Contributions of chemoautotrophic bacteria to the acid thermal waters of the geyser springs group in Yellowstone National Park
by James Alan Brierley

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Microbiology
Montana State University

Abstract:
The Geyser Springs Group in Yellowstone National Park was selected for a study of the thermal water chemistry and the influence of the chemosynthetic autotrophic bacteria on the properties of these waters. A comparison of the analyses of certain thermal springs with a description published in 1935 showed no appreciable difference.

Two acid water drainages from thermal springs were studied to determine the effect of the chemosynthetic autotrophic bacteria on the water chemistry of these drainages.

Strains of sulfur-oxidizing Thiobacillus and iron-oxidizing Thiobacillus were found in the drainage designated GS-VI, VII. Only sulfur-oxidizing thiobacilli were found in the second drainage, designated GS-I. The highest temperature at which sulfur-oxidizing bacteria were found in these drainages was 69.5°C. A sulfur-oxidizing bacterium with a spherical cell shape was isolated. It resisted heating at 80°C for 90 minutes. Retractile bodies, which stained with a spore stain, were present in the cells of this unusual bacterium. This indicated that this organism may be able to form spores.

The chemosynthetic autotrophic bacteria influence the composition of the thermal drainage waters by their energy metabolism. The sulfuric acid, produced as a result of the oxidation of sulfur compounds, decreased the pH and increased the titratable acidity of the drainage waters. The aluminum concentration was increased in the water of the GS-VI, VII drainage probably as a result of the chemosynthetic autotrophic bacterial activity. The acid produced solubilized the aluminum from the kaolinite of the drainage channel.

The activity of the chemosynthetic autotrophic sulfur-oxidizing bacteria, as determined by gas uptake, was contained in the drainage channel material and not in the water flowing down the drainage. The two acid water drainages differed in their bacterial activity. Samples of the GS-VI, VII drainage taken from locations at which the temperature was between 43 and 53°C and between 20 and 26.5°C had the same activity. On the other hand a sample from the GS-I drainage at the location which the temperature was between 50 and 53°C had no measurable activity. But, there was a low rate of gas uptake in a sample from a location of temperature between 34 and 42.5°C. It was suggested that the difference in activity between the two drainages was because of the difference in water chemistry.

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August, 1966

ACKNOWLEDGMENTS

The author would like to take this opportunity to express his gratitude to those who have been especially of help during the course of his graduate program.

Sincere thanks must be given to Dr. James E. Ogg of Colorado State University for without his confidence and encouragement this thesis would never have been accomplished.

The author expresses his gratitude to Dr. Kenneth L. Temple for his guidance in this study and help in the preparation of this thesis. Thanks are also due to Dr. John Wright for his support and suggestions throughout the duration of this project.

The cooperation extended by Mr. John McLaughlin, Superintendent, and Mr. John Good, Chief Park Naturalist, of Yellowstone National Park was greatly appreciated.

Sincere thanks are given to Dr. Bikash C. Raymahashay for his aid in the field and laboratory work and the information concerning the geochemical nature of the Geyser Springs Group.

Thanks to my wife, Corale, for her patience, excellent technical help, and aid in preparation of this thesis.

This project was supported by National Science Foundation Grant GB2062 and by Public Health Service Training Grant T1-WP-1, from the Division of Water Supply and Pollution Control.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

ABSTRACT

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Two acid water drainages from thermal springs were studied to determine the effect of the chemosynthetic autotrophic bacteria on the water chemistry of these drainages.

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INTRODUCTION

One of Nature's most unusual features is the discharge of thermal water in the form of hot springs and geysers. The beautiful colors of thermal water pools, the vivid sequence of coloration of drainages from hot springs and the eruptions of geysers are some of the striking features of thermal water phenomena. To the casual observer these spectacles provide impressions of a superficial nature. For those interested in the fundamental aspects of these phenomena, there is an area for study by a cross section of many scientific disciplines--physics, chemistry, geology, and biology. The study of thermal springs is especially interesting to the biologist, for here is an environment that provides bizarre conditions.

The major concentrations of thermal springs occur in five areas--Iceland, New Zealand, Japan, New Guinea, and Yellowstone National Park. The proximity of Yellowstone National Park to the laboratories at Montana State University, Bozeman, Montana, facilitates study of these thermal phenomena and almost makes it an obligation.

The most extensive chemical and physical description of Yellowstone National Park was published by Allen and Day (1935). They listed some characteristics of selected thermal springs of almost every thermal area within the Park. Their work will be quoted throughout this thesis for descriptive and comparative purposes. Recently the New Zealand thermal areas have been the subject of extensive chemical investigations by Ellis and Anderson (1961), Ellis and Sewell (1963), Golding and Speer (1961),

Mahon (1962, 1964, 1965), Ritchie (1961), and Surbutt (1964). These studies were concerned with the determination of the physical-chemical origin of the constituents of thermal waters. Uzumasa (1965) has reviewed the literature of the thermal water chemistry of Japanese thermal springs. His review presented analyses, classifications of thermal springs based on chemical constituents, and origins and mechanisms of flow of thermal springs.

Many forms of life are found in thermal springs and drainages from thermal springs. The early studies of thermal environments has been reviewed by Weed (1889 a, b).

The ecological studies of the blue-green algae of the thermal springs in Yellowstone National Park have received the most attention. Copeland (1936) and Nash (1938) made extensive descriptive surveys of the Myxophyceae.

The animal life of the hot springs of Yellowstone National Park and other hot springs in North America has been reviewed and described by Brues (1924, 1932). He listed the animal types found, but did little to relate these types to their environment. His description of the thermal springs was limited to water temperature, pH, and specific gravity.

Most studies of life in thermal waters are of a descriptive nature. However, some information has been obtained regarding upper temperature limits of life by the observations of biological activity in thermal waters. Setchell (1903) made observations and collected samples from the thermal springs of Yellowstone National Park and California to

determine this upper temperature limit. He found only blue-green algae and bacteria in water of temperatures above 45°C. He recorded 89°C as the highest temperature at which living organisms could exist, and he found these organisms to be filamentous bacteria. Setchell was able to find life only in alkaline waters and stated, "No organisms were found in springs reputed to have a decided acid reaction."

Kempner (1963), who studied upper temperature limits of life in Yellowstone National Park waters, found that 73°C was the upper limit for algal material scraped from the sides of pools and pool drainages. His evidence was based on the uptake of P^{32} labelled phosphate at 73°C. Although his evidence is considered reliable, his study was very limited. There is a wide divergence of thermal environments present in the Park. Some of the areas may yield organisms with an increased temperature tolerance. Temple (unpublished data) has found a filamentous bacterium apparently growing at 78.5°C in a small alkaline spring.

Brock and Brock (1966) determined that the optimum temperature for algal development in thermal springs of Yellowstone National Park is between 51° and 56°C. However, their investigation considered temperature as the only variable in thermal spring environments and they neglected the importance of water chemistry. It should also be pointed out that this temperature optimum is for algae of alkaline waters and not for algae of acid waters.

The study of the bacteria in thermal springs of Yellowstone National Park has not been extensive. Species of the genus Bacillus have been

found in springs of many thermal areas, (Walter 1952, Walter and Northam 1952, Marsh and Larsen 1953, Brierley and Walter 1963, and Brierly 1963). All of the bacteria were obtained from pools or pool and geyser drainages of alkaline pH except three, which were obtained from waters of acid reaction. All the studies with the exception of Walter (1952) were limited to the thermophilic species of Bacillus. The interaction of these bacteria with their environment has not been determined.

The bacteria and algae present in the thermal springs of Japan have probably received greater study than those of either New Zealand, Iceland or Yellowstone National Park. Most of the literature dealing with the life in the thermal springs of Japan is of a descriptive nature and gives little information about the activities of the organisms. Miyoshi's (1897) study of the Beggiatoa, Chromatium and sulfur bacteria was one of the earliest in this area. Molisch (1926) found Beggiatoa, Chromatium, Thiothrix, and iron bacteria, probably of the Leptothrix type. Algae, amoebae, infusoria, flagellates, rotifers and Anguillua of thermal springs were also described.

Emoto (1933a) made an extensive study to determine the presence or absence of thiobacilli in the thermal waters of Japan. He found four species--Thiobacillus thermitanus, T. crenatus, T. lobatus, and T. umbonatus. They were present only in water with detectable H_2S, however these organisms could tolerate a pH range of 1.4 to 7.5 and a temperature range of 5° to 80°C. T. thermitanus was the only species reported to exist in water of 80°C and pH 2.8.

The four species of thiobacilli described by Emoto have not been generally accepted, since their classification is based wholly on colony morphology. Vishniac and Santer (1957) believe that these four species are all T. thiooxidans. The Russian workers, Zavarzin and Zhilina (1964), studied the sulfur-oxidizing bacteria in the thermal areas of the Kunashir and Kurile Islands. They had hoped to find the Japanese species of Thiobacillus, but they were unable to isolate any bacteria which could be specifically identified. The bacteria which they isolated demonstrated a range of characteristics between T. thiooxidans and T. thioparus.

Emoto and Hirose (1942a) described 15 species of Cyanophyceae and two species of Chlorophyceae from the thermal springs of Narugo. They found Beggiatoa leptomitoformis in water having a pH of 6.6 to 6.7 and a temperature of 33° to 37°C. They also found the four Japanese species of Thiobacillus. T. thermitanus, T. crenatus, and T. lobatus were found at water temperatures between 33° and 76°C and T. umbonatus was found between the temperatures of 44° and 61°C. All of these species apparently live in waters having a pH between 3.0 to 7.1.

Emoto and Hirose (1942b) found Leptothrix ochraceae and three species of Thiobacillus in the thermal waters of the Onikobe springs. The Leptothrix was found only in a sample of water with a pH of 3.2 and at 43°C. T. thermitanus, T. crenatus and T. lobatus were only found in acid waters between pH 1.8 and 3.6 and at temperatures ranging from 31° to 69°C. In addition to the bacteria, there were 43 other forms of plant life which included members of the Cyanophyceae, Heterocontae, Chlorophyceae and

Conjugatae.

