

## UNIVERSITY GRANTS COMMISSION

Bahadur Shah Zafar Marg  
New Delhi-110 002.

Application for getting Financial Assistance to attend International Conference/Symposium under 'Travel Grants' Scheme.\*

### A. BIO-DATA

1. Name of the applicant (in BLOCK letters) *Prof. KRISHNA BAHADUR*
- (a) Date of birth and age on the date of applying *20<sup>th</sup> Jan. 1926  
63 years*
2. Designation *Retired Professor and Head, Chemistry Dept. Univ. of Allahabad.*
3. Official address with pin code *Chemistry Department, University of Allahabad.  
211002*
4. In case of College, the University to which it is affiliated *—*
5. Field of specialisation *Origin of Life (Biogenesis)*
6. List of publications in the specific field (attach separate sheets) *I have published about 225 scientific papers.*
7. Whether a member of National/International professional bodies (circle the appropriate word) *~~Yes~~  
Yes/No*
8. If 'YES' specify the name of the body *1. Full member of International Society for the Study of Origin of Life  
2. Life member of Nat. Acad. Sci. India*

\* (1) Four copies of the application along with all the enclosures should be sent to Secretary University Grants Commission New Delhi-110 002.

(2) The proposal will be processed only if

- (a) all the columns of this application form are filled in and
- (b) the necessary enclosures are sent along with the proposal

9. Date of appointment to the present post *Appointed on 14<sup>th</sup> Aug 1950*  
*Retired on 30.6.1986.*
10. Date of confirmation in the substantive post *—*

### B. CONFERENCE DETAILS

11. Title of the Conference to be attended *International Society for the Study of Origin of Life, VI. ISSL Meeting + 9<sup>th</sup> International Confere of origin of life.*
12. Name of the organisers with complete address *The Laboratory of Evolutionary Biology, Czechoslovak Academy of Science, Prague, Czechoslovak.*
13. Duration of the Conference *July 3<sup>rd</sup> to 8<sup>th</sup> 1989*
14. The role of the applicant in the Conference/Symposium (put a mark  under the relevant column) Yes/No
- (a) Presiding/Chairing a Session (If yes, attach documentary evidence)
- (b) Delivering a plenary lecture/invited talk (If 'Yes' attach documentary evidence along with 4 copies of the full text/Summary of the lecture/talk that was sent to conference organisers)
- (c) Presenting a paper. (*file papers*)
15. Whether the Paper has been accepted for presentation? (If 'Yes' attach documentary evidence and 4 copies of the paper sent to the organisers) *Yes*
16. (a) Indicate the mode of presentation (attach documentary evidence) Yes/No
- (i) Oral
- (ii) Poster
- (iii) Both
16. Indicate whether the paper has been co-authored
- (a) If 'YES' give their names along with address Yes/No
- 1. Dr (Mrs) S. Ranganayak } Chemistry Dept.  
 2. Dr. Suresh Jeet Singh } University of  
 3. Dr. Sybil Kurnat } Allahabad  
 4. Dr. (Mrs) Manohar B. Choudhary }  
 5. Vandana Mathur }*

17. Has the 'no-objection' certificate (s) from the Co-author (s) have been enclosed? (attach photo copy of the certificate (s))

I am the chief author

Yes/No ✓

18. Indicate the complete travel plan from the proposed date and time of departure (from the place of working to the Conference) and back

Shall leave of 30th Jun 1989 and be back from Prague on 12th July 1989.

19. Do the Conference authorities send the papers for review before accepting?

~~Yes/No~~ Do not know ✓

(a) If 'Yes' attach the documentary evidence and also the criteria followed by them, if any

20. Indicate the amount to be paid to the organisers and the amount required from the Commission on the following heads, (attach (i) the communication from the organisers and (ii) letter from the travel agent regarding air fare)

To be paid to organisers

Assistance required from the Commission

(a) Travel within India to reach the nearest airport

Rs 1200'00

(b) Air-fare (Both ways)

Rs 23080'00

(c) Registration fee

Rs 3200'00

(d) Pre-diem required ( indicate the number of (days and the rate)

Rs 7500'00

Total (in Rs.)

Rs 35880'00

21. Has the applicant approached the organisers/any other agency to

Yes/No ✓

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- (a) Waive Registration Fee?  
 (b) Support Air Travel?  
 (c) Get the maintenance Allowance?  
 (d) Support boarding and lodging?  
 (e) Any other? (specify)

22. If 'YES' to any one of the above item, indicate the latest position and the amount likely to be made available (attach documentary evidence).

Not applicable.

23. Indicate the agency/institution to whom applied for meeting the remaining (50%) cost involved in attending the conference.

Not applicable.

24. Has the applicant availed the financial assistance from UGC for attending seminar/conference/symposium etc., in the last 3 years prior to the date of the present conference?

No.

Yes/No

(a) If 'YES' give the details in the following table.

Name of the Conference attended	Place and dates of Conference	Total Financial assistance availed (in Rs.)	U.G.C. sanctioned letter No. with date.
/			

25. Proposed date of joining duty in the Institution

Not applicable.

26. Any other information the applicant would like to give in support of the case

As university does not give financial assistance to the retired teachers I am applying to you for full support.

I certify that (a) the details given above are correct (b) if the information supplied is found to be incorrect on later date I shall reimburse the entire money to the Commission, (c) the money received will be used for the purpose for which it is requested, (d) in case financial assistance is received from the organisers or any other agency I shall pay back the amount granted by the Commission, and (e) I shall abide by the decision of the Commission.

Place: Allahabad.

Date: 15.4.89

Krishna Bahadur  
(Signature of the applicant)

Forwarded

Kly ada  
**HEAD**

Certificate by the Head of the Institution:

I certify that:

- (i) The details given by the applicant are correct.
- (ii) The applicant has not availed the provision in the last 3 years
- (ii) The college has been declared fit to receive financial assistance under section 12B of the UGC Act.
- (iv) The applicant has enclosed all the relevant documents/paper.

**DEPARTMENT OF CHEMISTRY  
UNIVERSITY OF ALLAHABAD**

Signature

[Handwritten Signature]

Name in Block letters

**REGISTERAR  
UNIVERSITY  
ALLAHABAD** A. B. SINGH

Designation

Registrar

Address

University of Allahabad

Office Seal

ate 17-4-89

## UNIVERSITY GRANTS COMMISSION

Bahadur Shah Zafar Marg  
New Delhi-110002.

### GUIDELINES FOR AVAILING THE FINANCIAL ASSISTANCE UNDER THE TRAVEL GRANTS SCHEME.

1. The scheme is open only to (i) college teachers and (ii) Research Associates and Post-Doctoral Fellows working full-time in Universities and Colleges either in connection with the research work or for attending International Conference.
2. In case of College teachers, the financial assistance will be limited to 50% (fifty per cent) of the total expenditure involved in travel, Registration fee and per-diem allowance.
3. In case of Research Associates and Post-Doctoral Fellows the assistance will be 100% of the total expenditure on the items mentioned above. However, the number of awards in a financial year shall be restricted to only ten in these cases.
4. Mere submission of the proposal will not be automatically eligible for getting the financial assistance.
5. The proposals received will be reviewed by the subject experts and based on their recommendation a final decision will be taken. Therefore, mere acceptance of the paper for presentation by the Organisers of the Conference/Symposium will not mean acceptance of the proposal by the Commission, even though it is one of the prerequisite for considering the proposal.
6. A proposal will be processed only if it is sent in the prescribed application along with the necessary enclosures, if the proposal is sent without the necessary enclosures, it will be summarily rejected and no further correspondence will be entertained.
7. The proposal will be considered only if the paper has been accepted for presentation in the conference/symposium.
8. A proposal will be considered only if it is received one month prior to the date of commencement of the Conference. No relaxation will be given in this regard.
9. The application is to be sent to the Commission duly forwarded by the Principal or the Head of the Institution.
10. If the paper is Co-authored then a no-objection certificate (s) from the author(s) who is (are) not going abroad has (have) to be enclosed along with the application.

11. In order to ensure prompt action in releasing the grant, the following information/papers have to be sent to the Commission as soon as the teacher/scholar comes back to India:

- (a) Statement of accounts giving details of expenditure incurred on various items viz. travel, air port tax, registration fee and daily allowance alongwith an attested copy of the permit issued by the Reserve Bank of India allocating Foreign Exchange of visit abroad of the teacher concerned.
- (b) Utilization Certificate in the prescribed form from the College Auditor for the total expenditure incurred on the visit.
- (c) Details of assistance received or facilities provided by the Organisers of the Conference or any other similar Indian/foreign agency.
- (d) Amount made available by the College/University/State Govt. and other sources for meeting 50% of approved expenses. (Not applicable in the case of Research Associates & Post-Doctoral Fellows)
- (e) Amount payable for each item by the U.G.C. as per terms and conditions.
- (f) A brief note on the participation of the teacher concerned in the International Conference and on visit to other academic institutions if excursion ticket has been purchased for the visit

COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH, RAFI MARG  
NEW DELHI

Application for Grant under Retired Scientist Scheme

## SECTION A: GENERAL

1. (a) Name(s) of the applicant(s): A. PROF. KRISHNA BAHADUR  
B. DR(MRS) S. RANGANAYAKI
- (b) Date of Birth : A. 20th January, 1926.  
B. 17th June, 1930.
2. Title of the Research Proposal : Photochemical Splitting of Water.
- (a) Broad area/field classification : Organic Chemistry, Biopoesis.
- (b) Names & addresses of 3-4 research scientists of standing in the field in the other universities/ research institutions in India actively engaged in the same area of research to whom a reference could be made.
1. Prof. O.N. Perti,  
Retired Professor and Head  
Chemistry Department  
Motilal Nehru Regional  
Engineering College,  
Allahabad  
9, Balrampur House  
Allahabad-211002
  2. Prof. (Mrs) Indra Kumari  
Verma  
Professor of Chemistry  
I.I.T., Hauz Khas  
New Delhi
  3. Prof. Vishnu Chandra,  
Chemistry Department,  
Gorakhpur University  
Gorakhpur
  4. Prof. R.P. Rastogi  
Vice-Chancellor, BHU  
Varanasi.

CERTIFICATE

I/we have read the conditions of 'Grant to Retired Scientists' and agree to abide by these if a grant is made.

*Krishna Bahadur  
Ranganayaki 6/3/90*

Signature and Date

## Declaration &amp; Attestation

I certify that the application is made with my approval and support and I agree to provide all facilities to the applicants.

Date

*Kly adams  
7.3.1990*

The Head of the  
Chemistry Department  
University of Allahabad

*D. S. Yadav*  
Signature of the  
Head/Executive Authority of the Inst.  
(Seal and Address)

REGISTRAR  
ALLAHABAD UNIVERSITY

3. Duration of research : Three years proposal

4. Amount of Grant proposed  
(Detailed break-up, justification may be given in Section C)

		<u>Ist year</u>	<u>2nd year</u>	<u>3rd year</u>	<u>Total</u>
		Rs.	Rs.	Rs.	Rs.
<u>A-Staff</u>					
1. Chief Investigators (two)	1.	60,000	60,000	60,000	180,000
	2.	60,000	60,000	60,000	180,000
2. Research Associates (two)	1.	26,400	27,600	28,800	82,800
	2.	26,400	27,600	28,800	82,800
3. Senior Research Assistants (two)	1.	25,200	25,200	25,200	75,600
	2.	25,200	25,200	25,200	75,600
4. Junior Research Assistants (three)	1.	21,600	21,600	21,600	64,800
	2.	21,600	21,600	21,600	64,800
	3.	21,600	21,600	21,600	64,800
<b>Total</b>		288,000	290,400	292,800	871,200

B. Contingencies

1. Chief Investigators (Two)	1.	15,000	15,000	15,000	45,000
	2.	15,000	15,000	15,000	45,000
2. Research Associates (two)	1.	10,000	10,000	10,000	30,000
	2.	10,000	10,000	10,000	30,000
3. Senior Research Assistants (two)	1.	10,000	10,000	10,000	30,000
	2.	10,000	10,000	10,000	30,000
4. Junior Research Assistants (three)	1.	5,000	5,000	5,000	15,000
	2.	5,000	5,000	5,000	15,000
	3.	5,000	5,000	5,000	15,000
		85,000	85,000	85,000	255,000

(A) Staff = Rs. 8,71,200  
 Contingency = Rs. 2,55,000  
 Grand total = Rs. 11,26,200

Rs. 3,75,400 per year for 3 years

SECTION B : BIO-DATA OF THE SCIENTISTS

1. Name : Prof. Krishna Bahadur  
M.Sc., D.Phil, D.Sc., D.I.C. (Lond)

Designation : Retired Professor and Head  
Chemistry Department,  
University of Allahabad, Allahabad-211002

