PHOTOCHEMICAL FORMATION OF SELF-SUSTAINING COACERVATES

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The photochemical formation of amino acids using sunlight and ultra-violet rays has been investigated in detail. Formation of peptides in aqueous mixtures utilising different sources of energy and particularly, light has been recorded, as also the photochemical formation of microstructures with morphological look and having many chemicals of the present-day cells. This work was soon confirmed by Briggs (1965) and Mueller and Rudin (1970).

A simple process of photochemical formation of self-sustaining coacervates appeared in the journal of British Interplanetary Society in 1970, which can easily be performed as a laboratory exercise by the post-graduate students of Biochemistry, Photobiology, Cytology and Microbiology. These particles are synthesised in mixtures exposed to light and contain a number of aminoacids, peptides and material with enzyme-like activities. Nucleic acid bases as well as ribose and desoxyribose have been detected in these particles (under print in *J. Brit. Interplanet Soc.*). The particles have distinct boundary wall and their central region can be stained with gentian violet and the region between the boundary wall and the central region stains with eosin. This work is under print in *Zbl. Bakt.*

The boundary wall of the particles can be stained with sudan black. These particles on extraction with chloroform : methanol mixture yield a viscous yellow liquid which gives the tests of phospholipids.

Several micrographs of the particles showing the boundary wall and internal structures have been taken under optical and phase-contrast microscope.

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 technique of sterilization 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 (1908) and Ch. Lipman (1922) 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 the Earth to some other far distant planet.

The expressions of Mitcherlich, Engel, Huxley and others led to the present approach to the problem of origin of life finally taking definite shape by the postulate of Oparin (1924) and Haldane (1929) commonly known as Molecular or

Chemical Evolution theory. 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 taking place on earth (Bahadur 1967). 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 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 (Blum 1961; Bahadur *et al.* 1966).

The second reservation in the theory of molecular evolution is that it is taken for granted that the 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 (Bahadur 1967). At least so far as the size of molecules is concerned it may be quite probable. Today's cell has high molecular weight substances, like proteins and nucleic acids as its vital components and it is quite possible that other molecules existed in the earliest 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 (Bernal 1961).

As we know only one form of life on our earth, i.e. the protein life, it was considered quite probable that their precursors were made of at least amino acids and this initiated the study of the abiogenesis of natural amino acids in nature. With the same process of logic the abiogenesis 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 (1913) was the first to use electricity for the abiogenesis of amino acids. 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 (Miller 1953). Photo-chemical formation of amino acids in sterilized mixtures containing formaldehyde, ammoniacal or nitric nitrogen with iron or molybdenum as inorganic catalysts has been observed by Bahadur (1954) and Bahadur and Ranganayaki (1955). Pavlovskaya and Pasynskii (1957) obtained amino acids by exposing similar mixtures to ultra-violet rays. Many other sources of energy have been used for amino acid synthesis (Polstarff and Meyer 1912; Master 1957; Bahadur *et al.* 1958; Santamaria and Fleishmann 1966; Terenin 1959; Deschreider 1958; Cultrera and Ferrari 1960; Heyns *et al.* 1957; Paschke *et al.* 1957).

The formation of peptide bonds under natural conditions was another important aspect of abiogenesis. Fox and Harada (1958) 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 pockets near volcanoes.

Bahadur (1957) observed that peptides of low mol wt are formed in sterilized aqueous mixture containing amino acids and sugar as energy source and iron or molybdenum as inorganic catalysts on exposure to sunlight (Bahadur and Ranganayaki 1958; Bahadur *et al.* 1961 *a*, *b*, 1962; Bahadur and Pandey 1965). Formation of peptides in aqueous medium has also been studied by Calvin and Steinman using dicyanamide as condensing agent (Steinman *et al.* 1964, 1965).

Abiogenesis of other compounds of biological interests has been worked out and conditions leading to the formation of nucleic acid bases and sugars were discovered. Several detailed reviews of this are now available (Steinman and Kenyon 1969; Oparin 1963).

The important point in the origin of life is to investigate as how these molecules formed the earliest cells. The work on abiogenesis becomes pertinent in solving the problem of life synthesis only if living systems can be synthesised using these chemicals synthesised abiogenically.

Backward exploitation of the diversity of the phylology in abiogenic time suggests that to begin with there must have been few species (Pirie 1954). The work of 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 (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 functions more efficiently, but individually the cell loses its properties. Thus, though man is far ahead in evolution than a unicellular organism, no cell of his body has that many properties which a 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 (Bahadur 1964) and these were the precursors of cellular life.

