type that the constraint is partially annulled.

~~Duplication is an important aspect of the study of origin of life. In the living forms there are two types of duplications. One is on molecular level where the same molecules are getting formed more and more and another is at macro level when the organism duplicates as a whole and it is known as reproduction.~~ The nucleic acid assisted duplication of protein molecules is common in all the livings of the present day. ~~The other type of duplication i.e., the one without the help of nucleic acid does not exist in the living forms of the present day and one some times wonders whether there ever was any other type of duplication in the living forms. However the present forms of living are made of protein molecules and their duplication is assisted by nucleic acid, another molecule of very high molecular weight. The important point is,~~ Did living forms originate initially as a protein-nucleic acid system or were they made of some simple molecules of small molecular weight and the living forms of high molecular weight molecules are the evolutionery product of the earlier forms. It appears more probable that to begin with living forms were made of small molecules and the nucleic acid-protein made living forms resulted as the evolutionery product afterwards ~~(37, 39)~~. If so, the smaller molecules can duplicate by the quantum mechanical resonance interaction special stability force considerations only ~~(40,41,42,43)~~ (Jordan 1938 1939 1939 1940). The molecules of such living forms could duplicate by themselves without needing the help of nucleic acid. If any mild change in the duplicating molecule was affected by the physico-chemical conditions of the environment, the slightly modified form could also duplicate and this system if in equilibrium with its environment could adapt and such a system would have been capable of duplication.

hhh (Bahadur and Ranganayaki (1964), Bahadur (1967), Bahadur 1964)
(Bernal 1961, Bahadur 1964, Bahadur et al. 1966)

~~Many scientists are of the opinion that virus is a living molecule and synthesis of virus will solve the problem of origin of life. The virus is a molecule which duplicates in living environment. So only the cell plus virus can be called as a living system but the cell is living even without virus. So the living system is one which is capable of performing all the formations of living, has a system of energy release and is a group of molecules held together and performing functional properties which we call as the properties of a living system and not a single molecule of some organic systems.~~

One of the difficult aspects of origin of life is the question of defining life or living forms. Though it appears to be easy to differentiate a living from non-living form, it is very difficult if one wants to define it in scientific language. An attempt of this was made in one of the meetings of the International Symposium on the problem of origin of life on the Earth held at Moscow in Aug. 1957 with Pauling as the chairman. Haldane ~~(44)~~ (1954) defined living system as a self perpetuating system. According to Bernal ~~(45)~~ (1959) embodiment of the self perpetuating system with-in a boundary was the occasion of origin of life. This functional property of self perpetuation was not found satisfactory by genetists. Horowitz (1959) defined living system as a monomolecular system in polymolecular

~~Haldane (1954)~~

environment, capable of multiplication and hetero-catalysis ~~(46)~~. ~~The definition of Horowitg was not agreeable. According to~~ Konikova, ~~the Russian genetist, this definition can be made correct if we introduce term "living" after the polymolecular environment in the above definition but in so doing this certainly cannot be the definition of living because it itself has a word living in it.~~ Konikova (1957). suggested that a living system is a molecule or a complex which by the process of chemical reactions with the molecules of its environment accomplishes growth and multiplication. It remains to itself while yet changing not in the direction of death or decay ~~(47)~~. Pirie (1957) ~~(48)~~ however did not agree to the limitation of growth and multiplication for the definition of ~~origin of life, for he~~ living system. ~~suggested that nerve cells do not multiply and mules do not reproduce though both of them are established living systems on other considerations.~~

In 1967 the Physicist J.D. Bernal gave a definition of life in electronic language. According to him Life is a continuous, partial, multiform and conditionally inter active, self realisation of the potentialities of the atomic electron state.

It thus appears that it is very difficult to draw a line of demarkation between livings and non-livings and defining life is a hopeless job. However it became necessary to at least enumerate a few properties which a system must have before it can be included in the category of living system. Bahadur made an attempt in this direction in 1963. According to him if a system is capable of growth, multiplication and metabolic activity the system can be included in the category of living, where growth stands for the increase in the size of the system from within by actual synthesis of the material with which the system is made, inside the system, multiplication means the system increases in number and the newer units come in existence through the parent ones and metabolic activity denotes any series of chemical reactions taking place within a boundary the result of which is that at least a part of the environmental molecules entering the system are converted into the material with which the system is made and these chemical transformations provide the energy necessary for the various energy requirements of the system for its performing the above functions (Bahadur & Ranganayaki 1964, Bahadur 1967) ~~(38)~~. ~~It will be possible to get living systems with fewer properties than these and they are established living systems due to other considerations but if a system has all the above three properties of growth, multiplication and metabolic activity it may be considered as living. Inanimate objects having one or two of these properties can certainly be found but if a system has all these three properties it certainly can be taken as living. This enumeration of the properties as above is acceptable to many of the exobiologists.~~

~~In all these various discussions on the definition of life or living system it is interesting to note that none has laid any stress on the chemical nature of the living systems. Thus though genetists believe that the earliest living molecule was most probably virus even they did not define life as a system made of nucleic acid and protein molecules though there is no known living system which is not made of these chemicals as the chief components.~~

About the chemical nature of the living system

~~According to~~ Pirie (1977) ~~(49)~~ holds that proteins are necessary for the present day cell because they are enzymes and catalyse many biochemical reactions. But many metallic ions also show enzyme like activity and it is possible that to begin with these might have been performing the work of protein enzymes. Bernal (1957) ~~(45)~~ is of the opinion that there might have been organisms which had only inorganic catalysts in the place of proteins and such organisms might have been sluggish but could have certainly performed all the functions of life. According to Smirnova (1957) ~~(50)~~ many inorganic substances have physical properties commonly found in organic substances and it is quite possible to conceive organisms made of these inorganic substances only. Bernal ~~(8)~~ (1961) writes "unless it is desired to push back the doctrine of special creation to the creation of enzymes and co-enzymes (there is a school that would take one of these, namely the coenzymes in the polymerised form as nucleic acid) as the beginning of life, unless then we are prepared to take such an easy way out, we must assume that before there were enzymes to carry out the catalytic reactions in metabolism there were some other agents that did it, not so well, but sufficiently well for the slow time of the origin of life". These evolved to produce protein - nucleic acid cellular life which we now observe on the earth. Bahadur has suggested a probable locale for these forms of life ~~(37,38).~~ (Bahadur & Ranganayaki 1964, Bahadur, 1964, 1967 and Bahadur et. al. 1966)

~~Cell Models~~ :- One of the important aspect of the problem of origin of life is to study the physico-chemical factors which brought the molecules which formed the earliest cell together, kept them held together and arranged them in some specific pattern that could show the properties of biological order.

Oparin is of the opinion that life originated as coacervate particles. Formation of coacervate helps in the concentration of the material which might have been significant in life synthesis. ~~Oparin has studied the change of the properties of the enzymes when incorporated in coacervate particles. These particles are commonly synthesised by the biogenic materials as gum arabic and gelatine but many abiogenic material can also form coacervate particles and the formation of coacervate particles could have been useful in concentration of materials.~~ Complete account of the work on coacervates are availeble (33, 51, 52, 53).

Fox had been studying another type of particles known as microspheres prepared by boiling his thermal peptides in water for one minute and then cooling the mixture. Large number of spherical particles are formed in the mixture. These appear to have a boundary wall. ~~However these are short lived and coalesce after a day or so. These are made of solid, brittle material and they break radially on pressing. Fox has been studying the effect of different chemicals on these particles by incorporating them in the particles or putting them in theenvironment.~~ His efforts are to introduce the properties of growth, multiplication and metabolic activities by using suitable chemicals (54,55).

~~Bahadur et. al. 1963~~, Bahadur & Ranganayaki 1964, Bahadur 1964, ~~Bahadur 1963~~, 1967, Bahadur et. al 1964, [illegible])

In 1963 Bahadur synthesised a type of particles photochemically which he named as Jeewanu ~~(38, 56, 57)~~. In the sterilised aqueous mixture containing organic carbon, inorganic nitrogen and minerals commonly found in cells small spherical particles are formed which have definite boundary wall and intricate internal structures (Micrograph 1) ~~(5,37,58,59)~~. These particles are very similar to the present day cell in chemical composition and differ from the common micro-organism that they cannot be grown on any known bacterial culture medium (Bahadur. 1967, 1964, 1966, Bahadur and Ranganayaki 1970) ~~Bahadur 1966~~. These particles multiply by budding and the small buds ~~(Microscope~~ (Micrograph 2)

(~~Micrograph~~ 3 ~~and~~)

grown to maturity size and bud/. Bahadur emphasised that in water where organic materials and necessary inorganic substances were present sun light synthesised amino acids, peptides, sugars, and such other biochemicals and these organised in the form of microstructures and formed Jeewanu. These Jeewanu were capable of adaptability and so these evolved into the present day cellular life (~~60,5~~). (Bahadur et.al. 1963, Bahadur 1967) The work on Jeewanu was soon repeated by Briggs and his confirmations of this work was read in the 4th International Symposium on Photobiology held at Oxford in 1964. He further repeated some experiments of Bahadur and published another confirmation in space flight in 1965 ~~(61)~~. In 1979 the work on Jeewanu was further confirmed by Rudin and Muller which appeared in Current Topics in Bioenergetics ~~(62)~~. A complete review of the work on Jeewanu has appeared ~~(5, 39)~~. (Bahadur 1966, 1967)

~~In 1965 Fox claimed the properties of growth, multiplication and metabolic activity in his microspheres and in 1967 Oparin claimed these properties to be present in the coacervate particles. However in these particles specific chemicalsare needed to show each one of these properties and it is only after one such chemicals is removed and another added that the other property may be observed. A new set of experiments may be started using another set of chemicals to observe the other property in the particle. Whereas~~ In the Jeewanu the property of growth, multiplication and metabolic activity is observed in a natural way, once the experiments are set and no specific chemicals are needed for any specific property ~~(63)~~. (Bahadur & Ranganayaki (1966)

Bahadur and Ranganayaki (1970) photochemically produced self sustaining coacervates by the interaction of ammonium molybdate, diammonium hydrogen phosphate, minerals, commonly found in cells and formaldehyde in aqueous mixture ~~(58)~~. ② These particles have a number of amino acids in free form and in combined form as peptides and sugars as ribose, deoxyribose, fructose, and glucose. These particles have distinct boundary wall and intricate internal structure. ① The particles on separation from the mixture if extracted with chloroform methanol : 80:20 in a soxhlet yield a viscous yellow liquid. This contains an ethylalcohol soluble compound which on chromatography gives the test for phospholipids ~~(64)~~. (~~Bahadur 1972~~) & Singh 1972) The particles on hydrolysis with perchloric acid or formic acid in sealed tube gives the tests for nucleic acid bases as adenine, guanine, cytosin, thymine and uracil. If the particles are kept in 1 N sodium hydroxide for 24 hours, filtered and filtrate acidified with dilute acetic acid a white precipitate is obtained which on subsequent hydrolysis gives the test of desoxylibose nucleic acid ~~(58, 65)~~. (Bahadur and Ranganayaki 1970, Ranganayaki et.al. 1972)

② The experiment is so simple that it can be given as a class exercise for graduate and postgraduate students.

The particles can be fixed with chromic acid and subsequently stained with gentian violet and then eosin. In the central portion chromatine-like blue structures are obtained and the portion outside the central zone gets red stain with eosin like cytoplasm ~~(66)~~. (Bahadur and Gupta 1972)

It has been reported that the materials of the particles on digestion with hydrochloric acid show strong optical activity ~~(58)~~. (Bahadur and Ranganayaki 1970.)

The factors which are responsible for the natural formation of morphological looking objects have been discussed by Bahadur ~~(5, 37, 39)~~. (Bahadur 1967, Bahadur 1964, Bahadur et.al 1968), The molecules of different chemicals are brought together and are first held by coacervate forming factors. If this contains some macromolecules which have a number of molecules

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① ~~Micrograph ... shows a~~ The internal structure can be clearly viewed under high ~~power~~ magnification (Micrograph 4). Micrograph of the particle under phase contrast microscope reveal clearly the boundary wall and the internal structure of the particles (Micrograph 5) and also the budding (Micrograph 6 and 8). These particles multiply by budding (B29 Micrograph, 9, 10 and 11) attached to it by various intermolecular forces as van der Waals forces, hydrogen bonding, hydrophobic bonding, molecular bonding and others and also have many molecules adsorbed, absorbed and held tegether by electrostatic forces, when this macromolecule tries to crystallise, it forms a highly deformed crystal and the whole thing results in a molecular mesh having wide gaps and passages through which small environmental molecules have specific permeability. The various gaps of different molecules held in this deformed crystal structure remain active chemically and also catalytically. The whole structure in an attempt to acquire a spatio energetic pattern representing the state of minimum energy and it results in a morphological looking stnucture. The outer material forms a boundary wall and the aggregate appears to have an intricate internal structure (~~59~~). If this structure is present in an appropriate environment containing molecules which can form its body material and if it has a source of energy, may be of some physical nature as obtained by irradiation or evolved by some chemical transformations taking place in the mixture, the environmental molecules enter the aggregate through the appropriate passages in the boundary wall and in the outer material of the agregate, interact and finally result in the material with which the aggregate is formed (~~67~~). Bahadur et al 1965

All such abiogenic morphological structures may not be able to show the properties of biological order but those which happened to be in theenvironment appropriate for them could show the properties of biological order. Of such innumerable particles those which depended on such materials which were continuously getting formed in the mixture - say by photochemical process (Bahadur 67) continued their living activity and the rest ceased their functions soon after the supply of the necessary molecules was finished up (5, 68) (Bahadur 1967 and Bahadur ~~1967~~ et al. 1967)

~~Thus was formed a self perpetuating system, as desired by Haldane (3), which was a chemical complex and this by the process of chemical reactions with the molecules of its environment accomplished growth and multiplication. Such a system was in equilibrium with its environment so it could adapt and thus evolve. Thus this system remained to itself while yet changing not in the direction of death or decay as desired by Konikova (47).~~

Many such microstructures are observed in ores and rocks and are reported as silicate particles, found in sedimentary rocks and are described as microfossils and are observed in carbonaceous chondrites and are mentioned as organised elements. Many of these are the models of the earliest structures in which the life was expressed.