Date of Birth 20th January, 1926

2. Educational Qualifications :

Degree	Institution conferring	Field(s)	Year
B.Sc.	University of Allahabad	Chemistry, Zoology, Botany	1944
M.Sc.	-do-	Chemistry- specialisation in Organic Chemistry	1946
D.Phil	-do-	Chemistry	1949
D.Sc.	-do-	Chemistry	1956
D.I.C.	Imperial College of Science and Technology, London	Microbiology	1962

3. Research experience  
during the last 5 years  
of service career

Institution	Year	Nature of work done	No. of papers published (list of papers with reprints may be given as Annexure
University of Allahabad	1984 to 1989	Study of origin of life, photo- chemical splitting of water and fixation of N <sub>2</sub> and CO <sub>2</sub>	About 25 (list attached)

4. Research Specialisation      Organic Chemistry, Plant Chemistry, Microbiology, Fermentation, Enzymology, Biopoesis (Origin of life). Photochemical splitting of water and Fixation of Nitrogen.
5. Research support received during the last 3 years or pending from various sources\*
- |   | Title of the project including CSIR Sanction No., if any | Total amount of grant | Total period of support with dates |
|---|--|-----------------------|------------------------------------|
| (a) CSIR Support past/present/pending               | - Nil -  |                       |                                    |
| (b) Support from other sources past/present/pending | - Nil -  |                       |                                    |

SECTION B : BIO-DATA OF THE SCIENTIST

1. Name : Dr(Mrs) S.Ranganayaki  
D.Phil, D.Sc.

Designation : Reader, Chemistry Department,  
University of Allahabad, Allahabad-  
211002

Date of Birth : 17th June, 1930

2. Educational Qualifications

Degree	Institution Conferring	Field(s)	Year
B.Sc.	Annamalai University	Chemistry, Physics, English	1950
M.A.	-do-	Chemistry- Organic	1952
D.Phil	Allahabad University	Chemistry	1955
D.Sc.	-do-	Chemistry	1961

3. Research experience during the last 5 years of service career

Institution	Year	Nature of work done	No. of papers published (list of papers with reprints may be given as annexure)
University of Allahabad	1984 to 1989	Study of Origin of Life, Photochemical Splitting of water and fixation of $N_2$ and $CO_2$	About 25 papers (List attached)

4. Research Specialisation : Organic Chemistry, Plant Chemistry, Enzymology, Microbiology, Fermentation, Biopoesis (Origin of Life), Photochemical splitting of water and fixation of Nitrogen

5. Research support received during the last 3 years or pending from various sources

	Title of the project including CSIR Sanction No., if any	Total amount of grant	Total period of support with dates
(a) CSIR support past/present/pending		- Nil -	
(b) Support from other sources past/present/pending		- Nil -	

SECTION C : DETAILED RESEARCH PROPOSAL

(a) Title of the Investigation : Photochemical splitting of water

(b) Summary of the proposed work:

Organo-molybdenum microstructures (OMM), were examined for their possible ferredoxin-like activity. It is because these microstructures prepared by the action of sunlight on sterilised aqueous mixture of ammonium molybdate, diammonium hydrogen phosphate, biological minerals and formaldehyde have a boundary wall ~~(1)~~ and internal structures <sup>(1)</sup>. It was observed by D.O.Hall et.al that these microstructures can be used in the place of ferredoxin in the system of chloroplast-ferredoxin-hydrogenase which is found in the algae, which are capable of splitting water to hydrogen and oxygen in sunlight. In photochemical splitting of water, ferredoxin is reduced to ferredoxin ~~from~~ <sup>by</sup> the electron liberated from water by the action of sunlight in the green algae through photosystems I and II. The electron of the ferredoxin is then transported to hydrogenase which produces  $H_2$  from  $H^+$  and the membrane of chloroplast helps in charge separation. For a membrane acting in energy conservating manner, the flow of electron must be towards the redox potential with high energy rather than from high to low redox level. The action of light is to provide a channel for dissociation of energy on existing redox potential along the membrane. Chemical nature of hydrogenase is yet not very clear. Colloidal platinum also acts as hydrogenase and many ferredoxins have hydrogenase like properties. OMM has characteristic membrane and this becomes important in its ability of splitting water molecules. Thus according to Hall, Rao et.al, chloroplast - OMM-hydrogenase system does split water into hydrogen and oxygen and the ferredoxin-like material of OMM is not destroyed by light and oxygen, like natural ferredoxin (2).

## EXPERIMENTS ON THE ORIGIN OF CELLS

Michael H. Briggs, D.Sc., F.R.A.S., M.I.Biol. Fellow  
Feed Service Ltd., Hartham Park, Corsham, Wiltshire.

PUBLISHED IN: Spaceflight, I, (4), 129-131 (1965).

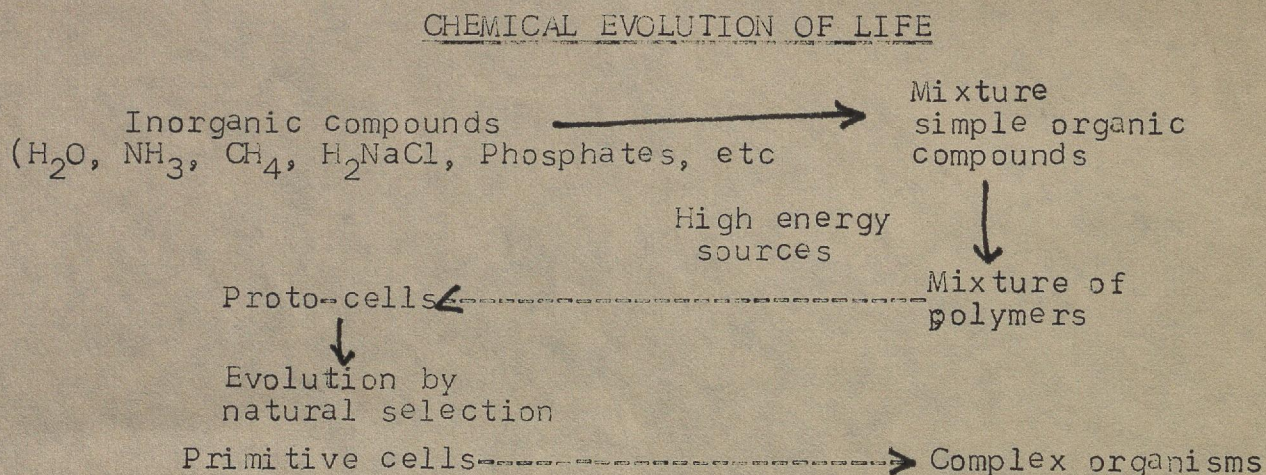
### INTRODUCTION

It is now generally agreed that the first process for the origination 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,  $\gamma$ -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 atmosphere 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.



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 cytoplasm. Reproduction appears to be entirely due to in growths 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) 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, etc. 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 thermal 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 sol. of molybdenum or iron. It is the purpose of this paper to report a confirmation and extension of this work.

#### THE LABORATORY SYNTHESIS OF PROTO-CELLS

A series of solutions was made up in 50 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 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 broth-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  $0.5 \mu$  to  $15 \mu$ . 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. Highest yields were from flasks 1 to 5, though objects formed in flasks 6 to <sup>9</sup> 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 precipitates 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, 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. No peptidase activity could be found.

Considered together, the results presented above demonstrate that microscopic objects in the 0.5 to 15  $\mu$  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. So also possess weak enzymic activity. There is some evidence that 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.

Table I

COMPOSITION OF SOLUTIONS

1	2	3
Citric acid 0.8% ferric oxide sol. 15% molybdenum oxide sol. 15%	Paraformaldehyde 0.2% molybdic acid 0.01% 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
i. 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	Vanilic acid, 3-hydroxy benzoic acid, 4-hydroxyphenyl-acetic acid.	
iv. Ureides	Urea	(i) Phenol-hypochlorite

REFERENCES

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20. S.W.Fox, "The origin of Prebiological Systems" Academic Press (1964).
21. K.Bahadur & S.Ranganayaki, Vijnana Parishad Anusandhan Patrika, 6, 63 (1965).
22. O.N.Perti, Agra Univ. J. Res. (Sci.) 12 (II) 1 (1963).
23. S.Natelson, "Microtechniques in Clinical Chemistry", Thomas : Springfield, III (1963).

GENETIC TAKEOVER AND THE MINERAL ORIGIN OF LIFE

by

A. G. Cairns Smith

Cambridge University Press, Cambridge (1982), p. 424

Evolutionary studies at all levels should give us further insights as to general evolutionary mechanisms, for example the roles of symbioses and of takeovers.

Bahadur and his school (see, for example, Bahadur & Ranganayaki, 1970; Smith, Folsome & Bahadur, 1981) have been studying microstructures-complex budding vesicles that Bahadur calls 'jeewanu' - which are generated when certain solutions are precipitated in sunlight (for example solutions containing molybdate, ammonia, phosphate and formaldehyde). Synthesis of a number of our biochemicals has been claimed. It will be interesting to know how these complex structures form. Is this a case of osmotic construction? To what extent is their compartmentalised structure relevant to photosynthetic activity? Perhaps the earliest photosynthesising organisms were a subclass of jeewanu, a kind whose structure was guided to some extent by inorganic crystal genes.

By the start of Chapter 9 we were beginning to think about the kinds of polymers that would have been available for structural purposes once the consistent synthesis of particular chirally homogeneous monomers could be presupposed. Insights into possible first uses for homopolymers and regular copolymers can come, as we saw, from studies of polysaccharides, sugar phosphate polymers, polynucleotides and 'proproteins'-both natural and synthetic. Could we design molecules for holding together clay particles? Or could we even invent a 'nucleic acid enzyme' (with oligonucleotide coenzymes to go with it)?

In considering first forms of nucleic acid replication and the evolution of our protein-synthetic machinery, we are

coming to the final stages of the evolution of our kind of life. But advances in understanding here could still be relevant to the question of genetic takeover. Such advances should tend to favour or refute the contention that the invention of our nucleic acid-protein control machinery could only have been made by organisms, highly evolved organisms, that had had to manage without any such machinery to begin with.

A useful life ? To come back to the very beginning, there are practical reasons for being interested in biogenesis if indeed first forms of life were mineral and could be recreated. Here would be a new technique of fabrication at the otherwise difficult, colloidal, size level-through artificial selection of replicating defect structures. For example, exceedingly finely engineered semiconductor devices would become available, made from the cheapest possible materials. It is such devices that are perhaps most needed to solve the problem of converting cheaply and efficiently solar energy into other forms.

## FORM D

## SECTION-B : BIODATA OF THE SCIENTIST

S.No. 4(a) List of honours & awards conferred on the I/c:  
About Prof. Krishna Bahadur

- (1) Nuffield Fellowship in 1960, worked in the Imperial College of Science and Technology, London for one year (This fellowship is awarded to 2 Indians every year and selection is made by the Nuffield Foundation, England).
- (2) Exchange Scientist in 1963, worked in the State University of Florida at Talahassec for seven months.
- (3) Canadian Commonwealth Travelling Research Fellowship in 1970. Worked in Sir George Williams University for one year (Fellowship is offered 2 Scientists only from whole of the Commonwealth countries)
- (4) Biography appeared in:
  - (a) Dictionary of International Biography, Seventh Edition (1970-71), Edited by Ernest Kay, Artillery Mansion, Victoria Street, London, S.W.1.(England)
  - (b) American Men of Science, 12th Edition, The Jacques Cattell Press, Tompe, Arizona, (U.S.A.)
  - (c) World Who's Who in Science  
(From Antiquity to the Present)  
Marquis Who's Who Incorporated,  
200 East Ohio Street, Chicago, Illinois (U.S.A.)  
The last one is concerning Scientists from anti-  
quity to the present of all over the world and  
has thus bibliography of Charak, Bhaskara, Socrates  
or Aristotle.
- (5) Written several books. The list of awarded books:
  - (a) Antibiotics - 1976-77, Uttar Pradesh Hindi Sansthan, Lucknow.
  - (b) Chandrama ki Yatra - Awarded by Govt. of India -  
Children's Book Award (*Hindi and English editions*)
  - (c) Naveentam Awishkar - Jet Viman  
Awarded by Uttar Pradesh Government.

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  - (c) World Who's Who in Science  
(From Antiquity to the Present)  
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Children's Book Award (English and Hindi editions)
  - (c) Naveentam Awishkar - Jet Viman  
Awarded by Uttar Pradesh Government.

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UNIVERSITY OF ALLAHABAD  
DEPARTMENT OF CHEMISTRY  
IN P. C. CAMPUS OF SPECIAL RESERVES

University Grants Commission  
Support for Science Research in the Universities  
Proposal for Support of Research

Applications of the Studies on Origin of Life

Photochemical Splitting of Water and Fixation of  
Nitrogen and Nitrogen Loss by Organo-  
Molybdenum Microstructures, the Molybdenum  
Jeevanu, model of the Prokaryotes.

By

Krishna Bahadur

M.Sc., D.Phil., D.Sc., D.I.C. (Lond.)

Chemistry Department  
University of Allahabad  
Allahabad.