The main problem of life synthesis is investigation of how such 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 nature we would have had only those earliest synthesised forms on our earth and not higher forms of life.

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 (Bahadur 1964, 1967; Bahadur and Ranganayaki 1964; Bahadur 1967) 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 duplication. 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 living beings 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 sometimes wonders whether there was ever 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 whether the living forms of high molecular weight molecules are the evolutionary 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 evolutionary product afterwards (Bahadur 1964; Bahadur *et al.* 1966). If so the smaller molecules can duplicate by the quantum mechanical resonance interaction special stability force considerations only (Jordon 1938; 1939 *a, b*, 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 effected 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.

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 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 to precisely define life 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 August, 1957 with Pauling as the chairman. Haldane (1954) defined a living system as a self-perpetuating system. According to Bernal (1959) embodiment of the self-perpetuating system within 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 environment, capable of multiplication and hetero-catalysis. According to Konikova, the Russian genetist, this definition can be made correct if we introduce the term 'living' after the polymolecular environment in the above definition but it obviously defeats its own purpose. Konikova 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 (Konikova 1959). Pirie (1959), however, did not agree to the limitation of growth and multiplication for the definition of origin of life for he 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 interactive, self-realisation of the potentialities of the atomic electron state.

It thus appears that it is very difficult to draw a line of demarcation between livings and non-livings and defining life is a hopeless job. However, it became necessary to atleast enumerate a few properties which a system must have before it can be called a 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 and Ranganayaki 1964). It will be possible to get living systems with fewer properties than these and they are established living systems due to other considerations and inanimate objects having one or two of these properties can certainly be found, but if a system has all the above three properties of growth, multiplication and metabolic activity, it may be considered as living.

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.

According to Pirie (1959) 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 (1959) 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 (1959) 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 (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 co-enzymes 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 (1964) and Bahadur and Ranganayaki (1964) suggested a probable locale for these forms of life.