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Synthesis of Jeewanu, the Protocell*

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The last two decades were spent on the synthetic approach to the problem of origin of life and having mostly agreed to the hypothesis that life originated by a process of molecular or chemical evolution, scientists, in different laboratories of the World, started investigation of the various natural conditions which probably existed on the Earth in its earlier history and could effect the synthesis of molecules which perhaps went to make the earliest molecular associations with the characteristics of the biological order. In the hectic search two important points were either missed or were postponed for consideration at some advanced stage of this investigation. One was that for effecting a particular synthesis any source of energy, no matter how strong or how difficult it might have been to get in nature, was used and whoever discovered a source which was helpful in some synthesis, emphasised the importance of that particular source in the origin of life. Another point was that the synthesis of the compounds which are found in the present cell was investigated in nature as a routine no matter many of these compounds might have formed in the cell itself during the course of its evolution and may not have anything to do with the formation of the earliest cells.

Abiogenesis of amino acids:

Proteins are the basic building materials of the present-day living systems. This stimulated the search for the natural conditions for the abiogenic formation of amino acids. In 1913 Loeb observed that if silent electric discharge is passed through a mixture of ammonia, formaldehyde and water, glycine and alanine are synthesised in the mixture(1). In 1953 Miller modified the electrical method of production of amino acids and obtained a few amino acids and some nitrogenous compounds on passing electric discharge in mixtures of gases as methane, hydrogen, ammonia and water(2). As there are evidences that these gases might have been present in the primitive atmospheres of the earth it was suggested that amino acids were formed by electric discharges taking place in the clouds of the primitive earth. However as suggested by Rubey(3) and referred by Bernal(4) the earth's primitive atmosphere might have had relatively little hydrocarbon, more carbon dioxide and a certain amount of ammonia or nitrogen and water.

In 1954 Bahadur (5,6,103) observed that if sterilised aqueous mixtures containing paraformaldehyde as the source of organic carbon, a source of fixed nitrogen and colloidal inorganic catalysts commonly found in soil are exposed to sun-light or artificial light from an electric bulb, glycine and alanine are synthesised in good quantity together with other natural amino acids in smaller amounts. The influence of the source of organic carbon (7,8), the hydrogen ion concentration of the irradiated mixtures (9,10), the source of light employed for exposure (11), the nature of catalyst used, the period of exposure on the formation of amino acids in the mixtures (12,13,35) and the mature of the surface of the vessels containing the irradiated mixtures (14) have been studied in detail (15).

It is of considerable interest to mention that the formation of amino acids in a mixture which had no initial fixed nitrogen has been observed. Here molecular nitrogen is fixed and a part of it appears in amino acids (16,17). This process has also been studied in detail(10,18,36,52).

Hasselstrom and co-workers(19) exposed aqueous solutions of ammonium acetate and observed the synthesis of glycine and aspartic

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* Under print in Zentralblatt fur Bakteriologie II. Abtg. (1966).

acid in the mixtures. The exposure of mixtures of gases, which probably were present in the atmosphere of the earth before the appearance of oxygen, to X-ray also produces amino acids (20). Groth (21) observed the formation of amino acids by exposing a mixture of methane, ammonia and water to radiations from a Krypton lamp which produces Schumann radiations. A number of experiments have been carried out demonstrating that simple gaseous mixtures when acted upon by ultra violet or electric discharges yield products as cyanide, amino acids, aldehydes and aromatic derivatives (22,23,24,25).

Abiogenesis of peptides:

The formation of peptides by the combination of amino acids is another important step in biogenesis. This is a dehydration, endothermal reaction. The addition of each amino acid in a preformed peptide chain involves the removal of one molecule of water, and since the cell is in aqueous medium, it would be expected that it will consititute an endergonic reaction. It is found that the formation of dipeptides by joining of two amino acid molecules involves positive free energies averaging around 2 to 5 kilogram calories per mole (26).

This gåve an idea to a few scientists that the formation of peptides could be possible only under conditions where moisture is absent and only with the help of a strong source of energy as heat. Fox(27) synthesised peptides by heating a mixture of amino acids in the absence of air at about 170°C for a few hours and named these peptidesas 'proteinoid', thereby meaning small protein molecules. It is difficult to get a dehydrating condition in nature and a high temperature as needed for this synthesis on the surface of the earth after the appearance of water, except near volcanoes. So it has been suggested that the formation of peptides took place near volcanoes. It has been further demonstrated that under anhydrous conditions and in presence of strong phosphoric acid, amino acids can be polymerised. The synthesis of peptides in aqueous mixtures containing a dehydrating agent as hydrogen cyanide has been effected (28).

Bahadur and Ranganayaki (29,30) reported a still simpler method viz., the photochemical formation of peptides in aqueous mixtures containing amino acids, a source of organic carbon and colloidal inorganic catalysts. Such sterilised mixtures on exposure to sun-light or artificial light show the formation of a number of peptides. The amino acid used in the mixture undergoes photolytic decomposition also and these decomposition products recombine forming new natural amino acids and peptides formation utilising these different amino acids takes place. This process of peptide formation can also be observed in the mixtures prepared for the study of photochemical formation of amino acids and are initially devoid of a source of amino acids, if longer exposures are given. The role of period of exposure, nature of amino acids and organic carbon present in the irradiated mixtures, influence of hydrogen ion concentration and nature of catalysts employed in this synthesis have been studied in detail (31,32,33,34).

Perti and co-workers (37,38,39) studied the photochemical formation of peptides in aqueous mixtures employing visible and ultra-violet light and succeeded in synthesising a number of peptides. Briggs(138) reported a similar synthesis of peptides in aqueous mixtures using visible light as the source of energy. Akabori(40) carried out the experiments of polymerising the simplest amino acids as glycine over a clay base with ultra-violet light and observed the formation of polyglycine. However, when ultra-violet light is used as the source of energy the products formed decompose quickly. The long ultra-violet present in the sun-light reaching the surface of the earth, is helpful in the synthesis of peptides and more peptides are formed when the mixtures of amino acids, water and colloidal inorganic catalysts are exposed to sun-light in quartz flasks after sterilisation. One such mixture, a saturated solution of glycine in water containing insoluble glycine, was sterilised and exposed to sun-light in quartz flask by us. Increasing concentration of peptide was observed in this mixture for over three years(41).

Abiogenesis of other compounds of biological interest:

In 1962 Oro observed the formation of heterocyclic bases using cyanides (42). Formaldehyde produce a large number of simple sugars in presence of lime (43) and Schramm demonstrated the synthesis of polynucleotides from ribose and purines in the presence of polyphosphate under anhydrous conditions (44). Ponnamperuma observed the formation of adenosine by irradiating ~~the~~ an aqueous solution of ribose and adenine with ultra-violet light (45). He further observed that if adenosine is irradiated with ultra-violet light absorbed by adenine in aqueous solution of pyrophosphate ester, adenylic acid and adenosisne triphosphate are formed (46).

Role of light is abiogenesis:

Thus during the last decade almost all possible sources of energy were used for the synthesis of amino acids and other compounds of biological interest and most of the sources tried yielded some result. However, one point should not be missed. The question is not that which source of energy can effect a particular synthesis but the real problem is that which is the weakest source of energy strong enough to effect the synthesis of these products. This consideration is essential for as Hull (47) pointed out, on thermodynamic considerations, the stronger the source of energy employed, the quicker will be the end products broken down and for the products thus formed to be effective in abiogenesis of the materials leading to the formation of the first molecular associations with characteristic of living order, an effective means of their protection becomes necessary. However, those sources of energy become more important which though effecting the synthesis are too weak to decompose the products formed at least for a period long enough that the products get removed from the field of reactions. It has been suggested that the products of the reaction may get concentrated by their absorption on the surface of the soil particles or surface active materials may get concentrated along the beach ~~of~~ by shore wind as described by Bernal(4). However these products will finally get adsorbed on soil particles and thus will become more prone to the destroying influence of the high energy radiations. Moreover the occurence of these energy sources would have been more of phenomenal nature on the surface of the earth in contrast to the energy obtained by sun-light which in all probability was available in abundance since the very beginning of the earth.

In all these, the molecules playing a vital part in cell activities were synthesised directly in the mixture using whatever source of energy that could result in their formation. It, however, remains yet to be solved whether most of these molecules constituting the present-day cell were abiogenically synthesised in nature in the beginning and were than incorporated in the molecular associations which started showing the properties of biological order or not. However, the other possibility is that the molecules as purines, pyrimidines, sugars, organic acids, adenosines and even the complete protein molecules as we see them to-day were synthesised within the proto-cells which initially were made of very simple peptides, amino acids and mineral molceules. Briggs(46) while confirming Bahadur's (49) observation on Jeewanu reported the presence of adenine, guanine, ribose, desoxyribose, glucose, fructose, a number of organic acids and peptides having about a dozens of natural amino acids in the protocells, Jeewanu structures, which are formed in sterilised aqueous mixtures containing a source of organic carbon and colloidal inorganic catalysts on exposure to artificial light froma 500 watts electric bulb. And the important point is that these molecules were present only in the body of the Jeewanu formed in the mixture and not in the environmental medium, indicating their formation in these molecular associations only and not inthe environmental medium.

Problem of energy transformation:

The energy transformation is one of the most important aspects of the study of the origin of life and it has been discussed in considerable detail by Blum (50). Firstly energy is required for the reduction of carbondioxide with water and this at present is mostly achieved by the photosynthesis taking place in green plants. Once these compounds are raised to higher thermodynamic level they can undergo slow degradation liberating energy for the processes taking place in the living system. The conversion of one organic molecule to another does not involve so much energy exchange as is necessary for the reduction of the carbon of carbon dioxide to organic carbon and on thermodynamic considerations alone a picture of transformation of certain organic molecules through successive stepwise degradations all needing little energy of activation, the need for which may be still diminished by suitable catalysts in the environment, can be imagined. Many such degradation of carbohydrate and proteins in plant and animal tissues are known to biochemists. These appear very much common in most of the living systems but a number of variations are known and even newer metabolic processes and stepwise degradations of organic molecules within existing living systems may come to our knowledge in future investigations. It is not difficult to imagine catalysts other than the present enzymes which may do this work or might have been doing this stepwise degradations in the earliets cells when the present metabolic processes of energy liberation had not evolved to the present stage.

These processes are important because these provide the energy necessary for the various biosyntheses taking place within their body. These degradations are in one way different from other thermodynamically permissible degradations of organic substances outside a living system. These laboratory degradations are usually fast and the energy is set free quickly. The heat thus produced burns down the intermediate products ultimately to carbondioxide and water. Thus a piece of ignited wood burns down to ash and a few gases. In the living system these degradations are slow, stepwise and the liberated energy is utilised at least in part for the various life-activities and other energy requirements of the organism. If a piece of wood would have burnt, as if in a slow motion picture, a chemist would have observed the cellulose and lignin molecules oxidising stepwise, producing different intermediate products each undergoing stepwise oxidation liberating energy. Thus in a living system enzymes have not only decreased the need of energy of activation but have also slowed down and controlled the degradations to liberate the energy in a form that could be utilised.

The process of raising the compound to higher thermodynamic level is different. At present most of the organic compounds on the surface of the earth have been synthesised at some stage or the other by photosynthesis taking place in the green plants. However, one thing is still not very well understood. The energy quanta necessary for the reduction of carbon dioxide with water to organic carbon is not present in the visible light which effects photosynthesis. Though the mechanism of photosynthesis in green plants is now fairly clear so far as the stepwise chemical reactions are concerned from the first detected organic molecule upto the formation of the complex carbohydrate structures, it is difficult to explain how the molecules theremodynamically at higher energy level can be formed with visible light which does not have the necessary quanta of energy for this process. Perhaps the single stage of the electron pair is raised to triplet stage by photochemical excitation and the energy of the triplet stage, by process of total transformation from one group to another finally reaches a spot which on going down from the triplet stage to singlet stage excitation liberated energy which is sufficient to effect the reduction of carbon dioxide to organic carbon or perhaps it is something like the tunnel effect of the semiconductors where even without the necessary potential for the flow of electron being reached, a few electrons pass through the potential barrier as if making a tunnel through the potential barrier.

Future investigations alone will clear this picture.

A reversible photochemical reaction :

However one thing about photosynthesis taking place in plants is important. The pigment in the photosynthesis is light sensitive and is oxidised in presence of light. The oxidised product changes back to reduced form during the darkness and is ready for another electron transfer during the next exposure(51). It has been observed by Bahadur that a molybdenum complex shows such a reversible photochemical transforatmon (100). A sterilised mixture of molybdic acid 0.05 per cent (w/v) and paraformaldehyde 0.5 per cent (w/v) when exposed to sunlight or artificial light from an electric bulb turns blue after a few days of exposure. After the mixture becomes distinctly blue, if the mixture is covered with thick black cloth and is kept in dark it becomes colourless again. This mixture on further exposure to light becomes blue and the colour can be discharged by keeping it in dark. The process can be repeated again and again and during this there is considerabke decrease in the pH of the mixture. If this exposure is done in a sealed tube filled upto the top the blue colour produced during exposure is not bleached on keeping in the dark. Hence this bleaching requires oxygen. During the exposure to light the molybdic form of molybdenum is reduced to molybdous form and blue colour is produced. This is not the molybdenum blue which does not become colourless in the dark. In appropriate chemical environment 'ous' form of molybdenum oxidises to 'ic' form again in dark and becomes ready to receive further quanta of light.

This reversible photochemical reduction-oxidation transformation of molybdenum complex is interesting because it is accompanied by simultaneous electron transfer in the environmental organic molecules(18). It is of further interest to mention that these mixtures show considerable fixation of molecular nitrogen(11), and also photochemical formation of amino acids (15).

