COMMONWEALTH OF AUSTRALIA.

DEPARTMENT OF NATIONAL DEVELOPMENT.

BUREAU OF MINERAL RESOURCES GEOLOGY AND GEOPHYSICS.

RECORDS:

1964/38

SYNGENESIS OF SULFIDE ORES: AN EVALUATION OF BIOCHEMICAL ASPECTS

by

Kenneth L. Temple

Dept. of Botany and Bacteriology, Montana State College
Bozeman, Montana.

* * * *

SYNGENESIS OF SULFIDE ORES: AN EVALUATION OF BIOCHEMICAL ASPECTS (1)

by

KENNETH L. TEMPLE.(2)

RECORDS 1964/38

ABSTRACT

Syngenetic theories of sulfide ore formation often include microbial sulfate-reduction as the source of sulfide. The conditions required for large scale reduction of sulfate, quantitative aspects, the potential and the limitations of microbial sulfate-reduction are sometimes not fully appreciated or may even be misunderstood by those participating in the debate on syngenesis of sulfides. The biochemical factors involved are considered separately and this is followed by a general discussion. Both qualitative and quantitative aspects of microbial sulfate-reduction are adequate for syngenetic theory. The conditions required for sulfate-reduction cannot be specified simply but are satisfied by various combinations of circumstances which result in oxygen depletion in a wide variety of physical environments. Metals entering such environments, whether adsorbed or not, are converted to metal sulfides. Sulfate-reduction does not explain the origin of the metal moiety. Separation and concentration of adsorbed metals and the identification of biochemicals in ore-bearing strata need further research. Objections to syngenesis based upon toxicity of metals are not valid. Biochemical factors cannot conclusively establish the origin of sulfide deposits but are consistent with a sedimentary origin. Microfossils in PreCambrian pyrite are not identifiable with modern sulfate-reducers but do contain organic matter. On biochemical grounds, it is extremely unlikely that microbial sulfate-reduction evolved later than the early PreCambrian.

INTRODUCTION

Syngenetic theories of formation for certain sulfide ores have been advanced for many years. Most of these theories received scant attention until recently. Geological evidence from such large commercial deposits as those at Mount Isa and Broken Hill in Australia and the Copperbelt in Northern Rhodesia has forced a reconsideration of the possibility of syngenesis and has resulted in a recasting of such theories (9, 11). Bastin in 1926, and most modern advocates of syngenesis, postulated bacterial sulfate-reduction as the source of sulfide (3). This introduces a number of biochemical considerations. The debate on syngenesis has generally been conducted without an adequate assessment of these biochemical factors. This paper attempts a critical evaluation of the biological sulfate-reducing system in-so-far as it relates to the overall problem

../2

(1) Published with the permission of the Director, Bureau of Mineral Resources, Geology and Geophysics, Canberra, and the Chief, Division of Plant Industry, C.S.I.R.O., Canberra.

(2) Dept. of Botany and Bacteriology, Montana State College, Bozeman, Montana.

of metal sulfide deposition. It is hoped that this evaluation will clarify both the value and the limitations of biological sulfate-reduction for syngenetic theory.

The biochemical aspects to be considered will first be identified and then discussed individually. A general discussion will then assemble these factors into a broad picture of sulfate-reduction in relation to syngenesis.

THE BIOCHEMICAL FACTORS PERTINENT TO SYNGENESIS

The formation of metal sulfides in a sediment at the time of, or shortly after, deposition of that sediment requires rather specific conditions. The gist of the problem is a means for bringing metals in some form into the proximity of a sulfide zone so that metal sulfides are produced and concentrated in a sediment. Various concentrations of different sulfides and of non-sulfide material must be explained. This is the portion of syngenetic theory in which biochemical factors are most important. However, it is also necessary to show that the subsequent history of the sediment and the mineralogical and geochemical evidence do not argue more convincingly for a replacement origin. Biochemical factors that are pertinent to this phase of syngenetic argument are the persistence of microfossils or degraded organic compounds of biological origin in sulfide ores and the microbial fractionation of isotopes.

For the purposes of this discussion, we may list the following factors:

(1) The environment in which sulfate-reduction takes place.

(2) Quantitative aspects of sulfate-reduction.

(3) Movement of microbially-produced sulfide.

(4) Microorganisms reducing sulfate; the age of biological sulfate-reduction.

(5) Natural factors limiting sulfate-reduction.

(6) Isotope fractionation by biochemical processes.

(7) Differential precipitation and concentration of sulfides.

(8) The forms of metals which will react to give sulfides under the conditions of microbial sulfate-reduction.

(9) Microfossils and relict organic compounds.

THE ENVIRONMENT OF BIOLOGICAL SULFATE-REDUCTION

The portion of the natural aqueous environment in which sulfate-reduction occurs has been described by numerous authors. The depiction by Baas-Becking et al. in terms of pH and Eh is most useful for our purpose. This consists of a graph with pH as the abscissa and Eh as the ordinate (Figure 1).

Oxidized (oxygenated) or well aerated waters have a high Eh value and are located at the upper part of the graph. Reduced waters with depleted oxygen or appreciable hydrogen sulfide have a low positive or a negative Eh value and are at the bottom of the graph. Acid waters are at the left and alkaline waters at the right. Similar graphs have been used by Garrels and others to define the stability zones of mineral species in aqueous media (12). Figure 1 superimposes a diagram from Garrels and one from Baas-Becking, Kaplan & Moore (1). The larger area enclosed by a solid line is the latter authors' conception of the actual boundaries of the natural aqueous environment at the earth's surface. The smaller area at the bottom enclosed by a solid line is that portion of the aqueous environment which is occupied by sulfate-reducing organisms. The dashed lines are the boundaries of mineral stability zones. According to this diagram, elemental or native Cu, an equilibrium mixture of S and CuS, CuS, and Cu_2S are all thermodynamically stable in part of the sulfate-reducing environment, but the largest part of this environment is occupied by the mineral Cu_2S. This is true only for the concentrations and the temperature and pressure given and in the absence of other chemicals. However, the effect of other variables can be calculated.

Similar diagrams can be prepared for any mineral desired. It will be found that metal sulfides are thermodynamically stable in at least part of the area where bacteria produce sulfide. This does not mean that an oxide, carbonate or silicate will necessarily be converted to the corresponding sulfide in such an environment because the diagram tells nothing about reaction rates. The laboratory preparation of metal sulfides will be discussed later. The position of boundary lines and the existence of some zones depends upon the concentrations of sulfide and carbon dioxide in the water. Garrels' text should be consulted for further examples and methods of calculating equilibria.