The most extensive survey of the acidic thermal waters of Japan was made by Negoro (1944), who gave chemical data of these thermal springs and presented information on the bacteria, algae and other plants of these waters. This paper presents the most complete descriptive survey of acidic thermal waters.

Schwabe (1936) made an extensive survey of the bacteria, algae, higher plants and insects of the thermal springs of Iceland. He supplemented his work by presenting physical descriptions, temperature conditions and chemical data for those thermal springs which were studied. Schwabe noted that bacteria were the only organisms in water at a temperature of 80°C and a pH of 9.4. This study dealt with alkaline waters which Schwabe says are the most important in Iceland's thermal areas.

There are many reports of a limited nature dealing with investigations of local thermal environments which occur throughout the world.

Czurda (1935) isolated two species of Thiobacterium (Thiobacillus) and a single species of Thiospirillum from the sediment of the Pystian hot spring in the Southern Moravian mountains of Czechoslovakia. In this spring drainage he found bacterial and blue-green algal growth at temperatures below 60°C.

Vouk (1960) reported that he had found an iron bacterium, Gallionella schmenzkii n. sp., in the thermal waters of Bad Gastein located in Austria.

A morphologically unusual bacterium was obtained from the hot springs of Tiberias (Israel) (Kahan, 1961). The cells were spherical, ovoid or

pearshaped and reproduction was by means of budding. These organisms grew in the laboratory between the temperatures of 37° and 50°C on low concentrations of yeast extract. The author believed that this organism was a member of the order Hyphomicrobiales.

A spore forming Thiobacillus, T. thermophilica n. sp., was isolated from the Bragunskie Hot Springs of Russia by Egorova and Derygina (1963). This chemoautotrophic bacterium has the characteristic of being the only Thiobacillus known to form spores. This bacterium grew at temperatures between 40° and 80°C, but only under alkaline conditions.

There are many references available regarding descriptive work on thermal waters. However, there is little information available regarding the interaction between the biological entities and their thermal environment. Weed (1889a) was probably the first worker to consider the role of life in thermal geochemistry. Weed speculated that plant life in the thermal springs of Mammoth Hot Springs in Yellowstone National Park causes the deposition of travertine. He also believed that vegetation of the alkaline thermal areas along the Firehole River in Yellowstone National Park were responsible for production of some deposits of siliceous sinter.

Kaplan (1956) surveyed the Rotorua-Taupo and White Island geothermal areas of New Zealand. His study determined the presence of sulfur-oxidizing bacteria, photosynthetic sulfur bacteria, sulphate-reducing bacteria, and algae of these thermal waters. He furthered his study by determining the conditions under which these populations exist. The

sulfur-oxidizing thiobacilli, which Kaplan studied, appeared to be the most geochemically active form of life present, and these bacteria may actually bring about the acidification of many pools and drainages. These sulfur-oxidizing thiobacilli may also be important in the formation of gypsum in the White Island area. It is known that gypsum is formed by the action of sulfuric acid on feldspar. Kaplan suggested that the gypsum, which was closely associated with sulfur, may be formed, at least in part, from sulfuric acid produced by the bacterial oxidation of sulfur.

Hariya and Kikuchi (1964) implicated the activity of bacteria in precipitating manganese as manganese hydroxide in hot and cold springs in Japan. The bacteria in their laboratory cultures were described as peritrichously flagellated rods, which were able to oxidize manganese as a source of energy. This organism is exceptional in that the group of organisms ordinarily associated with inorganic oxidations are of the order Pseudomanadales which all possess terminal flagella.

The bacteria of the Thiobacillus-Ferrobacillus group have been shown to catalyze a number of important geochemical transformations other than those which occur in thermal springs. The classic example (Razzell and Trussell, 1963) of degrading activity is the leaching of chalcopyrite ore by Thiobacillus ferrooxidans, which releases copper. Ehrlich (1964) demonstrated that a member of the Thiobacillus-Ferrobacillus group is able to catalyze the oxidation of arsenopyrite. During this oxidation there is a concurrent solubilization of arsenic as arsenite and arsenate. Ehrlich (1963) also demonstrated that orpiment can be oxidized by

Ferrobacillus ferrooxidans with a concurrent release of arsenite. T. ferrooxidans and T. thiooxidans have been shown to oxidize molybdenite with a simultaneous increase of molybdenum in solution. (Bhappu, et. al., 1965). Temple and Koehler (1954) demonstrated that T. thiooxidans and T. ferrooxidans can produce sulfuric acid by their oxidative attack on pyrite and marcasite in coal.

The purpose of this study is to describe some of the chemical and physical properties of a small thermal area within Yellowstone National Park, and to consider in detail its environment of acid thermal waters. The chemosynthetic autotrophic bacteria isolated from the acid thermal waters will be characterized. Any relationships, of a geochemical nature, between these bacterial populations and their environment will be explored.

MATERIALS AND METHODS

Water Chemistry

Water samples were collected from the sampling stations during all seasons from 1964 to 1966. The samples were filtered in the field through "Millipore" filters with a pore size of 0.45μ or "Flotronic" silver membrane filters with pore sizes of 0.2μ, 0.45μ, 0.8μ, and 1.2μ. After filtering, the samples were placed in 1 liter polypropylene plastic screw-cap bottles, which had been rinsed with a small amount of filtered sample. About 100 ml of each sample was also placed in clean glass stoppered pyrex bottles for iron analysis.

The samples were returned to the laboratory. When chemical analyses could not be done immediately after returning to the laboratory the samples were stored at 4°C.

The chemical analyses of all samples were conducted as follows: sodium, potassium, calcium and magnesium were determined with a Beckman Model DU Flame Spectrophotometer, following the procedures given in the Beckman Instruction Manual #334-A, and the magnesium of some samples was determined by atomic absorption spectroscopy using the Beckman Spectrophotometer; total alkalinity, aluminum, chloride, ferrous and total iron, fluoride, phosphate, silica, and sulfate determinations were made as described by the American Public Health Association (1960). The samples in which fluoride was determined, were distilled by procedure II of the American Public Health Association (1960). Ammonia was determined by the direct Nessler

method. The total or titratable acidity of acid water samples was determined by titration with 0.1 N NaOH to the phenolphthalein end point.

All laboratory pH values were taken with samples at 25°C using a Radiometer model 25 expanded scale pH meter which was standardized with two buffers to bracket the pH value of the sample. The laboratory Eh values were also determined using the Radiometer with the sample at 25°C.

The standard deviation (s) reported for the results were computed using the formula, $s=\sqrt{\frac{\Sigma x^2}{N-1}}$, in which $\Sigma x^2 = \frac{N\Sigma x^2 - (\Sigma x)^2}{N}$ (Crabtree, 1962).

Field Measurements

Field temperature measurements were taken using a "Tri-R" electronic temperature probe with a 20 foot lead.

Field pH and Eh determinations were made with Beckman or Corning high temperature glass electrodes, Beckman calomel and platinum electrodes. Readings were made with a "Keithley" VTVM and a standard reference EMF box, at the temperature of the water.

The rate of flow of water in one drainage was determined. A piece of pliable plastic was used to divert the water from the drainage into a 2 liter plastic beaker for a measured period of time determined with the sweep second hand of a wrist watch.

Bacteriological Studies

Samples for bacteriological analysis were collected in sterile, 100 ml, screw cap polypropylene bottles. Each sample consisted of the spring water and a portion of the drainage channel material at the site of collection. The samples were returned to the laboratory and stored at 4°C.

Microscopic observations were made with a Nikon phase-interference microscope.

Sulfur and iron-oxidizing bacteria were selected for inoculating enrichment media with 1 ml of sample. The enrichment media were incubated at temperatures which were within the range of temperature of the sample in the field. After three transfers in enrichment media, plates of sulfur and iron agar were streaked to obtain isolated colonies. Colony types were picked and restreaked at least three times to ensure purity of the culture.

Media

All media consisted of one of two basal salts solutions which were similar in composition to the water of the two acid thermal springs investigated.

GS-I basal medium:

Distilled water	1 L
$(NH_4)_2SO_4$	0.5 g
NaCl	0.3 g
KH_2PO_4	0.1 g
$Ca(NO_3)_2.4H_2O$	0.01 g
1 N H_2SO_4	2 ml
Trace element solution	0.5 ml
pH	2.7

GS-VI, VII basal medium

Distilled water	1 L
$(NH_4)_2SO_4$	0.3 g
NaCl	0.025g
KH_2PO_4	0.07 g
$Ca(NO_3)_2.4H_2O$	0.01 g
1N H_2SO_4	1.5 ml
Trace element solution	0.5 ml
pH	2.9

Appleby's trace element solution (Appleby, C. A. Division of Plant Industry, C. S. I. R. O., Canberra, Australia, personnel communication) was used in the above basal media.

Appleby's trace element solution

Distilled water	1 L
$FeCl_3.6H_2O$	3.60 g
H_3BO_3	0.57 g
$ZnSO_4.7H_2O$	0.44 g
$CoCl_2.6H_2O$	0.20 g
$CuSO_4.5H_2O$	0.02 g
$MnCl_2.4H_2O$	0.02 g
$Na_2MoO_4.2H_2O$	0.05 g

The enrichment media consisted of one of the basal salts solution and either sulfur or ferrous iron as an energy source.

The sulfur was sterilized by intermittent steaming for 4 consecutive days. Sulfur in 0.25 g quantities was steamed for 35 minutes and sulfur in 1 g quantities was steamed for 60 minutes. The basal salts solutions were dispensed in 50 ml quantities in 125 ml Erlenmeyer flasks and sterilized by autoclaving. The sterile 0.25 g quantity of sulfur was added to produce the sulfur enrichment medium. The 1 g quantities of sulfur were used for larger quantities of basal salts solution.

A solution of $FeSO_4.7H_2O$, 25 g in 100 ml of distilled water, was sterilized by autoclaving. Two ml of this solution was added to 50 ml of sterile basal salts solution in a 125 ml Erlenmeyer flask to provide the iron enrichment medium.