In this connection of availability of the energy on the surface of the earth for the formation of amino acids and peptides, it is of considerable interest to report the observation that in the photochemical processes some amino acids were detected even in the mixtures kept in dark (18), formation of peptides was observed in traces in the mixtures kept in dark (34) and considerable fixation of nitrogen was recorded in the unexposed mixtures also (11,18). As these mixtures were kept thoroughly covered with several folds of thick black cloth and kept near the exposed mixtures all the time during exposure, a search was made for the radiation which was effecting these chemical reactions, So in another series of experiments similar mixtures were kept in a lead chamber of one inch thick walls of solid lead. No formation of amino acids, peptides or fixation of nitrogen was observed in these mixtures even after a few years. These experiments show that radiations to which the black cloth is permeable also help in these processes in a feeble way and cosmic radiations or terrestrial radio-activity might be responsible for these reactions.

How the problem of going up on the thermodynamic level was achieved is not of great importance in origin of life on the earth because the earth had an initial stock of organic compounds abiogenically formed much before life came in existence and in all probability the earliest living systems were heterotrophic in nature(53,54,55) and they depended upon the organic substances already present in the environment which they simply assimilated there and by a series of stepwise degradations liberated energy necessary for their life processes. Autotrophs developed afterwards with pigments which could effect carbondioxide fixation. It is of interest to investigate what helped in enriching the supply of organic matter before the photosynthetic pigment and the accessory enzymes of the photosynthetic apparatus of the autotrophs took over this work.

It appears that there may be a possibility of exciting molecules with even the radiations which do not otherwise contain the necessary quanta. Nitrogen is regarded as photochemically inert in the Schumann range of radiations and needs much shorter wave lengths. However Russian scientists have proved the production of metastable nitrogen molecules. They have observed, that in nitrogen irradiated by wave lengths in Schumann range, the emission of bands belonging to such high excited level of nitrogen molecule that they could be reached by subsequent absorption of light by metastable molecules (56,57). These metastable nitrogen molecules possessing an extra ordinary large amount of energy of 9.76 eV can certainly display a definite chemical activity on colloision with hydrogen, methane or carbon monoxide.

Another important observation in this aspect is that the energy requirement for photolysis of a bond is considerably shifted to long wave length when the molecule is adsorbed on an active surface. Thus water molecules adsorbed on silicates are also decomposed in the range of 23oo A^o rather than in the Schumann ultra violet. The earlier photolysis of water molecules in these heterogeneous environment is explained by the fact that water forms at the surface of porous silicates, alumino silicates and oxides, superficial hydrates of the type - Si - OH, as observed by Terenin in infra red spectra (58). The splitting of OH radicals from these hydrates requires quanta of lesser magnitude than does the photodissociation of gaseous water (59).

The shift of photochemically sensitive spectral range towards longer wave lengths (amounting to Ca 1eV, or more than 20 K.cal, in energy) may be effecting the accessibility of the photochemical reactions of the compounds concerned to the ordinary visible light when the substrates are adsorbed over a surface.

Bernal(4) also emphasising the role of light as the chief source of energy for the earliest form of living organisms writes, "The major essential for metabolism , without which the enzymes and coenzymes could not have functioned is a source of energy and this energy can be provided either immediately by light or it can be drawn from stores in which energy has been stored by the previous section of light .... It happens that iron and other metal coordinate compounds are suited not only for carrying out enzyme action but also can absorb light and being coloured, can absorb it where it is much stronger in the visible region of solar spectrum".

The concentration of the amino acids photochemically synthesised in the oceans of the primitive earth would have concentrated by the mechanism of wave drift and adsorption in the mud as suggested by Bernal (4,60) and thus acquired a disposition for being quickly acted upon by sun-light forming peptides.

The experiments carried out so far only show that amino acids and peptides can be formed in aqueous mixtures by sun-light. Not much is so far known about the mechanism by which these have been synthesised. Unveiling of this mechanism may be quite a complicated place of physico chemical analysis.

The formation of amino acids and peptides by different methods discussed so far does not exhaust the possibility that there may be many other processes still unknown which might be possible on the earth at some stage or the other or might have occurred at least as phenomenal only but the speciality of the process of photochemical formation of amino acids and peptides is that it is quite in conformation with the two basic principles of science. The first of these is the uniformitarian principles used in the first place by Lyell in his analysis of geological phenomenon, namely as far as possible, by taking the past as if it were a backward extension of the present. It means than, except when we have positive reasons not to believe it, we much imagine that the same kind of phenomenon, that occurs now occured in the past and trace their possible consequence. The second principle is by

Henderson(61) introduced by him in his, "Fitness of Environment". This states that the subsequent stages of complicated systems make use of the inherent properties of the simple stages already present. This for instance limits life for practical purposes to the regions of 30° to 40°C (4).

The molecular evolution:

Thus abiogenesis developed considerably during the last 10-15 years but does it solve the problem of origin of life? As suggested by Blum(63) we would not try to describe an automobile by grinding up its various parts and subjecting them to chemical analysis, and we should not expect to learn all about the living machine by following, exclusively a similar approach. It seems hardly necessary to say this and yet we seem at times to go astray in just this direction whether we are trying to study the nature of viruses, the growth of cancers, the mechanism of genetic inheritance or as in the present case, the origin and evolution of life.

The term "Molecular evolution" or "Chemical evolution" is at present being used too frequently but until the complete self-replicating machine had emerged, evolution by natural selection, in the sense in which the word may properly be used was not possible; and here an error may creep into our thinking about the origin of the living systems. For natural selection is sometimes wrongly invoked, directly or tacitly, to explain the origin of the things which had to be present before evolution by natural selection could take place, that is to explain events leading upto the appearance of the self-replicating system in terms of things that could have happened only after such a system was already in existence. This incorrect application of the concept of natural selection may lead into the realm of teleology and finalism. For chemical changes to take place only thermodynamic and kinetic components are necessary but no spatial component in the sense of replicating pattern is required, and no system of replication is necessary. This lack seems to establish a sharp distinction between chemical reactions in non-living world and evolutionary adaptation in a living system.

There seems to be a tacit idea at various points that once there was present, a mixture of the component molecules life must spontaneously arise and evolve.

Nucleic acid and the problem of origin of life:

Nucleic acid molecule controls the sequence of amino acids in the protein molecules, and thus helps in the transfer of inheritance of genetic code and under suitable conditions is capable of duplication in the living system. The fossil record tells that the major molecular patterns of inheritance must have been pretty well established even before at least one billion years. But living systems had existed and evolution has been going on for a long time before the beginning of cambrian - possibly since three and half bellion years ago when living systems originated for the first time as suggested by Bernal(4), and the major themes of organic evolution were established in the course of this earlier period. And as suggested by Blum(63) it seems likely that strinctly physico-chemical factors played a more prominent role in setting limits upon the direction in which natural selection could take place then, than they did later.

A wrong use of the term "living Molecule" for virus has caused considerable mis-conception. The term was introduced to describe virus when it was crystallised for the first time. However, now the idea that virus constitutes a complete living system is quite fixed in the scientific as well as popular minds. The virus consists of a nucleic acid or nucleoprotein component surrounded by a protein jacket. The latter is left behind when nucleoprotein part enters into the host cell. After penetrating the cell nucleoprotein furnishes a pattern or template for replication by the cell. Most of the cell substance may be used up for the manufacture of virus particles including the protein sheath. This leads to the death of the host cell and release of the virus particles. Thus virus particle is not a self-replicating system but depends for its replication upon the metabolism of the host cell. Virus is not a living system and it is the cell-virus system which is the living system. However, the cell was a living system even without the virus. Virus is thus not a living system but it can be a part of the living system if it reaches a suitable living system. Thus Blum suggests (63) if the term "living molecule" is used to describe a virus, one runs the risk of having it accepted in a more complete sense than it should be.

Many scientists seem to think that if a virus particle was once formed or even if a nucleic acid molecule or long polynucleotide were formed then the life could be said to have originated. This system will be useless unless a living system is already in existence rather the appearance of any of these would indicate that the living system is already in existence. This living system will contain a thermodynamic and kinetic component as well as a template.

The modern concept of the gene seems to be that it is a template or a locus in nucleic acid molecule - the desoxyribose nucleic acid. If genes are considered as being self-replicating, the same difficulty will be faced which is met on considering viruses as living molecules.

Thus, as Bernal(4) suggested, unless we desire to push back the doctrine of special creation to the creation of enzymes and coenzymes - coenzyme in the polymerised form as nucleic acid - as the beginning of life, we must assume that before there were enzymes to carrt out the catalytic reactions in metabolism these were some other agents that did it, not as well but sufficiently well for the origin of life. Similarly, before there were any coenzymes there must have been some other and less efficient means of transferring energy from place to place. Calvin(64) and Nicolaev(65) have shown that iron coordination compounds are capable of speeding up many chemical reactions to several thousand times. Such compounds of iron or of other transition elements as copper, nickel, etc. would form spontaneously from any solution in which there are nitrogenous compounds as amino acids and these may have been the protoenzymes of the earliest living system.

The present day cell has usually organelles as chloroplasts responsible for photosynthesis, the mitochondria that carry out the enzymic changes which constitute the metabolism that synthesise protein and contain ribonucleic acid, desoxyribose nucleic acid-containing chromosomes etc. The evolution of the cell leading to the formation of these organelles will be considered later but here it may well be said that all these represent specialised versions of what was originally an all - in living system. This will be an aggregate or molecular association and not a single nucleic acid molecule which can do nothing by itself.

In the present living system some form of genetic information is transferred from parent to offspring by nucleic acid. The sequence Hypothesis suggests that this genetic information is stored and transmitted by the linear order of the bases in the nucleic acid molecules. This by some unequivocal process is expressed in the linear sequence of amino acid residues in proteins. This information is transmitted in one direction only that is from the base sequence of nucleic acid to amino acid sequences. According to Crick (66) the three dimensional configurations of proteins, as well as their aggregations and the specific interactions within the protein molecules and the molecules of its environment in a particular condition are due to the particular linear order of their constituent amino acids.

As there are about 20 natural amino acids in proteins and four bases in nucleic acids investigation of the 'cod-ing problem' has been started with a view to compile a time-independent dictionary expressing the sequence of nucleic acid bases which are translated into a specific amino acids sequence in proteins (67,68,69,70,71).

However no present theory of replication and protein synthesis suggests the origin of the genetic text which is being replicated, translated and expressed in functional proteins. The present theories of replication, coding and protein synthesis suggests that the information necessary to order the linear sequence of amino acids in proteins is provided by the genetic sequence (72). Thus most of the theories of replication, coding and protein synthesis employ the absence of rules, restrictions or expectation of tny order in the sequence of subunits in biological macro molecules, except when such order pre-exists in genetic sequence.

Generally genetists believe that origin of this genetic order which exists in linear sequences of base in nucleic acid is due to evolutionary process of natural selection from the random, arbitrary errors in memory or replication of pre-existing sequences or due to the random mixing of sequences in the process of reproduction. The initial origin of the genetic order is usually considered as a chance combination of the subunits which for some unknown reasons became capable of self-duplication. However, the theory of natural selection suggests only an eventual choice from a population of completed functionally distinguishable entities which can replicate but it does not in any way itself impose rules for the formation of any particular order during its synthesis and least the capacity to reproduce.

If we consider that before the present genetic control there was a disordered reservoir of molecular subunits, it is difficult to explain the degree and type of order which is now observed in biological macromolecules without considering at some step, a random or arbitrary choice of sequence of the ordering process,

As suggested by Muller (73) the processes of multiplication, variation, selection and also the adaptability produce genetic information which now results in choice of certain sequence, from some enormous number of alternative sequences. However, all this concerns the accumulation of genetic information to define a certain state but provides no information to produce this state under initial conditions to begin with.

Abiogenic origin of order in molecules.

According to Pattee (75) the possibility of conceivable sequences of even one small molecule of TMV protein in size is about 100^{200} where as it is difficult to even imagine the total number of macromolecules existing on the surface of the earth as more than 100^{60} if the age of the earth is considered as 10^{17} seconds and the generation time as one second and a volume of macromolecules on the earth equal to the entire volume of the surface of the earth one metre thick or 5×10^{23} cm^3, considering 10^{19} molecules per cm^3. If all conceivable linear order of typical biological macromolecules are equally probable except for genetic control there is no physically realizable selective process to reduce effectively the enormous number of sequence orders so as the formation of some particular sequence may become even an event.

A conceivable solution to this problem appears firstly that the genetic order originated with certain molecules which were much smaller in size than even the small protein molecules of the present-day and it has subsequently modified, to be able to produce bigger protein molecules and secondly that the nongenetic equiprobability of sequence must thermodynamically lead at some stage to specific choices between the possible sequence. Pattee's hypothesis for the origin of the degree and type of replicating order now found in biological macromolecules on computer feed-back mechanism appears to be highly feasible.

Biochemical macromolecules have characteristic sequence arrangement of their subunits. These arrangements are repeated by some order process withing the living cells by nucleic acid molecules. This in turn is controlled by the same degree of order in nucleic acid linear base sequences, which are replicated and express their order in the arrangement of amino acids sequence in protein molecules by a simple dictionary - type code. These codes are time-independent and contain no inter-systems restrictions. The information feed back is not considered on this ordering process and these codes are independent of the state of the growing configuration and its local environment. As these codes usually exclude any inherent restriction or expectation of order which did not arise from pre-existing genetic order, they do not in themselves lead to any explanation of the origin of this high degree of order which is being duplicated and decoded in protein, neither they lead to the explanation of the origin of the replication or coding process themselves. On the other hand the process of natural selection has no means of effectively increasing the significant probability by producing one positive sequence. Therefore the possibility is that the precursors of biological macromolecules were not of random sequence but crystal structures which were produced from the restrictions inherent in their growth similar to what is observed in all crystallisation processes. The general logical process of discrete-state computation in simple configurations with feed-back can assemble elaborate, repeating well ordered sequences, without requiring pre-existing sequences from which to copy. A molecular representation of such a computer by the process of stereospecific polymerisation, in which the choice of subunit is determined by conditional, state-dependent rules has been suggested by Pattee (74). From such redundantly ordered macromolecules replicating by fission, the evolutionary process of variation and selection may then effectively accumulate as much genetic information capacity as produces survival value.

It is then more reasonable to believe that the genetic mechanism themselves are the evolutionary result of the natural occurrences of ordered macromolecular sequences rather than making special assumption that in living systems ordered macromolecular sequence are the evolutionary end result of the spontaneous, chance origin of genetic system.