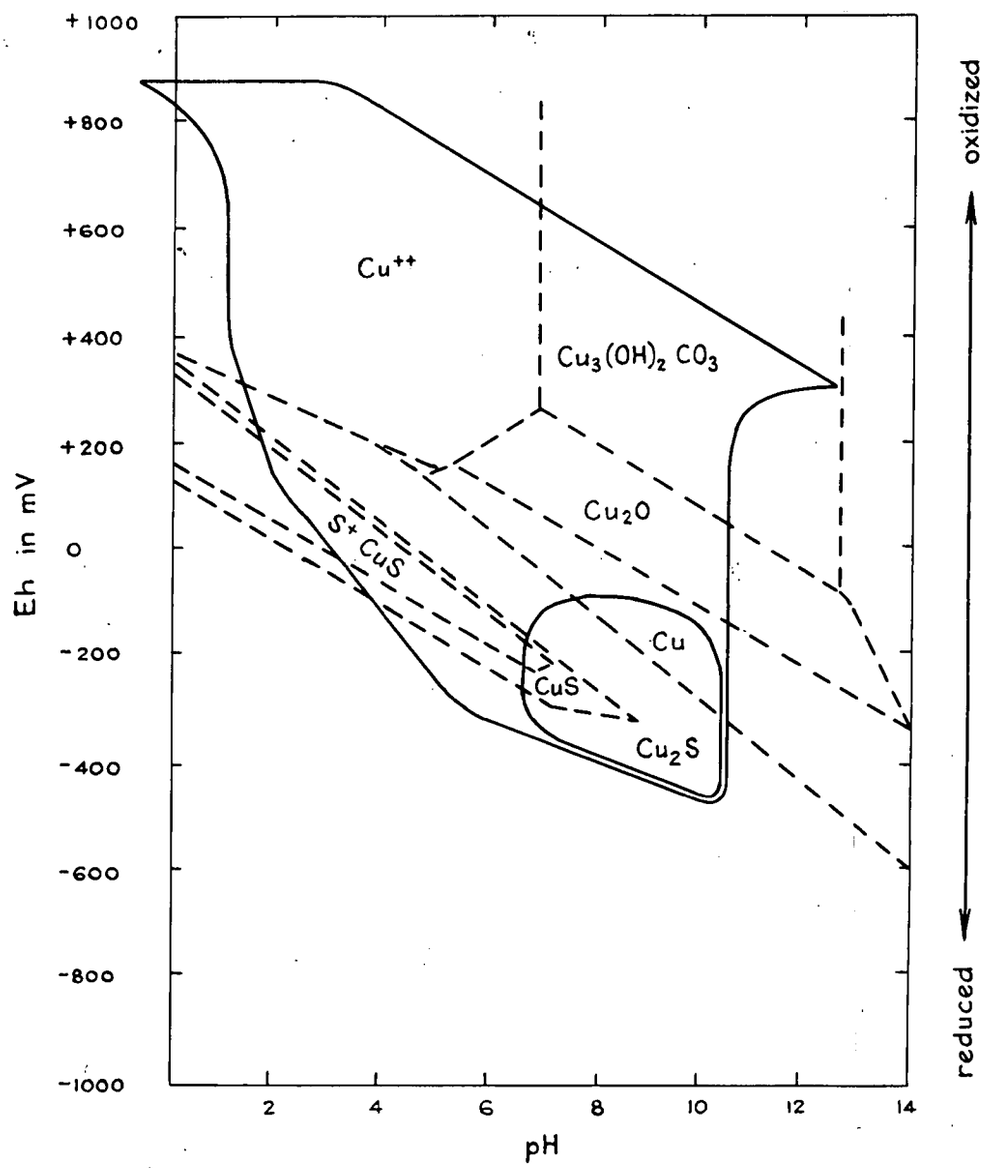
It is worth noting here that natural waters having a low Eh and a nearly neutral or slightly alkaline pH do in fact contain sulfide. The amounts of sulfide will be discussed under quantitative aspects.

The environment occupied by sulfate-reducing microbes is set by the peculiar nature of their metabolic reactions. Sulfate-reducing bacteria are obligate anaerobes, quite unable to grow in the presence of dissolved oxygen. In spite of this, they live by an oxidation reaction. Unable to use molecular O_2, they substitute oxygen atoms from the sulfate ion, SO_4--, and oxidize either organic compounds (i.e., by removing hydrogen) or elemental H_2. Sulfide is a by-product.

$$SO_4{}^{--} + 8\ H \qquad 4\ H_2O + S^{--} + \text{energy}$$

This reaction, the oxidation of hydrogen to water, using sulfate as the source of oxygen, and producing sulfide as a by-product, is the primary source of all energy for these organisms. The energy is needed to run the chemical factory that is the living cell. Biological sulfate-reduction begins in water that is already deficient in oxygen from some other physical, chemical or biological cause. Once underway, the reduction process lowers the Eh still more by reason of the sulfide produced. A sulfate-reducing zone of any size can then be recognized simply by its sulfide odor.

Fig. 1



Stability fields of copper compounds in the system $Cu-H_2O-O_2-S-CO_2$ at 25°C, 1 atm. total pressure, $P_{CO_2} = 10^{-3.5}$ [total S] $= 10^{-1}$, superimposed on the boundaries of the natural aqueous environment and the sulphate-reducing environment.

Outer solid line - natural aqueous environment

Inner solid line - sulphate-reducing environment

From Baas-Becking et al., 1960

To accompany Record 1964/38

Bureau of Mineral Resources Geology and Geophysics August 1964 M(Gb)/1

The environment of biological sulfate-reduction is found wherever the exchange of oxygen with the air is limited or the consumption of oxygen is very great. A limited exchange of oxygen with the air exists in deep basins, in basins with thermal layering or chemical layering, in basins with a large volume to flow ratio and under salt crusts. High oxygen consumption rates are usually due to the rapid decomposition of organic matter which occurs in estuaries, tidal flats, swamps, and intermittently flooded soils. In a well-aerated basin, sulfate-reducers are most apt to be found in the bottom mud and the water immediately above. The importance of oxygen removal in this combination is often underestimated. Sulfate-reduction is more apt to occur in a shallow basin with appreciable organic debris than in a deep basin with little organic matter. The examples discussed in the next section should help visualize the situation.

QUANTITATIVE ASPECTS OF SULFATE-REDUCTION

Laboratory experiments show that three criteria of sulfate-reduction, i.e. the rate, the concentration of sulfate attained, and the density of the bacterial population reached, depend upon (1) the amount of sulfate available, (2) the amount of reducing agent available, (3) the type of reducing agent (whether H_2 or any of several organic compounds furnishing hydrogen), (4) the presence of specific stimulatory organic compounds, (5) the salt concentration, (6) the temperature and (7) the degree of agitation or mixing. Sulfate-reducing bacteria are commonly encountered in cold water or sediments only a few degrees above freezing. But like other bacteria found in cold environments, these grow faster and are more active at temperatures of 20° to 30°C. Sulfate-reduction takes place in fresh, marine and saline waters but one percent NaCl concentration has been reported as optimum for many strains and we may expect that sulfate-reducers from any source will have a definite optimum salt concentration (20). Peptone has been used in laboratory media for growing sulfate-reducers with good success and Kadota & Miyoshi reported that nucleotides and amino acids improved growth (21). No attempt will be made to summarize the extensive data on growth of sulfate-reducers under controlled conditions, but the yields under typical laboratory conditions will be discussed and such comparative data as is available from natural environments will be covered.