A medium with thiosulfate as the energy source, employing either of the basal media, was devised. It was necessary to modify each of the basal media to bring their pH near neutrality. Acid conditions change

thiosulfate to sulfite and sulfur. The GS-I basal medium was modified by using 0.1 g K_2HPO_4 in place of KH_2PO_4 and adding 3 ml of 0.01 N H_2SO_4 instead of the 1N H_2SO_4. The GS-VI, VII basal medium was modified by using 0.07 g K_2HPO_4 in place of KH_2PO_4 and 10 ml of 0.01 N H_2SO_4 in place of 1N H_2SO_4. The pH of the modified basal media was near 7. Two ml of a filter sterilized $Na_2S_2O_3.5H_2O$ solution, 12.5 g in 100 ml of distilled water, was added to 50 ml of the sterile modified basal media.

Solid media with either thiosulfate, iron or sulfur as energy sources and with respective basal salts solutions were also used.

A 0.9% concentration of Oxoid "Ionagar" No. 2 was added to the modified GS-I or GS-VI, VII basal media which was then autoclaved and cooled to 50°C. Ten ml of a filter sterilized solution of $Na_2S_2O_3.5H_2O$, 50 g in 100 ml distilled water, was added to each liter of medium. Plates were then poured.

It was necessary to separately sterilize the components of ferrous iron agar. The components for 1 L of basal medium were dissolved in 250 ml of distilled water. Five grams of $FeSO_4.7H_2O$ was mixed in a second 250 ml quantity of distilled water, and 9 g of Oxoid "Ionagar" No. 2 was placed in a third 500 ml quantity of distilled water. All of the components were sterilized by autoclaving for 15 minutes at 121°C, cooled to 50°C, combined and plates poured.

A new method was devised for the incorporation of colloidal sulfur in agar to provide a solid medium for the isolation of sulfur-oxidizing bacteria. The method described below is rapid and the medium, unlike the

thiosulfate agar medium, can be adjusted to any desired pH.

The colloidal sulfur suspension was prepared as follows: 1 g of flowers of sulfur in 50 ml of acetone was heated using a hot plate. The mixture was boiled for 3 minutes with constant swirling. After boiling, undissolved sulfur was allowed to settle and the hot solution of sulfur in acetone was decanted into 500 ml of distilled water at room temperature. This produced a milky colloidal suspension of sulfur. The amount of sulfur in suspension was increased by repeating the boiling procedure with two more portions of hot acetone. This suspension of sulfur in water was autoclaved at 121°C for 15 minutes to remove the acetone and sterilize the sulfur suspension. It was important to form the suspension in distilled water only, as the presence of salts caused the colloidal particles to aggregate.

The colloidal sulfur suspension was used to prepare solid media by mixing the sulfur suspension with an appropriately prepared concentration of agar and salt solution when all of the components had cooled to 50°C following autoclaving. The agar solution was prepared by adding 9 g of Oxoid "Ionagar" No. 2 to 250 ml of distilled water. Either the GS-I or GS-VI, VII basal salt solutions was used. The salts for 1 liter of medium were dissolved in 250 ml of distilled water. This provided 1 liter of an acidified agar medium with finely divided sulfur for the energy source.

When 1 or more liters of liquid medium were used in an experiment the vessels were sparged with air which had passed through sterile cotton

filters. A "Dyna-Vac" pump was used to deliver air to the flasks. The pump was not reliable enough to provide a constant regulated supply of air although it was continuous and vigorous.

The heat resistance of two strains of the sulfur-oxidizing bacterial isolates was determined.

One hundred milliliters of culture with sulfur as an energy source was incubated for 7 days at the same temperature used for the isolation of the strains. Each culture was then placed in a sterile dilution bottle with an Escher stopper and vigorously shaken for 1 minute. Five milliliters of the mixed suspension was placed in sterile screw cap tubes, which measured 115 mm x 16 mm. The tubes were placed in a 1 L beaker with water at 80°C. A tube containing 5 ml of distilled water and a thermometer was also placed in the bath. When the water in the tube reached 80°C the timing was begun. It was possible to maintain the temperature of the water in the tube at 80$\pm$ 1°C by adjusting the flame of the Bunsen burner used for heating and by constant stirring of the bath with a glass rod. Tubes were removed at 10 minute intervals. Immediately after the removal of a culture tube from the bath, 1 ml was transferred to a sterile sulfur enrichment medium and incubated at the temperature at which the culture was grown. The sulfur enrichment cultures were examined for growth after 7 days of incubation. Microscopic observation of each flask was made. The pH of the medium of each flask was determined and compared with an uninoculated control flask.

The importance of the chemoautotrophic bacterial populations in causing changes in water chemistry was assessed. For this experiment the bacteria were grown in the presence of drainage bed material to determine if any biologically initiated alteration of this material could bring about a change in water chemistry.

Two and one-half liter Fernbach flasks, filled with 2 L or either GS-I or GS-VI, VII basal medium, were used as reaction vessels. Fifteen grams of dried drainage bed material from either the GS-I or the GS-VI, VII drainages was added to respective flasks with the corresponding basal medium. The flasks with media and drainage bed material were sterilized by autoclaving at 18# for 30 minutes. The flasks were cooled and either 3 g of sterile sulfur or a sterile solution of $FeSO_4.7H_2O$, 5 g in 100 ml of distilled water, was added. These compounds served as bacterial energy sources.

Each flask was stoppered and connected to an air pressure manifold. Figure 1 shows the arrangement of each flask. Air was bubbled through distilled water in order to saturate the air with water and to prevent evaporation from the reaction vessel. The air was passed from the flask containing distilled water, through a sterile cotton filter, and into the reaction vessel.

The flasks were incubated (at temperatures corresponding to the temperature at which the inoculum was originally obtained) for a period of 3 days to allow for equilibration of basal medium with the drainage bed material. The flasks were then inoculated with 50 ml of culture.

Enrichment culture samples were used for inoculation rather than pure cultures. It was believed that these cultures would more closely duplicate the population in the field environment than a pure culture.

Each flask was aseptically sampled immediately after inoculation by closing the exhaust tube and removing the tube cover of the sampling tube. The air from the sparger increased the pressure and forced the sample through the sampling tube. The exhaust was reopened and the sterile tube cover replaced over the sampling tube.

The reaction vessels, inoculated with iron enrichment cultures, were sparged with air immediately after inoculation. The reaction vessels with the sulfur enrichment cultures were sparged with air only after a 3 day

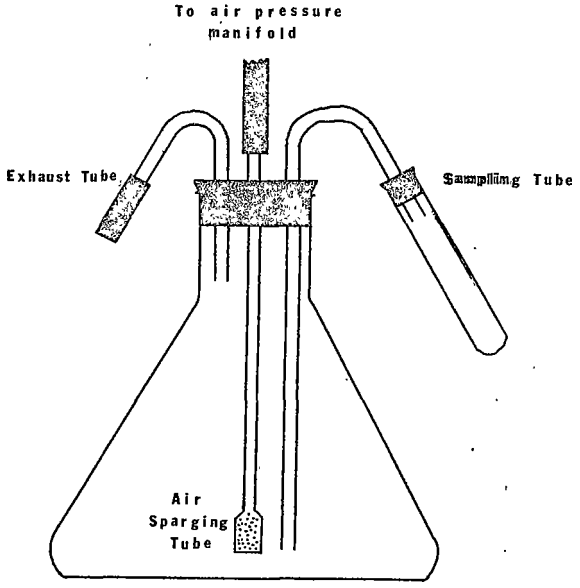


Figure 1. The reaction vessel used for determining geo-microbial activity.

incubation period. Cook (1964) showed that this period was necessary in order to establish growth. Immediate vigorous aeration or shaking prevents the contact of the bacterium with the sulfur and thus inhibits growth.

After 2 weeks of incubation, the final sample was removed for determination of the level of chemical constituents.

Both the initial and final samples were filtered through 0.45μ "Millipore" filters immediately after removal from the reaction vessels.

Manometric Measurements

Manometric experiments were performed with a Gilson Differential Respirometer. Single side arm reaction vessels were used.

Plug Sampler

It was necessary to compare samples from the thermal spring drainages to determine their relative biological activity. A sampling device was constructed so that equal sized samples of the drainage channel beds could be obtained. This device was used to obtain a "plug" of the channel bed material. The plug sampler (Figure 2) was assembled with a Bunsen burner, rubber stoppers, steel rod, and scrap metal. The core, which was 2.6 cm in diameter, was removed from a size 12 rubber stopper. The stopper was slipped over the bottom end of the Bunsen burner, so that 1 cm of the end of the Bunsen burner protruded. This stopper served as a collar to prevent sampling deeper than 1 cm. A size 6 rubber stopper was shaped to give a tight fit within the Bunsen burner. This rubber stopper was mounted on the end of a steel rod 3/8 inches in diameter and 9 inches long. The piston thus formed was placed in the Bunsen burner to