Origin of molecular associations:

According to Schrodin er (140) " A small molecule might be called as the germ of a solid". These small solid germs can build up larger and larger molecular associations. One is a dull way of repeating the same structure in three dimensions again and again as in crystal formation and once the periodicity is established there is no definite limit of the size of the aggregate. Another is a semi periodic way of building more and more extended aggregates with wide gaps which allow even passage of smaller molecules and without having the dull device of repetition. This is the case with complicated molecules or groups of molecules held together by various intermolecular forces as hydrogen bonding, hydrophobic bonding, electrostatic interactions etc., in which every atom or group of atoms plays an individual role, not equivalent to that of many others as in the case of a periodic structure. Thus by selecting suitable compounds and creating appropriate chemical environment, it is possible to make a molecular association of semi-periodic nature and the energy necessary for its formation may be derived during the achievement of a spatio-energetic pattern representing the state of minimum energy by the materials of this aggregate.

In such a process of formation of molecular association, the material is made of semi-solid nature, the growth from outside is inhibited and this starts inward from within, the gaps in semi-periodic structure already formed are big enough to permit the passage of the environmental substrate molecules selectively, the materials forming this aggregate~~d~~ are not present preformed in the environment but are synthesised during the course of the passage of the substrate molecules from the environment through the preformed portion by the catalytically active atoms and groups already present in the structure, this hypothetical structure will start growing from within by the synthesis of the material of the aggregate within this system.

Bahadur (75,100) has shown that the slow tendency of otherwise amorphous material to change into crystalline state yet not actually achieving it due to the restrictions caused by the physico-chemical nature of the environment and the chemical changes affecting the material concerned before and during this process under specific conditions results in the formation of molecular associations with characteristics of biological order and growth, multiplication and metabolic activity.

So far the considerations of abiogenesis of such materials which constitute the present form of living system, their energy transfer and transformation and possibility of establishment of some sort of order in otherwise random reservoir of molecules concerned have been made but in all these the basic discussions have been around the living systems of our earth only. It is worth while to consider the possibility, as far as the present evidences allow, whether there is life or living systems any where else in our solar system and then in universe and if so what variations may be expected in them.

Distribution of organic compounds:

Considerable evidences have accumulated to suggest that organic matter is common in the solar system. Briggs (76) has suggested that the clouds of Venus contain volatile organic compounds and according to Briggs (77), Sagan (78) and Wilson (79) such compouhds are trapped in the surface of the Moon. The colour changes of Jupiter has been explained by Briggs (80) as due to the abiogenic organic systhesis. Saturn, Uranus and Neptune contain a large quantity of methane. Briggs (81) and Guerin (82) have independently suggested that the 'blue haze' of Mars is due to small droplets of volatile plant products. According to Briggs (83) the earth must have acquired about 10^{13} gm. of organic matter from carbonaceous coondrites alone.

Possibility of extra terrestrial life:

It is important to investigate as on what planet in our solar system life actually exists and recently this aspect has been the focus of considerable attention of the exobiologists. There are tentative evidences of existence of life on the "maria" of Mars and on no other planet or satellite. The evidences for life on Mars are - the physico-chemical properties of the atmosphere and surface can permit the existence of life - forms based on a terrestrial type biochemistry have been demonstrated in the laboratory (84,85,86), the maria has rapid regenerative properties (87), the infra-red absorption of the Martian maria, but not of the desert show absorption at 3.5 u (88), certain similarity between the reflection spectra of maria and those of terrestrial vegetation adapted to high and cold habitats (89) and the seasonal changes in the polarization of light reflected from the maria somewhat resemble that observed from some vegetation (90). However, it is debatable whether terrestrial astronomical method can ever provide conclusive evidence of extra-terrestrial life.

To have a correct concept of the problem of origin of life processes it is necessary to consider the abundance of distribution of life in the universe as a whole. The order of the number of stars in our galaxy in about 10^{11}. About 14 percent of these are stars of G-type similar to our sun, and 75 percent of the these are binaries. The orbital dynamics of binaries has been studied only with a few systems (91) to indicate the presence of planets in these stars. If the planets arose from condensation of an equitorial nubula of the primitive sun (92,93) it will result in slowing of the solar rotation. According to this view rate of rotation of any star could be used as a criterion of the presence of planets (94). About two-third of the galactic population are slow-rotating stars and may therefore be accompanied by planets. Our sun is a second generation star in the galaxy and only such late stars will have sufficient elements of high atomic weight to yield high density planets as Earth. First generation stars may have planets of H, Hi, C, N, O etc. Only and may be unsuitable abodes for life.

Thus Briggs (95) is of the opinion that the Universe is so large that it is a statistical certainty that extra-terrestrial life, even intelligent life, exists elsewhere.

The Chemical nature of living systems:

The chemical nature of the earliest forms of living system is also difficult to assess for as Haldane (96,97) suggested chemistry and physics may have been significantly different in the Precambrian. The consideration of the formation of the polymers of the present form of living system and the control of subunit sequence in these macromolecules have been made. Protein plays an important role in them for it is the basic building material of the living systems known. Probably the earliest living systems were also made of proteins but we do not know whether all the living systems in existence elsewhere in the universe are also proteinous. According to Pirie(98) proteins are useful to an organism because they are enzymes that catalyse metabolic reactions very efficiently. But there are innumerable non-protein catalysts of similar action e.g., oxidation can be catalysed by many metals and by thiourea, some of the rare elements are esterase and so on. An organism using such systems might be sluggish but it would be conceivable.

There is a possibility of existence of such living systems in the prebiological era which might have been made of materials other than proteins and were consuming such nutrition the supply of which was limited, together with such living systems which on subseuqnent evolution produced the present form of living system(100). There might have been a period when such non-proteinous and proteinous form might have existed side by side. However, those living systems which depended on such nutrition which was limited in a locality would have finished their life activity soon after all their nutrients in that locality was booked in their body. But proteinous living system continued their life activities because their nutrition supply was photochemically furnished through the course of time. The possibility of more than one form of living system has been suggested by Pirie also (98). According to him the evidence of evolution and Linnaean system of classification support the idea that the number of species existing at any time has been getting large. Projecting this principle back it seems reasonable to think that all the present living systems sprang from one or a few common ancestors. But it is illogical to think that this ancestor was the original and one of the only type of organism. And it is correctly doubted whether the problem of 'origin of life' should not be modified as 'origin of lives'.

Many inorganic substances resemble organic substances in many physical oproperties as high molecular weight, optical activity, viscosity, stability, sensitivity to pH and temperature of the environment as suggested by Sminnon (99). One wonders why these molecules cannot form living systems which may be devoid of protein or even organic material. This aspect of the problem has been discussed by Bahadur (75, 100) in considerable detail and experimental evidences of the existence of such systems has been provided.

Mentioning the possibility of existence of such living systems Bernal had said (102) " .... There may have been other radically incompatible forms on the earth at earlier times and these or others we may succeed in making artificially. When life exists on other planets it is most likely that it will be radically incompatible with ours though we may suspect that it will show striking parallels, biochemically as well as structurally".

The problem of defining 'life' and living system:

Still more difficult is the problem of defining 'life' or 'living system' and the ideal definition of life is still awaited. The problem of differentiating a living system from a non-living becomes a hopeless job particularly when we consider the border line cases between living and non-living. However it is necessary to have a clear understanding as what we are looking for when we are looking for it all over the universe and studying the cause and action of its origin.

According to Pirie (104) a rigid devision between living and non-living is not possible and he considers life as not a definable quality but a statement of one's attitude of mind towards a system. Haldane (105) defined living system as " a self perpetuating pattern of chemical reactions and Bernal (103) and Haldane (105) suggested that the embodiment with a boundary of a self maintaining chemical process was the occasion of the origin of life.

Konikova (106) proposed substitution of 'self maintaining' word by 'self-developing'. Thus he holds that a living system is a chemical substance or a complex which by process of chemical reactions with the substances of its environment accomplishes its reproduction and development i.e., it remains to itself while yet changing not in the direction of death or decay. Life is the attainment, by a chemical system, of the ability to rebuild itself by interacting with other substances, remaining alive itself and beginning to be different from itself,

Horowitz's (107) definition of life is more specific and he believes that a living system is a self-reproducing system with ability to mutate rendomly and to reproduce in this new form. He attributes three essential properties to a living system - self-reproduction, mutation and heterocatalysis.

In a sectional meeting during the First International Symposium on Origin of Life on the Earth held at Moscow in August, 1957, the problem of defining 'life' and 'living system' was discussed under the Chairmanship of Prof. Pauling. In this Pirie (108) opposed the idea of mutation to be included in the sense it is understood in biological context on the ground that to demand that a living system shall mutate is a purely linguistic restriction and it leads to absurdities. Only small fraction of the organisms that are universally accepted as living have been shown to mutate though many of them probably do. But some very ancient species do not seem to mutate much. Thus according to him it is important to distinguish more clearly than Horowitz does between at least three types of multiplications:-

Multiplications in many celled organism where the process can fail as in worker bees and mules although the organism is obviously still alive; multiplication in single cells of an organism or a single-celled organism which can be absent as in the brain or the nerve cells although the tissue is obviously still alive and the multiplication of the cell components such as genes and virus which are simple lifeless molecules which can do nothing by themselves. It is probably the system which makes the other gene rather than the genes which merely act as template for producing a copy. If these types of multiplication or reproduction are accepted it is unlikely that the ability to reproduce could be used as an adequate criterion of life or living system.

Brownshtein (109) opposed the idea of a mono-molecular living system on the ground that if desoxyribose nucleic acid of genes is supposed to be alive because they have power of self-reproduction and self-development, viral nucleic acid could be said to be alive. The idea of Horowitz's minimum requirement for a living system is "a living molecule in a multi-molecular environment' Brownshtein commented that if this definition is to describe the position accurately one word must be added so as to read, " a living molecule in a multi-molecular living environment", but by doing so the idea contained in Horowitz's definition is negated.

Pauling (110) tried to establish a compromise between these two different types of definitions proposed for a living system and said, "It is sometime easier to study a subject than to define it", for as Pirie pointed out even with existing objects a rigid division into the living and non-living is not possible.

Though it is difficult to draw the line of demarkation between living and non-living systems it becomes necessary to have a few such clearly defined properties which when present in a system should qualify it for being admitted to the category of livings, particularly when systematic study of the formation of molecular associations resembling biological system is attempted and this work has considerably advanced with extremely interesting results (111,112,113,114, 101).

According to Bahadur (112,100) a living system is one which is capable of growth, multiplication and metabolic activity where growth means increase in the size of the system from within by the actual synthesis of the material with which it is made, within the system, multiplication stands for the increase in the number of the system where newer units come in existence through the parent one and the term metabolic activity stands for any series of chemical reactions taking place within a boundary the result of which is that at least a part of the environmental molecules entering the system are converted into the material with which the system is made. This certainly is not an effort to define a living system but simply to enumerate the essential properties which if present together in a system will qualify it for admission to the category of the living and these properties have been selected by going more towards the living side of the line of demarkation between living and non-living where as it will be possible to have a few living systems which are accepted as living on other considerations but not having all these three properties together and it may be conceiveable to have living systems with lesser properties than these but it will be agreeable to almost all the scientists that if a system shows these properties it can be classified as living and accomplishment of these functions as described by Bahadur (112) will be achieved by transfer and transformation of energy and transformation and communication of information if the system is to function for any appreciable time and these have been described as the essential characteristics of a living system by Calvin (62).

This functional approach to the characteristics of a living system does not include any comment on the chemical nature of the system concerned for we have yet no information for or against the existence of living systems of enitirely different chemical composition than ours else where ~~where~~ and the logical possibility of their existence is enormous.

The structure and the properties of the earliest cells.

Before we think of synthesising a living system we cannot avoid thinking of a cell which is the basic unit of all the living systems. Evolution of higher multicellular organisms from the earliest unicellular organisms has been studied in detail by the biologists but usually a biologist stops at uni-cellular stage and when it comes to the synthesis of a living system be unconsciously assumes that one is discussing the synthesis of a cell as he knows it. There is consoderable evidence of evolutionary cytology to support the view that the complicated structure of the cell which we see to day is the product of evolution which this cell has under gone during its existence since last three and half billions years which is the estimate of Bernal of the time since when life came in existence (4).

A model cell possesses highly organised inclusions known as organelles as nucleus, mitochondria, microsome, ribosome, chromosomes etc. This model cellis the end-product of evolution which not only produced the associations of cells as multicellular organisms but there is considerable evidence for the evolution of these organelles also (115). The cytology of simple modern organisms provides enough proof of this evolution. Thus the nuclear apparatus of vertebrate cells is far more complicated than that of even the most highly developed plants and the cells of seed-plants lack astral rays and well developed centrioles. The sulphur bacterium, Beggiatoa lacks discrete nucleus but possesses numerous chromatin granules scattered through out the cytoplasm and the reproduction takes place entirely due to ingrowths of the cell walls (116,117). In somewhat more complex bacterial Rhodobacteriales, *chromatium* the chromatin granules are in association but not covered by a nuclear membrane (116,117). Distinct chromatomes are detected only in the organisms of the complexity of *Euglena* and even in this organism spindle threads and interzonal strands are lacking (118).

Slow evolutionary evidence of other organelles of the cells can also be traced. Thus in the blue-green algae, the photosynthetic pigments are dispersed throughout the cytoplasm and no distinct chloroplasts are present whereas purple sulphur bacteeria have granular pigment structures lacking the detailed micromorphology of higher plant chloroplasts. Mitochondria are totally absent in blue-green algae while minute granules possessing all the metabolic functions of mitochondria can be obtained on ultracentrifugation of many species of true bacteria (120). True mitochondria though present in yeast cells are of very simple structure with few internal folds only (121).

By the evidence of comparative cytology Dillon (123) concluded that origin of organelles are evolutionary specialization and that the earliest cells lacked such structures, their functions being carried out by the undifferentiated cytoplasm. Briggs (115) is of the opinion that the organization of the primeval organic solutions into a relatively inefficient molecular organisation does not present the problem of intra-cellular organization and specialization. The latter is a problem of cellular evolution and not of biopoesis.