In stationary cultures, Miller obtained H_2S concentrations up to 2500 mg H_2S/liter. By adding metals to precipitate the sulfide as it was formed, the total sulfide yield could be stepped up even more (23, 24, 25). More recent studies with three liter stationary culture vessels yielded 200 to 600 mg H_2S/liter depending upon the amount of calcium sulfate present (4). Freke & Tate used a three liter vessel which was stirred and in which the liquid medium was steadily replaced at

A mixture of ferrous and ferric sulfates was used to remove the H_2S as iron sulfide. A precipitate analyzed as $2FeS \cdot Fe_2S_3$ was formed at the rate of 150 mg/liter/hour and had a settling rate of 10 inches/min. (10). Butlin & Postgate reported results from a continuous flow culture apparatus in which replacement of the medium was complete every 24 hours. Their sulfide yields were 135 to 175 mg of sulfide sulfur/liter when a nutritionally simple medium was used and were boosted to 2000-3000 mg/liter only when special nutrients,

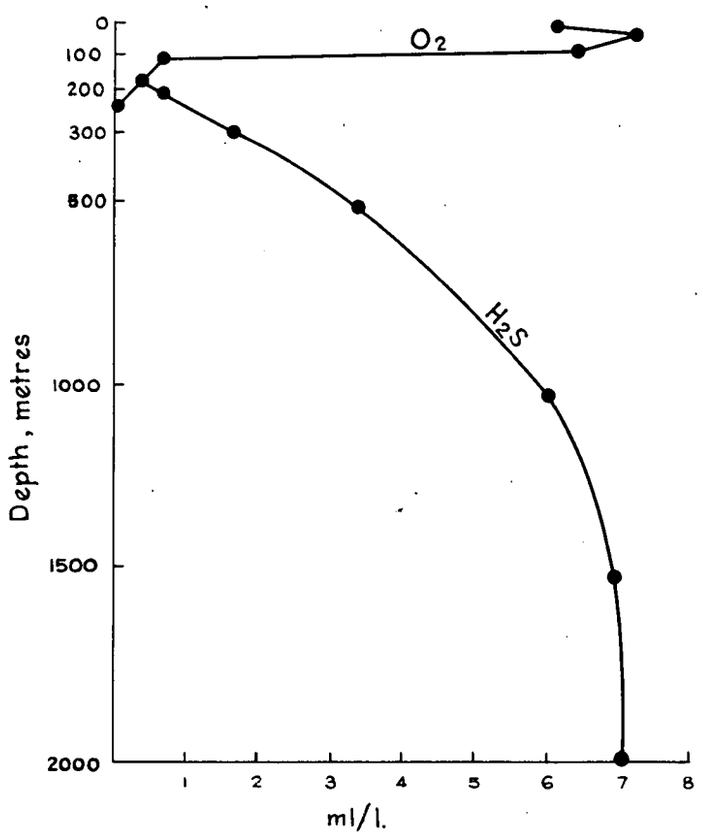
yeast extract and tryptone, were added (5). The percent of sulfate reduced was only 24 to 31 percent in the simple medium. However, this low efficiency would give an iron sulfide deposition rate of 203 to 262 mg Fe_4S_5/liter/hr compared to the 150 mg cited above. Temple & LeRoux found that mature sulfate-reducing cultures which had entirely exhausted their supply of organic matter but which had an unlimited supply of H_2 brought about 100 percent reduction of sulfate in stationary cultures to which copper sulfate was added (31). Continuous flow cultures can be adjusted on the basis of any of several criteria and no doubt complete reduction is possible with them using simple media.

Laboratory experiments such as those just mentioned should be more efficient than the uncontrolled reactions of natural systems. In particular, one might expect the amount and kind of organic matter to be suboptimal in natural waters. This is not critical however, as the following examples will show.

The average H_2S content of the Black Sea is shown in Figure 2. The maximum concentrations of 5.97 to 6.97 ml H_2S/liter (ca 4 mg H_2S/liter) are quite low compared to laboratory results. Although the Black Sea is the classical example of a sulfate-reducing basin, or has been so considered, it is not the most impressive one. A Libyan lake, Ain-ez-Zauia, had 15 to 20 mg H_2S/liter at the surface and 108 mg H_2S/liter at the bottom (6). Lake Faro, a small stagnant lake, had frequent values of approximately 60 to 70 mg/liter (13, 14). Genovese recorded a complete disappearance of sulfide in Lake Faro after a canal to the Tyrrhenian Sea had been open for a month. After closing this canal from September to 11 November, the hydrogen sulfide value had returned to 17.35 mg/liter. This is probably more indicative of the time required for an anaerobic environment to be regenerated than it is of sulfate-reduction rates. An estimate of minimum sulfate-reduction rates can be made from data for Lake Ain-ez-Zauia by Butlin & Postgate (5, 6). In this and nearby lakes, the sulfide formed from sulfate was undergoing oxidation to elemental sulfur, either chemically or through the action of photosynthetic sulfur bacteria which were present in large masses. The sulfur settled to the lake bottom and was harvested annually. A lake volume of 500,000 gallons yielded 100 tons of sulfur in a year. Since the oxidation of sulfide to sulfur cannot be expected to be 100 percent efficient, the rate of reduction of sulfate to sulfide must have been in excess of this value.

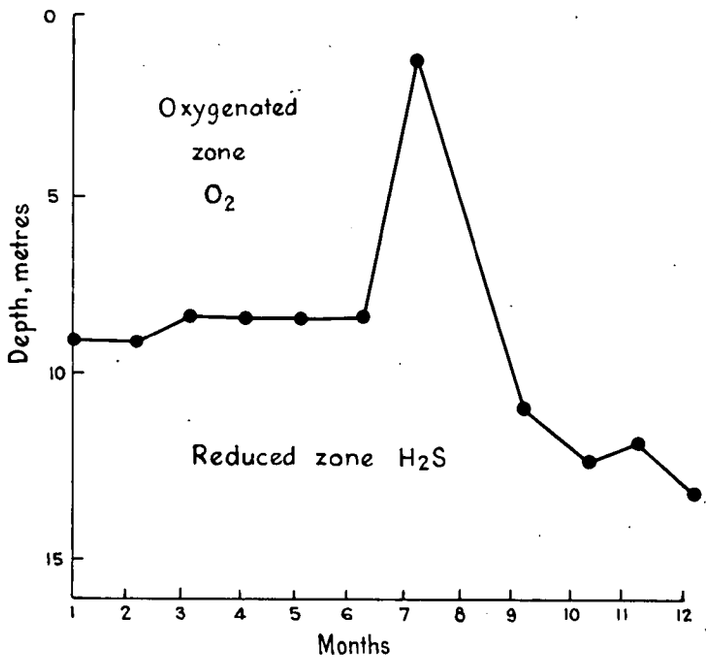
Gunkel & Oppenheimer conducted experiments on sediments in situ by inserting plastic tubes into the soft sediments of Port Aransas, closing the tubes and withdrawing tubes at intervals for analysis. Sediments from Texas and from the North Sea were also collected, homogenized and incubated in the laboratory (15). These sediments had a high organic matter and some of the observed sulfide production was due to this organic sulfur. In the undisturbed sediment, sulfate reduction amounted to 0.577 mg sulfate-S/gram wet sediment/11 weeks. Taking one gram of wet sediment as approximately one ml, this equals 7.96 mg H_2S/liter/day. However, not all of the vanished sulfate appeared as sulfide.