UNIVERSITY OF ALLAHABAD  
DEPARTMENT OF CHEMISTRY  
( U. G. C. Centre of Special Assistance )

University Grants Commission  
Support for Science Research in the Universities  
Proposal for Support of Research

1. Title of the project :

Applications of the Studies on Origin of Life.

Sub-title: Photo<sup>chemical</sup> splitting of water and fixation of nitrogen and nitrogen loss by Organo-molybdenum microstructures, the molybdenum Jeevana, model of the Proto cells.

2(a) Institution and address: ←

University of Allahabad, ~~India~~ Allahabad, India.

(b) Department: Chemistry Department, University of Allahabad, India.

(c) Principal Investigator and other members of the research group if any :

Principal Investigator: — Prof. Krishna Bahadur, <sup>Retired Professor and</sup> Head Chemistry Department, University of Allahabad, Allahabad.

To be Associated: — Dr (Mrs). S. Ranganayaki, <sup>Retired Professor</sup> ~~Assistant~~, Chemistry Department, University of Allahabad, Allahabad.



# UNIVERSITY OF ALLAHABAD

DEPARTMENT OF CHEMISTRY

( U. G. C. Centre of Special Assistance )

2

3. Academic qualifications and research experience of the investigators and publications related to the above research projects. Please indicate the research schemes already undertaken and in-force with funds from Commission or any other agency, giving details of the grants and number of persons employed, duration etc.

(A) INFORMATION REGARDING THE PRINCIPAL INVESTIGATOR

Retired Professor and Head

- (a) Name & Designation : Dr. Krishna Bahadur, ~~Reader~~ Chemistry Department, University of Allahabad, Allahabad 211 002
- (b) Brief Bio-data indicating his specialised interest particularly in relation to proposed research work : Born on 20th Jan. 1926, educational qualifications : M.Sc., D.Phil., D.Sc., D.I.C. (Lond.). Have published more than 300 scientific papers and 45 students have been awarded D.Phil. degree under me. Nuffield Fellow (1960), Exchange Scientist (1963), Canadian Commonwealth Research Fellow (1968). Have been working on <sup>Chemical</sup> Photolytic splitting of water ~~by~~ and fixation of nitrogen since last 5 Yrs. I have 34 Years of experience of teaching and guiding research.
- (c) List of Important publications in this or related field : Attached ~~Appendix~~
- (d) List of research scheme : None being carried out by principal Investigator with financial support from various agencies/ organisations

by organo-molybdenum microstructures

(B) INFORMATION REGARDING OTHER RESEARCH SCIENTISTS TO BE ASSOCIATED WITH THE INVESTIGATION

- (a) Name and designation : Dr. (Mrs) S. Ranganayaki, D.Sc., ~~Reader~~ <sup>Retired Professor</sup> Chemistry Department University of Allahabad
- (b) Brief Bio-data indicating her specialised interest particularly in relation to proposed research work : She is D.Phil. and D.Sc. in chemistry and guiding research since last 25 Years. About 25 research students have been awarded D.Phil under her and she has published about 150 scientific papers. She is working on this problem for the last several years. ~~and is retiring in June, 1978.~~
- (c) List of publications in this or related field : In most of my papers she is Co-author.
- (d) List of other research scheme with which the scientists is associated : None

(4) Brief Outline of Objective or work and its importance. In case it has any R and D importance please substantiate: (3A)

Organised microstructures are synthesised in certain sterilised aqueous mixtures of simple organic and inorganic substances when these are exposed to sunlight or other sources of energy. ~~These~~ These microstructures have ~~low~~ boundary wall and internal structures and under specific conditions may show the properties of growth from within, multiplication by budding and some metabolic activity. <sup>(see the attached copy of the P.A. M.H. Briggs)</sup> Of the several types of such microstructures investigated by our school the organo molybdenum microstructures, the molybdenum Jeevans are specially interesting because they have many of the biochemicals of the present-day cells.

← Study of the functional properties of organo molybdenum microstructures (OMM) as model for Protocells :-

OMM, a type of model of protocells, have been examined for ferredoxin - like activity. It is because these microstructures prepared by the action of light on sterilised aqueous mixture of ammonium molybdate, diammonium hydrogen phosphate, mineral solution and formaldehyde, have boundary wall, a central mass and chemicals of interest in the study of origin of life (1,2,3,4). That they have ferredoxin - like material is evident because they can be used in the place of ferredoxin in the photobiological system, chloroplast-ferredoxin - hydrogenase (5,6). These microstructures were tested for nitrogenase-like activity. It was observed that if an aqueous mixture of OMM having acetylene in the overhead space is exposed to light from a Xenon lamp, the amount of acetylene decreases and ethylene increases with the period of exposure (7).

If OMM and D<sub>2</sub>O mixture is exposed to the radiation from Rayonet UPR 2337 nm keeping acetylene in the overhead

(3B)

space it is converted into  $\text{CHD} = \text{CHD}$  indicating that the protons come from water and that these particles are capable of splitting water (8). On irradiation of an aqueous mixture of OMM and  $\text{NaH}^{14}\text{CO}_3$ , with 254 mm,  $^{14}\text{C}$  was found in the organic material formed in the mixture (8) indicating fixation of carbon.

Bubbles of gas are observed to be evolved when an aqueous mixture of OMM is exposed to sunlight. The evolution of gas continues for 4 or 5 days and then ceases but it stops when sunlight is cut off. If the mixture is allowed to stand in dark overnight and then exposed to sunlight on the next day, again gas is evolved.

Warburg's study indicated that on exposure of OMM suspended in water to sunlight, hydrogen and oxygen are produced. As OMM have nitrogenase-like material the hydrogen produced combines with nitrogen in the ratio of 1 vol. of nitrogen to 1 vol. of hydrogen indicating the formation of diimide ( $\text{NH} = \text{NH}$ ) as an intermediate compound. If nitrogen fixation is inhibited a mixture of hydrogen and oxygen is obtained.

(5) Actual plan of the work proposed;

Experimental approach :- On treatment of the OMM with 1% sodium carbonate solution a fraction of OMM dissolves in it and insoluble bluish rod-like structures are left in the mixture. The dissolved fraction can be precipitated with

HCl as a yellow precipitate. This is some complex of molybdenum which is capable of splitting water and fixing nitrogen.

In another specific mixture in which OMM are produced on exposure to sunlight, in the beginning for a few days only spherical microstructures are formed. *after 18 hr. of exposure to sunlight in three* Then ~~one~~ <sup>days</sup>, after the day's exposure, the mixture shows the presence of only blue-rod structures which appear like crystals under the microscope. On keeping this mixture in dark during the night the next morning the mixture is full of blue, spherical particles of 1 to 2  $\mu$  in diameter. These show distinct boundary wall and some dense mass in the center under phase contrast microscope. When this mixture is exposed to sunlight on the next day, by the evening the mixture again has only rectangular blue structures which appear like crystals under the microscope. This mixture, on standing in dark during night is again full of spherical particles the next mornings and the process can be repeated for about one week when finally this interchange stops and only blue, rod shaped microstructures are left in the mixture. <sup>we</sup> I wish to investigate the relationship between blue ~~rod~~ rod structures like crystals and the spherical OMM with boundary wall, both of which in preliminary examination by X-ray diffraction appear non-crystalline, employing extensive X-ray diffraction and other technics. <sup>we</sup> I want to determine the chemical-physical factors that underlie the OMM structure and functional attributes.

Microstructures with a specific morphology, with ability to utilise the energy of sunlight for not only their formation but even for splitting of water in sunlight is of considerable interest in the study of origin of life because this process will set up an energy flow through the system and this will provide additional stability. If to the mixture in which OMM are produced on exposure to sunlight, some soluble salts of semi-conductors and/or transitional elements is added some of these are incorporated in the OMM formed and their properties of photochemical splitting of water and fixation of nitrogen is significantly improved. By incorporating suitable amount of Mn, Cd, Co, Zn, La and Tc in the mixture of OMM efficient OMM can be prepared which may become of commercial interest. We have already done some work on these lines.

Some quantitative idea of photochemical splitting of water and fixation of nitrogen by the OMM :-

6

Incorporation of lanthanum and then titanium in OMM renders them more efficient. The following table indicate the pressure changes observed on exposing 20 mg. of OMM suspended in 5 ml. water taken in a conical Warburg's flask (WF) of 14.5 ml. capacity and bottom area  $9.5 \text{ cm}^2$ . The pressure increase on an exposure of 30 minutes to sunlight (i), and the pressure decrease during this period as estimated by the decrease in pressure during the next 30 minutes on keeping the flask in shade (ii) were recorded. From this the amount of hydrogen set free per day per square meter using 20 gm. of the OMM on six hours of exposure (iii) and also the hydrogen consumed during this period in grams (iv) were calculated, assuming that the mixture is changed after each 30 minutes exposure.

Reference number of OMM	Condition	Pressure in W.F. in cm. of mercury in 30 min exposure (i)	Decrease in pressure in 30 min in shade in cm of mercury (ii) in WF	Net increase in process in cm of mercury by photolysis of water in the WF	g of H <sub>2</sub> produced in g / m <sup>2</sup> /day (iii)	g of H <sub>2</sub> used up in N <sub>2</sub> fixation in g/m <sup>2</sup> /day
HM (Modi) J (Ac) 60	Oxygenic	4.75	-1.0	5.75	4.00	0.70
HM (Modi) J (Ac) La60	Oxygenic	5.57	-5.0	10.50	7.60	4.57
HM (Modi) J (Ac) La Ti60	-do-	8.30	-3.5	11.80	8.32	2.50
HMJ60	anoxxygenic	8.00	-6.00	14.00	14.70	11.00

The gas transformation of the modified OMM when their aqueous mixture is exposed to sunlight and subsequently kept in shade will be investigated using gas chromatograph and Warburg's apparatus with time and some scanning will be done to see whether the process of water splitting and nitrogen fixation can be enhanced in some of these OMM at some particular pH using suitable buffer.

~~Actual plan of work proposed;~~

88

In presence of OMM the fixation of nitrogen continues even after the exposure is stopped if the hydrogen set free by splitting of water is available under the condition when rapid fixation of nitrogen takes place it is usually followed by loss of nitrogen. The ability of OMM of photo~~lytic~~chemical splitting of water has been considerably increased by adding titanium sulphate and acetic acid in the mixture used for the preparation of OMM. *we* wish to prepare OMM by introducing silicon in these by adding soluble sodium orthosilicate in the mixture before exposure and to examine the OMM produced for their ability of splitting water in sunlight and fixation

of nitrogen. For this the time lapse study of the gas exchanges in sunlight and subsequently in shade will be carried out using gas chromatograph. *we* also wish to explore the possibility of utilising the OMM or some of its modified variety for the commercial exploitation of solar energy.

(a) the sponsoring institutions

We have been investigating the photolytic <sup>chemical</sup> splitting of water by the molybdenum <sup>Ten</sup> ~~six~~ years and have prepared several modified MJ incorporating transitional elements and semi-conductor elements in these structures. During this investigation which was carried out, using Warburg's apparatus and chemical estimation, it was found that some of these microstructures are strong nitrogen fixers on exposure to sunlight. A few of these show good reductive fixation of nitrogen whereas some are good oxidative fixers of nitrogen.

(b) Research work done and progress in India

This work is not in progress anywhere else in India.

(c) Research work done and progress in abroad

We formed a collaboration with Dr. D.O. Hall of the London University, King's College, London and in this collaboration we discovered that OMM have ferredoxin-like material and these can substitute ferredoxin in the photobiological system, Chloroplast-ferredoxin-hydrogenase, found in some algae which split water to hydrogen and oxygen in sunlight. The work has been published (5).

We then formed a collaboration with Dr. A. Smith of NASA Ames Research Center, Moffett Field, California and Dr. C. Folsome of Hawaii University to investigate the nitrogenase-like properties in the OMM. It was observed that if  $D_2O$  and MJ mixture is exposed to light keeping acetylene in the overhead space,  $CHD = CHD$  is formed in the overhead space of the mixture. It was further observed that an aqueous mixture of OMM and  $NaH^{14}CO_3$  on exposure to light shows the presence of  $^{14}C$  in the organic materials synthesised in the mixture indicating that OMM has nitrogenase-like material and is also capable of fixing inorganic carbon to organic carbon. The work has been published (8). It was subsequently observed chemically that OMM are capable of fixing molecular nitrogen and carbondioxide (9,10).

Even earlier in 1957 we had formed a collaboration with prof. L. Santamaria of the Institute of General Pathology Milano (Italy) to investigate the problem of nitrogen fixation utilising the energy of light employing colloidal molybdic acid as catalyst. The work was published in Nature (11). This work was confirmed recently by the work of Schrauzer (6) who reported that molybdenum has nitrogenase-like activity.

TECHNICAL PROGRAMME

10

(a) Phase one : STUDY OF THE REDUCTIVE FIXATION OF NITROGEN

(i) Several molybdenum Jeewanu will be examined for their ability of fixing nitrogen in the form of diimide, hydrazine and ammonia using Warburg's apparatus and by chemical estimation.