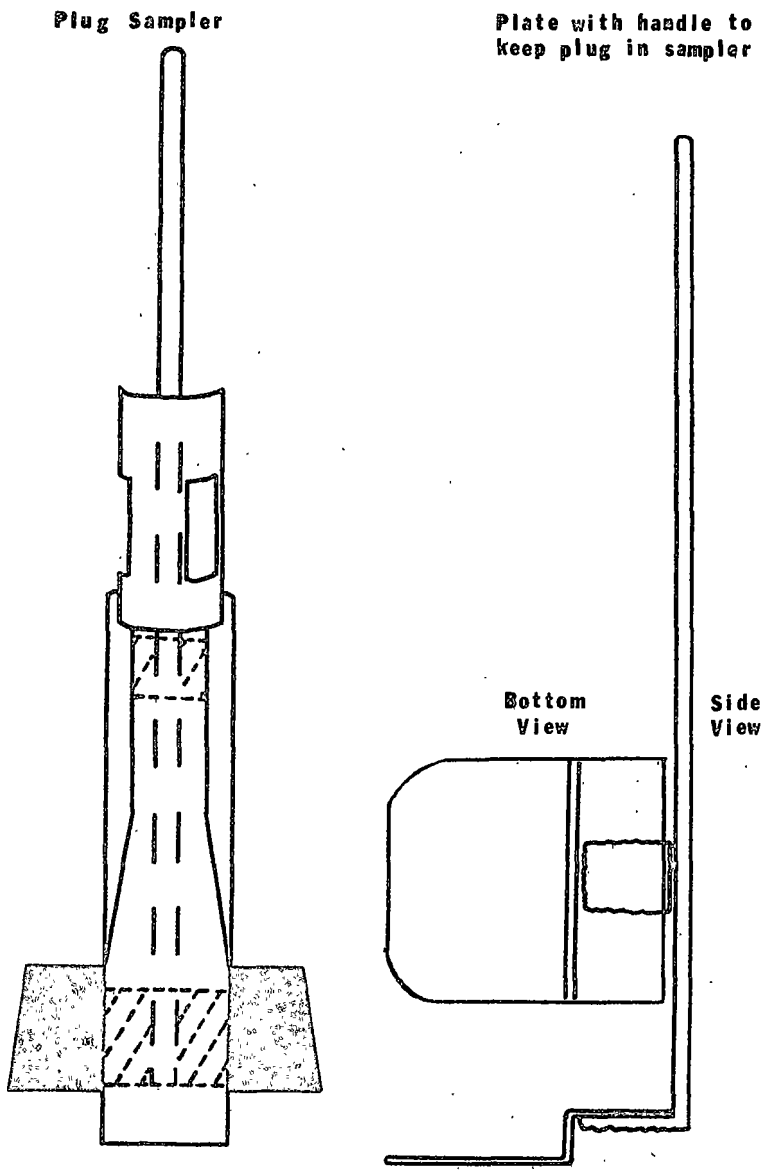


Figure 2. The plug sampler used for obtaining uniform samples from drainage beds.

provide a means for removing the sample plug. A small rubber stopper with the center removed was placed in the Bunsen burner opposite the sampling end. This stopper served as a guide for the piston. A metal plate with a handle (Figure 1) was used to close the sampling end to prevent the sample plug from dropping out when the sample was removed. The plugs were placed in sterile glass jars. This sampler removed a plug with a 5.3 cm^2 surface area and 5.3 cm^3 volume.

The following procedure was used to compare the activity of the sulfur-oxidizing chemoautotrophic bacteria in the two thermal water drainages investigated.

Each plug sample was mixed with 20 ml of sterile GS-I or GS-VI, VII basal medium, depending on the drainage from which the plug was obtained. The drainage material and basal medium were vigorously shaken for 1 minute. Ten milliliters of this suspension was sterilized by autoclaving at 18# for 15 minutes. Three milliliters of the basal medium with the plug sample and 3 ml of the sterilized suspension were placed in respective manometric flasks. The pH of the suspensions in each flask was stabilized at pH 7 by adding 1.5 ml of sterile 0.005 M Na_2CO_3. One-half milliliter of a sterile solution of $Na_2S_2O_3.5H_2O$, 0.125 g/ml, was added to each flask to provide an energy source. To serve as a control for endogenous respiration, one manometric flask contained 0.5 ml of sterile distilled water in place of the thiosulfate. The manometric flasks were then connected to the Gilson Differential Respirometer and the flasks were allowed to equilibrate with the water bath temperature for 15 minutes before the

start of each experiment. The change of gas pressure which was measured, was probably caused by the uptake of O_2 during the oxidation of thiosulfate. However, some of this change may have been due to the uptake of CO_2 since the chemoautotrophic sulfur-oxidizing bacteria are able to fix CO_2.

EXPERIMENTS AND RESULTS

Description of Thermal Area

The Geyser Springs Group was selected for extensive study of the thermal water chemistry and the chemoautotrophic populations. This thermal area is located in the Gibbon Geyser Basin on the eastern side of Paintpot Hill. It is reached by following Artists Paintpots Trail to the thermal area of the same name and then leaving the trail to follow around the base of Paintpot Hill on its northern side to the Geyser Springs Group.

This area possessed desirable characteristics for research work as it is secluded, seldom visited by tourists, and is not visible from any traveled route. It is approximately one mile from Grand Loop Road.

Geyser Creek Basin has not changed noticeably since 1935 when it was described by Allen and Day (1935). Their description, quoted below, is suitable for a present day description.

> On the east side of Paint Pot (sic) Hill are two small connecting basins draining into Geyser Creek. . . .Deep and narrow (65 yards wide), the northern of the two depressions extends in a direction nearly north and south for 175 yards on both sides of the creek, continuing on toward the northwest for 100 yards farther in a mere strip of ground 30 or 40 yards wide. Rhyolite gravel surrounds the basin. The floor, more or less flooded with water and extensively colored by algal growth, is, in places, encrusted with a thin sheet of sinter concealing hot mud and affording a very insecure footing. Free sulphur is rather scarce. . .
>
> A short distance up Geyser Creek to the south, on a higher bench, is a smaller basin 50 by 100 to 125 yards, of similar character. . .In this little area are half a dozen large alkaline springs, heavily lined and bordered with silica.

South of the upper bench are several springs, which form a small tributary joining Geyser Creek. Two hundred and eighty feet south of the

place where the tributary joins Geyser Creek is a small area with several thermal springs which contribute water to the creek.

The entire Geyser Springs Group has over 100 individual springs and spring complexes, springs so closely associated that the individual ones could not be differentiated. There are three geysers and a few fumaroles.

Twenty stations were established for periodic sampling of waters for chemical analysis. Figure 3 is a map of Geyser Springs Group (drawn by K. L. Temple, Montana State University, Bozeman, Montana), which shows the approximate location of these stations.

Station one. This sampling site is located on Geyser Creek at the lower end of the thermal area. The water samples from this station showed the contribution of all thermal springs drainaing into Geyser Creek. The creek at this station is about 2 feet wide and 6 to 10 inches deep. The water is clear and covering the stream bed was a mass of reddish gray material. The water temperature varied between 44° and 46°C.

Station two. This is an alkaline water pool, measuring approximately 7 feet by 4 feet (Figure 1, Appendix). The water is clear and the walls of the pool are dark gray sinter with evidence of sulfur at the air-water junction. The activity of this pool is cyclic. The water level rises and lowers approximately 6 inches every 60 minutes. The pool is quiescent at its lowest level, but during its rise there is a constant emission of gas causing bubbling at the center of the pool. The temperature was 84°C when the water level was lowest and increased to 88°C at the highest level.

Station three. A very large pool (Figure 2, Appendix), measuring 15 feet north-south by 10 feet east-west, was selected for sampling. This

pool was also designated GS-I, meaning Geyser Springs pool I. It has a beautiful yellow-green color. Sulfur is deposited on the sides of the pool and the drainage bed. The pool is turbid with a constant emission of gas near its center. The water is acid and the temperature varied between 71° and 73.5°C.

Station four. This station is an acid water pool which was also designated GS-III (Figure 3, Appendix). In the water fine gray clay and small pebbles are kept in suspension by the violent motion brought about by gas emission. The clay which is suspended by the water, is subsequently deposited in the drainage of this thermal spring. The pool is about 3 feet in diameter. The temperature varied between 88° and 93.5°C.

Station five. This thermal pool, (Figure 4, Appendix), also known as GS-II, is a constant bubbler with an unusual cone located near one edge. This cone resembles a small geyser when it intermittently spurts small quantities of water about 1 foot into the air. The pool water is alkaline and its color varies from "crystal" clear to turbid when fine grayish black particles are held in suspension. The sinter forming the walls and bottom of the spring and drainage is dark gray. The coloration of the sinter lining the drainage, changes and sometimes becomes a dull reddish gray color. Pyrite grains were observed in the pool. The temperature varied between 84.5° and 88°C.

Station six. This pool was also called "The Bone Pool" (Figure 5, Appendix) because of the bleached animal bones visible on the bottom. This pool is large and has a beautiful blue color. A thin sinter crust

around the edge is deceptive because it gives an appearance of solid ground. A constant emission of gas agitates the center of the pool. The water is alkaline and the temperature was between 70.5° and 80°C.

Station seven. This station was established on the drainage of two springs, GS-VI and GS-VII (Picture 6, Appendix), which is the tributary near the southern end of the basin. The location of this station is 300 feet from GS-VI, which is the pool at the head of the tributary. The drainage runs down the side of a steep hill in a narrow "V" shaped gully. The water is acid and the temperature varied between 21.5° and 28.7°C.

Station eight. This station which was also called GS-VII, (Figure 7, Appendix) was located in the drainage originating from the GS-VI pool and 100 feet downstream from this pool. The pool is about 4 feet in diameter. This station is actually a composite of 3 pools. The main portion of the pool is relatively quiet with little evolution of gas. The two areas adjacent to the main pool have an active evolution of gas and a variable water flow. These areas were dry during the late summer but at all other times there was an abundance of water. The water temperature of the main portion of the pool varied between 31° and 45° C. The temperature of one of the adjacent pools was 69°C. The water is acid.

Station nine. This pool, also designated GS-VI (Figure 8, Appendix), is an acid water pool of temperature between 85° and 91°C. The pool is constantly agitated by gas issuing from the place where the pool meets a steep bank of Paintpot Hill. The motion of the water maintains gray white clay-like particles in suspension. The rocks in the pool have a

green color where they are in the water and a red color where exposed to the air.

Station ten. This station was located on Geyser Creek just upstream from the point where it first receives any known thermal spring water from the Geyser Springs Group. Lush vegetation surrounds the area and grows within a small pool formed in the creek. The water is alkaline and the water temperature varied between 17° and 23.5°C.

Station eleven. The drainage (Figure 9, Appendix) of the GS-I pool was sampled at a point 80 feet below the lip of the pool. The water temperature at this location varied between 37.5° and 41.2°C.

Station twelve. This station was established on Geyser Creek 12 feet below the place where the drainage of GS-VI and GS-VII flow into the creek. The creek is roily between the inflow of GS-VI and GS-VII drainage and this station. It is believed that the creek and drainage are mixed at the sampling location. The water is acid and the temperature varied between 30.5° and 35°C.

Station thirteen. GS-VII is a grayish black, turbid pool of alkaline water which bubbles violently. A drainage with a bed of grayish black leads from the pool to Geyser Creek. The temperature of the pool varied between 85° and 87.5°C.

