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Copy of
Briggs Confirmation

Confirmation of the work on JEEWANU

By
Dr. M. H. Briggs.

" More recently Bahadur and Perti have described the formation of a series of cell-like microstructures (named by them "JEEWANU", a sanskrit word for "particles of life) by the action of sunlight or an UV lamp on sterilised solutions containing citric acid and a colloidal salt of molybdenum or iron. IT IS THE PURPOSE OF THIS PAPER TO REPORT A CONFIRMATION AND EXTENSION OF THIS WORK"

(page 129 line 18 to 22)

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Jet Propulsion Lab.

EXPERIMENTS ON THE ORIGIN OF CELLS

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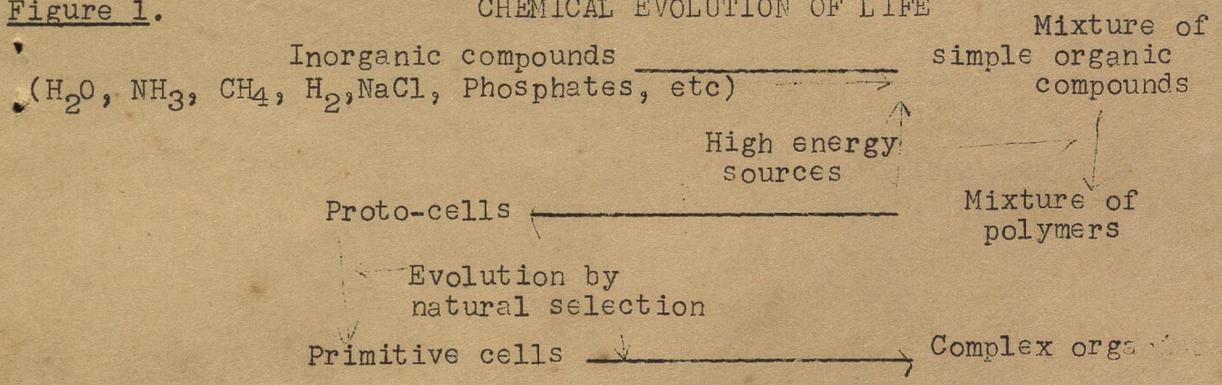
INTRODUCTION

It is now generally agreed that the first process for the originati of living cells with a terrestrial-type biochemistry is the formation of relatively large quantities of organic substances on a primitive and lifeless planet (1,2,3,4). It has been conclusively demonstrated by many workers that complex mixtures of organic compounds can be produced by the action of high-energy sources (UV, X-radiation, heat, Y-radiation, electrical discharges, etc.) on mixtures of simple gases such as methane, ammonia, hydrogen and water(4,5,6,7,8). It is known that the atmospheres of the outer planets are composed of just such gaseous mixtures, and there are strong reasons to believe that the primitive atmospheres of the inner planets had a similar composition(4,6).

It has been shown that simple sugars can be converted to polysaccharides(9), amino acids to polypeptides (10,11) and nucleotides to simple polynucleotides(12) by a continuation of the same mechanisms. The origin of life by chemical evolution can consequently be represented by Figure 1.

The outstanding problem at the present time is to account for the formation of the first molecular associations that would be recognisable as living cells.

Figure 1. CHEMICAL EVOLUTION OF LIFE



MORPHOLOGY OF PROTO-CELLS

When a simple mixture of organic polymers is compared to the complexity of any cell from within a living organism, tremendous differences are immediately apparent. A modern cell possesses highly organised inclusions, known as organelles (nucleus, mitochondria, microsomes, etc.) However modern cells are the end-products of several thousand, million years of evolution and not only have associations of cells (i.e., organisms) evolved but there is considerable evidence for an evolution of organelles(13).

Thus, the cytology of simple, modern organisms often provides evidence of this evolution.

For example, the nuclear apparatus of vertebrate cells is far more complex than that of even the most highly developed plants, in that the cells of seed-plants lack astral rays and well developed centrioles. When the nuclear cytology of the sulphur bacterium, Beggiatoa, is considered this organism lacks a discrete nucleus, but possesses numerous chromatin granules scattered throughout the cytoplasms. Reproduction appears to be entirely due to ingrowths of the cell-walls (14,15).

The somewhat more complex Rhodobacteriales (i.e., Chromatium) have their chromatin granules in association, but not covered by a nuclear membrane(14,15).

It is not until organism of the complexity of Euglena are examined that distinct chromosomes can be detected, and even with this organism spindle threads and interzonal strands are lacking (16).

Similar evidence of a slow evolution can be found for other organelles. Thus in the blue-green algae, the photosynthetic pigments are dispersed throughout the cytoplasm and chloroplasts are lacking. Granular pigment structures, lacking the detailed micromorphology of higher plant chloroplasts, can be found in the purple sulphur bacteria(17).

The mitochondrion shows a similar evolution. This organelle is totally absent from the blue-green algae, while minute granules possessing all the metabolic functions of mitochondria can be obtained by ultracentrifugation of many species of true bacteria(18). True mitochondria are present in yeasts, but are very simple structures with few internal folds(19).