(ii) The optimum pH for the reductive fixation of nitrogen will be investigated using Warburg's apparatus and gas analysis by gas chromatogram.

(iii) The effect of presence and absence of oxygen in the reductive fixation of nitrogen will be investigated.

(iv) After determining the favourable conditions for reductive fixation of nitrogen as observed by the above experiments the amount of nitrogen fixed will be estimated by chemical methods.

(v) Gas chromatographic estimation of the various constituents formed during the exposure of various irradiation mixtures will be done.

(b) Phase two : STUDY OF OXIDATIVE FIXATION OF NITROGEN

(i) The effect of pH on the oxidative fixation of nitrogen will be determined and optimum pH for this fixation of a few samples of OMM will be undertaken using Warburg's apparatus.

(ii) The amount of nitric nitrogen fixed by different OMM under optimum conditions will be determined using :

(a) Warburg's apparatus

(b) Chemical methods.

(iii) Experiments will be planned to segregate alkaline nitrogen fixation products from the acidic fractions to avoid formation of  $NH_4NO_2$  by devising suitable apparatus. To begin with two lobed Warburg's flask will be used and study will be made using Warburg's apparatus. Subsequently simple apparatus for this will be devised.

(c) Phase three : SEARCH FOR SUITABLE INHIBITORS OF NITROGEN LOSS REACTIONS

(i) Investigation of suitable inhibitors for nitrogen loss due to decomposition of diimide will be undertaken. Usual inorganic inhibitors as iodide, mercuric ions, copper ions, cyanide ions and others will be examined.

(ii) Inhibitors of loss of nitrogen via ammonia nitrite will be searched.

(iii) If the effort of searching suitable nitrogen loss inhibitors fail, an effort to segregate the primary components of the final decomposition product will be undertaken.

Anticipated results and their potential impact in understanding  
the origin of life :-

12

Organo-molybdenum microstructures (OMM) are models for protocells which have several chemicals of the present-day cell but are basically inorganic and have about 44.50% of molybdenum (9). These have <sup>some</sup> ~~certain~~ functional properties as splitting of water in sun light and fixation of nitrogen. By incorporating semiconductors and transitional elements in these we may be able to produce particles which are efficient in these functional properties. If the secondary atmosphere of the prebiological era was made of carbon dioxide, nitrogen and water vapours and there was not enough organic matter on the surface of the earth or in the ocean, particles like these could act as chemical precursors to photoautotrophs. If efficient OMM could be prepared may be

these could be used for commercial splitting of water utilising the energy of sunlight and the study of origin of life could be put to some economic use. (See the last page of Cairns-Smith's book "Genetic Takeover" attached.)

(b) Duration of the project: Three years.

(7) Detailed estimates of the expenditure to be incurred on the project (on annual basis and not to exceed three years in First phase):

(a) Non recurring: ~~Nil~~ Nil.

(b) Recurring per year: Additional facilities required which are chargeable to the scheme per year.

- (i) Equipment and apparatus (Purchase of carrier gas and other accessories) - Rs. 20,000
- (ii) Land/livestock - Nil
- (iii) Laboratory and Office facilities, etc. - Chemical and glasswares Rs. 20,000  
Stationary Rs. 5,000  
Postage, travelling, etc. Rs. 45,000

DURATIONS: Three years

Personnel:  
STAFF REQUIREMENTS

Sl. No.	Designation of post	No. of post	Scale	Qualifications prescribed
1.	<u>Junior</u> Research Fellow	2	Rs. 1800/- p.m. (fixed)	M.Sc. in Chemistry
2.	Senior Research Fellow	2	Rs. 2100/- p.m. (fixed)	M.Sc. in Chemistry with Research experience
3.	Research Associate	2	Rs. 2200/- p.m. (fixed) <del>2700/- p.m.</del> @ Rs. 100/- Annual increment	D.Phil.
4.	Prof. Krishna Bahadur (Chief investigator)	1	Rs. 5000/p.m.	Qualification mentioned
5.	Dr. (Mrs.) S. Ranganayagi	1	Rs. 5000/p.m.	

ESTIMATED COSTS

Name of Post	Scale	Ist year	IIInd year	IIIrd year	Total
Chief investigator -	Rs. 1500/-	18,000	18,000	18,000	54,000
Research Fellow(2)	Rs. 800/-	19,200	19,200	19,200	57,000
Research Associate	Rs. 1200/-	14,400	14,400	14,400	43,200
		Rs. 51,600	Rs. 51,600	Rs. 51,600	154,800

(14)

5/2/4

(14) Working expenses;

	per year in Rs.	in three years Rs.
1. Pay of officers per year	Rs. <del>1,54,800.00</del> Rs. 51,600	154800.00
2. Pay of establishment One Office assistant @ 1000/p.m. One lab attendant @ 800/-p.m.	12,000.00	36,000.00
3. Allowances and honoraria	Nil	Nil
A. Dearness Allowances	Per rule	per rule
B. House Rent	Nil	Nil
C. City Compensatory Allowance	Nil	Nil
D. Other Allowance	Nil	Nil
E. Travelling Allowances	Rs. 5,000.00	15,000.00
4. Contingencies		
A. Recurring	Rs. 45,000.00	135,000.00
B. Non-recurring	Nil	
<b>Total</b>	Rs. <del>2,10,800.00</del> Rs. 2,13,600	340,800.00

Total Pay of officials	Recurring	Non-recurring	Total per year
Rs. <del>1,54,800.00</del> Rs. 51,600	Rs. 62,000.00 p.a.	Nil	Rs. 2,13,600 p.a.

28  
330625  
57

Total Recurring <sup>expenditure</sup> for the duration of the project

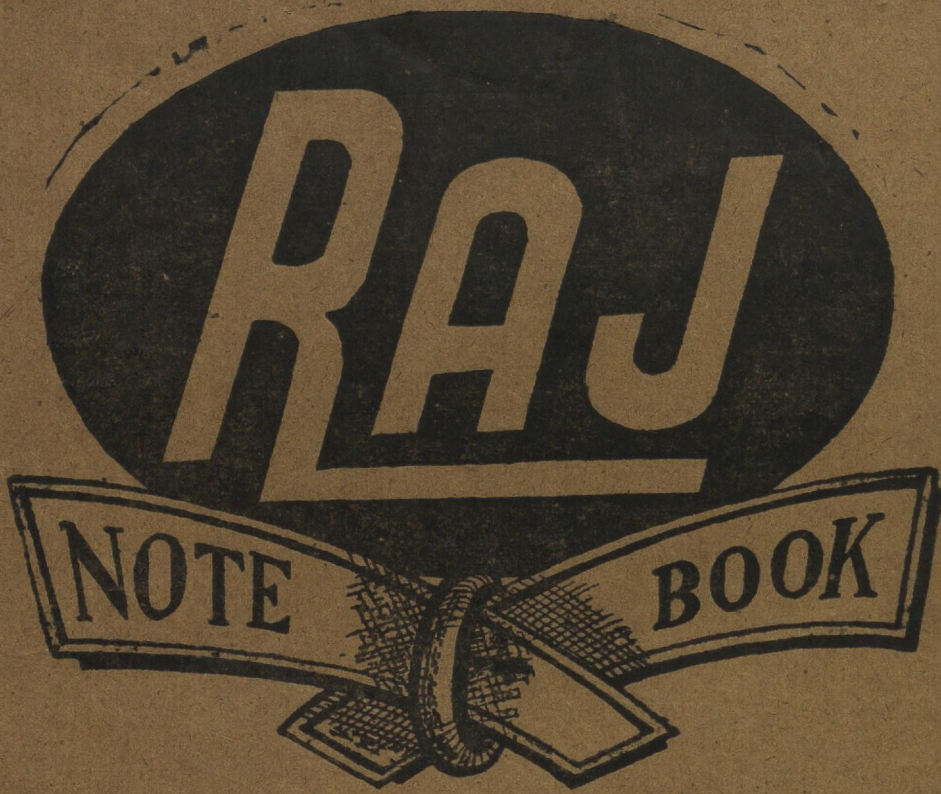
Per year Rs 1,13,600.00

In three years Rs 3,40,800.00

Total Non-recurring and recurring <sup>expenditure</sup> Rs 3,40,800.00  
in three years.

Please give names and address of three persons in other universities actively engaged in your area of research interest to whom a reference may be made regarding the research project:

1. Prof. O. N. Perti,  
Retired Professor and Head  
Chemistry Department  
Motilal Nehru Regional  
Engineering College,  
Allahabad.  
9, Balrampur House,  
Allahabad-211002.
2. Prof. (Mrs) Indra Kumari  
Verma  
Professor of Chemistry  
I. I. T. Hauz Khas, Khas  
New Delhi.
3. Prof. Vishnu Chandra,  
Chemistry Department,  
Gorakhpur University  
Gorakhpur.
4. Prof. R. P. Rastogi  
Vice-Chancellor, BHU  
Varanasi.



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• \_\_\_\_\_

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Prices 1.10



## Brief Outline and Actual Plan of work

In the foregoing section our contribution in the field of Origin of life has been described. We synthesise amino acids and peptides with the help of light. Under certain conditions, micro-structures capable of growth from within, multiplication by budding and having metabolic ~~condition~~ activity have been synthesised in these irradiated mixtures. ~~These~~ mixtures ~~have~~ A number of biochemicals <sup>are</sup> synthesised in these mixtures <sup>and</sup> ~~amongst these~~ these are many of enzyme-like materials in them and also substances with ferredoxin-like activity. These particles can be fixed with biological ~~dyes~~ fixatives and stained with a number of biological dyes. It is drawing attention of scientists all over the world (Amexh. B.C. 2000). Now the work has developed to an extent that four fruitful lines of investigation can be undertaken. We propose to ~~and~~ study the problem under the following major headings:

(1) Study of the problem of Origin of life with a view to utilise the enzyme-like material formed for the various economic processes.

(2) Investigation of the possibility of exploiting the photochemical formation of amino acid for the commercial production of amino acids.

(3) Use of the Geewann for photolytic decomposition of water for the producing hydrogen to be used as fuel.

(4) Using photochemical processes employing molybdenum & iron for the formation of amino acids utilising the nitrogen fixation.

1. Briggs, M.H. (1965), *Spaceflight*, I, 129-31.
2. Bahadur, K. (1967), *Zbl. Bakt.* II 121 (~~121~~) 291-319
3. Bahadur, K. and Ranganayaki, S. (1970)  
*J. Brit. Interplanetary Soc.* 23, 813-829.

(1) Study of the problem of Origin of life - Jeewanu prepared by exposing simple <sup>aqueous mixture of</sup> organic and inorganic substances <sup>the presence of</sup> show material with esterase, catalase and phosphatase - like activities (1,2). The Jeewanu prepared by exposing sterilised aqueous mixtures containing ammonium molybdate, diammonium hydrogen phosphate, biological mineral and formaldehyde show the presence of materials with urease -, ATP-ase and peroxidase - like activities (3).

It has been observed that formaldehyde of this mixture can partially be substituted with simple organic compounds as methyl alcohol, ethyl alcohol, acetic acid or sugars as lactose or sucrose and this produces modified particles on irradiation ~~mixture~~. These particles have different fixative ability and are chemically different <sup>from</sup> the particles ~~with~~ produced in mixtures having only formaldehyde as the source of <sup>organic</sup> carbon. These particles have to be examined for their enzyme-like properties.

The particles have a molybdenum complex produced by the action of formaldehyde on molybdate in presence of ~~formaldehyde~~ <sup>strong visible light</sup>. This blue complex becomes oxidised to Coloured ~~&~~ molybdate form by the atmospheric oxygen in dark. This process of conversion of ic to one form of molybdenum in light in presence of formaldehyde and the back reaction of one form being ic in dark by the atmospheric oxygen in the ~~at~~ presence of formaldehyde produces an interesting photochemical reversible electron transfer. This photochemical electron ~~transfer~~ <sup>transfer</sup> ~~with~~ system together with enzyme-like material ~~with~~ can make a useful combination of chemical transfer

7. Nayya H.K., (1977) "Studies in the Abiogenesis of Amino Acids", D.Phil. Thesis, Chemistry Department Allahabad University

8. Srivastava, M. (1977) "Studies in Abiogenesis of Amino Acids", D.Phil. Thesis, Chemistry Department, Allahabad University

~~9. <sup>Kumari</sup> ~~Sarma~~, S.~~

9. Kumari, S. (1977) "Studies in the Estimations of Amino Acids", D.Phil. Thesis, Chemistry Department, Allahabad University

further observed that if <sup>aqueous</sup> ~~aqueous~~ mixture of ammonium molybdate, diammonium hydrogen phosphate, biological mineral and formaldehyde is exposed to sunlight the formation of amino acids is considerably enhanced. The factors influencing the formation of amino acids have been investigated in our laboratory since last few years (7, 8) and their yield considerably improved. The conditions favouring the formation of cysteine have been investigated (9). It has been observed that ~~an~~ yield of upto 5 gm per liter has been reported in a few days. The ~~work~~ problem of separation of these amino acids from the mixture presents considerable difficulty because of the presence of formaldehyde in the mixture and this work is in progress in our laboratory.