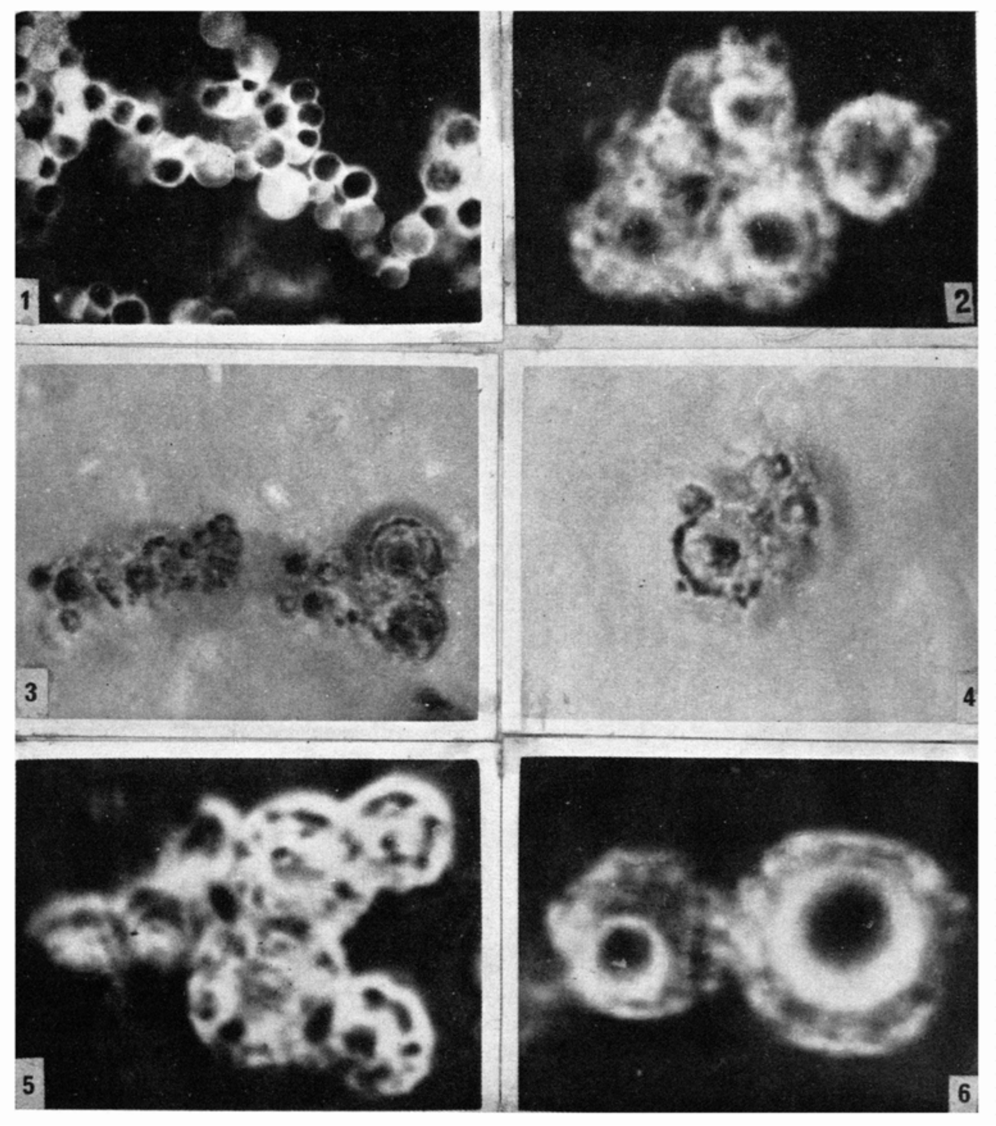
Cell Models

One of the important aspects 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 materials 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 is available (Oparin 1957 *a, b*, 1959, 1963).

Fox has 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 the environment. His efforts are to introduce the properties of growth, multiplication and metabolic activities by using suitable chemicals (Fox 1959; Fox *et al.* 1964).

Bahadur and co-workers (1964) synthesised a type of particles photo-chemically which they named as Jeewanu. In the sterilized 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 (Bahadur 1964, 1966, 1967; Bahadur and Ranganayaki 1970). Fig. 1 shows a general view of the particles. The internal structures can be clearly viewed under high magnification (Fig. 2). Micrograph of the particles under phase contrast microscope reveal clearly the boundary wall and the internal structure of the particles (Fig.3) and also the budding (Figs. 4 and 6). 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. These particles multiply by budding (Fig. 7) and the small buds grow to maturity size (Figs. 8 and 9) and bud (Fig. 5). Under the phase contrast microscope the structure and the boundary wall of the mother and daughter cells can be observed (Fig. 6). 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 microstructure and formed Jeewanu. These Jeewanu were capable of adaptability and so these evolved into the present day cellular life (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



FIGS. 1–6. 1, Micrograph showing clusters of the self-sustaining coacervates. × 1500; 2, Micrograph showing the internal structures of the particles. × 1500 and negative magnified; 3, Micrograph of the self-sustaining coacervates taken in phase-contrast microscope on coloured film. Boundary wall and internal structures of the particles on the top left are seen clearly. × 1500 and negative magnified in black and white. 4, Micrograph of the particles under phase-contrast microscope on coloured film showing boundary wall and internal structures × 1500 and negative magnified in black and white; 5, Micrograph of a group of particles under phase-contrast microscope showing boundary wall and internal structures. × 1500 and negative magnified; 6, Micrograph of two particles under phase-contrast microscope showing boundary wall and internal structures. × 1500 and negative magnified.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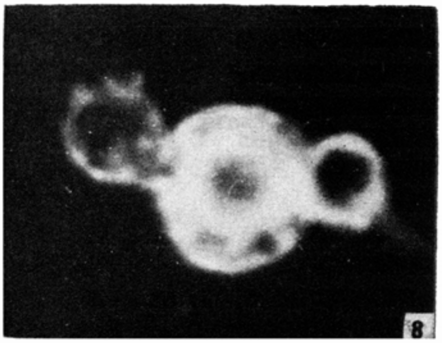
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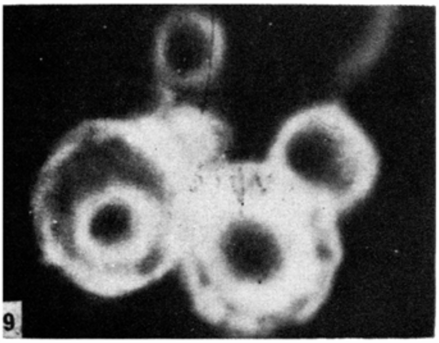
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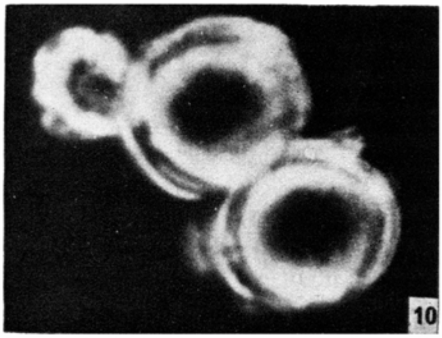
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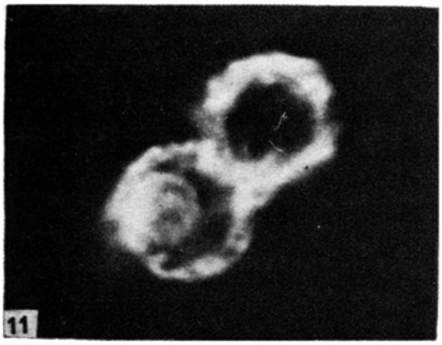
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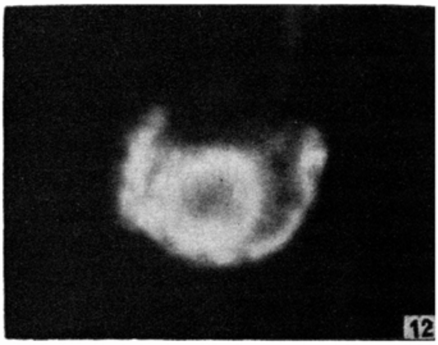
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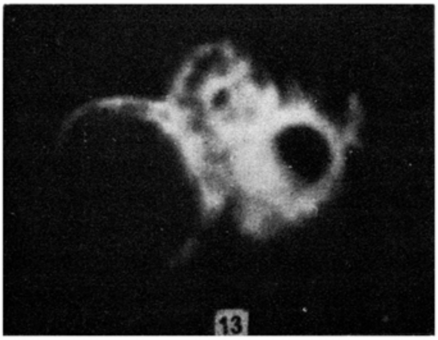
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Photobiology held at Oxford in 1964. He further repeated some experiments of Bahadur and published another confirmation in space flight in 1965. The work on Jeewanu was further confirmed by Mueller and Rudin, (1970). A complete review of the work on Jeewanu has been published (Bahadur 1967; Bahadur *et al.* 1966).