Fig. 2



Average H_2S and oxygen content of the Black Sea

Fig. 3



Variation in the boundary of oxygenated and reduced zones in Lake Faro over a 12month period. After Genovese (14)

To accompany Record 1964/38

Ivanov investigated the rate of sulfate-reduction in waters taken from bores in the Carpathian sulfur deposits (16, 17). His technique was to add radioactively labeled sodium sulfate, $Na_2S^{35}O_4$, to the water samples, stopper to exclude air, incubate about 24 hours and determine the distribution of S^{35} in the sulfate, sulfide and total sulfur fractions. The rate of sulfate-reduction varied with the formation from which the water came and with different bore holes in a given formation. Results were: 0.20 to 0.25 mg H_2S/liter/day at Nemirov, 0.54 mg/liter/day at Vektii and 0.029 to 2.015 mg/liter/day at Rozdol. These figures represent the capacity of these waters to reduce sulfate added to them. They do not necessarily indicate the rate of sulfate-reduction actually occurring in the waters underground. Moreover, these samples are not from the type of water basin in which we are most interested. Nevertheless, the use of isotopes and a short incubation time suggests that the results are reliable and may apply to other sulfate-reducing waters.

The implications of these several estimates of quantitative sulfate-reduction will be examined in the general discussion.

MOVEMENT OF MICROBIALLY-PRODUCED SULFIDE

The term 'movement' is here used in respect to processes regulating the distribution of sulfide in the vicinity of an anaerobic zone, and does not refer to transport of metal sulfides or later displacement mechanisms.

Throughout this paper the word 'sulfide,' unless otherwise modified, connotes the most reduced form of sulfur without specifying any molecular or ionic entity. The particular form of sulfide which predominates depends upon the equilibrium of hydrogen sulfide, hydrosulfide ion and sulfide ion, and is controlled primarily by the total sulfide concentration and the pH.

$$H_2S \rightleftharpoons HS^- + H^+ \rightleftharpoons S^{--} + H^+$$

All three forms are probably present to some extent in water containing any one of them (some people dispute this) and all of them diffuse. H_2S is also lost to the atmosphere by volatilization. Calculated sulfur balances of fresh water runoff and the sulfate expected from weathering leave a large discrepancy. The missing sulphur has been converted to H_2S, some of which escapes into the atmosphere (8). Isotope data lend credence to this explanation (18).

Regardless of the exact amount of H_2S that is volatilized, it is certain that microbially produced sulfide diffuses in all directions and passes into the gas phase on reaching a gas-water interface. If a tube of copper sulfate is suspended over a culture of sulfate-reducing bacteria, covellite forms in the tube (our results). In Lake Ain-ez-Zauia, upward diffusion reached the surface but in the Black Sea the upward diffusion limit was about 150 meters below the surface. When upward diffusion has a sharp limit, this is due to the removal of sulfide by photosynthetic sulfide-utilizing bacteria. Since these bacteria are anaerobic, they are usually found below the

surface. Since they are photosynthetic, they are found above the zone of sulfate-reduction and in reach of sunlight. Because of the reddish pigment that some of these bacteria possess, they may form a visible zone of red water at the lower boundary of the oxygenated water and the upper boundary of the sulfide zone. Figure 3 by Genovese shows how the red zone can change position over a year's time (14). In the laboratory, microbially or chemically produced sulfide will diffuse through a column of agar and displace metals from a clay on which they are adsorbed (32).

It is unreasonable to expect sulfide to be moved very far from the zone of its productinn unless present in sufficient quantity to maintain a reducing Eh. Movement by processes other than simple diffusion cannot be expected because a turbulent basin contains too much oxygen for sulfide production in the first place.

A sulfate-reducing basin can act as a sulfur trap in which the sulfur is continuously recycled without much loss from either precipitation or volatilization and is therefore concentrated. Green Lake, in New York is an example of this (7). This water basin is 59 meters deep and the stagnant volume below 20 meters is 47.5 percent of the total. The upper boundary of the stagnant zone is marked by the presence of magenta-colored photosynthetic sulfide-utilizing bacteria which trap the rising sulfide and prevent escape through volatilization. There is no iron or other heavy metal in appreciable quantities and the bottom sediment has no detectable sulfur. The lake has a sulfate content of 441 mg sulfate-S/liter and an H_2S content in the sulfate-reducing zone of over 30 mg H_2S/liter. It is an outstanding example of the failure to precipitate sulfides when the metals in the entering water are in very low concentration.

Ostroumov & Shilov studied the distribution of H_2S and iron sulfides in sediments of the Kuril-Kamchatkan and Aleutian deeps (26). In contrast to open ocean sediments, those deep sediments had a sulfate-reducing zone that began near the top of the sediment (at about 5 cm depth) and free H_2S was present as well as hydrotoilite. Two factors, the presence of organic matter and the relative absence of oxygen, were assumed to be responsible for the greater prevalence of sulfate-reduction in these deeps than in most open ocean sediments. However, the relation between organic matter and sulfide in cores was not consistent. This can only mean that sulfide was diffusing through the sediment from its place of origin or that some factor other than organic matter was limiting. Local stringers of sulfide-rich mud were found and a sulfide maximum was commonly present at 30 to 40 cm. Diffusion rates were evidently unequal and less than sulfate-reduction rates.

The movement of microbially produced sulfide is therefore highly restricted and massive deposits of metal sulfides formed from it must be deposited originally in the near vicinity of the sulfate-reducing zone. Depending upon the settling rate of the metal sulfides, some movement could take place subsequent to metal sulfide formation. Any postulated model of an anaerobic basin with metal carrying waters flowing into it must have volume and flow proportions such that the anaerobic nature of the basin is not disturbed.

MICROORGANISMS REDUCING SULFATE : THE AGE OF SULFATE REDUCTION

Identification of the sulfate-reducers has been deferred so that their origin and evolution could be discussed against a background of their activity. Desulfovibrio is the name currently applied to the genus of bacteria mainly responsible for sulfate-reduction in soil and water. Desulfovibrio desulfuricans is the best known species and is a small, motile, curved rod or vibrio which does not form spores and which grows best at moderate temperatures. Other species are D. aestuarii and D. orientis (spore forming). Older names for these same bacteria are Spirillum desulfuricans, Microspira aestuarii, Vibrio thermodesulfuricans and Sporovibrio desulfuricans. An entirely different bacterium, Clostridium nigrificans, also reduces sulfate but is not typically found in sulfate-reducing waters. Desulfovibrio has a quite universal distribution in soil and water and quickly develops to appreciable numbers whenever oxygen becomes deficient.