We propose to investigate the factors which govern the formation of some particular amino acids, then work out the condition which favours the formation of essential amino acids, increase their yield and devise easy procedures for their isolation. As there is world shortage of ~~a~~ essential amino acid, the effort, if successful, will provide an easy method of preparing them in large quantity cheaply. For this, analysis of the irradiation mixture under different conditions for their amino acid content in amino acid analyses will be done and separation of the amino acids will be done first ~~by~~ <sup>by</sup> low pressure and temperature drying of the irradiated product and then ~~a~~ <sup>their</sup> separation ~~by~~ <sup>subjecting it to</sup> column chromatographic separation.

(3) Using Jeewanu for photolytic dissociation of water: The microstructures produced by exposing

(10) Hall, D.O., Rao, K.K. and Cammack, R., (1975),  
Sci. Prog. ~~of~~. (1975), 62, 285-317.

a mixture of ammonium molybdate, diammonium hydrogen phosphate biological minerals as sodium chloride, potassium sulphate, calcium acetate, magnesium sulphate, manganese sulphate or, ferric sulphate and potassium dihydrogen phosphate and formaldehyde has been found to contain ferredoxin-like properties (Munaxum  $\text{O}_2$  and  $\text{F}$ ).

Ferredoxin-like properties in Geewanna thus prepared is of interest because ferredoxin ~~to~~ helps in oxidation of  $\text{NADH}_2$  using  $\text{H}_2\text{O}$  as substrate, oxidase oxidation of xanthine and aldehydes, hydroxylation, pyridine nucleotide reduction and photophosphorylation, phosphoclastic reactions, synthesis of L-keto acids employing  $\text{CO}_2$  fixation, one carbon metabolism, hydrogen metabolism nitrogen fixation, pyridine nucleotide reduction, photosynthetic electron transfer in bacteria and  $\omega$ -hydroxylation of hydrocarbons (10). As there is general shortage of fuel, using sunlight for the photolytic dissociation of water producing hydrogen is being tried in several laboratories all over the world. We want to investigate the possibilities of using Geewanna for this process. Two lines of approach ~~are~~ are to be tried.

(a) It has been observed that if <sup>intact</sup> chloroplast with its intact membrane and ~~the~~ chlorophyll is exposed to light in presence of ferredoxin and hydrogenase,  $\text{H}_2\text{O}$  by molecule is ~~the~~ decomposed liberating hydrogen and oxygen. Several chloroplasts, ferredoxins and hydrogenases from different sources have to be examined. The ~~to~~ oxygen produced in the reaction inactivates chloroplast membrane, ferredoxin and hydrogenase and the reaction soon stops. Some work is in progress

(11) Chen, J. S. and Mortenson, L. E. (1973), *Biochim.*  
*Biophys. Acta*, 371, 283-298.

using oxygen scavengers in the mixture. In this experiment Gevannu have been found to be useful as ferredoxin component, ~~and~~ their advantage being that they are ~~so~~ fairly stable in oxygen and light (Annexure ~~G~~) and ~~according to~~ appears to have potentiality of being used as useful catalysts in photo dissociation of water. We wish to prepare a series of Gevannu with greater efficiency in this process and their possible exploitation in decomposition of water with light for commercial production of hydrogen to be used as future ~~fuel~~ non polluting fuel.

As Gevannu consists of a molybdenum complex which shows  $\uparrow$  reversible photochemical electron transfer, the particles have boundary wall ~~the~~ containing phospholipids and having selective permeability. They also  $\uparrow$  have ferredoxin-like property and thus are capable of electron transfer and ferredoxin ~~to~~ itself has hydrogenase activity (10). ~~An~~ An effort therefore will be made to study the conditions which may ~~enable~~ help in the photodissociation of water in presence of Gevannu alone. As almost all the constituents needed for the biological photodissociation of water are present in Gevannu ~~alone~~ itself, may be this line of investigation proves fruitful. Work on this line is already in progress in Concordia University, Montreal <sup>Canada</sup> and in Ames Research Center, Moffet field, U.S.A. (Annexure ~~G~~ F)

We have observed that ~~the~~ if Gevannu ~~mixed~~ mixed with distilled water are exposed

- (12) Bahadur, K., Ranganayaki, S. and Santamaria, L., (1958),  
182, 1668.
- (13) Mortenson, L.E., Zumft, E.G., Huang, T.C.,  
and Palmer, G., (1973), Biochem. Soc. Trans., 1, 35
- (14) Eady, R.R., Smith, B.E., Thorneley, R.N.F., Ware, D.A. and  
Postgate, J.R., (1973), Biochem. Soc. Trans., 1, 37.
- (15) Burns, R.C., Holsten, R.D. and Hardy, R.W.F.,  
(1970), Biochem. Biophys. Res. Comm., 39, 90
- (16) Bonemann, J.R., Yoch, D.C., Valentine, R.C. and Arnon,  
D.J., (1971), Biochem. Biophys. Acta, 226, 205,
- (17) Smith, R.V., Noy, R.J. and Evans, M.C.W., (1971)  
Biochem. Biophys. Res. Comm., 253, 104,
- (18) Winter, H.C. and Arnon, D.J., (1970), Biochem. Biophys.  
Acta, 197, 170.
- (19) Evans, M.C.W., Telfer, A. and Smith, R.V. (1973),  
Biochem. Biophys. Acta, 310, 344.
- (20) Schrauzer, G.N., ~~A.~~ (1975), Angew. Chem. internat.  
Edit., 14, (8), 514-522.

to light the pH of the medium drops with time. The indication of the presence of hydrogenase-like activity in the mixture ~~are present~~ <sup>is that the</sup> and ~~the~~ methylene blue if present in these mixtures is quickly bleached. We wish to investigate the conditions <sup>necessary</sup> for this photodissociation of water producing hydrogen.

Photochemical fixation of nitrogen:  
(4) In 1958 we observed that sterilised aqueous mixture of paraformaldehyde ~~and~~ containing colloidal molybdic acid as catalyst on exposure to ~~nitrogen~~ sunlight indicates the formation of a number of amino acids. Here the nitrogen of the amino acids comes ~~from~~ from fixation of atmospheric nitrogen (12). Apart from the fact that ferredoxin has nitrogenase activity also, molybdoferredoxin is an important constituent of the nitrogenase enzyme catalysing  $N_2$  fixation in anaerobes (13), facultative anaerobes (14) and aerobes (15, 16). Nitrogenase catalysed  $N_2$  fixation occurs also in ~~many~~ blue-green algae (17) and in the red photosynthetic bacterium Chromatium (18, 19). The fixation of nitrogen requires the co-operation of ~~the~~ molybdoferredoxin and another Fe-S protein called azoferredoxin.

Schrauzer simulated most of the reactions of the nitrogen reducing enzymes under nonenzymatic conditions (20). The key reactions of the substrates of nitrogenase occur at a molybdenum active site. The non-heme iron bound to sulphur of protein-S mediate the transport of electron to molybdenum active site but does not participate in the reduction

(21) Bahadur, K., and K.M.L. Agarwal (1962),  
Journal of Scientific and Industrial Research.  
Vol. 21 B, No. 7, pp 336, 37

of the substrate directly. ATP is required for the acceleration of the reduction and activation of the molybdenum site and is hydrolyzed to ADP and inorganic phosphate.

It has been observed by us that ~~the~~ a mixture of colloidal molybdic oxide and colloidal ferric oxide is a far better catalyst in photochemical fixation of nitrogen using formaldehyde as the source of organic carbon (21). The work of the photochemical fixation of nitrogen and formation of amino acids in the mixture using colloidal  $\text{MoO}_3$  and  $\text{Fe}_2\text{O}_3$  which was started in our laboratory has already been started in the Ames Research Center, Moffettfield, California (Annexure ~~to~~ H)

The particles, Jernann, have <sup>not only high concentration</sup> ~~not only about 99%~~ of ~~the~~ Mo but they also have ATP-ase like property and ferredoxin-like compound with Mo-Fe-S + nucleus. It is therefore of interest to investigate their possible role in photochemical fixation of nitrogen.

We propose to study the nitrogen fixation using  $\text{N}^{15}$  and investigate the possible exploitation of the process for commercial fixation of nitrogen and commercial manufacture of nitrogen fertilizer.

The problem of origin of life is not only an important aspect of the study of Biological Sciences for it enables us to understand the basic urge of the physico-chemical environment around us which produced something capable of evolution and created conscious living system from the life less matter ~~around~~ <sup>around</sup> us and

thus enabled us to understand our environment in a way which proposes a model of our behaviour in this Earth consisting of ~~inanimate~~ <sup>inanimate</sup> materials and the ~~inanimate~~ <sup>inanimate</sup> synthesised by them; and the work <sup>on</sup> of Jeevanu, which are capable of growth, multiplication, metabolic activity and innumerable chemical transformations places it ahead of the work on Origin of life elsewhere and its extended study will ~~reveal~~ <sup>reveal</sup> information of considerable value to humanity in solving many of its ~~religio-social~~ <sup>socio-economic</sup> economic problems it faces to-day. The time is ripe for exploiting this work for the production of food as amino acids, energy liberation of hydrogen to be used as <sup>a much</sup> needed ~~so~~ fuel by photodissociation of water and photochemical fixation of nitrogen which is the limiting factor in solving the food production to sustain the growing population of the Earth.

I request that ~~the~~ <sup>the</sup> ~~be~~ <sup>be</sup> ~~given~~ <sup>given</sup> facilities to investigate these problems, be given to us.

(Krishna Bahadur)

7

(a) Grant needed for the purchase of instruments:

	Rs
Deep freeze	10,000
pH meter	15,000
✓ Thermostat	15,000
Autoclave	10,000
Microscope with photographic attachments	50,000
Hemogeniser	10,000
Lipholyser	60,000
Ultra centrifuge	15,000
Fraction Collector	15,000
<del>Low pressure</del> Vacuum pump	20,000
Glass fitting for vacuum apparatus	10,000
U.V. Spectrometer with automatic scanner	50,000
Chemicals	50,000
Glass apparatus	50,000

Rs 380,000

Rs. 420,000

Books  
 Strengthening workshop facilities  
 (Mx) Research Associates - 2  
 Pay ~~Rs 1000~~ Rs 14,00 per month

For 12 months in Rs.  
 33,600/-

Senior Fellows (Post doctoral) - 4  
 @ Rs 8,000 per month

38,400/-

Junior Fellowship - 6  
 @ Rs 4,000 per month.

28,800/-

Rs. 1,00,800/-

Ministerial Staff

Technical assistant - 1  
 @ Rs 425-15-500 ~~Rs 425~~

5,100/-

Office assistant - 1  
 @ Rs 330-10-380

3,960/-

Rs

4 Laboratory assistants - 2  
@ 260-6-336

Rs  
6,240

Rs. 15,300

4 Plus dearness allowance as may be permissible.

Pay of the Staff -

1<sup>st</sup> year

Rs 1,16,000 ✓

2<sup>nd</sup> year

Rs 1,16,000 ✓

4 increment of the ministerial  
staff ~~with~~

Rs ~~444~~ ✓  
Rs 1,16,444

3<sup>rd</sup> year

Rs 1,16,544

increment of the ministerial staff.

444

1,16,988

Total pay of the staff for three years Rs. 3,49,632 ✓

Service, travel and contingency for 3 yrs.  
@ 1,50,000 per year

4,50,000 ✓

Rs. 7,99,632

(ii) Service charges for amino acid analysis,  
EPR  
NMR, Electron paramagnetic resonance spectra, electron micrographs  
etc.

50,000

Travel grants, symposia and visit  
of scientists from abroad to this laboratory  
and for the laboratory to other places.

50,000

Contingency grant for the purchase of  
chemicals, glass apparatus, repair, postage  
typing and stationary

50,000

1,50,000

Thus Non recurring Rs 4,20,000

Recurring for total of  
three years.

7,99,632

Rs

12,19,632

(ii)

1. Dr. A. E. Smith, 239-9,  
National Aeronautics and Space Administration (NASA)  
Ames Research Center,  
Moffett field,  
California - 94035. (U.S.A).

(2) Dr. D.O. Hall,  
University of London King's College,  
Department of Plant Sciences,  
School of Biological Sciences,  
68, Half Moon Lane,  
London - SE24 9JF (England)

(3) Dr. J. S. Rawat,  
Prof. of Biochemistry,  
U. P. College of Veterinary Sciences,  
Mathura.

1-

(1) Study of the problem of Origin of Life and its application in amino acid manufacture, photolytic decomposition of water and photochemical fixation of nitrogen.

(2) (a) Allahabad University

(b) Chemistry

(c) Dr. Krishna Bahadur and Dr. (Mrs.) S. Ranganayaki.

(3) (i) Dr. Krishna Bahadur, D. Sc.

Has published about 170 scientific papers.