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 chemicals are needed to show each one of these properties and it is only after one such chemical 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 no specific chemicals are needed for any specific property (Bahadur *et al.* 1966).

Bahadur and Ranganayaki photochemically produced self-sustaining coacervates by the interaction of ammonium molybdate, diammonium hydrogen phosphate, minerals commonly found in cells and formaldehyde, in aqueous mixtures (Bahadur and Ranganayaki 1970). 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. 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 ethyl alcohol-soluble compound which on chromatography gives the test for phospholipids (Bahadur and Singh 1971). The particles on hydrolysis with perchloric acid or formic acid in a sealed tube give 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 hr, filtered and filtrate acidified with dilute acetic acida white precipitate is obtained which on subsequent hydrolysis gives the test of desoxyribose nucleic acid (Bahadur and Ranganayaki 1970; Ranganayaki *et al.* 1972).

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 (Bahadur and Gupta 1973).

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

The factors which are responsible for the natural formation of morphological looking objects have been discussed by Bahadur (1964, 1967) and Bahadur *et al.* (1966).

FIGS. 7–13. 7, Micrograph of the particles, one on the right has its wall broken at the top and the particle at the left shows a small bud at the top. × 1500 and negative magnified; 8, Micrograph of the particles apparent unit with two daughter units fairly grown showing boundary wall and internal structures. × 1500 and negative magnified; 9, Micrograph of the particles showing thick boundary wall and dense central portion each particle having a bud one bud can be observed separately. × 1500 and negative magnified; 10, Micrograph showing the boundary wall of the particles. × 1500 and negative magnified; 11, Micrograph of the particles showing thick boundary wall × 1500 and negative magnified; 12, Micrograph of the particles showing broken boundary wall × 1500 and negative magnified; 13, Micrograph showing broken boundary wall of a particle the internal material of which has gone out and only the broken boundary wall is seen like the broken shell of an egg. × 1500 and negative magnified.

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 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 together 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 results in a morphological looking structure. The outer material forms a boundary wall and the aggregate appears to have an intricate internal structure (Bahadur 1966). The boundary wall of a parent unit, a daughter unit and a grand daughter unit can be clearly seen in Fig. 10. The boundary wall can be broken (Fig. 12). Fig. 11 shows a cell with the internal material intact and another from which the internal material has flowed out. In this, a part of the internal material is still inside whereas, when all the internal material goes out the broken wall of the cell is observed to be like the broken shell of an egg as in Fig. 13. If these structures are present in an appropriate environment containing molecules which can form its body material and if these have 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 (Perti *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 the environment 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 — continued their living activity and the rest ceased their functions soon after the supply of the necessary molecules was finished up (Bahadur 1967; Bahadur *et al.* 1967).

Thus, was formed a self perpetuating system, as desired by Haldane (1929), 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 (1959).

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