Just as a consideration of the cytological evolution of the organelles of the modern cell is necessary to have some ideas of the internal structure of the organisms which were synthesised when the life began on our earth, it is also necesszry to study whether the loss or gain in the capacity of the earliest cells took place with the progress of evolution. Lwoff (123) by his extensive work on micro-organisms produced convincing evidence that much of the evolution is accomplished by the selective loss and not gain in the capacity of the cell. Thus though a simple cell-like organism developed more and more complicated organelles with evolution making it more fit for performing a particular function in the specific prevailing environment, the cell as a whole lost its initial capacity or potentiality for performing very many types of vital functions in many probable diverse environments which it originally had. Even in the present organisms no cell of the body of a multicellular organism has the capacity of performing as many functions as a single cell of a unicellular organism can do.

Keeping the above mentioned evolutionary changes in view if one goes back in the time-cloch to examine the earliest living cells he expects to find them very simple in structure yet full of capacity of performing the various functions of biological order. These functions are limited and it can be said that the initial living systems had very simple structures and were full of properties within a certain limit. The synthesis of these molecular associations with properties of biological order is the outstanding problem of the origin of life and this has been successfully solved by Bahadur (112,113,114, 100, 75).

Origin of enzymic activity

Before we conceive of any molecular association with characteristics of living order the problem of some sort of metabolism liberating the energy necessary for the various activities of this aggregate becomes necessary. The metabolic processes observed in the present day organisms might have been present in some of the earlist forms of life but there is an equal probability that these too have evolved from some very simple processes of the earliest living systems. Whatever the form of the metabolism might have been, it is certain that these must have been using some sort of catalysts. The origin of enzymic activity in the prebiological era is of interest particularly when there is no possibility of there being elaborate protein structures of the type we have to-day.

In the absence of typical enzyme like molecules co-ordinate compounds of metals, various metallic ions and organic molecules would have acted as the initial enzymes as suggested by Calvin. A search of enzymic activity in the sterilised aqueous mixtures containing metallic catalysts and organic molecules was made after irradiation with interesting results (124). It has been observed that considerable phosphatase activity is produced in these mixtures and it is destroyed on boiling. The phosphatase activity is dependent on the nature of catalyst employed, organic molecules used in the mixtures, the period of exposure and hydrogen ion concentration of the medium (125), It has been observed that the phosphatase activity is lost on prolonged exposure when protecell like structures called Jeewanu make their appearance in the mixture and is then confined in the material of these molceular associations only. If diffused artificial light is used as the source of irradiation the phosphatase activity continues to increase with increasing period of irradiation for a long time and the formation of molecular association is delayed (124). Recently Briggs (115) confirmed the observation on phosphatase activity in Jeewanu synthesised photochemically. He observed mild esterase activity also inthe irradiated mixtures.

Synthesis of molecular association:

In the formation of the molecular association which may resemble a cell model the work of Oparin (126) and Booji and De Jong (127) on coacervate formation is important. The coacervate although made from materials of biological origin as gelatine and gum arabic are simple systems and do possess limiting membrane with osmotic properties. These have selective absorption for certain compounds. Binary fission of the coacervate droplets occurs under suitable conditions, while vacuole formation is common. A study of coacervate formation by polymeric products of abiogenic reactions may yield interesting findings relevant to the origin of cells.

Microspheres have been prepared by treatment of thermally synthesised peptides with water (128). These microspheres retain their integrity on centrifucation, show volume changes in hypertonic salt solutions and can be made in various shape by incorporating other substances such as lipids and nucleic acids. It seems possible that surface of these microspheres possess some properties in common with cell membrane. Bahadur prepared Jeewanu from ammonium molybdate - peptide complexes in presence of ascorbic acid and biological minerals and the peptides used were thermally prepared. These Jeewanu show the properties of growth, multiplication and metabolic activity (114). In contrast with microspheres which have a comparatively short life and normally coalesce in a day or so forming clums of irregular shape at the bottom of the mixture and are made of brittle material which break radically on pressure, Jeewanu retain their shape indefinitely and are made of a viscous, honey like material(114).

For coacervates, microspheres and many other objects produced by various scientists during the experimentation to duplicate the morphology of cells Briggs in his paper, "The formation of cell-like structures by the action of light on a possible primitiv earth hydrosphere, "read in the Fourth International Photobiology Congress held at Oxford in 1964 writes, "Several authors as described in the review by Oparin (126), have conducted experiments to duplicate the morphology of cells by interaction of simple inorganic and organic compounds. While there isno doubt that the products obtained by many of these workers bear a morphological resemblance to living cell, this is the only feature in common, in that the products are dissimilar in chemical composition are metabolically inert, do not grow or reproduce etc. Moreover, most of these aftefacts are produced from substances and under conditions that were probably quite absent from the primitive Earth. The only interesting products are those of Fox (128) who has shown that theremally synthesised proteinoids produce microspheres in water".

"However, more recently Bahadur (111) and Perti (129) have described the formation of a series of cell-like microspheres (named by them Jeewanu, a sanskrit word for particles of life) by action of sunlight or an ultra violet on sterilised solutions containing citric acid and a colloidal salt of molybdenum and iron. It is the purpose of this paper to report a confirmation and extension of this work". The synthesis of Jeewanu will be described later in this article.

Inherent properties of matter

Consider the earliest living systems with simple internal structures but full of properties and capacities within the limits of biological order. The formation of such molecular associations has been the most important aspect of the origin of life. If we take properties of say iron hydroxide and after enumerating them all ask as how will a cell-like small structure with simple internal structures can be formed full of properties within these limits, the answer is simple. Any method of preparating particles of the desired size may be used with iron hydroxide as the body material of the particles. Thus in short, if the material concerned has the properties which are sought in the particles, it is easy to prepare the particles of such material with simple structures having the properties within the particular limits described. If thematter concerned had the properties which we include as the properties within biological order, it is easy to imagine the fommation of particles with these properties. However, this is just an over-simplified example of the idea and this simily extends only this for and no further for in the above example the consideration of chemical and physical properties only were made which any piece of the chemical concerned will show, but in the living system we are concerned with the functional properties as growth, multiplication and metabolic activity and many coordinated functions of different factors, will be necessary to achieve this result.

Bahadur suggested that matter has inherent properties of duplication and adaptability (100,112). Thus under suitable conditions matter has inherent property of duplication and if a system of matter in equilibrium is subjected to constraint a change occurs in the system if possible of such a kind that the constraint is partially annulled. By the term duplication of matter under suitable condition it is meant that if a system at dynamic equilibrium has the possibility of formation of several molecular species at the same thermodynamic level and needing the same energy of activstion and if in this system one of the possible synthesiseable molecular structures is introduced, more of this molecular structure will be synthesised in the system than other possible structures. On quantum mechanical resonance interaction special stability consideration a system AA where A and A are identical will be more stable than AB where A and B are similar but not identical (130). Thus if a molecule A is introduced in a system which has all the constituents for its formation and which has the necessary available energy on thermodynamic and activation energy consideration, more of A will be formed in the system than other possible molecular structures needing the same energy of activation and on the same thermodynamic level. In a simple way this property can be expressed as "under suitable conditions matter has an inherent property of duplication".

According to Pauling and Delbruck (131) the special quantum mechanical resonance interaction stability force will be negligible for larger molecule of the size of the protein as compared to the molecular interaction energy of the identical and unidentical molecules and their duplication is not possible on this consideration. But if is more reasonable to believe that the initial living systems were built of smaller molecules and in them the molecules could have duplicated by the quantum mechanical resonance interaction force. When these polymers became bigger in size this process was taken over by nucleic acid assisted duplication process where the duplication is a two step process instead of one step as condidered here. The one step process of duplication is the more probable process to begin with, before coming to our present day nucleic acid assisted two step duplication as observed inthe present form of living. Gurwitsch (132) has described duplication of sample molecules in specific environments.

The phenomenon of adaptability is of great importance in evolution and may be considered as an important property of the living system. Adaptation as far as it involves the alteration or variation of the characteristics of a species, is a facet of evolution and all heritable adaptaive changes are evolutionary changes The reverse statement namely that all evolutionary changes are adaptive is not true.

There are two important aspects of evolution. Firstly that which exemplified simply by changes and secondly that which leads to creation of new species. The bulk of the evolutionary changes fall within the former aspect of evolution and these are the changes that a species or a population of individuals belonging to that species undergo in response to the environmental changes. These changes which if made, enable the population to continue its existence.

Natural selection does not cause the disparity between the parents and the rest of the population, it is this disparity itself. If a systematic slow environmental change occurs, then natural selection may lead to adaptive changes in the population.

Thus evolution has been defined as the sum of adaptations. It can be expressed more simply as "adaptation is evolution" (133).

Population of individuals adapt to new environment through systematic alteration in the frequencies of various genes carried by the population. In bacterial population where certain environmental constraint can be met by single-gene changes and where in general the descendents of a given individual are genetically identical with the parent individual, adaptation of the population comes sbout in a single generation and the response is absolute. In diploid, cross-fertilising individuals, as the result of gene recombination the connection between the phenotype of parents and offsprings is much less than that in bacteria.

According to the computer model described earlier, the accumulation of genetic information by adaptive natural selection necessary, leads to sequence with ever increasing information capacity in the feed back path. And as Patte (74) has suggested it is quite conceivable that this capacity may have grown so large that the autonomous computation that now remains is through the entire process of adaptive variations and selection, which may be considered as an information feed-back loop from functioning protein molecules to nucleic acid sequences. Phenomenon of adaptabil ty can be explained in these terms, if a living system is subjected to a constraint a change occurs in the organism if possible of such akind that the constraint is partially annulled, A living system is a system in equilibrium. Thus the above statement may be modified as - if a system in equilibrium issubjected to a constraint a change occurs in the system if possible of such a kind that the constraint is partially annulled. But this is Le Chateleir's principle itself as proposed by him in 1888.

This adaptability is observed not only in living systems but can be observed in many systems in equilibrium. The study of the adaptability in enzyme has been observed by Bahadur and Saxena (134, 124) during the investigation of the inhibition of urease by metallic ions.

If the constraint is strong enough to cause minor structural damage and the repair of this damage can be called as a change of a kind that the constraint on the system is partially annulled and thus this can be called as adaptability particularly in such cases as destruction of the tertiary or the quarternary structures of the protein molecules by boiling or other denaturating processes and their subsequent regeneration under suitable conditions involving the process where repair does not necessarily include formation of new molecules but merely establishment of an orderly folding from an uncoiled random linear structure of the peptide molecules formed due to the distnrbance of the tertiary and quarternary structure of the protein molecules not even provoking primary valency, such adaptability has been observed in the case of many enzymes. It has been reported that one can inactivate an enzyme and show that this inactivation involves the destruction of the tertiary or quarternary structures, in which subunits are packed together but not linked by primary valency. But by suitablyyincubating the inactive material, as much as 95 percent of the enzymatic activity can be recovered. This means that the tertiary and quarternary structures depending on the enzyme concerned are feformed. One can carry this denaturation clear down to the random coil level - that is go down all the way to the primary structure and can climb almost all theway back up through the alpha helix into the tertiary folding and even into the quarternary aggregation. This last has been achieved in the case of aldolase (135).

Many of these processes can be explained on the thermodynamic stability of these specific molecular folding but these show adaptability in the sense that the damage caused by the constraint is being annulled. Such changes also take place in living system during the constraint caused by the changes in enviromnent and the adaptives changes in living system can also be explained theremodynamically.

Synthesis of Jeewanu

If adaptability and duplication are inherent properties of the matter it remains the matter of only creating suitable conditions under which these properties of the matter concerned could be observed. Under these conditions a particular set of matter in appropriate environment will show growth - multiplication on molecular level, multiplication - multiplication on macrolevel and metabolic activity-stepwise thermodynamic processes liberating energy for the above two processes of multiplication from the structural units available, degrading a part to liberate the necessary amount of energy and utilising a part to provide the building material for the above mentioned growth and multiplication. The synthesis of such systems has been achieved in the laboratory (75,100,111,112, 114, 129, 136). The particles thus formed grow in size. After achieving a maturity size these reproduce by budding; the newly formed but may separate out of may remain attached to the parent unit and after achieving maturity size buds again, These particles can be subcultured in their specific environmental medium. I have named these particles as "JEEWANU" a sanskrit word for the particles of life (111).

A number of procedures for their preparation have been described (111,113,114,75,129,136,137). These particles have a boundary wall and complicated internal structures. These are made of materials which are not present in the environment but are synthesised within their body from the environmental molecules which enter them. Thus where the environmental medium may contain only a few free amino acids photo-chemically formed in the ~~aqueous~~ mixture containing a source of organic carbon, inorganic catalysts and biological minerals etc. the body material of the Jeewanu shows the presence of only a few free amino acids but on hydrolysis a large number of natural amino acids are produced thereby showing that their body structure is made of material very similar to protein. The body material of these Jeewanu show phosphatase activity. Time-lapse photomicrographs showing growth and multiplication have been successfully taken (100,101). Dr M.H.Briggs confirmed the work on the formation of Jeewanu independently in his laboratory. In the paper entitled "The formation of cell-like structure by the action of light on a possible primitive hydrosphere", "read in the Fourth International Congress on Photobiology held at Oxford in 1964, in the following of words. A similar paper entitled "Experiments on the Origin of cells," has been published in Space Flight(138).

"Laboratory synthesis of Protocells

"The primitive hydrosphere was stimulated by ashing 1.0 Kg. of compressed yeast at 500°C. for 12 hours. The resulting ash was examined for cellular remains and for organic matters neither were detected. The ash was then redissolved in 1.0 litre of triple distilled water."

"A variety of organic mixtures was tested, including formaldehyde + ammonium nitrate, acetaldehyde + ammonium nitrate, paraformaldehyde + ammonium phosphate, tyrosine alone, citric acid + ammonium phosphate,, a sacein-hydrolysate alone. In some experiments the effects of added inorganic suspensions (ferris oxide sol., molybdic oxide sol., alumina) were also investigated."