Station fourteen. The large thermal spring GS-IX is a turbid grayish green alkaline water pool which bubbled violently. This pool also drained into Geyser Creek. The pool had a temperature between 85.5° and 88°C.

Station fifteen. The water of Geyser Creek was sampled immediately

before the creek entered the lower bench of the Geyser Springs Group. Since the lower bench comprises the main thermal area and also contributes the largest quantity of thermal water, it was necessary to establish this station in order to collect a sample which showed the total contribution of all thermal waters prior to the lower bench. The temperature of the water varied between 37.3° and 41.5°C and the water was alkaline.

Station sixteen. Geyser Creek was again sampled about midway in the lower bench. The water is alkaline and the temperature at this location varied between 52° and 52.3°C.

Station seventeen. The main geyser of Geyser Springs was sampled during one of its eruptions. This geyser ". . .rises in a heap of rough rhyolite fragments situated 10 or 15 feet up the steep bank, overflows when it erupts and again drops out of sight after the short period of action is over. . ." (Allen and Day, 1935). The geyser erupts approximately every 8 to 10 minutes with a duration of about 2.5 minutes. The sample was collected from a small pool which filled with water during the eruption. The temperature probe was placed in one of the orifices in the mound of rocks from which the water erupted. The temperature remained at 51°C after the eruption and was followed by a rapid rise to 70°C heralding the next eruption and then another rise to 94°C as the water erupted. The water of this geyser is alkaline.

Station eighteen. GS-XII is a beautiful clear pool with yellow colored walls. The drainage, which sinks into the ground without reaching Geyser Creek, is brilliant red. This alkaline water pool is approximately

7 feet by 4 feet, and it is deep with perpendicular walls. The temperature at the bottom of the pool, almost 20 feet below the surface, was 70°C and at the surface it was 63°C.

Station nineteen. This station was established on one of the two drainages which carried alkaline water from the numerous springs of the upper bench. The samples were collected from the drainage in which small "bubblers" released hydrogen sulfide gas. Dark green filamentous algae were attached to the drainage bed. The water temperature at the sampling location varied between 60° and 61.5°C.

Station twenty. This sampling station was located on the second of the two alkaline drainages of the upper bench. The drainage was a bright red and orange color, and the water temperature at this location varied between 48° and 53°C.

These stations were selected to provide a representation of springs throughout the thermal area and to show changes which occur in thermal spring drainage water chemistry and the water chemistry of Geyser Creek. Not all of the stations were sampled during each field trip. Some of the last stations which were described, were sampled only once or twice. These stations were added to complete the water chemistry picture of the Geyser Springs Group.

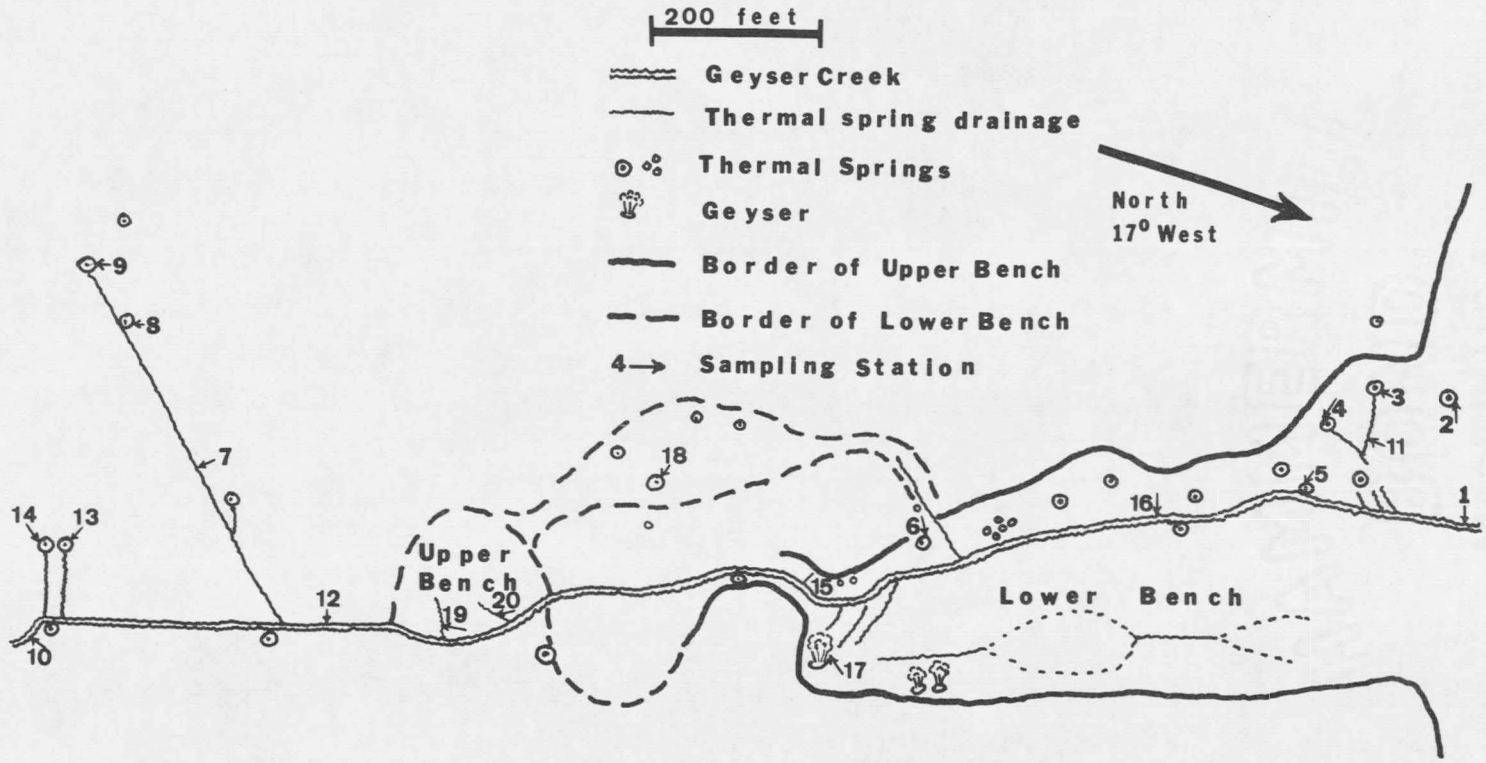
Table I is the schedule of sampling of the twenty stations from June through August, 1965, and also presents the number of samples collected from each station during this period.

Table I. Sampling schedule for chemical analyses 1965

Station no.	Dates of Sampling 6-24-65	7-2-65	7-13-65	7-22-65	8-3-65	8-12-65	8-24-65	Number of Samples Collected
1	+	+	+	+	+	-	+	6
2	+	+	+	+	-	-	-	4
3	+	+	+	+	+	-	+	6
4	+	+	+	+	+	-	-	5
5	+	+	+	+	+	-	-	5
6	+	+	+	+	+	-	-	5
7	+	+	+	+	+	-	+	6
8	+	+	+	+	+	-	+	6
9	+	+	+	+	+	-	+	6
10	+	+	+	+	+	-	+	6
11	-	+	+	+	+	-	+	5
12	-	+	+	+	+	-	-	4
13	-	+	+	+	+	-	-	4
14	-	+	+	+	+	-	-	4
15	-	-	+	+	-	-	+	3
16	-	-	-	-	+	-	+	2
17	-	-	-	-	-	+	-	1
18	-	-	-	-	-	+	-	1
19	-	-	-	-	-	+	+	2
20	-	-	-	-	-	+	+	2

Figure 3

Geyser Springs Group



The average of the pH readings and the average of the Eh values from the field readings and samples returned for laboratory analysis from each station are presented in Table II. There is an agreement between field and laboratory pH values, particularly for the acid samples; any differences are probably due to temperature differences between the field and laboratory determinations. All of the laboratory determinations were made at 25°C. The Eh values show marked differences between field and laboratory values. The field values are probably more reliable than laboratory values as changes in samples such as oxidation of sulfide, gas solubility and oxidation-reduction reactions could have occurred in the laboratory samples.

The chemical analyses of the samples are presented in Table III. The results have been averaged and the standard deviation computed. The waters may be classified in four types. Two of these are high chloride, low sulfate, and alkaline, e.g. stations 1, 2, 5, 6, 15, 16, 17, 18, 19, and 20; high chloride, high sulfate, and acid, e.g. stations 3, 4, and 11. All of these stations lie in the area of the lower and the upper bench. The stations south of the upper bench are of low chloride, low sulfate, and alkaline, e.g. stations 10, 13, and 14; low chloride, high sulfate, and acid, e.g. stations 7, 8, 9, and 12.

The drainages and pools of GS-I, GS-VI and GS-VII were selected for more extensive study of water chemistry and the chemoautotrophic bacteria. These two drainages and their respective pools were selected because the waters are acid, the drainages are long, well defined, and a thermal gradient was established down each drainage.

Table II. Average of pH and Eh values of samples and at field stations.

Station	Field pH	Standard Deviation	Laboratory pH	Standard Deviation	Field Eh +V.	Standard Deviation	Laboratory Eh +V.	Standard Deviation
1	7.13	0.16	7.67	0.15	0.27	0.02	0.77	0.01
2	7.88	0.39	7.44	0.12	0.15	0	0.78	0.01
3	2.61	0.15	2.48	0.02	0.40	0.05	0.53	0.02
4	5.28	0.27	6.25	0.02	0.20	0.04	0.78	0.02
5	6.95	0.18	7.65	0.13	0.14	0.08	0.77	0.07
6	6.98	0.31	7.54	0.09	0.22	0.05	0.78	0.01
7	2.72	0.13	2.73	0.03	0.59	0.04	0.49	0.07
8	2.71	0.16	2.76	0.03	0.35	0.07	0.53	0.06
9	2.81	0.26	2.82	0.03	0.41	0.04	0.57	0.02
10	6.19	0.22	6.90	0.48	0.37	0.03	0.75	0.04
11	2.49	0.23	2.43	0.03	0.51	0.09	0.54	0.02
12	2.88	0.09	2.71	0.45	0.54	0.03	0.50	0.08
13	6.96	0.50	7.32	0.21	0.20	0.19	0.81	0.02
14	6.44	0.45	6.44	0.05	0.24	0.02	0.78	0.03
15	7.52	0.12	8.32	0.47	0.29	0.01	0.83	0.01
16	6.55	0.07	7.74	0.44	0.26	0.08	*0.78	--
17	N.D.	--	*8.54	--	N.D.	--	*0.82	--
18	*7.70	--	*7.43	--	*0.28	--	*0.77	--
19	8.15	0.07	8.48	0.06	0.13	0.01	*0.80	--
20	8.33	0.11	8.77	0.05	0.19	0.01	*0.84	--

* Only one value
N.D.-Not determined

Table III. Chemical analysis of water samples from Geyser Springs Group.