The ecological niche occupied by Desulfovibrio is a narrow and highly specialized one which explains the virtual lack of competitors with similar abilities. Although narrow, this niche has certainly existed continuously throughout most of geologic time ever since the evolution of life on earth. The theories of the origin of life which postulate a reducing environment permit the hypothesis that sulfate-reduction by some biological agent is one of the most primitive biochemical mechanisms. Bacteria in general (Desulfovibrio included) have a rather complicated internal metabolism closely related to that of higher living forms and not necessarily very close to the real primitive cells. Since bacteria do not leave a reliable fossil record (see further under microfossils) it is impossible to do more than speculate on their origin. The unicellular organism has definite competitive advantages in rapidity of response and adaptability to changing circumstances. Unicellular organisms of necessity precede multicellular ones. Consequently it is reasonable supposition that some sulfate-reducing microbe has been in existence since early in the history of life. The extension of the fossil record into the PreCambrian, however scantily, carries with it the presumption that microbial sulfate-reduction is at least that old and probably much older. It is possible that early specialization never led to extinction for sulfate-reducers and that Desulfovibrio is a very ancient genus.

The only evidence pertinent to this topic is indirect, from isotope data. Desulfovibrio preferentially reduces the lighter mass isotopes of sulfur (as described later). Assuming that deposits of sulfate, sulfur or sulfide reflect this isotope fractionation, Thode estimated that the maximum age of biological sulfate-reduction was 700 to 800 million years (33). Vinogradov disagreed with the basis for this estimate (36). The accuracy of this date could be important for syngenetic theory if we accept the idea that Desulfovibrio or another organism which selectively used sulfur isotopes is required.

NATURAL FACTORS LIMITING SULFATE-REDUCTION

Oxygen is the commonest inhibitor of sulfate-reduction and of the growth of Desulfovibrio. In laboratory experiments with pure cultures, a small bubble of air is enough to prevent growth. Mixed cultures of sulfate-reducers and other bacteria are much more tolerant to oxygen. This is because the other bacteria remove the oxygen.

The study of oxygen transport in water basins is very complicated, involving not only solubility and diffusion but thermal layering, salinity layering, other chemical layering, turbulence and changes in pressure and temperature. It is sufficient to acknowledge that many deep basins contain appreciable dissolved oxygen and that this fact alone adequately explains the lack of sulfate-reduction in these basins. Eh and dissolved oxygen measurements indicate exygenated water in some Californaia coastal basins at depths of thousands of meters contrasted to anaerobic zones at 35 meters in Green Lake and 200 meters in the Black Sea. Oxygen and hydrogen sulfide concentrations are inversely related in the definite manner shown in Figure 2 redrawn from Skopintsev (29).

Without facultatively anaerobic bacteria and aerobic microorganisms of various sorts, oxygen inhibition would be more extensive. The example of mixed culture effect found in laboratory cultures holds for natural environments. Decomposition of organic matter is carried out initially by aerobic and facultatively anaerobic organisms (those able to grow either in air or its absence). These microorganisms use oxygen to break down the organic molecules. If a large mass of organic matter is decomposed, the entire area becomes at least temporarily depleted of oxygen. If smaller amounts of organic matter are present and the environment is not thoroughly mixed by turbulence currents, microscopic anaerobic pockets develop. Sequences of microbial populations are measured on a time scale of hours so that anerobic conditions can be established, or re-established, in a matter of minutes.

Sulfide itself is toxic to most organisms including many bacteria but does not interfere with the activities of the Desulfovibrio which produce it (24).

The ionized forms of heavy metals are general metabolic poisons and one might expect that this would be a stumbling block for syngenetic theory. However, numerous examples are known of microbes tolerant to heavy concentrations of copper and other metals and some thrive in saturated copper sulfate solution. In the case of Desulfovibrio, however, the sulfide zone enveloping the bacteria protect them from metal toxicity (31).

No other substances are important inhibitors of sulfate-reduction in natural environments. Both sulfate and organic matter (or H_2) must be limiting factors. The variation in sulfate content of waters is too well known to require comment. Organic matter is much more suspect as a limiting factor in practice. No quantitative data is available. As stated, amino acids, nucleotides and specific

are not supported by the observations of sulfate-reduction in Libyan lakes wherein Butlin and Postgate commented on the low organic matter content and the high sulfur turnover (6). Still, productivity must eventually rest upon the available energy and the energy-yielding reactions for Desulfovibrio require either organic matter or H_2. Organic matter is the most probable immediate external source of energy. This brings us to the conclusion that extensive sulfate-reduction is not limited to those basins where there is an abundant supply of decomposing higher plants, and that adequate organic matter for large amounts of sulfide formation exists in water supplied with zooplankton and phytoplankton as the major organic material.

ISOTOPE FRACTIONATION BY BIOCHEMICAL PROCESSES

When sulfate is reduced to sulfide by microorganisms, the lighter mass isotopes are reduced at a slightly faster rate than the heavier isotopes. Sulfur has stable isotopes of weight S^{32}, S^{33}, S^{34}, and S^{36}. The isotopic composition of sulfur compounds is usually reported in terms of S^{32} and S^{34} which together account for over 99 percent of the total. Data are often expressed as the simple ratio S^{32}/S^{34}. Interpretation requires a comparison of the isotopic composition with that of some reference sulfur material. The meteoritic troilite from Canon Diablo has recently been established as having an agreed S^{32}/S^{34} ratio of 22.220 (19). One means of expressing the deviation in isotopic composition from such a reference material is the delta S^{34} per mil value:

$$S^{34}\ {}^{0}/_{00} = \frac{(S^{34}/S^{32})_{\text{sample}} - (S^{34}/S^{32})_{\text{standard}}}{(S^{34}/S^{32})_{\text{standard}}} \times 1000$$

Actual S^{32}/S^{34} values can be calculated from delta per mil values if the standard is known. Other ratios have been used, particularly in laboratory experiments using a starting material of known isotope composition. Another valuable comparison is the range of isotope ratios encountered in a given deposit.

The degree of isotope fractionation by Desulfovibrio in experiments is variable from nil to about 2.7 percent. Fractionation is enhanced by slow reaction rates, high sulfate concentration and mechanical agitation of the medium (20). Interpretation of isotope ratios of natural materials with an uncertain history is difficult. Assumptions regarding the isotope ratio of the hypothetical starting material are hazardous, since the ratio in a closed basin continuously and progressively changes. Deviations from standard ratios and variations in ratios within a single deposit, if large enough to be convincing, indicate a fractionation mechanism at some stage but they do not reveal the stepwise history of the sample. Isotope ratios have received general acceptance as evidence for bacterial fractionation during the formation of sulfur domes and in the atmospheric sulfur cycle (20, 8, 18). Isotope ratios also strongly support the theory of microbial fractionation for many sulfide deposits but no attempt will be made to evaluate the many conflicting interpretations. A good example of the complexity of interpretation for a single area is that for the Sudbury district by Thode et al. (34). The maximum fractionation reported for a present basin is Green Lake with a value of 1.0575 or a delta per mil of 57.5.

Carbon isotopes are used as indicators of the mineral or organic source of carbon. C^{12} and C^{13} are the relevant isotopes and results are usually given as delta per mil of C^{13}. Organic matter exists in the shales and in the "so called" graphite of sulfide ores but the author is not aware of any carbon isotope studies of those materials.