"The solutions were placed in quartz flasks, which were sealed sterilised by autoclave. Each solution was represented by four flasks in each experiment. Two flasks of each solution were immediately covered with thick dark cloths and placed in a locked cupboard, while the other two were exposed to the light of a 500 watt continuously for a period of four to six months. In duplicate experiments flasks were exposed to a high pressure mercury UV light with a filter removing radiation below 300 m u. The flasks were opened and samples of the contents immediately examined microscopically. Some of the samples of the contents of each flask were inoculated into a series of sterile microbial growth-media and agar slopes. These were then sealed and incubated for 2 weeks at 37°C. No growth was detected in any medium or on any slope, indicating the absence of microbial contamination in the flasks."

"Microscopic examination of samples from the flasks stored in darkness failed to reveal any microstructures, but samples from all the flasks exposed to light revealed numerous globular structures ranging in size from about 0.5 u to 15 u. Most of these structures were solitary, but some showed budding, while others were associated in groups ranging from 3 to 15. Similar objects, though in differing quantities, were seen in all flasks."

"Larger samples of the light-exposed solutions were now centrifuged at 5000 r.p.m. for 30 minutes, when the solutions separated into a precipitate and a clear supernatant. Samples of the supernatant and the washed precipitate were subjected to amino acid analysis by high-voltage paper electropheresis. Samples of solutions kept in the dark were similarly examined. No amino acids were detected in any of the solutions (to which no amino acids had been originally added) stored in darkness. However, all flasks exposed to light originally containing aldehydes + ammonium salts now contained free amino acids. Glycine, glutamate, aspartate and alanine were detected in all. Approximately 0.1% of the added aldehydes were present as free amino acids after 4 months irradiation."

"Examination of the precipitates from all flasks revealed the presence of the same four free amino acids. However, the precipitates also showed several peptide spots on paper that stained with bromophenol bluel Hydrolysis of the precipitates with 5N Hcl, released numerous amino acids that were readily detected on the paper electrophoratograms. From Rf values in several solvents the following amino acids were tentatively identified : glycine, alanine, glutamate, aspartate, histidine, lysine, arginine, serine, threonine, phenylalanine, leucine, valine."

"The hydrolysates were also examined by paper chromatography using spraying reagents specific for particular classes of organic compounds. The silver chromate and mercuric nitrate-ammonium sulphate techniques gave positive reactions with areas on the chromatograms with Rf values similar to adenine and guanine. Ammoniacal silver nitrate and aniline-diphenylamine were used for tentatively identifying glucose, fructose, ribose and 2-deoxyribose. Phenol-hypochlorite reacted with a compound on all chromatograms with the Rf of urea, while diazotisation reactions showed many spots of which three were tentatively identified as vanillic acid, 3-hydroxybenzoic acid and 4-hydroxyphenulacetic acid.

For the irradiation periods used, approximately 1% to 9% of the originally added organic compounds had been converted to other substances.

Tests of the precipitates for enzymatic activity have also been conducted. Esterase, peptidase and phosphatase were searched for in the precipitates using routine micro-clinical assays. Detectable levels of esterase activity were found in some precipitates, while phosphatase activity was found in others. The levels of activity were very low, but were quite repeatable, though they were undetectable in precipitates heated to 100°C for 5 minutes. No peptidase activity was found in any precipitate.

"Detailed microscopic investigation of the more interesting microstructures synthesised by the above techniques revealed the presence of considerable internal architecture. Vacuoles, spherical solid inclusions, and apparent wall-structures were seen in many. Some of the objects fluoresced in the UV, while many were stainable with biological dyes (e.g. acidic carmine, azure II, eosin, haematoxylin-eosin, Janus green B. methylene blue, nuetral red, ninhydrin, saphranin, and toluidine blue).

Conclusions

"Considered together, the results presented above demonstrate that microscopic objects in the 0.5 to 15 u size range can be formed by the prolonged action of light on solutions of simple organic compounds in a mineral medium stimulating the primitive hydrosphere. Some of these objects possess a morphology similar to that of simple cells. The objects contain many of the organic compounds found in protoplasm. Some also appear to possess weak enzymatic activity."

"While the definitions of 'life' and 'living' are difficult problems, 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 oceans of the primitive Earth and were the immediate precursors of cellular life".

The observations on Jeewanu pose many problems of interest in exobiology. Are these Jeewanu formed from protein like materials only or can other substances also form Jeewanu ? The suggestion that matter has inherent properties of duplication and adaptability open the possibility that if suitable conditions are created objects made of many types of substances but showing the properties of growth, multiplication and metabolic activity can be synthesised. Jeewanu of quite different materials have been prepared (75).

Jeewanu described in the above photo-chemical preparations are different from a typical unicellular organism in the sense that the presence of nucleic acid molecules or all the components of nucleic acid molecules has not been detected in them so far. The tests for the presence of thyamine and cytosine hsve given negative results so far. However, as adenine, guanine, ribose and desoxyribose are present the presence of some rudimentary form of nucleic acid substituting the pyrimudines with some other bases may be imagined. All the attempts to grow these in bacterial culture media have given negative results and these can be subcultured in their specific media only.

An attempt was mde to prepare Jeewanu of basically inorganic materials as cuprous oxide (75, 100, 101). It has been observed that cuprous oxide Jeewanu can be prepared by heating a mixture of Fehling solution, biological minerals, ammonium molybdate, gum arabic, sucrose and traces of glucose. The details of these preparation have been described else where (95,101). Small spherules are prepared by boiling this mixture and these may be used for seeding other similar unboiled mixture. These spherical objects range from 0.2 to 3 u in size. These multiply by budding and have metabolic activity (95,101). These can be kept indefinitely in active stage by providing necessary nutrition at appropriate intervals. These particles on analysis were found to yield about 88 percent ash. They contain about 64 percent of cuprous oxide, 4.2 percent of carbon, 0.28 percent of nitrogen and rest is hydrogen, oxygen, biological minerals and molybdate. The body material of these cuprous oxide Jeewanu shows considerable catalase activity which is destroyed on boiling (95,101). Time-lapse photomicrographs of these Jeewanu showing growth and multiplication by budding have been taken (75,100,101). These units have complicated internal structure with distinct boundary wall and several photomicrographs showing the internal structures have been taken (75,101), some of these resemble coccolith (139). Interesting adaptive changes have been observed in these particles and growth and multiplication of such changed Jeewanu have also been recorded on time-lapse photomicrographs(75,101). Because of these properties of biological order these hzve been named as Cuprous oxide Jeewanu. These are different from metabolically inert cuprous oxide particles formed by reducing Fehling solution with glucose. Particles thus formed do not show growth or multiplication; further more these particles are only of cuprous oxide and do not have catalase activity as in cuprous oxide Jeewanu.

Cuprous oxide Jeewanu have been trained to utilise mannitol as the source of carbon. These trained Jeewanu need only Fehling solution, biological minerals, ammonium molybdate and mannitol as their nutrition. In the environmental medium of these constituents these trained Jeewanu have been grown for over one year.

Summary

Evidence has been produced that basic constituents of the present day living system as amino acids and peptides are formed in sterilised aqueous mixture containing organic carbon and colloidal inorganic catalysts on exposure to sunlight or artificial light from an electric bulb. The polymers separate out from the mixture if rendered insoluble by either increase in size of the molecules or by the chemical reaction with the environmental molecules. The solid material thus separated is viscous like honey and it tends to crystallise. If the process of crystallization is hindered by the nature of the material concerned or the chemical reaction by the molecules of the environment with this material the amorphous state tending to crystallise accomplishes only partial success. The effort to achieve a spatio-energetic pattern represenging the state of minimum energy helps in establishing a subunit sequence by computer feed-back mechanism establishing some sort of order and decrease in entropy in otherwise random aggregate of molecules. A semi-permeable boundary is soon formed. In the enclosed semi-solid viscour material the molecules do not remain only randomly held up but get arranged in certain pattern depending on inter molecular forces as van der Waal's forces, molecular interactions, electrostatic interactions, hydrophobic bonds, hydrogen bonds etc. and when this aggregate was of smaller molecules quantam mechanical resonance interaction played a very important role. If this order was destroyed by energy liberated by certain chemical reactions, amongst some molecules when these came together in this viscous mass, they again tended to achieve the spationergetic pattern represenging a state of minimum energy and if the environment continually supplied such molecules to this mass, a dynamic state will result in these particles. Complicated internal movement amongst organelles of Jeewanu can be observed under microscope.

Matter has inherent properties of duplication and adaptability which can be observed under suitable conditions in any piece of matter. If the molecular associations as described above, is constituted of smaller molecules and the constituents necessary for its formation and the energy needed on thermodynamic and energy of activation considerations are available the molecules of this aggregate will get so arranged that they facilitate the process of multiplication of the molecules whose multiplication is permissible - others acting as catalysts and the building meterial of the structure needed for this prupose. The multiplications may not be restricted to one but a number of molceules may thus duplicate.

This molecular association will assimilate nutrition from the environment and will use it for liberation of energy and building its body material and will grow in size and reproduce by budding. As this has the property of adaptation it will evolve in mildly changing environments. In the course of evolution with time the various molecular species constituting this system which initially had specific position in this system because of their physico-chemical properties will acquire more definite position resulting in the formation of the molecular organelles. First even the arrangement of molecular organelles would have been mostly determined by the physico-chemical properties of the molecules concerned and the thermodynamic consideration but evolution in progress these gradually acquired shape and position which was more guided by the better function of these for achieving a particular effect for the system in an efficient manner. Thus originated the first living systems JEEWANU, which in the couse of evolution formed the present-day cells.

The Jeewanu of several types have been prepared all showing the properties of growth, multiplication and metabolic activity. Several photomicrographs showing the internal structure of Jeewanu and their boundary wall have been taken.

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ORIGIN OF LIFE

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INTRODUCTION :

Limitation of Molecular Evolution, Abiogenesis, what is life, Functional Properties of Life, Evolution by Natural Selection. Variation and Differentiation. Formation of Specific Structures, Estimation of the Appearance of the Earliest Living System, Evolution and Properties of Individual Cells.

Chapter - I The Living Phenomenon

Life in a Wider-Sense, Natural Selection in Cosmogenic Sense, Birth of a Star; C-N-O Cycle, Metabolism in Stars, Origin of Mg and Fe and other elements, Size of Main Sequence Stars and Possibility of Life Synthesis, Distribution of Organic Compound in Interstellar Space. Interstellar Free Radicals.

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Prebiotic Interrelationship Between Polynucleotides and Poly amino acids, Base Stacked Configuration, The two Type of Autocatalytic Cycle, Reflexive Catalysts, Cycle of Molecular Replication, Beginning of Protoplasm, Molecular Replication by Reflexive Catalysts.

Chapter - XIV Formation of Peptides

Thermal Peptides, Photochemical Formation of Peptides in Aqueous Mixtures, Peptide ~~of~~ Formation Over Clay Base, Dicyanamide Condensation, Active Site Sequence, Autocatalysis, Orde~~n~~risation in Polymerisation of Amino acids.

Chapter - XV Macromolecular Sequence and Automata

Origin of Genetic Text, Generation of Genetic Information, Mutation, Replication and Natural Selection, Ordenisation Process For the Formation of Living System, Autonomous Computers, Sequence Interaction in Growing Chain, Stereo Specific Polymerisation, Evolution of Genetic Control, Biological Macromolecules, The Feed-back, Discrete-State Computer Model, Origin of Life, Evolutionary Theory, Biological Order from Quantum Mechanical Laws, Automata Theory, Natural Conditions For Molecular Automata, Prevalence and Organisation, Hereditary Organisation, Biological Organisation, Creative Processes, Growth of Crystals, Life Synthesis, Genetic Metamorphosis, A Possible Primitive Molecular Biology, The Genetic Crystals, Silicates as the Primitive Genetic Crystals, Biochemical Transformation on Clay, Origin of Life Through Genetic Crystals.

Chapter - XVI Matter Contrives to be Alive

Molecular Evolution, The Chief Reasons for Belief in, Abiogenesis of Amino Acids, Abiogenesis of Peptides, Problem of Energy Transformation, A Reversible Photochemical Reaction, The

Problem of Defining Life and Living System, The Structure and the Properties of the Earliest Cells, Origin of Enzymic Activity, Nucleic acid and the Problem of Origin of Life, Abiogenic Origin of Order in Molecules, Distribution of Organic Compounds, Possibility of Extra-terrestrial Life, Life Detection on Mars, Chemical Nature of Living System, Life Synthesis, Jeewanu, the Particle of Life, Possibility of Inorganic Life, Mechanism of Growth, Cell Models, Jeewanu The Protocells, Laboratory Synthesis of Protocells, Photochemical Formation of Self-sustaining Coacervates.

Chapter -XVII Detection of Some Significant Biological Functions in Abiogenic Microstructures.

Detection of Phospholipids, Self-sustaining Coacervates, Development of Spots, The Boundary Wall, Use of Molybdenum in the Abiogenic Experiments, Molybdenum in Rocks, Distribution of Molybdenum in Soil, Concentration of Molybdenum in Fresh and Sea Water, Molybdenum in Sediments, Biological Distribution, Directed Panspermia, Banin and Nawrat Observations, Transitional Metal Complexes, Sensitivity of Jeewanu to Antibiotics and Sulphur Drugs, Physical Foundation of Biogenesis, Ammonium thiocyanate-formaldehyde Microstructures, Extension of the Work on Ammonium Thiocyanate-formaldehyde Particles, Some Inorganic Cell Models, Formation of Morphological Looking Microstructures, A Simple Experiment for the Preparation of Cuprous Oxide Jeewanu.

Chapter - XVIII Neobiogenesis.

Living Yet not a Cell, Neobiogenesis in the Light of Recent Development, Appearance of Organised Structures in Sterilised Media, Cinatl Calcar~~i~~eous Structures, Autosynthetic Cells, Simple Forms of

Life, Self-organisation of Biochemicals, Neobiogenesis Pertaining to Origin of Life, Development of Virus, Mycoplasma-like Organisms, Introduction of Viruses into Biosphe~~r~~res, Cellular Defences Indicative of Virus Synthesis in Tissues, Reorganisation of the Degradation Products of Jeewanu.

Chapter - XIX <u>Early Cell Evolution</u>.