(ii) Dr. (Mrs.) S. Ranganayaki, D. Sc.

Has published about 45 scientific papers.

The list of their publications concerning the subject of this project is attached.

(4) + (5). Attached.

(6) Applied for three years in the first phase.

(7) (a) + (b) typist: Please put here the matter written earlier under 7

(8) Annexure A, B, C, D, E, ~~F~~, G, ~~H~~ and H.

Section 'A'.

All the necessary information about the Chemistry Department of Allahabad University is with the U.G.C.

- 1 Technical Assistant - 425-15-500 EB-15-560-20-700  
1 Office assistant - 330-10-380 EB-12-500 EB-15-570  
2 Lab. Assistants - 260-6-326-EB-8-350

UNIVERSITY GRANTS COMMISSION

Support for Science Research in the  
Universities

PROPOSAL FOR SUPPORT OF RESEARCH

Applications of the Studies on Origin of Life

Photolytic splitting of water and fixation of Nitrogen  
and Nitrogen Loss by Organo Molybdenum Microstructures, the  
Molybdenum Jeewanu, model of the Protocells.

by

Krishna Bahadur  
M.Sc., D.Phil., D.Sc., D.I.C. (London)  
Chemistry Department  
University of Allahabad  
ALLAHABAD.

Dec. 1985.

UNIVERSITY GRANTS COMMISSION  
Support for Science Research in the Universities  
Proposal for support of Research

1. Title of the Project : Applications of the studies on Origin of Life.

Sub-title : Photolytic splitting of water and fixation of nitrogen and nitrogen loss by Organo Molybdenum microstructures : the molybdenum Jeevanu, model of the Protocells.

2. (a) Institution and address : University of Allahabad,  
Allahabad, India.

(b) Department : Chemistry Department, University of Allahabad,  
INDIA.

- (c) Principal Investigator and other members of the research group if any :

Principal Investigator : Prof. Krishna Bahadur, Head,  
Chemistry Department, University  
of Allahabad.

To be assisted : Dr. (Mrs.) S. Ranganayaki, Reader, Chemistry  
Department, University of Allahabad.

3. Academic qualifications and research experience of the investigators and publication related to the above research projects. Please indicate the research schemes already under taken and in force with funds from commission or any other agency, giving details of the grants and number of persons employed, duration etc.

(A) INFORMATION REGARDING THE PRINCIPAL INVESTIGATOR :

(a) Name & Designation : Dr. Krishna Bahadur, Reader  
Chemistry Department, University of  
Allahabad, Allahabad - 211 002.

(b) Brief Bio-data indicating his specialised interest particularly in relation to proposed research work :

Born on 20th Jan. 1926, educational qualifications : M.Sc., D.Phil., D.Sc., D.I.C. (Lond.). Have published more than 200 scientific papers and 40 students have been awarded D.Phil. degree under me. Nuffield Fellow (1960), Exchange Scientist (1963), Canadian

Commonwealth Research Fellow (1968).  
Have been working on Photolytic splitting  
of water by fixation of nitrogen since  
last 5 years. I have 34 years of  
experience of teaching and guiding  
research.

- (c) List of Important : Attached.  
publications in  
this or related  
field
- (d) List of research : None.  
scheme being  
carried out by  
principal Investi-  
gator with finan-  
cial support from  
various agencies/  
organisations

(B) INFORMATION REGARDING OTHER RESEARCH SCIENTISTS TO BE ASSOCIATED  
WITH THE INVESTIGATION

- (a) Name and designation : Dr. (Mrs) S. Ranganayaki, D.Sc.,
- (b) Brief Bio-data indica- : Reader, Chemistry Department,  
ting his specialised University of Allahabad. She is  
interest particularly D.Phil. and D.Sc. in Chemistry  
in relation to propo- and guiding research since last  
sed research work 20 years. About 15 research  
students have been awarded D.Phil  
under her and she has published  
about 100 scientific papers. She  
is working on this problem for the  
last several years.
- (c) List of publications : In most of my papers she is Co-  
in this of related author.  
field
- (d) List of other research : None.  
scheme with which the  
scientists is associa-  
ted
4. Brief outline of objective or work and its importance. In  
case it has any R and D importance, please substantiate :

Organised microstructures are synthesised in certain  
sterilised aqueous mixtures of simple organic and inorganic  
substances when these are exposed to sunlight or other sources  
of energy. These microstructures have been boundary well and  
internal structures and under specific conditions may show the

properties of growth from within, multiplication by budding and some metabolic activity (see the attached confirmation of Prof. M.H. Briggs). Of the several types of such microstructures investigated by our school the organo molybdenum microstructures, the molybdenum Jeevanu are specially interesting because they have many of the biochemicals of the present-day cells.

Study of the functional properties of organo molybdenum microstructures (OMM) as model for Protocells:-

OMM, a type of model of protocells, have been examined for ferredoxin - like activity. It is because these microstructures prepared by the action of light on sterilised aqueous mixture of ammonium molybdate, diammonium hydrogen phosphate, mineral solution and formaldehyde, have boundary wall, a central mass and chemicals of interest in the study of origin of life (1,2,3,4). That they have ferredoxin - like material is evident because they can be used in the place of ferredoxin in the photobiological system, chloroplastferredoxin-hydrogenase (5,6). These microstructures were tested for nitrogenase - like activity. It was observed that if an aqueous mixture of OMM having acetylene in the overhead space is exposed to light from a Xenon lamp, the amount of acetylene decreases and ethylene increases with the period of exposure (7).

If OMM and  $D_2O$  mixture is exposed to the radiation from Rayonet GPR 2337<sup>2</sup>nm keeping acetylene in the overhead space it is converted into  $CND = CND = CND$  indicating that the protons come from water and that these particles are capable of splitting water (8). On irradiation of an aqueous mixture of OMM and  $NH^{14}CO_3$ , with 254 nm,  $^{14}C$  was found in the organic material formed in the mixture (9) indicating fixation of carbon.

Bubbles of gas are observed to be evolved when an aqueous mixture of OMM is exposed to sunlight. The evolution of gas continues for 4 or 5 days and then ceases but it stops when sunlight is cut off. If the mixture is allowed to stand in dark overnight and then exposed to sunlight on the next day, again gas is evolved.

Warburg's study indicated that on exposure of OMM suspended in water to sunlight, hydrogen and oxygen are produced. As OMM have nitrogenase-like material the hydrogen produced combined with nitrogen in the ratio of 1 vol. of diimide ( $NH = NH$ ) as an intermediate compound. If nitrogen fixation is inhibited a mixture of hydrogen and oxygen is obtained.

5. Actual plan of the work proposed ; Experimental approach :-

On treatment of the OMM with 1% sodium Carbonate solution a fraction of OMM dissolves in it and insoluble bluish rod-like

structures are left in the mixture. The dissolved fraction can be precipitated with HCl as a yellow precipitate. This is some complex of molybdenum which is capable of splitting water and fixing nitrogen.

In another specific mixture in which OMM are produced on exposure to sunlight, in the beginning for a few days only spherical microstructures are formed. Then after 16 hr of exposure to sunlight in three days, after the day's exposure, the mixture shows the presence of only blue rod structures which appear like crystals under the microscope. On keeping this mixture in dark during the night the next morning the mixture is full of blue, spherical particles of 1 to 2 in diameter. These show distinct boundary wall and some dense mass in the center under phase contrast microscope. When this mixture is exposed to sunlight on the next day, by the evening the mixture again has only rectangular blue structure which appear like crystals under the microscope. This mixture, on standing in dark during night is again full of spherical particles the next mornings and the process can be repeated for about one week when finally this interchange stops and only blue, rod shaped microstructures are left in the mixture. I wish to investigate the relationship between blue rod structures like crystals and the spherical OMM with boundary wall, both of which in preliminary examination by X-ray diffraction appear non-crystalline, employing extensive X-ray diffraction and other technics. I want to determine the chemical-physical factors that underlie the OMM structure and functional attributes.

Microstructures with a specific morphology, with ability to utilise the energy of sunlight for not only their formation but even for splitting of water in sunlight is of considerable interest in the study of origin of life because this process will set up an energy flow through the system and this will provide additional stability. If to the mixture in which OMM are produced on exposure to sunlight, some soluble salts of semi.conductors and or transitional elements is added some of these are incorporated in the OMM formed and their properties of photochemical splitting of water and fixation of nitrogen is significantly improved. By incorporating suitable amount of Mn, Cd, Co, Zn, La and Tc in the mixture of OMM efficient OMM can be prepared which may become of commercial interest. I have already done some work on these lines.

Some quantitative idea of photochemical splitting of water and fixation of nitrogen by the OMM :-

Incorporation of lanthanum and then titanium in OMM renders them more efficient. The following table indicate the pressure changes observed on exposing 20 mg. of OMM suspended in 5 ml. water

taken in a conical Warburg's flask (WF) of 14.5 ml capacity and bottom area 9.5 cm<sup>2</sup>. The pressure increase on an exposure of 30 minutes to sunlight (i), and the pressure decrease during this period as estimated by the decrease in pressure during the next 30 minutes on keeping the flask in shade (ii) were recorded. From this the amount of hydrogen set free per day per square meter using 20 gm of the OMM on six hours of exposure (iii) and also the hydrogen consumed during this period in grams (iv) were calculated, assuming that the mixture is changed after each 30 minutes exposure.

Reference number of OMM	Condition	Pressure in W.F. in cm. of mercury in 30 min exposure (i)	Decrease in pressure in 30 min in shade in cm of mercury lys. of (ii) in water in WF	Net increase in pressure in 30 min of mercury by photo (iii) in water in the WF	G of H <sub>2</sub> produced in g/m/day (iii)	g of H <sub>2</sub> used up in N <sub>2</sub> fixation g/m <sup>2</sup> /day
HM (Modi) J (Ac) 60	Oxygenic	4.75	-1.0	5.75	4.00	0.70
HM (Modi) J (Ac) La 60	Oxygenic	5.57	-5.0	10.50	7.60	4.57
HM (Modi) J (Ac) La Tl 60	-do-	8.30	-3.5	11.80	8.32	2.50
HM J 60	anoxygenic	8.00	-6.00	14.00	14.70	11.00

The gas transformation of the modified OMM when their aqueous mixture is exposed to sunlight and subsequently kept in shade will be investigated using gas chromatograph and Warburg's apparatus with time and some scanning will be done to see whether the process of water splitting and nitrogen fixation can be enhanced in some of these OMM at some particular pH using suitable buffer.

In presence of OMM the fixation of nitrogen continues even after the exposure is stopped if the hydrogen set free by splitting of water is available under the condition when rapid fixation of nitrogen takes place it is usually followed by loss of nitrogen. The ability of OMM of photolytic splitting of water has been considerably increased by adding titanium sulphate and

acetic acid in the mixture used for the preparation of OMM. I wish to prepare OMM by introducing silicon in these by adding soluble sodium orthosilicate in the mixture before exposure and to examine the OMM produced for their ability of splitting water in sunlight and fixation of nitrogen. For this the time lapse study of the gas exchanges in sunlight and subsequently in shade will be carried out using gas chromatograph. I also wish to explore the possibility of utilising the OMM or some of its modified variety for the commercial exploitation of solar energy.

#### Research work done at the sponsoring institutions

We have been investigating the photolytic splitting of water by the molybdenum gevesau since last six years and have prepared several modified MO incorporating transitional elements and semi conductor elements in these structures. During this investigation which was carried out, using Warburg's apparatus and chemical estimation, it was found that some of these microstructures are strong nitrogen fixers on exposure to sunlight. A few of these show good reductive fixation of nitrogen whereas some are good oxidative fixers of nitrogen.

#### (b) Research work done and progress in India

This work is not in progress anywhere else in India.

#### (c) Research work done and progress in abroad

We formed a collaboration with Dr. D.O. Hall of the London University, King's College, London and in this collaboration we discovered that OMM have ferredoxin-like material and these can substitute ferredoxin in the photobiological system, Chloroplast-ferredoxin-hydrogenase, found in some algae which split water to hydrogen and oxygen in sunlight. The work has been published(5).

We then formed a collaboration with Dr. A. Smith of NASA Ames Research Center, Moffett Field, California and Dr. C. Folesome of Hawaii University to investigate the nitrogenase-like properties in the OMM. It was observed that if  $D_2O$  and  $N_2$  mixture is exposed to sunlight keeping acetylene in the overhead space  $CND = CND$  is formed in the over head space of the mixture. It was further observed that an aqueous mixture of OMM and  $H_2^{14}CO_2$  on exposure to light shows the presence of  $^{14}C$  in the organic materials synthesised in the mixture indicating that OMM has nitrogenase-like material and is also capable of fixing inorganic carbon to organic carbon. The work has been published (8). It was subsequently observed chemically that OMM are capable of fixing molecular nitrogen and carbon dioxide (9,10).