DIFFERENTIAL PRECIPITATION AND CONCENTRATION OF SULFIDES

Sulfate-reducing bacteria do not of themselves concentrate, select or precipitate metals. Metals do not enter the cell in geologically significant amounts. The sulfate-reducers merely act as hydrogen sulfide generators. If one postulates a continuous or an intermittent transport of metals into an anaerobic basin, then the metals will be precipitated nearly quantitatively. The site of deposition will depend upon the individual settling rates of the several metal sulfides and the existence of water currents. Desorption of metals adsorbed on kaolinite has been demonstrated (32). Existing metal deposits are susceptible to conversion to the sulfide form in the presence of bacterially produced sulfide. These factors are not biochemical but are inorganic and physical chemical aspects of syngenetic theory. They are important, are eminently suitable for detailed quantitative experimentation and have been insufficiently investigated. It is possible to visualize a biological concentration of metals but no handy examples that can be combined with an anaerobic sulfate-reducing environment are available. Speculation of this type is probably useless.

THE FORMS OF METALS WHICH WILL REACT TO GIVE SULFIDES UNDER THE CONDITIONS OF MICROBIAL SULFATE-REDUCTION

Biogenesis of metal sulfides in the laboratory is well documented. The term biogenesis has been questioned since the metal sulfide synthesis proper is an inorganic reaction. However, the fact that the necessary sulfide is generated by bacteria, and the further fact that no metal sulfides would be formed in the same circumstances without these bacteria, justifies the terminology. In nature, the formation of hydrotroilite and its conversion to pyrite and marcasite, all dependent upon microbially produced sulfide, is not seriously questioned. Only the other metal sulfides arouse controversy.

Baas-Becking et al. obtained ferrous sulfide from steel wool, covellite from malachite and from chrysocolla, digenite from artificially prepared cuprous oxide, argentite from the chloride and the carbonate, galena from the carbonate and hydroxycarbonate, sphalerite from zinc wire and from Smithsonite (2). Miller's earlier work on biogenesis was undoubtedly correct, although his experiments lacked the authority of X-Ray diffraction identifications (25). The present author confirmed Baas-Becking's results for lead, copper and zinc using carbonate as starting materials. Positive diffraction pattern identifications were also realized for bismuth sulfide from the carbonate and for covellite from the sulfate. Not all substances gave such clear cut results. Nickel and tin both yielded completely amorphous products while arsenic trioxide, antimony trioxide and cobalt carbonate gave patterns typical of the starting compound. However, all substances tried underwent color changes indicative of sulfide formation and gave positive chemical tests for sulfide. Probably these materials reacted only at the immediate surface or formed amorphous sulfides. Whether identifiable crystalline sulfides would result from longer exposure to sulfate-reducing cultures than the two to three week period used is problematical.

It is evident that not only soluble metal salts but also many relatively insoluble oxides, carbonates and silicates can be converted to sulfides in the presence of a

sulfate-reducing culture at atmospheric pressure and room temperature in a short time. There remains the problem of the formation of mixed sulfides, some specific simple sulfides, and the ratio of metal sulfides found in ores. These considerations are not biochemical and will not be considered at length here. But we should note that the biochemical experiments on biogenesis dovetail neatly with some recent mineralogical research on low temperature sulfide synthesis. Roberts reported the formation of the mixed sulfide chalcopyrite from chemically prepared chalcocite and pyrrhotite at low temperatures and the reversible conversion of chalcopyrite to bornite (28). Under pressures of 2000 atmospheres but a temperature of only 120°C, recrystallization gave an intergrowth of bornite and chalcopyrite with a peripheral area of digenite and dispersed masses of covellite. The biogenetic and mineralogical experiments, together, form a preliminary model for the formation of mineral assemblages similar to those in known ore bodies, starting from metals introduced into an anaerobic sediment and subsequently subjected to low temperature metamorphosis.

MICROFOSSILS AND RELICT ORGANIC COMPOUNDS

Several of the sulfide ore bodies, for which a syngenetic origin is claimed, are PreCambrian. The fossil record is very weak at this point in time but evidence for algal life in the PreCambrian is now reasonable definite and this implies that single celled soft bodies microorganisms are still older. Sulfate-reducing bacteria have no hard structures and decay rapidly. The cell wall of bacteria is a composite of carbohydrate, protein and lipid materials that break down far more easily and rapidly than cellulosic plant walls. None of the many other types of microorganisms associated with any type of sulfur metabolism can be expected to be very resistant to decay. Bacterial remains in sediments are possible if there is rapid sedimentation in an anaerobic environment and such cells could be pyritized but it would be almost impossible to identify the organisms. Pyritized microfossils have been claimed to be present in sedimentary rocks of all ages. Only two examples will be mentioned. The "fossilized bacteria" first described by Schneiderhohn for the Mansfeld copper shales have been most critically treated by Ramdohr (27). Love & Zimmerman reported fossil microorganisms from the Mount Isa shale (22). These materials are a step beyond the customary province of the micropalaeontologist, i.e. they are not recognizable as either pollen grains or single-celled plants by standard microscopical methods.

These are real structures and undoubtedly represent something. Moreover, not all of them can be easily explained as artifacts of colloid chemistry. On the other hand, the many casual statements of resemblance to known sulfur bacteria are unwarranted by the actual appearance of these structures. They do not obviously resemble or suggest any sulfur microbes, and certainly not sulfate-reducers. Love & Zimmerman's technique for digesting pyrite and siliceous material and leaving an apparently organic residue is easily reproducible. Research on the organic nature of "microfossils" from Mount Isa microspherular pyrite has been conducted at the C.S.I.R.O. laboratory in Canberra (Loach, to be published).

Many microorganisms growing on sulfide (not producing sulfide) accumulate globules of sulfur in the cell and some of these cells are large enough to approximate the size of "vererzte Bakterien". Dead cells settling into the sediment of the

sulfate-reducing zone might decay slowly with reduction of the sulfur to sulfide acting as a nucleus for pyritization. Unlike sulfate-reducers, sulfide users are quickly killed by heavy metals (30). Intermittent introduction of metals into an anaerobic basin could result in the accumulation of these bacteria in the sediment. But it is unnecessary to suppose that "microfossils" were originally sulfur bacteria of any kind. Slowly decaying tissue forms a locus for pyritization as can be observed in today's swamps. Microspheres suggestive of microspherular pyrite have been observed in present sediments by Vallentyne & Swabey (35).

Powerful analytical and physical-chemical methods are available for determining the functional groups and the type structure of organic compounds in small samples. These techniques are extensively used in coal and petroleum research and in studies of the kerogen type of organic matter in sediments. The same methods can be applied to organic matter from ore bearing formations. Definite results are not yet available. If it were possible to identify remnants of typical biochemical organic compounds in ore bearing strata, the argument against replacement would be strengthened. If the syngeneticist is correct, metamorphosis in these formations has been a low temperature process that would degrade but not destroy organic compounds. (Incidentally, the term graphite should not be applied to the slick carbonaceous organic matter found in these formations).

The difficulty of positively identifying microbial remains will be apparent to anyone who has followed the recent controversy over evidences of life in meteoritic fragments. However, the organic matter of sulfide ore formations has not been adequately investigated. Definite results are possible and are worth the effort. Isotope ratios of the stable mass isotopes of carbon in some of these formations might also be investigated.