It seems an entirely reasonable assumption that the proto-cells of the primitive earth were very simple structures lacking most of the organelles found in the cells of modern organisms.

MECHANISMS FOR THE ORIGIN OF PROTO-CELLS

Several authors (see review by Oparin[4]) have conducted experiments to duplicate the morphology of cells by interactions of simple inorganic and organic mixtures. While there is no doubt that the products obtained by many of these workers do bear a morphological resemblance to living cells, this is the only feature in common, in that the products are dissimilar in chemical composition, are metabolically inert, do not grow or reproduce, et Moreover most of these artefacts are produced from substances and under conditions that were probably quite absent from the primitive Earth. The only interesting products are those of Fox[20] who has shown that thermall synthesised proteionoids produce microspheres in water.

However, more recently Bahadur[21] and Perti[22] HAVE DESCRIBED THE formation of a series of cell-like microstructures (named by them "Jeewanu a Sanskrit word for "particles of life") by the action of sunlight or an UV lamp on sterilised solutions containing citric acid and a colloidal sal of molybdenum or iron. It is the purpose of this paper to report a confir mation and extension of this work.

THE LABORATORY SYNTHESIS OF PROTO-CELLS

A series of solutions was made up in 150 ml. conical flasks. Each solution was represented by four flasks. The composition of the various solutions is given in Table I. Each flask was plugged with cotton wool and then sterilised by autoclave. The cotton plugs in the flasks were th covered with polythene sheet and cellotape. 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 bulb continuously for a period of four months. Samples were taken using aseptic techniques at various intervals.

After this time the flasks were opened and samples of the contents 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 of 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 μ to 15 μ. 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 differeing quantities, were seen in all flasks. Highest yields were from flasks 1 to 5, though objects formed in flasks 6 to 9 showed more detailed micromorphology.

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 electrophoresis. Samples of solutions kept in the dark were similarly examined. Results are shown in Table II and indicate the fixation of atmospheric nitrogen for flasks 1 to 3.

Analyses of the precipitate by paper chromatography were also conducted for purines, pyrimidines, aromatic compounds, reducing sugars and urea. Table III presents a summary of those compounds tentatively identified.

Tests of the precipitates for enzymic activity have also been conducted. Esterase, peptiase 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. No peptidase activity could be found.

Considered together, the results presented above demonstrate that microscopic objects in the 0.5 to 15 μ size range can be formed by the prolonged action of light on solutions of simple compounds. Some of these objects possess a morphology similar to that of simple cells. The objects are composed of organic matter very similar to protoplasm. Some also possess weak enzymic activity. There is some evidence that

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the objects reproduce by budding and are not merely formed continuously from dissolved organic matter.

CONCLUSIONS

While the definition of "life" and "living" is a difficult problem, it can be said that these microscopic objects satisfy many of the criteria of living cells. It seems entirely probable that objects similar to those observed in the present experiments were formed in abundance in the oceans of the primitive Earth and were the immediate precursors of cellular life.

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COMPOSITION OF SOLUTIONS

Table I

1	2	3
Citric acid 0.8% ferric oxide sol. 15% molybdenum oxide sol.	Paraformaldehyde 0.2% molybdic acid 1.01% ferric chloride 0.01%	tartaric acid 1% molybdic acid 0.01% ferric chloride 0.01%
4	5	6
L-tyrosine 0.05% molybdic acid 0.01%	ferric chloride 0.01% L-tyrosine 0.05%	tartaric acid 1% ashed yeast* 0.1% diammonium phosphate 0.1%
7	8	9
L-tyrosine 0.05% ashed yeast 0.1%	paraformaldehyde 0.2% ashed yeast 0.1% diammonium phosphate 0.1%	citric acid 0.8% ashed yeast 0.1% diammonium phosphate 0.1%

* ashed yeast, with no organic contents, was used to simulate the primitive hydrosphere.

Table II AMINO ACID ANALYSIS

Solution in dark.	Solutions exposed to light			
	Supernatant	Precipitate	Hydrolysed supernatant	Hydrolysed Precipitate
no ninhydrin positive compounds	glycine alanine glutamic acid	glycine alanine glutamic acid aspartic acid + several peptides	glycine alanine glutamic acid	glycine, alanine glutamic acid aspartic acid histidine lysine arginine serine threonine phenylalanine tyrosine leucine valine

Table III COMPOUNDS TENTATIVELY IDENTIFIED IN MICROSTRUCTURES

Class of compound	Compounds	Detection Reagents on Paper Chromatograms
1. Purines	Adenine Guanine	(i) Silver chromate (ii) mercuric nitrate-ammonium sulphide
II. Reducing sugars	Glucose Fructose	(i) ammoniacal silver nitrate (ii) acid potassium permanganate (iii) aniline-diphenylamine
III. Aromatic compounds	Vanillic acid 3-hydroxy benzoic acid 4-hydroxyphenyl-acetic acid	(i) diazotisation
IV. Ureides	Urea	(i) phenol-hypochlorite

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