Even earlier in 1957 we had formed a collaboration with Prof. L. Santamaris of the Institute of General Pathology Milano (Italy) to investigate the problem of nitrogen fixation utilizing the energy of light employing colloidal molybdic acid as catalyst. The work was published in Nature (1). This work was confirmed recently by the work of Schrauzer (6) who reported that molybdenum has nitrogenase-like activity.

#### TECHNICAL PROGRAMME

##### (a) Phase one : Study of the Reductive Fixation of nitrogen

- (i) Several molybdenum Jeevanu will be examined for their ability of fixing nitrogen in the form of diimide, hydrazine and ammonia using Warburg's apparatus and by chemical estimation.
- (ii) The optimum pH for the reductive fixation of nitrogen will be investigated using Warburg's apparatus and gas analysis by gaschromatogram.
- (iii) The effect of presence and absence of oxygen in the reductive fixation of nitrogen will be investigated.
- (iv) After determining the favourable conditions for reductive fixation of nitrogen as observed by the above experiments the amount of nitrogen fixed will be estimated by chemical methods.
- (v) Gas chromatographic estimation of the various constituents formed during the exposure of various irradiation mixtures will be done.

##### (b) Phase two : Study of oxidative fixation of nitrogen

- (i) The effect of pH on the oxidative fixation of nitrogen will be determined and optimum pH for this fixation of a few samples of OMM will be undertaken using Warburg's apparatus.
- (ii) The amount of nitric nitrogen fixed by different OMM under optimum conditions will be determined using :
  - (a) Warburg's apparatus
  - (b) Chemical methods.
- (iii) Experiments will be planned to segregate alkaline nitrogen fixation products from the acidic fractions to avoid formation of  $\text{NH}_4\text{NO}_3$  by devising suitable apparatus. To begin with the lobed Warburg's flask will be used and study will be made using Warburg's apparatus. Subsequently simple apparatus for this will be devised.

##### (c) Phase three : Search for suitable inhibitors of nitrogen loss Reactions.

- (i) Investigation of suitable inhibitors for nitrogen loss due to decomposition of diimide will be undertaken.

Usual inorganic inhibitors as iodide, mercuric ions, copper ions, cyanide ions and others will be examined.

- (ii) Inhibitors of loss of nitrogen via ammonia nitrite will be searched.
- (iii) If the effort of searching suitable nitrogen loss inhibitors fail, an effort to segregate the primary components of the final decomposition product will be undertaken.

Anticipated results and their potential impact in understanding the origin of life :-

Organo molybdenum microstructures (OMM) are models for protocells which have several chemicals of the present day cell but are basically inorganic and have about 44.50% of molybdenum(9). These have some functional properties as splitting of water in sun light and fixation of nitrogen. By incorporating semiconductor and transitional elements in these we may be able to produce particles which are efficient in these functional properties. If the secondary atmosphere of the prebiological era was made of carbon dioxide, nitrogen and water vapours and there was not enough organic matter on the surface of the earth or in the ocean, particles like these could act as chemical precursors to photoautotrophs. If efficient OMM could be prepared may be these could be used for commercial splitting of water utilising the energy of sunlight and the study of origin of life could be put to some economic use (see the last page of Cairns-Smith's book "Genetic Takeover" attached).

- (6) Duration of the project : Five years.
- (7) Detailed estimates of the expenditure to be incurred on the project (on annual basis and not to exceed three years in first phase) :
  - (a) Non recurring : Nil
  - (b) Recurring per year : £.

Additional facilities required which are chargeable to the scheme per year :

(i)	Equipment and apparatus (purchase of carrier gas and other accessories.	-	£. 20,000
(ii)	Land/livestock	-	Nil.
(iii)	Laboratory and Office facilities, etc.	-	Chemical and glasswares - £. 20,000
		-	Stationery, postage, travelling, etc. - £. 5,000
			<hr/> £. 45,000 <hr/>

Personnel :

Staff Requirements

Sl. No.	Designation of post	No. of post	Scale	Qualifications prescribed
1.	Research Fellow	2	Rs. 800/-p.m. (fixed)	M.Sc. in Chemistry
2.	Research Associate	1	Rs. 1200/-p.m. (fixed)	D.Phil.
3.	Prof. Krishna Bahadur (Chief Investigator)	1	Rs. 1500/-p.m.	qualification mentioned.

ESTIMATED COSTS

Name of post	Scale	Ist year	IInd year	IIIRD year	Total
Chief Investigator	Rs. 1500/-	18,000	18,000	18,000	54,000
Research Fellow(2)	Rs. 800/-	19,200	19,200	19,200	57,000
Research Associate	Rs. 1200/-	14,400	14,400	14,400	43,200
		Rs. 51,600	Rs. 51,600	Rs. 51,600	Rs. 154,800

(ii) Working expenses :

	per year in (Rs.)	per three year in (Rs.)		
1. Pay of officers per year	51,500-00	1,54,800-00		
2. Pay of establishment	X			
One office assistant @ 600/-p.m.	X 12,000-00	36,000-00		
One lab attendant @ 400/-p.m.	X			
3. Allowances and honoraria	Nil	Nil		
A. Dearness Allowances	Per rule	Per rule		
B. House rent	Nil	Nil		
C. City Compensatory Allowance	Nil	Nil		
D. Other Allowance	Nil	Nil		
E. Travelling Allowances	Rs. 5,000-00	15,000-00		
4. <u>Contingencies</u>				
A. Recurring	45,000-00	1,35,000-00		
B. Non-recurring	Nil	Nil		
	Rs. 1,13,600-00	Rs. 3,40,800-00		
<u>Total pay of officials</u>	<u>Recurring</u>	<u>Non-recurring</u>	<u>Total per year</u>	
	Rs. 51,600-00	Rs. 62,000-00	Nil	Rs. 1,13,600-00p.a.

Total Recurring expenditure for the duration of the Project

Per year Rs. 1,13,600-00  
On three years Rs. 3,40,800-00

Total Non-recurring and recurring expenditure Rs. 3,40,800-00  
in three years.

Please give names and address of three persons in other universities actively engaged in your area of research interest to whom a reference may be made regarding the research project :

1. Prof. O.N.Perti,  
Retired Professor and Head  
Chemistry Department,  
Motilal Nehru Regional Engineering College,  
Allahabad.

9, Balrampur House  
Allahabad-211002.

2. Prof. (Mrs.) Indra Kumari Verma  
Professor of Chemistry  
I.I.T. New Delhi,  
New Delhi.

3. Prof. Vishnu Chandra,  
Chemistry Department,  
Gorakhpur University,  
GORAKHPUR.

4. Prof. R.P.Rastogi  
Vice-Chancellor, BHU,  
VARANASI.

8. Any other information useful in the evaluation of the Project:

I will be retiring from the University of Allahabad, Chemistry Department, where I am Professor and Head on 20th Jan. 1986 when I will complete my sixty years of age.

9. Certified that :

(a) General facilities required such as laboratories, equipment etc. are available in the department.

(b) No foreign exchange is required for taking up this project

(c) This research proposal has not been submitted to any other agency for assistance.

(Signature)

Principal Investigator  
Head of the Department  
Department  
University

Krishna Bahadur  
Krishna Bahadur  
Chemistry  
University of Allahabad, Allahabad-211002

HEAD

DEPARTMENT OF CHEMISTRY  
UNIVERSITY OF ALLAHABAD

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UNIVERSITY GRANTS COMMISSION

Proforma for submission of proposals for support of Research.

1. Title of the project : Photosynthesis of Amino Acids
2. (a) Institution and address : University of Allahabad,  
Allahabad-211002.
- (b) Department : Department of Chemistry
- (c) Principal Investigator: Dr. (Smt.) S. Ranganayaki  
M.A., D.Phil., D.Sc.
3. Academic qualification and research experience of the Investigator, existing facilities and suitability of the project : (Please indicate the research scheme already undertaken and inforce with funds from commission or any other agency) -
- (a) Qualification : M.A., D.Phil., D.Sc.
- (b) Research Experience : 25 years, Published 50 scientific papers.
- (c) Facilities : All routine facilities are available and the project can be handled without any further substantial requirements of equipment.
- (d) Scheme in progress : -
4. Brief outline of objective of work and its importance :  
Kindly see <sup>Annex</sup> 'Enclosure-A'.
5. Actual plan of work proposed : Kindly see <sup>Annex</sup> 'Enclosure B'  
(Technical programme and plan of work)
6. Duration of the project : 3 years.
7. Detailed estimate of the expenditure to be incurred on the project (on annual basis and not to exceed 3 years) :
- (a) Staff :
- |                                  |   |                                     |
|----------------------------------|---|-------------------------------------|
| (i) One Senior Research Fellow   | - | Rs. 500/- p.m. (fixed)              |
| (ii) Two Junior Research Fellows | - | Rs. 400/- p.m. (fixed)              |
| (iii) One Laboratory Attendant   | - | Rs. 150/- p.m. (fixed)              |
|                                  |   | Total - <u>Rs. 17400/- per year</u> |
- (b) Contingency including : Rs. 5000/- per year  
stationary, reprints etc. \_\_\_\_\_
- GRAND TOTAL: Rs. 22,400/- per year

Thus the total grant needed for three years will be Rs. 22,400x3  
= Rs. 67,200/-.

(Note : I want one Senior and Two Junior research fellows as well as a lab. assistant. Their pay and contingency may be modified according to the prevailing rules of UCC and requisite calculations for 3 years may be made if this is not found in order.)

8. Any other information useful in evaluation of the project :

This method of formation of amino acid would be cheap and financially viable. It also may lead to solving the problem of protein deficiency in developing nations.

9. Certified that :

- (a) Facilities required such as laboratories, equipment etc. are available in the department.
- (b) No foreign exchange is required for taking up this project.
- (c) This research proposal has not been submitted to any other agency for assistance.
- (d) The additional staff to be recruited under the project will be on the same conditions as prevalent for similar categories of the staff in the University/College.

(Signature)

REGISTRAR  
UNIVERSITY OF ALLAHABAD

PRINCIPAL INVESTIGATOR  
DEPARTMENT OF CHEMISTRY  
UNIVERSITY OF ALLAHABAD

HEAD OF THE  
DEPARTMENT OF  
CHEMISTRY,  
ALLAHABAD UNIVERSITY

## ANNEXURE A

The search for abiogenesis of amino acids started because amino acids are building blocks of proteins and proteins are building material of the present form of life on the earth. The modern study of origin of life started with the search of abiogenic process which could form amino acids under natural conditions and good reviews of work on amino acids synthesis are available (1,2). Of all <sup>the</sup> processes of photochemical synthesis of amino acids using formaldehyde, ammonical <sup>a</sup> nitrogen and inorganic catalysts are being investigated by us.

Photochemical formation of amino acids <sup>s</sup> have been observed in sterilised aqueous mixtures containing formaldehyde as a source of organic carbon, ammonical <sup>a</sup> nitrogen as source of fixed nitrogen and inorganic catalysts in colloidal state (3,4,5). The effect of period of exposure, hydrogen ion concentration, nature of catalyst and source of nitrogen on the formation of amino acids have been studied in detail (6,7,8). This method of formation of amino acids has been considerably improved and it has been observed that in aqueous mixtures of ammonium molybdate, diammonium hydrogen phosphate, biological minerals and formaldehyde the formation of amino acid is so much in amount that it may be tried on commercial scale (9, 10, 11). The synthesis of as much as 11 gm. amino acid per l. has been observed in our laboratory (12).

The isolation of individual amino acids from these mixtures present <sup>s</sup> a difficult problem. Preliminary estimation of amino acids by titrimetric methods (13, 14) indicate that sufficient

amount of amino acids are found in the mixture. Chromatographic separation of amino acids has been carried out using column chromatography and a total of about 3 gm. of amino acids per litre of the irradiated mixture has been obtained (15) but the products obtained were not pure and further purification and perfection of the separation by column chromatography is to be tried.

It has been observed that the nature of amino acids synthesized can be influenced by the change of the concentration of the reactants (16, 17) and under specific conditions essential amino acids can be synthesised in appreciable amounts (18).

Amino acids are expensive commodity and a cheap method of their production is of immense utility. This procedure of photochemical formation of amino acids will provide a cheap source of amino acids. One of the many problems faced by developing countries like India is shortage of proteins and this leads to malnutrition of the growing generation and the availability of amino acids in large quantities, particularly the essential amino acids, will greatly contribute towards solving this problem and may be a major break through in the nutrition problem of the whole world.

The method of separation of amino acids from hydrolysate of proteins is well known but the separation of amino acids which have formaldehyde in the mixture presents difficulty. We plan to achieve this separation by column chromatography

after removing excess of amino acid by low temperature (below 5°C) distillation. For subsequent analysis of amino acid, paper chromatography, titrimetric method modified for titration of specific amino acids in pressure<sup>enc</sup> of other natural amino acids (19, 20, 21, 22), gas chromatography, mass spectrometry and amino acid analysers will be used.

The point is not to develop a method for isolation of amino acid and their subsequent analysis which are well known but to develop method which give good yields of amino acid in the process of photochemical formation of amino acids which we are investigating since last 20 years and isolate these amino acids and try to make this process commercial specially for the essential amino acids for which there is world shortage.