Station	Ca^{++} meq/L		Mg^{++} meq/L		K^{+} meq/L		Na^{+} meq/L		Al^{+++} meq/L	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
1	0.057	0.014	0.15*	-	0.858	0.051	14.66	1.22	0.0049	0.008
2	0.087	0.007	0*	-	0.968	0.011	17.75	0.85	0	0
3	0.016	0.014	0*	-	0.381	0.010	6.05	0.59	0.54	0.075
4	0.026	0.007	0*	-	0.342	0.002	9.14	0.13	0.019	0.006
5	0.09	0.02	0*	-	0.957	0.039	17.98	0.85	0	0
6	0.097	0.023	0*	-	1.097	0.033	19.16	0.97	0	0
7	0.014	0.009	0.10*	-	0.579	0.040	1.02	0.21	1.19	0.19
8	0.024	0.014	0.10*	-	0.585	0.037	0.90	0.12	0.397	0.049
9	0.030	0.013	0.10*	-	0.556	0.042	0.87	0.10	0.374	0.065
10	0.057	0.010	0.20*	-	0.241	0.056	0.51	0.16	0.008	0.008
11	0.007	0.005	0.10*	-	0.417	0.049	6.24	0.31	0.56	0.05
12	0.0125	0.005	0.15*	-	0.587	0.059	1.27	0.15	0.481	0.091
13	0.006	0.004	0*	-	0.70	0.04	2.15	0.54	0.014	0.008
14	0.01	0	0*	-	0.685	0.013	2.77	0.21	0.007	0.008
15	0.021	0.014	0*	-	0.696	0.036	12.85	0.44	0.0027	0.0027
16	0.063	0.017	0*	-	0.900	0.025	15.0	0	0	0
17	0.075*	-	0*	-	1.175*	-	20.0*	-	0*	-
18	0.15*	-	0*	-	0.862*	-	20.0*	-	0*	-
19	0.0127	0.0004	0*	-	0.918	0.011	21.55	1.34	0*	-
20	0.012	0.030	0*	-	1.031	0.031	25.55	0.05	0*	-

*Single determinations
0-Not detectable by method used.
N.D.-Not determined.

Station	Total Fe mg/L		Cl^- meq/L		$SO_4^=$ meq/L		$PO_4^=$ meq/L		SiO_2 mg/L	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
1	0.25	0.08	11.43	0.21	3.08	0.21	0.043	0.010	234.6	22.2
2	0.08	0.11	14.43	0.39	3.10	0.22	0.015	0.002	247.2	9.0
3	0.50	0.14	3.94	1.07	8.76	0.99	0.017	0.004	234	11.4
4	0.08	0.11	5.06	0.45	5.52	0.68	0.009	0.004	186.6	19.8
5	0.08	0.11	15.28	0.38	2.17	0.29	0.038	0.012	220.2	41.4
6	0.006	0.008	16.80	0.57	2.63	0.51	0.074	0.011	237.6	46.2
7	1.76	0.17	0.67	0.36	5.36	0.45	0	0	220.2	16.2
8	1.98	0.42	0.38	0.16	4.28	0.44	0.0005	0.0012	223.8	21.6
9	1.59	0.42	0.46	0.33	3.99	0.38	0.0005	0.0012	209.4	23.4
10	0.028	0.028	0.04	0.03	0.39	0.08	0.003	0.004	97.2	28.8
11	*0.42	-	3.49	0.16	8.72	1.31	0.013	0.002	243.6	20.4
12	1.95	0.37	0.53	0.28	4.02	0.64	0	0	202.2	11.4
13	0.028	0.056	0.17	0.23	2.09	0.29	0.007	0.005	249.6	12.0
14	0.002	0.0008	0.47	0.44	3.37	0.20	0.005	0.005	259.8	19.2
15	0.47	0.028	7.09	0.52	3.04	0.81	0.055	0.0013	207.0	2.4
16	0.22	0.028	11.30	0.13	2.92	0.05	0.065	0.001	220.8	19.8
17	*0.25	-	*17.1	-	*1.82	-	*0.034	-	*114.6	-
18	*0.038	-	*15.8	-	*2.37	-	*0.078	-	*202.2	-
19	0.148	0.069	11.45	0.63	3.00	0.14	0.058	0.007	144.6	24.6
20	0.08	0.028	12.92	0.09	2.36	0.09	0.056	0.0	127.2	17.4

* Single determinations.
0 - Not detectable by method used.
N.D. - Not determined.

Station	Fe^{++}* mg/L	$CO_3^{=}$* meq/L	HCO_3^{-}* meq/L
1	0.2	0	0.7
2	N.D.	0	1.60
3	0.75	N.D.	N.D.
4	0.15	0	1.25
5	N.D.	0	1.45
6	N.D.	0	1.55
7	1.90	N.D.	N.D.
8	1.92	N.D.	N.D.
9	1.42	N.D.	N.D.
10	N.D.	0	0.6
11	N.D.	N.D.	N.D.
12	N.D.	N.D.	N.D.
13	N.D.	0	0.90
14	N.D.	0	0.25
15	N.D.	0	1.6
16	N.D.	N.D.	N.D.
17	N.D.	0.50	1.25
18	N.D.	0	1.40
19	N.D.	0.4	7.2
20	N.D.	1.0	8.3

* Single determinations.
0 - Not detectable by method used.
N.D. - Not determined.

The GS-I pool was station 3, and station 11 was located 80 feet downstream on the drainage issuing from this pool (Figure 4). The pool and drainage lie in the main area or lower bench of the thermal basin. About 50 feet down the drainage algal filaments have developed. The filaments have a yellow color because of a coating of sulfur carried by the water. The main channel is well defined throughout the entire drainage length but between 20 and 55 feet from the pool the drainage spreads out. However, the main drainage channel is well defined even in this zone. A dense algal mat lies beside the main drainage in the area between 60 to 70 feet below the pool.

Table IV presents the change in water chemistry which occurred between the spring and station 11. The changes over the 80 foot distance were generally small. There was a slight increase in acidity as shown by both the field and laboratory pH values and by titratable acidity. There was an increase of 0.68 meq of acid during the 80 foot interval.

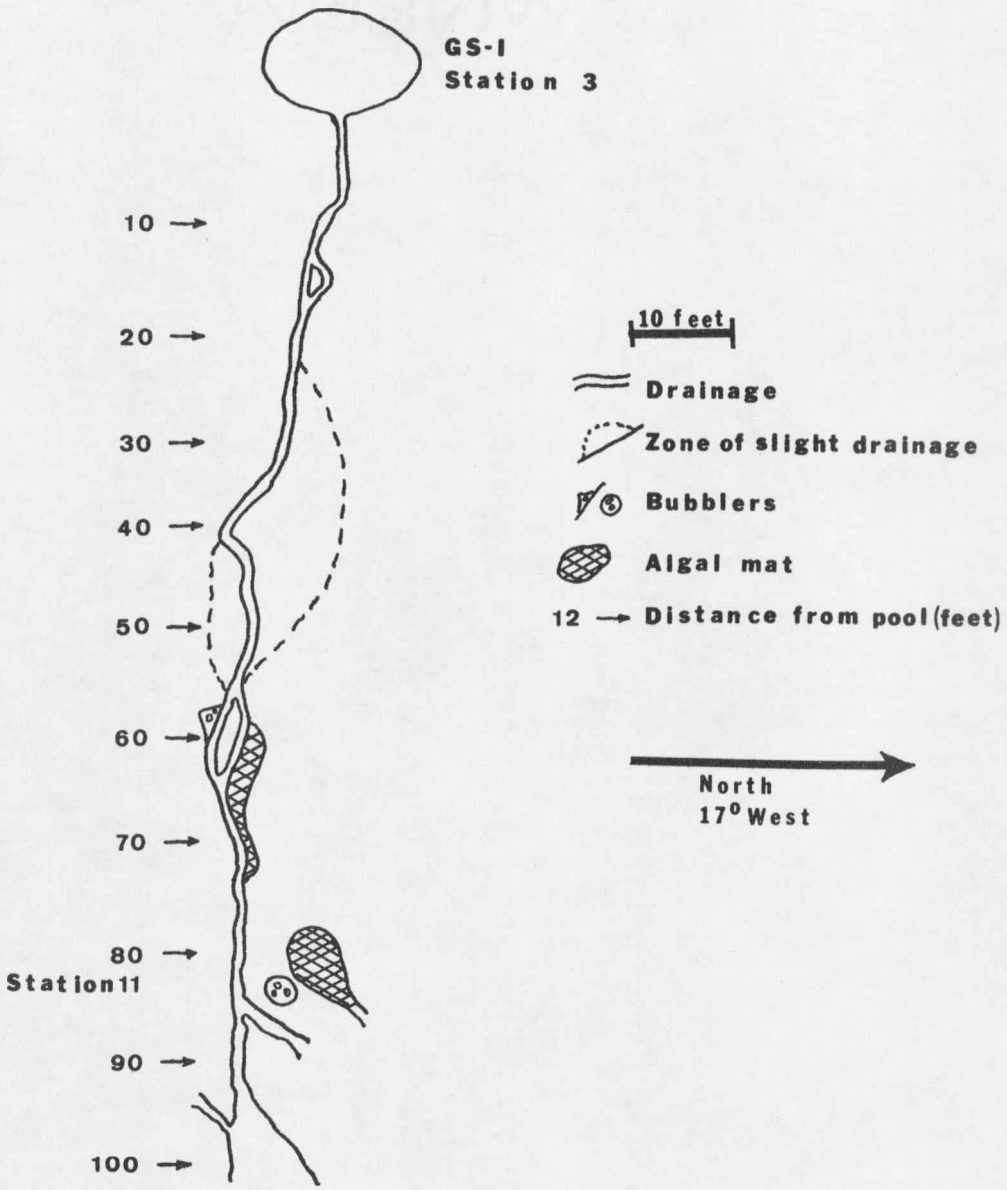
The fluoride content of the water at station 3 was determined to be 3.48 mg/L.