GENERAL DISCUSSION

Both qualitatively and quantitatively, biological sulfate-reduction is a satisfactory element in the syngenetic theory of sulfide ore formation. Sulfate-reduction is not limited to any single physical type of basin but is determined by a combination of factors that can be summed up in terms of oxygen supply and oxygen consumption. Sulfate-reduction occurs in some but not all instances of the following environments: deep basins, shallow basins, sediments, open waters, fresh water, sea water, salt lake water, sands, heavy soils, estuaries, ocean deeps. The rate of sulfate-reduction is sufficient to explain the most massive ore deposits. If the Ain-ez-Zauia sulfur production is accepted as a basis for sulfide estimates, a water volume of 242 cu. ft. would produce one ton of CuS in a year, provided the H_2S could be converted to CuS quantitatively. A similar estimate based on the reduction of sulfate in Port Aransas sediment would require 39 cu. ft. of wet sediment to produce a ton of CuS/year. Since not all of the reduced sulfate appeared as fulfide in the Port Aransas experiments, the two estimates are probably fairly close. Extrapolation of laboratory experiments gives quite different results. E.G., the experiment on formation of Fe_4S_5 indicated that only 0.55 liters or 0.019 cu.ft. would supply enough sulfide to make a ton of CuS in a year. This is a sulfide production rate about 10,000 times that estimated from Ain-ez-Zauia. This difference in order of magnitude is encouraging. It shows that sulfide production figures estimated from natural environments are much below those under ideal laboratory conditions but are still adequate for the precipitation of great masses of metal sulfides.

The more realistic rate is the one estimated from natural environments. But even this rate would not be attained unless sufficient metal were present to precipitate the sulfide and prevent volatilization losses. On the other hand, it is a general observation that addition to sulfate-reducing cultures of heavy metals enhances sulfate-reduction, provided a continuous source of sulfate is available. These considerations all make more attractive those syngenetic theories in which the water is somehow enriched in heavy metals over the ordinary sea water level. They do not preclude precipitation of metal in very dilute solution and the actual requirements in a given case can only be judged by an estimate of sedimentation conditions prevailing at the time of formation, particularly the relative quantities of non-sulfide material.

Strong currents combined with a large volume of flow are incompatible with the stagnant condition of sulfate-reducing basins. Precipitated sulfides should be close to the source of hydrogen sulfide. Sulfides of different metals have different settling rates. This, along with the complexities of precipitation of soluble, colloidally suspended or variously adsorbed metals, suggests experiments on zonation and metal ratios. A beginning in this direction has been made by Garlick, but much more data is needed and could be readily obtained.

The antiquity of biological sulfate-reduction is more important to syngenetic argument than is the identity of the sulfate-reducing organism. Desulfovibrio or other sulfate-reducers could have persisted since the earliest times of biological evolution. The extremely widespread occurrence of Desulfovibrio in soil and water demonstrates that large anaerobic zones are not needed for the activity and preservation of this organism. Even large scale shifts from predominantly reducing to predominantly oxidizing conditions would not eliminate Desulfovibrio. As long as any life existed, decomposition would produce microscopic anaerobic pockets suitable for its survival. If life evolved in an anaerobic environment, sulfate-reduction could have arisen as one of the earliest terminal respiration mechanisms. Biochemically, the normal supposition is that microbial sulfate-reduction by some organism or other is older than the PreCambrian sulfide ores. Isotope fractionation by microbes and isotope ratios of sulfur, sulfate and sulfide deposits furnish the only direct evidence of the age of microbial sulfate-reduction. Considering the activity in this field, it should be possible to be more positive about isotope fractionation in sulfide ores in a few years. The supposed microfossils are not particularly suggestive of sulfur bacteria on any morphological basis and their significance is not yet certain. Microspherular pyrite is, however, strongly suggestive of microscopic organisms of some type, particularly because of the organic residue left on digestion of mineral components. This organic matter, and other organic matter associated with ore bearing strata, need further study. It is possible that recognizable biochemical compounds or identifiable degradation products therefrom could survive. A search for these substances is practical but would require several years' effort.

Objections to syngenesis based on copper toxicity or other metal toxicity are not serious. Neither are objections based upon the fact that metals are often transported in water as adsorbates very important. Desulfovibrio is not harmed by heavy metals because it does not come into contact with them; the sulfide envelope protects the organism. A massive influx of metals

could precipitate all of the sulfide and kill the bacteria but this is unlikely in any basin large enough to be useful in explaining an ore body. Adsorbed metals are desorbed and precipitated as sulfides on entering a sulfide zone. Metals do kill other organisms in a sulfate-reducing zone as shown by Suckow & Schwarz. This might lead to an interaction due to the loss of organic matter produced by these other microbes. If sulfide utilizing bacteria are killed by heavy metals and are hence unable to produce organic matter, Desulfovibrio might run low on organic matter. This is very hypothetical but some experimental work could be done to ascertain the dependence of Desulfovibrio upon other organisms in a sulfuretum.

The chief objection to syngenesis that arises from a scrutiny of the biochemical aspects is that it explains too much. The successful experiments on laboratory biogenesis leave no doubt that metal sulfides can be formed in a natural sulfate-reducing system if a source of metal is at hand. One might expect ore bodies to be forming today at a great rate. Sulfate-reducing bacteria are so widespread and so active that it seems odd if they do not act as biogeochemical agents for metal sulfide production just as they do for elemental sulfur production. The present author considers that this is a serious objection to syngenetic theories which do not include a means of enriching the water in metals. The hypothesis of metal sulfide precipitation from unenriched fresh and salt waters should not be abandoned, however, until further work has been done on the concentration of metals by adsorption. This should take cognizance of the variation in type of adsorbent predominating in different rivers. This difference is quite marked in respect to both clay type and suspended colloidal organic matter.

Aside from iron sulfides, no examples of large scale metal sulfide precipitation in anaerobic basins are known at present. Moreover, the occurrence of concentrated metal sulfides is much more infrequent than the occurrence of sulfate-reducing basins in the same sedimentary strata must have been. It is difficult to escape the conclusion that natural biogenesis of sulfide ores requires a conjunction of factors. Green Lake has been given as an example of a reducing basin that is a sulfur trap but not a metal trap. The simplest postulate for biogenesis is therefore the conjunction of metal-enriched water and an anaerobic basin.

At this point the microbiologist must bow out. He cannot solve the problems of the geologist who is considering a syngenetic origin for certain sulfide ores. A few areas of biochemical research that still need attention have been pointed out. When these results are in, the most encouraging and positive results can only affirm that syngenesis is a possibility. Acceptance or rejection of this possibility for a given deposit can only be determined by examining all of the evidence from structural, mineralogical, geochemical and biochemical studies.

ACKNOWLEDGEMENT

Studies on the geobiology of sulfide ores were jointly supported by the Mining Industry Research Association, the Bureau of Mineral Resources, Geology and Geophysics, and the Commonwealth Scientific and Industrial Research Organization. X-Ray diffraction studies were conducted by the B.M.R. laboratory in Canberra under the direction of Mr. Roberts. Chemical tests on biogenic sulfides were made by Mr. LeRoux of the B.M.R. Laboratory. The author is indebted to Mr. Haddon King and to numerous individuals at the B.M.R. for stimulating discussions, to personnel of Lake George Mines, Mount Isa Mines and the several mining companies at Broken Hill for a patient introduction to the geology of those areas.

