24 JUL 1962

DRAFT

MEMORANDUM FOR: THE RECORD

SUBJECT : Continuation of MKULTRA, Subproject 133

1. The purpose of MKULTRA, Subproject 133 is to enable TSD/BB to utilize the services of [redacted] Associate Professor of Biology at Rensselaer Polytechnic Institute, Troy, New York.

2. During the first year of the program [redacted] has made significant contributions to the all too scanty knowledge of the mechanisms of mineral transformations. A technical discussion of these accomplishments is attached hereto, with an outline of proposed work for the coming year. It is possible that these investigations may well lead to new approaches for energy transfer systems (bio-batteries) and deterioration of metals.

3. [redacted] functioned as cover and cutout during the first year of this project. This service will heretofore be furnished by the Geschickter Fund for Medical Research. The cost of this program for the second year will be $9,000.00 to which must be added $360.00 which represents a 4% service charge to be retained by the cutout. The total cost of the program, therefore, will not exceed $9,360.00. Charges should be made against Allotment No. 3125-1390-3902.

(b)(3)

4. It is not anticipated that permanent equipment other than that listed in the budget will be required for this program. Title to the

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equipment listed will be retained by the Institute in lieu of higher overhead rates.

5. Documentation and accounting for travel expenses which are reimbursable by [redacted] will conform to the accepted practice of that organization.

6. [redacted] has been cleared COVERTLY and is unwitting and will remain unwitting of the true nature of the sponsor.

[redacted]

TSD/[redacted]

[redacted]

CHIEF

TSD/[redacted]

APPROVED FOR OBLIGATION OF FUNDS:

____

AC/TSD

DATE: ____________________

Attached:
- Budget
- Project Summary and Proposal

Distribution:
- Original only

1-55-55

[redacted]

Research Division B

25 June 1962

B The Geschicter Fund
1834 Connecticut Avenue
Washington, D. C.

Attention: [redacted]

Subject: Proposal entitled "[redacted] C

Dear [redacted]

Please find enclosed one copy of the subject proposal submitted on behalf of [redacted]

B [redacted]

The proposal is not being submitted elsewhere for possible support.

Your consideration of our proposal will be appreciated and we look forward to hearing from you.

Very truly yours,

B [redacted]

Assistant Director

[redacted]ep B
Enclosure

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[redacted]

Proposal entitled

[redacted]

Submitted on behalf of

[redacted]

June 1962

DEPARTMENT OF BIOLOGY

[redacted]

June 14, 1962

[redacted]

Purpose of Study:

The purpose of this proposal is a request for financial support to continue an investigation of microbial action on marine manganese nodules and terrigenous mineral sulfides, which the principal investigator has been pursuing since 1958. Very intensive work on these materials is being carried on by him, with fruitful results, during the current year, 1961-62, under a grant from the [redacted] of Stanford University, California. Since relatively little is known about microbial mineral transformation, and in view of current academic and practical interest of microbiologists, geologists, mining engineers, soil scientists, oceanographers, etc., in the subject, this research should make a valuable contribution to science.

Summary of Past Work:

a. Bacteriology of mineral sulfides.

Attempts were made to evaluate the microbial flora isolable from unsterilized, crushed sulfide minerals by enrichment in mineral solution. The following minerals were studied: alabandite, arsenopyrite, bornite, chalcocite, chalcopyrite, cinnabar, cobaltite, covellite, enargite, galena, marcasite, orpiment, pyrite, pyrrhotite, realgar, and sphalerite. Of these minerals, only cobaltite, enargite, galena, pyrite, pyrrhotite, realgar, and sphalerite yielded microorganisms. For the most part these organisms were heterotrophic and probably represented contaminants. However, Hyphomicrobium, isolated from realgar, a pink yeast repea from sphalerite, and Arthrobacter, isolated from cobaltite, galena, pyrrhotite, realgar, and sphalerite may constitute part of a normal flora. The action of any of these organisms with respect to the mineral with which they were found associated remains to be established.

After surface-sterilization, some of the above mineral sulfides, when enriched in mineral solution, have yielded iron-oxidizing autotrophs. These minerals include arsenopyrite, pyrite, pyrrhotite, chalcopyrite, enargite, galena, marcasite, and sphalerite. At least some of the isolated bacterial strains are not restricted to a diet of iron for energy, but can use sulfur or, probably, some other oxidizable metals.

The ability to grow on any of the above sulfide minerals was tested by inoculating surface-sterile samples in oxidizing columns with Ferrobacillus ferrooxidans, and attempting to recover the organism from effluent feeding solution over a period of two months or more. So far, positive results have been obtained with arsenopyrite, enargite, chalcopyrite, marcasite, galena, pyrite, pyrrhotite, and sphalerite. Negative results have been obtained with alabandite, bornite, cobaltite, covellite, chalcocite, and one sample of galena. Cinnabar, orpiment, and realgar are being currently investigated.