The ammonium ion in the water was determined for a single sample also. Station 3 had 1.12 mg/L and station 11 had 1.18 mg/L.

Figure 5 shows the temperature profile obtained for the GS-I drainage. The rate of temperature decrease for the first 60 feet was -0.52°C/ft, and the rate for the next 40 feet was -0.18°C/ft. The range or total temperature variation observed at the source was 2.5°C and the range increased near the end of the drainage, reaching 11.5°C at 100 feet. This increase

Figure 4

GS-I Spring and Drainage



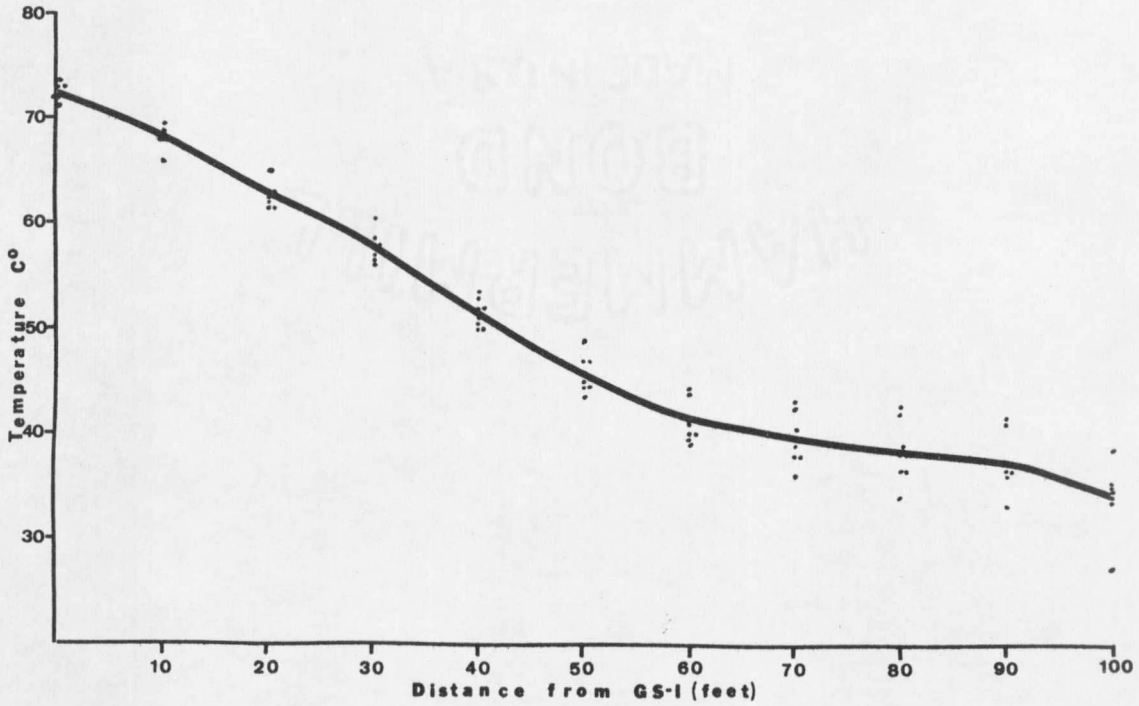


Figure 5. Temperature gradient down GS-I drainage. The solid line represents an average value.

in range is attributed to the increased effect of air temperature at greater distances from the pool.

The walls of the GS-I pool contained kaolinite, (approximately 30%), and alunite, $K_2SO_4.Al_2(SO_4)_3.4Al(OH)_3$, (approximately 10%) in addition to quartz and opal-cristobalite. There was no unaltered feldspar. The turbid suspension at the center of the pool was composed of pure, granular quartz. The drainage near the pool consisted mostly of silica, opal-cristobalite, quartz and minor amounts of kaolinite and a large proportion of crystalline sulfur. The drainage, 80 feet from the spring, contained silica minerals, opal-cristobalite and quartz (personal communication from Bikash Raymahashay, Harvard University).

Table IV. Change of water chemistry occurring in the GS-I drainage.

	Units	Spring	80 feet from spring	Change
		$\bar{x}$	$\bar{x}$	
Ca^{++}	meq/L	0.016	0.007	-0.009
Mg^{++}	"	0.10	0.10	0
K^{+}	"	0.381	0.417	+0.036
Na^{+}	"	6.05	6.24	+0.19
Al^{+++}	"	0.54	0.56	+0.02
Total Fe	mg/L	0.50	0.42	-0.08
Cl^{-}	meq/L	3.94	3.49	-0.45
$SO_4^{=}$	"	8.76	8.72	-0.04
$PO_4^{\equiv}$	"	0.017	0.013	-0.004
SiO_2	mg/L	234	243.6	+9.6
Field pH		2.61	2.49	-0.12
Lab pH		2.48	2.43	-0.05
Field Eh	v	0.40	0.51	+0.11
Lab Eh	v	0.53	0.54	+0.01
Titratable acidity	meq/L	5.52	6.20	+0.68

The second acid thermal environment selected for study consisted of the pools GS-VI and GS-VII and their common drainage. The GS-VI pool was station 9, the GS-VII pool was station 8, and station 7 was located 300 feet down the drainage from GS-VI. Figure 6 shows the detail of this drainage and the pools. The drainage runs down a steep heavily forested "V" shaped gully. The drainage is a few inches wide and quite shallow. Crisscrossing the drainage in many places are fallen trees. About 175 feet below the GS-VI pool algal streamers of a brilliant green color become visible and are evident in the drainage to 300 feet below GS-VI.

Thermal water from a small pool about 200 feet below GS-VI and thermal water from the "Frying pan" entering at 350 feet below GS-VI increase the temperature of the drainage water as shown by the temperature profile, Figure 7.

The changes which occurred in water chemistry, are shown in Table V. The acidity of the water increased from station 9 to 8 as shown by the decrease in pH and the increase of titratable acidity. The titratable acidity increased as the water proceeded from station 8 to 7. The laboratory pH decreased, but the field pH showed a slight increase. The Ca^{++}, K^{+}, Mg^{++}, Na^{+}, total Fe, Cl^{-}, $PO_4^{\equiv}$, and SiO_2 showed small or no changes as the water proceeded down the drainage. The $SO_4^{\equiv}$ and Al^{+++} were substantially increased.

Fluoride was determined on individual samples from stations 7, 8, and 9. Station 9 had 0.70 mg/l, station 8, 0.63 mg/l, and station 7, 0.53 mg/l. Thus, there was also a decrease of fluoride in the water

Table V. Change of water chemistry occurring in the GS-VI, VII drainage.

	Units	Spring Station 9	100 feet from spring Station 8	Change over 100 ft	300 feet from spring Station 7	Change between 100 & 300 ft
Ca^{++}	meq/L	0.030	0.024	-0.006	0.014	-0.008
Mg^{++}	"	0.10	0.10	0	0.10	0
K^{+}	"	0.556	0.585	+0.029	0.579	-0.006
Na^{+}	"	0.87	0.90	+0.03	1.02	+0.12
Al^{+++}	"	0.374	0.397	+0.023	1.19	+0.793
Total Fe	mg/L	1.59	1.98	+0.39	1.76	-0.12
Cl^{-}	meq/L	0.46	0.38	-0.08	0.67	+0.29
$SO_4^{=}$	"	3.99	4.28	+0.29	5.36	+1.08
$PO_4^{\equiv}$	"	0.0005	0.0005	0	0	-0.0005
SiO_2	mg/L	209.4	223.8	+14.4	220.2	-3.6
Field pH		2.81	2.71	-0.10	2.72	+0.01
Lab pH		2.82	2.76	-0.06	2.73	-0.01
Field Eh	v	0.41	0.35	-0.06	0.59	+0.24
Lab Eh	v	0.57	0.53	-0.04	0.49	-0.04
Titratable Acidity	meq/L	2.62	2.92	+0.30	3.67	+0.75