There had been significant development in the work during the last two years. It had been observed that in the above irradiation<sup>ed</sup> mixtures microstructures known as Jeewanu are synthesised in large numbers (9). These particles have ferredoxin-like activity (23). It have<sup>s</sup> been further observed that the particles thus formed have nitrogenase-like activity. Thus a mixture of acetylene if kept over the atmosphere of these particles in water and mixture exposed to light, acetylene is converted to ethylene (24). Using healthy<sup>v</sup> water in the experiment it has been observed that the ethylene formed is CHD=CHD, thus indicating splitting of water molecule by these particles with sunlight. The particles are also able to fix carbon dioxide to organic carbon (25). Experiments with H<sup>14</sup>CO<sub>3</sub> ions in the mixture showed

that the particles are capable of reducing inorganic  $^{14}\text{C}$  to organic  $^{14}\text{C}$  and in this process water is split by the micro-structure (25).

Thus the mixtures we want to investigate will not only provide a method for producing amino acids from the cheap starting material but also suggest a method of utilising the solar energy for the fixation of nitrogen and carbon dioxide and splitting water to hydrogen and <sup>oxygen</sup> water. In this field of work we are pioneers in the world and I request you to please sanction this scheme so that we keep up the pace with the world.

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-

ANNEXURE B

1. We will first investigate the conditions which help in
  - (a) the optimum formation of natural amino acids
  - (b) the optimum formation of essential amino acids as Lysine, histidine, arginine and proline and cysteine which are normally synthesised in appreciable amount in our mixture.
  
2. We will develop quick methods of estimation of natural amino acids and particularly of essential amino acids because quick and accurate estimation of these amino acids is necessary to follow the reactions.
  
3. A method for the isolation of amino acids from the mixture of many substances produced in the irradiated mixture is to be developed. For this we will try
  - (a) Column chromatography
  - (b) chemical separation.

The separation of amino acids from those irradiated mixtures is an extremely difficult matter not only because there are many biochemicals found in them but also because formaldehyde and phosphoric acid are present in the reaction mixture.

---

PREPARATION OF SILICA JEEWANU, 121111 J 24

Following solutions are prepared :

1. 4% ammonium molybdate solution is prepared by dissolving 4 gm of the salt in 100 ml of distilled water.
2. 3% diammonium hydrogen phosphate solution is prepared by dissolving 3 gm of the salt in 100 ml of distilled water.
3. Mineral solution is prepared by dissolving 20 mg of each of sodium chloride, potassium sulphate, calcium acetate, magnesium sulphate, manganese sulphate and potassium dihydrogen phosphate in about 85 ml of distilled water. One salt is completely dissolved before adding another salt.

In a separate test-tube 10 ml of distilled water is taken and to this 50 mg of ferrous sulphate is added with a few drops of dilute sulphuric acid to avoid hydrolysis. When a complete solution is obtained it is mixed with the above 85 ml solution of other salts and the total volume is made to 100 ml with distilled water.

4. 36% formaldehyde solution is used in the experiment.
5. 3% sodium chloride solution is prepared by dissolving 3 gm of the salt in 100 ml of distilled water.
6. 5% soluble sodium silicate (used in soap industry) <sup>is prepared by</sup> is dissolving <sup>5 gm of the salt</sup> in 100 ml of distilled water.

PROCEDURE FOR PREPARING PARENTAL ENVIRONMENTAL MEDIUM (PEM):

50 ml of the ammonium molybdate solution, 100 ml of the diammonium hydrogen phosphate solution, 50 ml of the mineral solution, 50 ml of the sodium chloride solution, 50 ml of the sodium silicate and 50 ml of formaldehyde are taken in a 500 ml pyrex conical flask. The flask

is cotton plugged and gently shaken. It is exposed to sunlight for 24 hours giving 4 hours exposure each day. The mixture is gently shaken once or twice each day to disperse the sediment of Jeevanu getting formed throughout the mixture.

The mixture becomes blue in about 2 hours of exposure. After a few hours of exposure a little of the mixture can be examined under 1500 magnification in a microscope to see the Jeevanu getting formed. After 24 hours of exposure the Jeevanu are separated by filtration or centrifugation, washed with 2 ml of distilled water two or three times and dried in vacuum desiccator. Yield = 1.2 gm.

We started exposing a similar mixture from light obtained from 1000 watts tungsten bulb keeping it at 1 m distance from the bulb. The mixture has become just slightly bluish in 100 hours of exposure and no Jeevanu are formed in it so far. So use sunlight as source of exposure.

EXPERIMENTS ON THE ORIGIN OF CELLS

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Feed Service Ltd., Hartham Park, Corsham, Wiltshire.

PUBLISHED IN: Spaceflight, I, (4), 129-131(1965).

INTRODUCTION

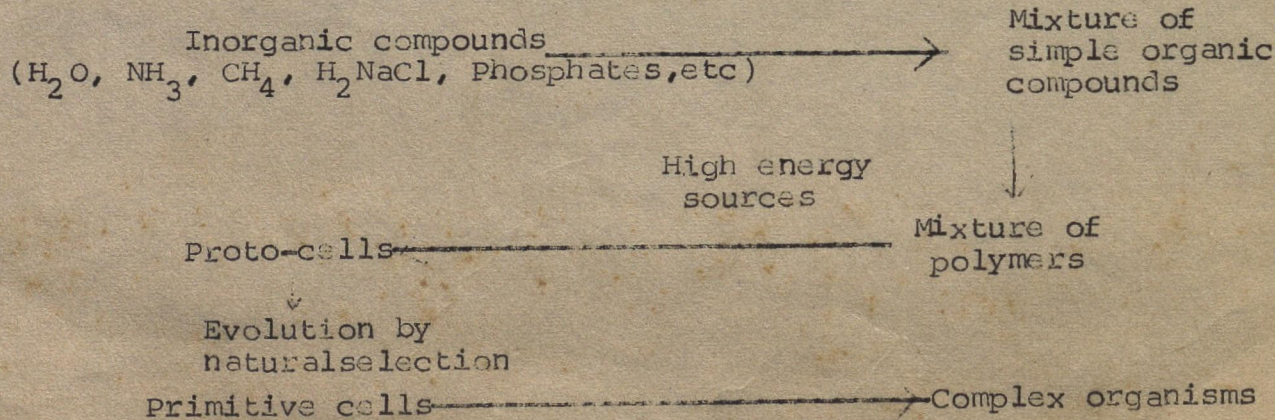
It is now generally agreed that the first process for the origination of living cells with a terrestrial-type biochemistry is the formation of relatively large quantities of organic substances on a primitive and lifeless planet (1,2,3,4). It has been conclusively demonstrated by many workers that complex 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



(2)

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 cytoplasm. Reproduction appears to be entirely due to ingrowths of the cell-walls (14,15).

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

It is not until organisms 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

(3)

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) 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, etc. 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 thermal 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

(4)

(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 confirmation and  
extension of this work.

#### THE LABORATORY SYNTHESIS OF PROTO-CELLS

A series of solutions was made up in 50 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 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  $\mu$  to 15  $\mu$ . 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. 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, 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. No peptidase activity could be found.

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 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. So also possess weak enzymic activity. There is some evidence that the objects

(6)

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.

#### ACKNOWLEDGEMENTS

I am most grateful to Professors K. Bahadur and C. N. Perti for their helpful suggestions.

Table I

#### COMPOSITION OF SOLUTIONS

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

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

Table II

(7)  
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

<u>Class of compound</u>	<u>Compounds</u>	<u>Detection Reagents on Paper Chromatograms</u>
I. Purines	Adenine Guanine	(i) Silverchromate (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.	
IV. Ureides	Urea	(i) phenol-hypochlorite

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UNIVERSITY GRANTS COMMISSION

SUPPORT FOR SCIENCE RESEARCH IN THE UNIVERSITIES

PROPOSAL FOR SUPPORT OF RESEARCH

1. Title of the project. : Study of the problem of origin of life and its application in amino acid manufacture, photolytic decomposition of water and photochemical fixation of nitrogen.
2. (a) Institution and address : Allahabad University.  
(b) Department. : Chemistry.  
(c) Principal Investigator and other members of research group if any. : Dr. Krishna Bahadur and Dr. (Mrs.) S. Ranganayaki.
3. Academic qualifications and research experience of the Investigator(s) and publications related to the above research project. Please indicate the research schemes already undertaken and in force with funds from Commission or any other agency, giving details of grants and number of persons employed, duration etc. : (i) Dr. Krishna Bahadur, D.Sc.  
Has published about 170 scientific papers.  
(ii) Dr. (Mrs.) S. Ranganayaki, D.Sc.  
Has published about 45 scientific papers.  
The list of their publications concerning the subject of this project is attached.
4. Brief outline of objective of work and its importance. In case it has any R & D importance, please substantiate. : Attached.
5. Actual plan of work proposed : Attached.
6. Duration of the project. : Applied for three years in the first phase.
7. Detailed estimates of the expenditure to be incurred on the project. ( On annual basis and not to exceed three years in First phase):
- (a) Non recurring : Expenditure.

Grant needed for the purchase of instruments.

	Rs.
Deep freeze	- 10,000
pH meter	- 15,000
Thermostat	- 15,000

( give a list, with necessary justification and also any foreign exchange required).	Autoclave	-	10,000
	Microscope with photographic attachments.	-	50,000
	Homogeniser.	-	10,000
	Lypholyser	-	60,000
	Ultracentrifuge	-	15,000
	Fraction collector	-	15,000
	Vacuum pump	-	20,000
	Glass fittings for vacuum apparatus	-	10,000
	U.V. Spectrometer with automatic recorder.	-	50,000
	Chemicals	-	50,000
Glass apparatus	-	50,000	
			Rs. 3,80,000

(iii) Scientific journals and books.	:	30,000
(iv) Strengthening of workshop facilities	:	10,000
Total	:	Rs. 4,20,000/-

( Please indicate what portion of the above could be met from the normal development grants in V Plan).

(b) Recurring: per annum :

No.	Expenditure	
(1) Personnel: <u>Research personnel</u>	For 12 months in Rs.	
Research Associates - 2		33,600/-
Pay @ Rs. 1,400/- p.m.		
Senior Research Fellows (Post doctoral) - 4		
@ Rs. 800/- p.m.		38,400/-
Junior Research Follows- 6		
@ Rs. 400/- p.m.		28,800/-
		1,00,800/-

Supporting technical personnel:

Technical assistant - 1		
@ Rs. 425-15-500		5,100/-
Office Assistant - 1		
@ Rs. 330-10-380		3,960/-

Laboratory Assistants -2  
@ Rs. 260-6-336. Rs. 6,240/-  
Plus dearness allowance as may be permissible. Rs. 15,300/-  
(ii) Working expenses:

Service charges for amino acid analysis, NMR, EPR spectra, electromicrographs etc. 50,000/-

Travel grants, symposia and visit of scientists from abroad to this laboratory and from the laboratory to other places. 50,000/-

Contingency grant for the purchase of chemicals, glass apparatus, repair, postage typing and stationary. 50,000/-

1,50,000/-

**Pay of the Staff -**

1st year Rs. 1,16,100/-

2nd year Rs. 1,16,100/-

increment of the ministerial staff. Rs. 444/-  
1,16,544/-

3rd year Rs. 1,16,544/-

increment of the ministerial staff. Rs. 444/-  
1,16,988/-

Total Rs. per annum. Rs. 2,66,100/-

**Total Recurring for the duration of the project:**

Total pay of the staff for three years. Rs. 3,49,632/-

Service, travel and contingency for three years. @ Rs.1,50,000/-p.a. Rs. 4,50,000/-  
7,99,632/-

**Total Non-recurring and recurring:**

Non-recurring: Rs. 4,20,000/-

Recurring for the total of three years. Rs. 7,99,632/-

12,19,632/-

Please give names and addresses of three persons in other universities/ research institutes actively engaged in your area of research interest, to whom a reference may be made regarding the research project.

1. Dr. A. E. Smith, 239-9, National Aeronautics and Space Administration (NASA) Ames Research Center, Moffett field, California - 94035. ( U.S.A. )

2. Dr. D. O. Hall, University of London King's College, Department of Plant Sciences, School of Biological Sciences, 68, Half Moon Lane, London- SE 24 9 JF (England)

3. Dr. J. S. Rawat, Prof. of Biochemistry, U.P. College of Veterinary Sciences, Mathura.

8. Any other information useful in the evaluation of the project : Annexures A, B, C, D, E, F, G and H.

9. Certified that:

- (a) General facilities required such as laboratories, equipment etc. are available in the department.
- (b) No foreign exchange is required for taking up this project/ foreign exchange is required; please indicate specific items:
- (c) This research proposal has not been submitted to any other agency for assistance.

( Signature )

Principal Investigator

Head of the department.

Department

University

SECTION 'A'

All the necessary information about the Chemistry Department of Allahabad University is with the U.G.C.