Physiology and Ecology Influence on Microbial Evolution, Microbial Record of Sediments, Evolution of Chloroplast, Atmospheric Oxygen and UV as mutagens, Morphology and Microbial Evolution, Evolution of Blue-green Algae, Derivatives of Chlorophyll in Sediments, An Outline of Cellular Evolution, Serial Symbiosis, Classical and Symbiotic View, Difference Between Prokaryotes and Eu Karyotes, Microbial Evolution.

" SCOPE OF THE PROBLEM OF ORIGIN OF LIFE"

'Origin of Life' may be more correctly mentioned as 'Life Coming in Being' because it was a gradual process operating on the Earth over a long span of time.

The important steps in this process are:
Inorganic molecules----organic molecules----polymerisation----molecular associations-----living system.

Earlier views on origin of life were displaced by the materialistic view suggested by Huxley, Spencer, Engels and Oparin. The term "Life" has clusters of misconceptions associated with it.

The first living systems were perhaps unicellular microorganisms much simpler than the unicellular organisms of the present age and originated from the 'hot thin soup' (Haldane) of the primordial ocean from coacervate or colloid particles.

Pasteur's work demolished the foundation of the theory of spontaneous Generation of Life but it did not say whether nonliving matter can be converted into living objects. It is still not clear whether this conversion happened once (Hackel) or is happening at all time neobiogenesis (Keosian).

Kayanau:

"There is no scientific basis that life may not be originating continuously upon the earth. The fact that we have no evidence of such de novo origin is of no particular significance, for if there is such origin we must anticipate that it would be in units far too small to be treated in the manner in which we are accustomed to dealing with organisms................"

Charles Darwin (1871) expressed a similar view in a letter (uncovered by G.Hardin):

"It is often said that all the conditions for the first formation of a living organism ard now present, which could even have been present. But if (and Oh! What a big if) we could conceive in some warm little pond, with all sorts of ammonia and phosphoric salts, light, heat, electricity etc. present, that a protein molecule was chemically formed ready to undergo still more complex change, at the present day, such matter would be instantly devoured or absorbed, which would not have been the case before living creatures were formed." What if Life? It is the expression of the new property of organic matter acquired in the course of evolution?

or

Is it some thing which resulted from the organisation of organic matter?

The fundamental properties of living systems might have appeared in the protein like macromolecules antedating the appearance of cellular life. Duplication and growth of DNA or Virus needs enzymes. Being unable to furnish the free energy needed for synthesis associated with their reproduction and ability to metabolise these nucleoproteins cannot be conceived as living systems.

Origin and Evolution of enzymic activity:

Cytochrome activity of Fe^{+++}, iron-haem complex and iron containing cytochrome (Calvin 1957)

Transamination reactions of pyridoxal in presence of metallic ions(Snell(1954)

The Energy liberating systems:
Adenosine phosphates, Heterotrophs - autotrophs, Chemoautotrophs and photoautotrophs (Braunstein 1957)

Morgulis (1952): Life could have originated not as the end of a consecutive chain of abonts by simultaneous coordination of several factors.

Cell: Both the atomic and the cellular theories have become obsolete. The cell like the 'indivisible' atom is now recognised as a highly organised integrated system built from extremely small and distinct particles. The concept of a cell as the unit of life has been thrown out of the window together with the atom.

- Nucleus →
  - nucleolus
  - Chromosomes - genes) Poor in enzymes, shows only synthesis of nucleic acid.
- Cell → Cytoplasm →
  - mitochondria--1/3 of the bulk-liponucleoproteins, functionally power house of the cell & seat of synthesis.
  - microsomes
  - Golgi Bodies
  - Chloroplasts etc.

Cellular symbiosis.
Molecular symbiosis.

Virus → Plant virus RNA, animal virus RNA and DNA.

Genes → DNA.

Themodynamically guided the molecular evolution would have continued without the formation of systems with living properties. Only when organic matter achieved a certain degree of organisation in some molecular association and acquired diverse potentialities the primordial life emerged as a new dimension in nature:

" Matter perpetuating its own Organisation".

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EVOLUTION OF THE CONCEPT OF ORIGION OF LIFE

Ancient Hindus were of the opinion that life originated by natural means through the interplay of primary elements.

Regveda, I, 164, 36; Atharvaveda, IX, 10,17.

According to them life originated in water:

Atharvaveda, IV, 2,6.

Ionian School (600 B.C.)

Organisms originated in sea slime by the action of heat, sun and air.

Thales, Greek school:

Living things deveoped from the amonphous slime under the influence of heat.

Anaximander (611-547 B.C.)

Living things arise in sea ooze and they go through a succession of stages in their deveopment.

Xenophane (560-480 B.C.)

Life originated from earth and water.

Anaxagorus (510-428 B.C.)

Animals and man all came from the earth's slime by a fructifying factor- the ethercal embryo, which is carried into the earth from the air with rain water.

Empedocles (490-44 B.C.)

Plants and animals can originate from lifeless materials and fructifying principles for the process are warmth of the sun, earth and rainfall from the sky.

Epicurus (341-270 B.C.)

Worms and innumerable other animals arose from the earth or manure under the influence of the moist heat of the sun and rain.

Aristotb (384-322 B.C.)

. ..Living things can be formed from lifeless materials by the action of 'Matter' and 'Form' where 'Form' is the energy of soul which endows matter with life and keeps it alive._ ____" The theory of Spontaneous Generation."

Basilius (315-379 A.D.)

Once upon a time earth produced living things by the command of God and to this day this ability has been retained in full force.

Saint Augustine (354-430 A.D.)

Living things are produced from Lifeless materials by the will of the God.

The mention of "Geese Tree" by Damiani-reported by Neckam (1157-1217)

'Vegetable lamb'-Pordenone (1331)

Recipe for the preparation of Lomunculus-Paracelsus(1493-1541)

Recipe for the preparation of mice - Van Helmont (1577-1544)

Harvey (1578-1657)

Living things originate by the action of special forces liborated in decay and analogous processes.

Descartes (1596-1650):

Spontaneous generation is a natural process occuring under still imperfectly understood circumstances, particularly when moist earth is exposed to sunlight or when there is decay.

Newton (1643-1726):

Plants were produced from the attenuated emanation from tail of comets.

First evidence against the theory of Spontaneous Generation was produced by Redi (1626-1697)

Leeuwenhoek (1632-1723) discovered the world of microorganisms and bacteria.

Buffon (1707-1788):

Introduced the hypothesis of the existence of a special vital principle and its fructifying role in the formation of microorganisms.

Needham (1713-1781):

"There is inherent in every microscopic pastide of organic matter a special "Vital force" which vitalized the organic matter.

Spallanzani (1765):

Discovered process of sterilisation and refuted the experiments of Buffon and Needham.

Pouchet (1859):

Tried to prove experimentally the possibility of spontaneous generation.

He deveoped the vitalistic theory.

The French Academy of Science offered a prize for exact and convincing experiments which would throw light upon the question of the autogeneration of living things (1859).

The prizes was awarded to Louis Pasteur in (1862):

P.T.O.-----2

Theory of Continuity of Life:

Lord Kelvin (1871):

Life is eternal, it only changes its form but is never created from dead substances.

I. The Theory of Eternity of Life - Preyer 1880
II. The Theory of Cosmozoa - Richter 1870
(vax Helmholtz-1884; Lipman 1932)
III. The Theory of Panspermia - Arrhenius - 1903

Life Originated at some distant period:

Haeckel (1866): The theory of Archegony.

Pfluger (1875): He identified proteins as indissociable essence of the vital process and emphasised the importance of cyanogen radicle.

Allen (1899):

He assumed that CO_2 was the primary organic compound.

All these theories have a common understanding that Life is endowed with absolute autonomy determined by special principles and forced applicable to organisms alone, the nature of which is radically different from the principles and forces operating in the inanimate Kingdom.- and thus all these persons felt an unabridgable gap between the Kingdoms of organisms and of inorganic nature.

Materialistic approach was devcoped by Huxlay, Spender, Mitchell, Schafer, Engel and Oparin.

Engels (1933)

"Life has neither arisen spontaneously nor has it existed eternally. It must have therefore resulted from a long evolution of matter, its origin being merely one step in the course of its historical development."

Oparins Work 1926, 1936, 1957, 1962.

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ENVIRONMENT OF BIOPOESIS

To-day scientists talk about life as a phenomenon like any other physico-chemical phenomenon.

In space age Astrobiology, Exobiology and Exoticchemistry are recognised branches of science and are the frontiers of science.

The phenomenon of duplication has been studies in detail by L.S.Pen/rose (1962). He concludes:

1. Replication leads to accumulation of structural energy and the stimulus for replication is derived directly from the pattern of replicating unit itse

2. Two changes- relevent and irrelevent occur during the duplication and the relevent changes are similar to mutation.

3. Two types of replicating systems are possible

(a) Desoxy nucleic acid replication of protein's as suggested by Watson and Crick (1953) is a two step process.

(b) Single step replication is fairly common. Theory of single chain replication has been built up involving the degree of self-reproductiveness of any structure in a special environment over a given period by Penrose (1962) and Good (1961). The problem of entropy in biopoesis has been studies by Schrodinger (1944). According to him metabolic activity frees a system from all entropy or disorder and it cannot help reproducing while functioning. He suggests two essentials for a living system: (a) boundry (b) metabolic activity with the formation of the material of the system.

Origin of the Universe: Coleman (1963)

1. The Primeval Atom Theory - Gamow (Big Bang Theory)
2. The Steady state Theory - Hoyle (Little Pop)

Origion of Planets: Shpley (1960) described the 15 theories about origion of planets.

Hot and Cold Origin of the Earth has been discussed in detail by Vinogradov (1959)

Many astronomers and a few geologists believe in Hot Origin of the Earth whil many geologists and a few stronomers believe in Cold Origin of the earth.

Possibilities of life bearing Planets:

Our galaxy which is one in millions of galaxies has about 150,000 million stars and these have about 100 million plants on which life can exist.

M.H. Briggs (1962) writes "Existence of many types of extraterrestrial life in millions of planets of our galaxy is highly probable."

Coleman (1963) dedicates his book on universe as follows: "This book is dedicated to all the youths on all planets in all Solar Systems throughout the Universe."

Radio Astronomy - Drake.

When did the earth originate?

The age of the earth as estimated by different methods is about 4.5. billions ye

By the rate of cooling of the earth - 2 to 4 billion years.

By the study of the moon asuming moon originated from the earth - 4 billion years.

By the study of the radioactive materials of the oldest rocks - 3.4 to 4.6 billion years.

By the study of meteorites - 4.5 billion years.

By the formation of elements-4.6 billion years.

However it is difficult to explain the loss of sediment from the ocean bottom and decreased salinity of sea water (Pirie 1957)

The pulse of the Earth - Prof. Umbgrove in 250 million years.

P.T.O.

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ABIOGENESIS AND EOBIOGENESIS

Amino acid Synthesis:

Loeb(1913)- silent electirc discharge in NH_3, CHO_2 and H_2O

Miller's Experiment (1953)- H_2,CO, NH_3, $CH_4$and Water-electirc discharge--- amino acids.

Abelson (1956) Using CO and CO_2 in the alre mixtures concluded the necessity of reducing atmosphere for electrical synthesis.

Pavlovs Kaya and Pasynskii(1957): Synthesised amino acids with electric discharge and U.V. radiation.

Bahadur (1954) Photochemical formation of natural amino acids in sterilised aquears mixtures containing organic sulotanus and inorganic catalysts in presence of fixed nitrogen.

Bahadur Ranganayaki and Santamaria (1958) Photochemical formation of amino acids involving nitrogen fixation.

PavlousKaya and Pasynskii (1957): formation of amino acids in aqueans mixtures of CH_2O and ammonium salts by irradiation with U.V.

Oro (1959) formation of amino acids by heating aqueas mixture of CH_2O and hydroxylamine to 70°C.

formation of amino acids in mixture containing 3N aqueans ammonia saturated with HCN by heating it to 70°C for 25 days.

Fox and Harada (1964) Formation of amino acids by passing a mixt~~ing~~ur NH_3, CH_4 and H_2O through a silica tube heated to 900-1000°C.

Lowe, Rees and Markham (1963): Formation of amino acids by heating a mixturing HCN and aqueas ammonia to 90°C for 18 hours.

Briggs (1964): Formation of amino acids in aqueas mixture containing organic substances and molybdinum and iron as catalysts (involving nitrogen fixatio

Formation of peptides:

Fox (1955): by heating a mixture of 18 natural amino acids to 180°C for 10 hours in limited supply of air.

Bahadur and Ranganayaki (1957) Formation of peptides in aqueas mixture by exposing sterilised aqueas mixtures containing amino acids and inorganic catalysts to light.

Perti (1960):Formation of peptides photochemically in aqueas solution.

Briggs (1964): Formation of peptides in aqueas solutions containing organic substances and inorganic latalysts by ceposux/to visible light.

Hull(1962) Consideration of the sources of energy for the formation of peptides and other macromolecules.

Bernal (1962) suggests possible process which might save the macromolecules from destruction under the influence of strong souces of energy.

Sugar Synthesis:

Oro' and Cox (1962) obtained 2-desoxyritose by condensing CH_2O and CH_3CHO or CH_3CHO and glyceraldehyde in aqueas salt solutions.

Ponnamperuma (1963) synthesised desoxyribose by exposing aqueas mixtures of formaldehyde and water to U.V. light.

Briggs (1964)obtained glucose, fructose, ritox and desoxyritose in specific molecular associations formedin sterilised aqueas mixtures containing organic substances and inorganic catalysts on exposux to visible light.

Purines and Pyrimidines synthesis:

Oro (1960) obtained adenine by heating solution of ammonium cyanide in water to 90°C for 24 hours.

Forx (1961) obtained uracil by heating a mixture of malic acid, urea and polyphosphoric acid.

Ponnamperuma (1963) observed the formation of purines and pyrimidines in the copolymerisation of amino acids.