REFERENCES

1. Baas-Becking, L.G.M., Kaplan, I.R., and Moore, D., 1960, Limits of the natural environment in terms of pH and oxidation-reduction potentials: J. Geol., v.68, pp.243-284.

2. Baas-Becking, L.G.M., and Moore, D., 1961, Biogenic sulfides: Econ. Geol., v.56 pp.259-272.

3. Bastin, E.S., 1926, A hypothesis of bacterial influence in the genesis of certain sulphide ore deposits: J. Geol., v.34, pp. 773-792.

4. Basu, S.K., and Ghose T.K., 1961, Bacterial sulphide production from sulphate-enriched spent distillery liquor-II: J. Biochem. Microbiol. Tech. Eng., v.III, pp. 181-197.

5. Butlin, K.R., and Postgate, J.R., 1953, Microbiological formation of sulphide and sulphur: VIth Int. Congr.Microbiol., Symposium on Microbial Metabolism, pp.126-143.

6. Butlin, K.R., and Postgate, J.R., 1954, The microbiological formation of sulphur in Cyrenaican lakes: in Biology of Deserts, Cloudsley-Thompson, J.L., Editor, Inst. Biol., London, pp.113-122.

7. Deevey, E.S., Jr., Nakai, N., and Stuiver, M., 1963, Fractionation of sulfur and carbon isotopes in a meromictic lake: Science, v.139, pp.407-408.

8. Eriksson, E., 1959, The yearly circulation of chloride and sulfur in nature; meteorological, geochemical and pedological implications. Part I.: Tellus, v.XI, pp.375-403.

9. Fisher, N.H., 1960, Review of evidence of genesis of Mt. Isa orebodies: Int. Geol. Congr., Part XVI, pp. 99-111.

10. Freke, A.M., and Tate, D., 1961, The formation of magnetic iron sulphide by bacterial reduction of iron solutions: J. Biochem. Microbiol. Tech. Eng., v. III, pp.29-39.

11. Garlick, W.G., 1961, The syngenetic theory: in the Geology of the Rhodesian Copperbelt, Mendelsohn, F., Editor, Macdonald, London, pp.146-165.

12. Garrels, R.M., 1960, Mineral Equilibria at Low Temperature and Pressure: Harper, N.Y., 254 pp.

13. Genovese, S., 1962, Sulle condizione fisico-chimiche dello stagno di Faro in di Scienze Fisiche Matematiche e Naturali, v.VIII, pp.65-72.

14. Genovese, S., 1963, The distribution of the H_2S in the Lake of Faro (Messina) with particular regard to the presence of "red water": in Marine Microbiology, Oppenheimer, C.H., Editor, Charles C. Thomas, Springfield, Illinois, pp.194-204.

15. Gunkel, W., and Oppenheimer, C.H., 1963, Experiments regarding the sulfide formation in sediments of the Texas Gulf Coast: in Marine Microbiology, Oppenheimer, C.H., Editor, Charles C. Thomas, Springfield, Illinois, pp.674-684.

16. Ivanov, M.V., 1960, Microbiological investigations of Carpathian sulfur deposits. I. Investigations of the Nemirov and Luben' deposits: Microbiology, v.29, pp.81-84. (A.I.B.S. Eng. trans of Mikrobiologiya)

17. Ivanov, M.V., 1960, Microbiological investigations of Carpathian sulfur deposits. II. Study of the microbiological process of sulfate reduction in the Rozdol sulfur deposit: Microbiology, v.30, PP.180-183. (A.I.B.S. Eng trans. of Mikrobiologiya)

18. Jensen, M.L., and Nakai, N., 1961, Sources and isotopic composition of atmospheric sulfur: Science, v.134, pp.2102-2104.

19. Jensen, M.L., 1962, Sulfur isotopes: Science, v.137, pp.470-472.

20. Jones, G.E., and Starkey, R.L., 1957, Fractionation of stable isotopes of sulfur by microorganisms and their role in the deposition of native sulfur: Applied Microbiol., v. 5, pp.111-118.

21. Kadota, H., and Miyoshi, H., 1963, Organic factors responsible for the stimulation of growth of Desulfovibrio desulfuricans: in Marine Microbiology, Oppenheimer, C.H., Editor, Charles C. Thomas, Springfield, Illinois, pp.442-452.

22. Love, L.G., and Zimmerman, D.O., 1961, Bedded pyrite and micro-organisms from the Mount Isa shale: Econ. Geol., v.56, pp.873-896.

23. Miller, L.P., 1949, Rapid formation of high concentrations of hydrogen sulfide: Contrib. Boyce Thompson Inst., v.16 pp.73-88

24. Miller, L.P., 1950, Tolerance of sulfate-reducing bacteria to hydrogen sulfide: Contrib. Boyce Thompson Inst., v.16, pp.73-83.

25. Miller, L.P., 1950, Formation of metal sulfides through the activities of sulfate-reducing bacteria: Contrib. Boyce Thompson Inst., v.16, pp.85-89.

26. Ostroumov, E.A., and Shilov, V.M., 1956, Distribution of sulfides of iron and hydrogen in deep sediments in the northwestern Pacific Ocean: Geochemistry, v.1956 No. 7, pp.669-683.

27. Ramdohr, P., 1953, Mineralbestand, Strukturen und Genesis der Rammelsberg Lagerstatte: Geol. Jb., v.67, pp.367-494.

28. Roberts, W.M.B., 1963, The low temperature synthesis in aqueous solution of chalcopyrite and bornite: Econ. Geol., v.58,pp.52-61.

29. Skopintsev, B.A., 1961, Recent work on the hydrochemistry of the Black Sea: Okeanologiya (Eng.Trans), 1961, pp.243-250.

30. Suckow, R., and Schwarz, W., 1963, Redox conditions and precipitation of iron and copper in sulphureta: in Marine Microbiology, Oppenheimer, C.H., Editor, Charles C. Thomas, Springfield, Illinois, pp.187-193.

31. Temple, K.L., and LeRoux, N., in press, Syngenesis of sulfide ores: sulfate-reducing bacteria and copper toxicity: Econ. Geol.

32. Temple, K.L., and LeRoux, N., in press, Syngenesis of sulfide ores: desorption of adsorbed metal ions and their precipitation as sulfides: Econ. Geol.

33. Thode, H.G., Macnamara, J., and Fleming, W.H., 1953, Sulphur isotope fractionation in nature and geological and biological time scales: Geochim.Cosmochim. Acta, v.3, pp.235-243.

34. Thode, H.G., Dunford, H.B., and Shima, M., 1962, Sulfur isotope abundance in rocks of the Sudbury district and their geological significance: Econ. Geol., v.57, pp.565-578.

35. Vallentyne, J.R., and Swabey, Y.S., 1955, A reinvestigation of the history of Lower Linsley Pond, Conn.: Amer. J. Sci., v.253, pp.313-340.

36. Vinogradov, A.P., 1956, The isotopic composition of sulfur in connection with the growth of pyrites of sedimentary origin: Geochemistry, v.1956 No. 1, pp.97-1 08.

---oOo---