In addition to the foregoing qualitative work, quantitative studies on the rates of oxidation of synthetic Cu_2S and natural arsenopyrite are presently being undertaken. From these studies it has become clear that synthetic Cu_2S can be oxidized at least 4x as fast by bacteria than by autoxidation, and that arsenopyrite can be more rapidly oxidized by bacteria than by autoxidation. Results with the latter material are not yet sufficient to establish an exact rate comparison. The precise mechanism of bacterial oxidation remains to be established. The work with synthetic Cu_2S proves, what some other workers seem to doubt, that F. ferrooxidans can oxidize metals other than iron.

b. Manganese Nodules

Oceanographers have felt pretty strongly in the past that the origin and development of manganese nodules in the oceans is attributable to purely physicochemical processes. However, [redacted] on finding organic nitrogen in nodules, concluded that biological agents were involved in nodule genesis. At his suggestion, the principal investigator attempted to find out if bacteria might play a role in this. He found that bacteria were indeed present in the nodular substance after surface-sterilization (a rough estimate at present is 10^4 per gram). They included Achromobacter, Arthrobacter, Bacillus, Brevibacterium, Staphylococcus, Vibrio, an unidentified rod, and an unidentified coccus. The principal investigator showed in quantitative experiments that nodular substance can adsorb manganous ion from sea water, and that this adsorption is accelerated by bacteria that grow from the nodular material. The acceleration of manganous ion adsorption is explainable on the basis that the bacteria oxidize the adsorbed manganese, which facilitates further adsorption of manganous manganese. The acceleration requires the presence of peptone, to permit bacterial development. If peptone and glucose are present, manganese is released from the nodular substance rather than adsorbed, at least in a net effect. Since some nodules were apparently initiated around shark's teeth, ear bones of whales, pumice, etc., in the sea, attempts were made to see if oyster shells can adsorb manganous manganese and thus serve as possible foci of nodules.

It was found that they do adsorb it and that peptone did not stimulate this adsorption (no bacteria were present!). As far as a survey of the literature has gone, these observations with respect to manganese nodules have not been reported before.

Pertinent literature:

The early literature dealing with microbial action on minerals has been covered by Alexander (1). A review by Lyalikova summarizes much of the past important work on Thiobacillus ferrooxidans and Ferrobacillus ferrooxidans (2). An intimate association of iron-oxidizing autotrophs with natural mineral sulfides has been indicated by the work of [redacted] and by that of Lyalikova (5). Differences of opinion exist between Bryner and Anderson (6), Malouf and Prater (7), and Ivanov, Nargirvyak, and Stepanov on the one hand, and [redacted] (4) [redacted] (8) on the other about a mechanism of mineral sulfide oxidation of chalcopyrite, molybdenite, chalcocite, and sphalerite, for instance. No previous studies on bacteria in manganese nodules has been reported. However, bacterial manganese oxidation and reduction by soil bacteria has been known for some time. An important quantitative study on large-scale bacterial manganese metabolism is that of Mann and Quastel (9). Descriptions of manganese nodules are given by Murray (10) and Dietz (11). A chemical and physical study of nodules was made by Buser and Gruetter (12). The finding of organic nitrogen in nodules was first reported by Graham (13) and Graham and Cooper (14), who also suggested a biological origin of the nodules on this basis.

References:

1. Alexander, M., INTRODUCTION TO SOIL MICROBIOLOGY, John Wiley & Sons Co., 1961.

2. Lyalikova, N. N., Mikrobiologiya 29: 773-779 (1960).

3. [redacted]

4. [redacted] (1962) in press.

5. Lyalikova, N. N., Mikrobiologiya, 30: 135-139 (1961).

6. Bryner, L. C., and R. Anderson, Ind. Eng. Chem. 49: 1721-1724 (1957).

7. Malouf, E. E., and J. D.Prater, J. Metals 13: 353-356 (1959).

8. Razzell, W. E., Annual Western Meeting, Vancouver, Oct. 1960 Transactions, LXV, 1962 pp. 135-136.

9. Mann, G., and J. H. Quastel, Nature 158: 154-156 (1946).

10. Murray, J., VOYAGE OF H.M.S. CHALLENGER. DEEP SEA DEPOSITS. Her Majesty's Stationary Office. 1891.

11. Dietz, R. S., J. Calif. Mines and Geol. 51: 209-220, (1955).

12. Buser, W. and A. Gruetter, Schweiz. mineralog. petrogr. Mitt. 36: 49-62, (1956).

13. Graham, J. W., Science 129: 1428-1429 (1959).

14. Graham, J. W. and Susan Cooper, Nature 183: 1050-1051.

Pertinent Publications by Principal Investigator:

[redacted] (1962) in press.

[redacted]

[redacted]

Proposed New Work:

Continuation of present lines of investigation:

a. Mineral Sulfides:
   1. Continuation of survey of natural sulfide minerals for a normal flora, with particular emphasis on large-scale microbial action on minerals.

   2. Characterization of isolates (physiological and morphological).

   3. Examination of isolates for specific mineralizing activities.

   4. Elucidation of biochemical mechanisms of mineral transformation.

b. Manganese Nodules:
   1. Qualitative and quantitative bacteriological comparison of manganese nodules from different oceans.

   2. Study of the biochemical mechanism of manganous oxidation and MnO_2 reduction in the various bacteria isolated.

   3. Determination of the mechanism of iron-incorporation into manganese nodules.

The methods to be used in these studies will be adaptations of standard procedures of bacteriology, physiology, and biochemistry.

Personnel:

leave in

Principal Investigator: [redacted]ssoc. Prof. of Biology.

Technician: [redacted]

Graduate Student: [redacted] not presently supported)

Undergraduate student:
(NSF undergraduate research fellow) [redacted] (summer 1962)
(summer 1963)

Proposed Budget:

PERSONNEL		
Principal Investigator		
1/8 time-academic year	$ 1,050	
1/4 time-summer months	700	
Technician-full time	4,155	
		$ 5,905
PERMANENT EQUIPMENT		
Fluorimeter		900
CONSUMABLE SUPPLIES		
Chemicals	$ 300	
Glassware	340	
		640
TRAVEL		
To scientific meetings		250
OTHER EXPENSES		
Publication	$ 180	
Telephone	20	
		200
	Total Direct Cost	$ 7,895
INDIRECT COST		
@ 14% of Total Direct Cost		1,105
	Total Cost	$ 9,000