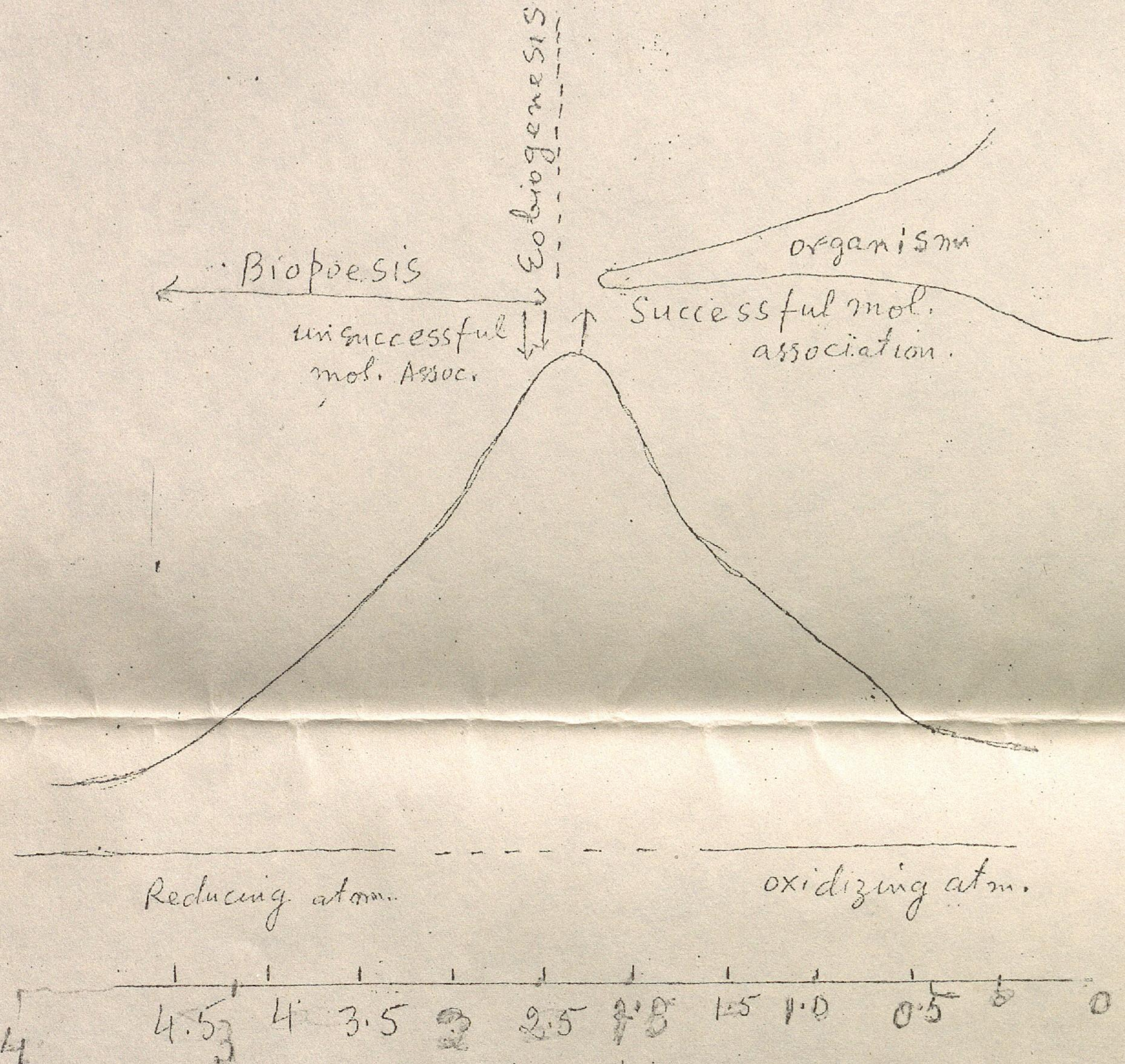
Briggs (1964): Observed the formation of adenine and guanine by exposing sterilised aqueas mixtures of organic substances and inorganic catalysts to visible light.

Study of the molecular associations:

Traube (1864): Copper sulphate crystals in aqueas solutions of potassium ferrocyanide.

Butschli (1892) By rubbing potassium hydroxide on olive oil.
Rhumbler (1906): Modified Butschii's experiment showing reproduction, feeding and movements.
Leduc(1907): $CaCl_2$ in saturated solution of KON and potassium phosphate.
Synthetic Biology.
Kuckuck: Glycerol & gelatine and sodium chloride in presence of radium salts.
Oparin (1936) Coaceruntes: Gum arabic and gelatine.
Fox (1959) Microspheres: Thermal peptides and water.
Kalinenko (1963): Zn and Cu couple in sea water.

MILESTONES IN THE ORIGIN OF LIFE.



Keosian (1964).

SYNTHESIS OF EOBIONTS OR PROTOCELLS

How was the first cell synthesised? Esperical origion of Life. Consideration of the idea with the theory of probability.

The cell which we see to-day has an evolutionary history of about 2 billions years.

THE EFFECT OF EVOLUTION ON THE CELL:

Lwoff (1943): Evolution is the loss of properties of individual cell.
Dillon (1962): The origin of organelles of the present day cell is the problem of evolutionary cytology and not of biopoesis. The earliest cells must have had a very simple internal structure (Bahadur & Ranganayaki 1963)

The idea was confirmed by Briggs (1964) who put forward the following points showing evolution at work:

1. The nuclear apparatus of vertebrate cells are far more complex than those of even most highly deveoped plants. Thus cells of seed plants lack: (i) astral rays (ii) well developed centrioles.

2. The nucleus of sulphur bacterium, Beggiatoa lacks discrete nucleus and possesses numerous chromatin granules scattered throughout the cytoplasm. Reproduction is entirely due to the ingrowth of the cell walls (Delaporte 1939-40)

3. The chromatin granules of Rhodobacillus (Chromatium) are in assocation and have no nuclear membrane.

4. Englena has distinct chromosomes but (i) lack in spindle threads (ii) and interzonal strands (Krichenbauer 1937)

5. In blue green algae the photosynthetic pigments are dispersed throughout the cytoplasm and lack chloroplasts.

6. The pusple sulphur bacteria have granular pigment structures lacking in detailed micromorphology of higher plants' Chloroplasts (Schacheman 1952)

7. Mitochondria is totally absent in blue-green algae while minute granules possessing all the metabolic functions of mitochondria can be obtained by ultracentrifugation of many species of true bacteria (Mudd 1953)

8. Ture mitochondria but of very simple structures with only a few internal folds are observed in yeast cells (Agar and Dauglas, 1957)

Thus Briggs concludes (1964):

It seems an entirely reasonable assumption that the protocells of the primitive earth were very simple structures lacking most of the organalles found in the cells of modern organism. Thus the protocells had:

(i) very simple inner structures.
(ii) and were full of properties.

INHERENT PROPERTIES OF MATTER:

Bahadur and Ranganayaki (1964);

Matter has inherent properties of duplication and adaptability and these are manifested under suitable conditions:

Thus: (a) Under suitable conditions matter duplicates itself (b) if system of matter in equilibrium is subjected to a constraint, a change occures if possible of such a kind that constraint is partially annulled.

Jordan (1938-40): On quantum mechanical resonance interaction special stability force consideration a systen AA is more stable than AB and B are similar but not identical.

Le Chatelier's Principle (1880) is the same as (b) described above.

If these are the properties of matter it should be possible to prepare objects showing the properites of growth, multiplication and maklolic activity.

Such units have been synthesised and have been named as Jeewanu:.

"Synthesis of Jeewanu, units capable of growth multiplication and metablic activity", Bahadur, K. et.al.. Zbl. Bakt., 117, 567-602 (1964)

These units contain a few free amino acids, a number of natural amino acids present in the form of peptides and the material of the units show esterase and phosphotase activities.

These units multiply by budding and their environmental medium contains only a few free amino acids photosynthesised in the mixture, the added organic substrate and inorganic salts.

The materials of the units are synthesised within the units, so the metablic activity.

A complete understanding of the process is published by Bahadur, K, 2bl. Bakt., 118, 671 (1964) under the title "Conversion of lifeless materials into living system."

The work is characterised by:

(i) a simple method for the aliogenic formation of the materials under the most common conditions of the nature without the need of any assumed condition of the earth.

(ii) and explanation for the formation of molecular associations with certain specific properties.

In April, 1964, Professor Briggs was invited by the British Interplanetary Society to give a talk on Jeewanu. In response he presented a paper in the socidty confirming the work on Jeewanu (1964).

M.H. Briggs presented another paper describing another series of experiments he performed to verify the work on Jeewanu, in the International Congress on Photobiology, Oxford (July 1964)

Confirming the work on Jeewanu Briggs reported the presence of following subtances in Jeewanu.

(a) peptides of about a dozen natural amino acids.
(b) sugars are glucose, fructose, ritose and desoxysibose.
(c) Purines as adenine and guanine.
(d) Urea and a number of amino acids.

He further reported that:

(i) These Jeewanu are stainable with biological dyes.
(ii) and gow and multiply.

Briggs Concludes (1964):

> "While the definitions of 'life' and 'living' are difficult problems, it can be said that these microscopic objects satisfy many of the criteria of living cells. It seems entirely probably that objects similar to those observed in the present experiments were formed in abundance in oceans of the primitive earth and were the immediate precursors of cellular life"

......oOo......

Some interesting photochemical reactions leading to the formation of morphological structures , Jeewanu.

It is interesting to observe the influence of Sun-light(1) on the following sterilised(2) mixtures. The mixture show the appearance of turbidity after certain hours of exposure to sunlight. This on microscopic examination show the formation of Jeewanu.

I Citric acid 1.60 gm, colloidal ferric oxide(3) 40 ml., colloial molybdenum oxide(4) 40 ml. and distilled water 140 ml.

The mixture was divided in two equal parts (one for light and another for dark) and sterilised in 250 ml conical flasks. One mixture was kept for exposure in Sun light and another one was covered with thick black cloth and kept near the exposed mixture all the time as a control. After 400 hours of exposure a turbidity appears in the exposed mixture which should be examined under the microscope. The turbidity increases with increasing period of irradation.(It is of interest to follow this for 1000 hours or more)

**II Tartaric acid 0.8 gm. , Colloidal ferric oxide 10 ml., colloidal molybdenum oxide 10 ml, buffer solution(5) of potassium dihydrogen phosphate and sodium hydroxide of 6.0 value 20 ml. and distilled water 60 ml.

The mixture was divided into two equal parts and one was exposed to light and the other one was kept in dark, after sterilisation in 150 ml conical flasks. A turbidity is observed after 600 hours of exposure,(Follow this turbidity under microscope)

III Citixaric acid 0.04 gm. , molybdehum oxide colloid 10 ml., ferric oxide colloid 10 ml., NaOH + KH_2PO_4 buffer solution of 6.0 pH value, 10 ml., mineral solution(6) 10 ml and distilled water 10 ml.

Two such mixtures were prepared . One was kept for exposure and another was for dark. After sterilisation ~~the mixtures were exposed~~ the mixtures were exposed in conical flasks of 150 ml capacity. ~~First~~ First day the mixture was exposed for one hour and then allowed to stand in the laoboratory for the night. Next day the mixture was exposed to sun-light for 8 hours and kept in the lab. Overnight. The turbidity was observed next morning.(Follow this turbidity microscopically for 15 days)

IV. Citric acid 0.04 gm, colloidal molybdenum oxide 10 ml, colloidal ferric oxide 10 ml., mineral solution 10 ml. and distilled water 20 ml.

The exposed mixtures show turbidity after 12 days.(It is of interest to follow the turbidity and its increase microscopically for one month or more)

V. Citric acid 3.2 mg., buffer solution of 6.0 pH value 40 ml., mineral solution 80 ml. and distilled water 280 ml.

The mixture was divided equally in eight different 150 ml. conical flasks. 0.08 gm. of anthracene wasdissolved in 5 ml of acetone. 1 ml. of this solution was added in each flask. The mixtures were sterilising Half of the sterilised mixtures were exposed to sun-light and half of these were kept in dark. The turbidity was observed after four days of exposure for 8 hours a day(Followthe turbidity for one month or more under the microspope)

---

(1) For all the above experiments sunlight was employed for 8 hours a day. The mixture were kept in dark for the rest of the day. The exposed and unexposed mixtures were kept near each other for all the time. These observations were exceedingly slow when visible light from 1000 Watts electric bulb was used as the source of irradiation.

(2) The flasks containing these mixtures were cotton plugged with cloth lined cotton plugs and sterilised at 15 lbs pressure for 30 minutes. The mouth of the flasks were then sealed with polythene paper before the experiments were started.

(3) Preparation of ferric oxide sol : The method adopted was one described by Debray and Krecke(1,2) which consist of hydrolysis of ferric chloride by slowly pouring concentrated solution of the salt in boiling water followed by dialysis in hot to remove hydrochloric acid and thus to avoid the reversal of the hydrolytic reaction. A concentrated solution of ferric chloride was added drop by drop in boiling water. The resulting deep red coloured sol was dialysed for seven days.

Conc. of ferric oxide in sol 0.80 gm./litre.

……………………………………………………………

(1) Debray, Compt. rend., 68 , 914 (1869)

(2) Krecke, J.prak. chem.(2) 3 , 286,295(1871)

(4) Preparation of molybdenum oxide colloid: Molybdenum trioxide sol was prepared by the method described by Graham(3). To a 5% solution of potassium molybdate a conc. solution of hydrochloric acid was added deop by drop with constant stirrinkg till a slight excess. Thw whole content was dailysed for 15 days and the sol was then found free from nitrogenous impurtties.

(3) Graham, Ann. , 135 , 65 (1865)

(5) Preparation of buffer solution of pH value 6.0. : 5.70 ml of 0.2 N sodium hydroxide and 50 ml. of 0.2 M acid potassium phosphate were mixed toget~~t~~her to give 55.70 ml of the buffer solution of pH 6.0.

(6) Preparation of the mineral solution: For preparing the mineral solution following chemicals were dissolved in 100 ml of distilled watee. Potassium sulphate 0.02 gm., sodium chloride 0.02 gm., calcium acetate 0.02 gm., magnesium sulphate 0.02 gm., zinc sulphate 0.002 gm.,

After dissolving the above minerals completely 0.02 gm. of potassium dihydrogen phosphate ~~solutin~~ was added to it and the mixture was shaken till whole thing went in solution.

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