Figure 6

Drainage of GS-VI and GS-VII Springs

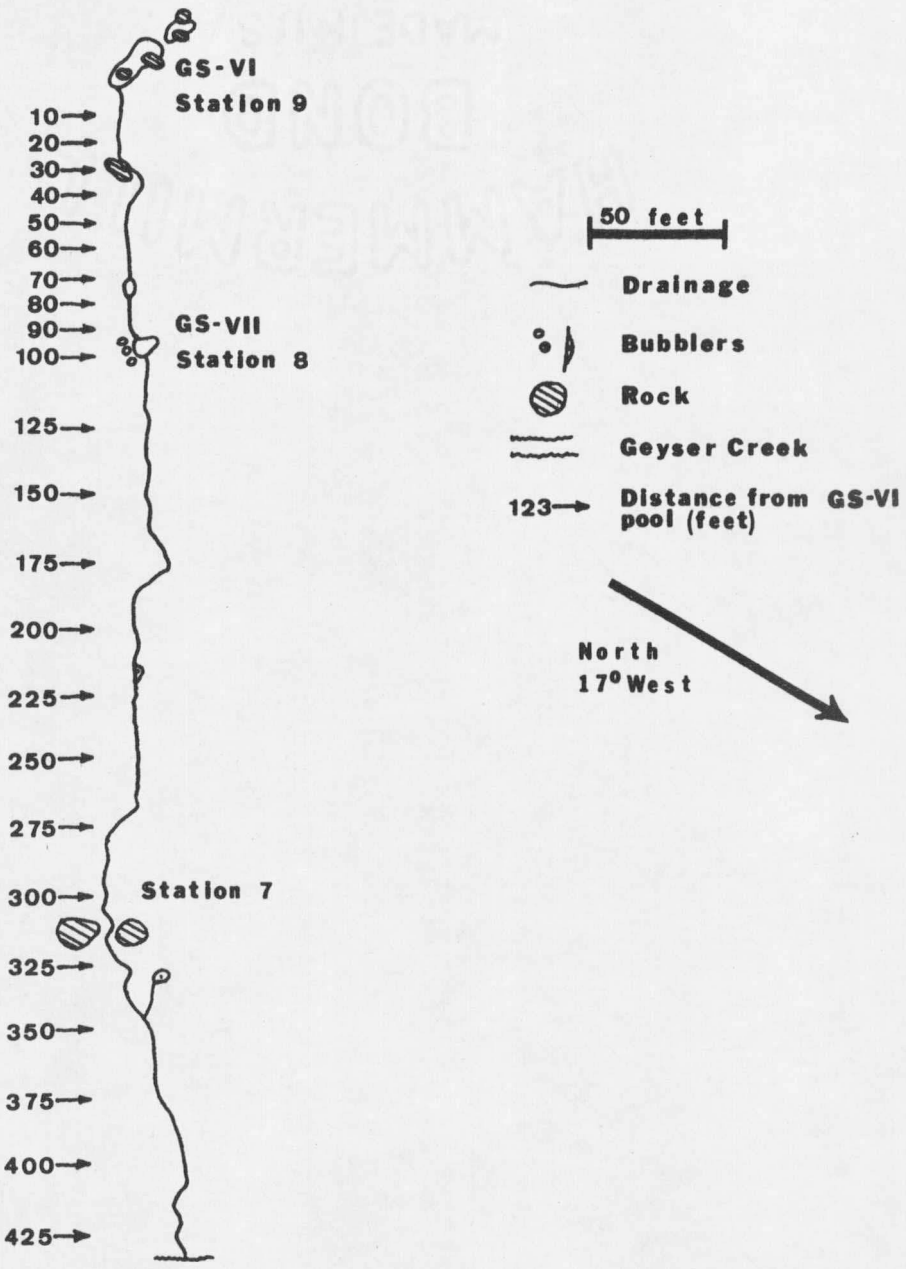


Figure 6. The drainage of the GS-VI and GS-VII pools overlooking station 7. The bottles were used for collection of water samples.

going down the drainage.

The ammonium ion was also determined for a single sample from each station. Station 9 had 1.56 mg/l; station 8, 1.54 mg/l and station 7, 1.27 mg/l.

The thermal gradient (Figure 7) for the first 60 feet of the drainage below GS-VI had an overall temperature decrease rate of -0.44°C/ft, and for the next 40 feet of the drainage the rate was -0.15°C/ft. These rates were similar to the rates of decrease for the GS-I drainage. The thermal gradient from 100 to 425 feet showed a rate of -0.037°C/ft; however, there was much variation within this zone. The temperature range at GS-VI was 5°C, and at the end of the drainage it was 16°C.

The rate of flow volume of the drainage of GS-VI and VII was determined 300 feet below GS-VI at various times during the year (Table VI). The greatest volume of flow occurred, as expected, during the spring thaw. The smallest amount occurred November 11, 1965, when the supply of ground water was decreased. The volume of flow did not appear to vary greatly during the summer and fall months. The results presented are not the true total flow values. The technique employed did not divert quite all of the water from the drainage into the calibrated measuring bucket, although it attempted to do so.

The walls of the GS-VI pool are composed of fresh country rock containing feldspar, $(Na,K)_2OAl_2O_3.6SiO_2$, silica minerals, quartz and opal-cristobalite. Nearly 50% of the rock is volcanic glass. The mud at the outlet contains unaltered feldspar, silica minerals and kaolinite,

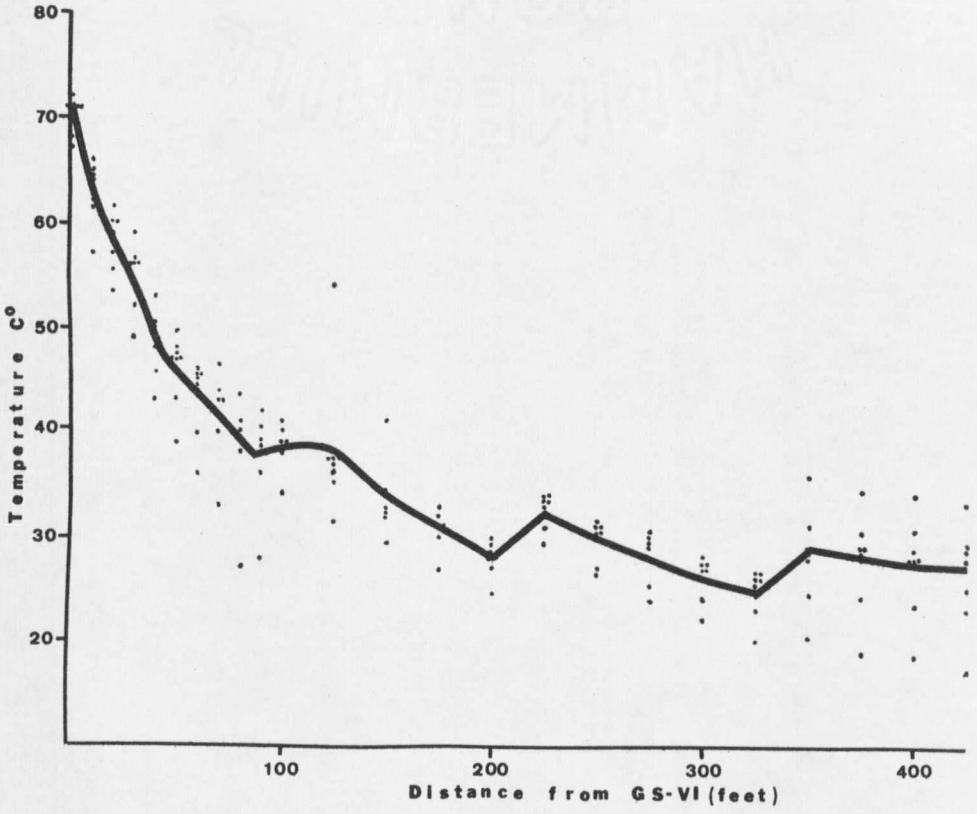


Figure 7. Temperature gradient down GS-VI, VII drainage. The solid line represents an average value.

Table VI. Flow Volume and Rate for GS-VI, VII Drainage.

Date	Flow ml/sec
April 4, 1965	234
July 24, 1965	97
August 3, 1965	123
August 24, 1965	119
October 7, 1965	109
November 11, 1965	86

$Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O$ (approximately 10%). There is little change in mineralogy in the drainage. The mud at GS-VII contains mostly feldspar and silica minerals with small amounts of kaolinite. The mud in the drainage 300 feet below GS-VI contains kaolinite, feldspar and silica minerals (personal communication from Bikash Raymahashay, Harvard University).

Bacteriology of the Acid Thermal Waters

Samples for determination of the chemoautotrophic bacterial populations were collected from the drainages of GS-I and GS-VI, VII at 10 foot intervals beginning at the lip of each pool and continuing to 100 feet down the drainage from the pool. Samples were collected at 25 foot intervals from 100 feet to 425 feet from the drainage of GS-VI, VII. The interval of these samples was increased because of the length of the drainage channel.

The GS-I Drainage

A hanging drop wet mount was prepared for each of the samples collected at 10 foot intervals from the GS-I drainage. These preparations were observed microscopically at 1000X diameters. The observations are listed in Table VII.

The most obvious form of life was the green alga which has been tentatively identified as Zygogonium. The description in Smith (1950) resembles this alga except for the conjugation tubes which have not been observed.

The blue-green algae, which were found in this drainage, were of the Phormidium-Oscillatoria type.

Table VII. Microscopic Observation of Samples from GS-I Drainage.

Distance from GS-I Pool in feet	Morphological Biotypes
0	None observed.
10	Rod shaped bacteria; single.
20	Rod shaped bacteria; single, short filaments.
30	Rod shaped bacteria; single, short filaments.
40	Rod shaped bacteria; single, short filaments.
50	Rod shaped bacteria; single, short filaments Green algal filaments, Zygogonium and Mougoetia, blue-green filamentous algae.
60 to 70	Rod shaped bacteria; single, short and long filaments. Green algal filaments, Zygogonium and Mougoetia. Blue-green filamentous algae. Circular green algae, single and clusters with irregular single chloroplasts, Protococcus Protozoan, Euglena. Blue-green circular alga, possibly Pluto.
80	Rod shaped bacteria; single, short and long filaments. Green algal filaments, Zygogonium.
90	Rod shaped bacteria; single, short and long filaments. Green algal filaments, Zygogonium. Circular green alga with an irregular chloroplast.
100	Rod shaped bacteria; single, short and long filaments. Green algal filaments, Zygogonium.

The first forms of life to be observed in the drainage were the bacteria which were found between 0 and 10 feet from the GS-I pool.

The GS-VI, VII Drainage

A hanging drop wet mount was prepared for each sample collected at 10 foot intervals for the first 100 feet and then of samples collected at 125, 150 and 300 feet from the GS-VI, VII drainage. The observations are listed in Table VII.

The first forms of life to appear in this drainage, as in the GS-I drainage, were the bacteria. Bacterial cells were found at the lip of the pool. The alga, tentatively identified as Zygogonium, appeared to be the most abundant form of life in this drainage also.

The diatoms living in these waters were identified as Eunotia exigua by Theodore Roeder.

The blue-green algae of this drainage were also of the Phormidium-Oscillatoria type.

The Chemoautotrophic Bacteria.

In order to determine if the bacteria, observed in the samples from GS-I and GS-VI, VII drainages, were of the chemoautotrophic type, sulfur and iron enrichment cultures were inoculated with 1 ml of sample and incubated at a temperature near the field temperature.

The samples from GS-I drainages were inoculated into GS-I sulfur and GS-I iron enrichment media. The results obtained after two weeks incubation at respective temperatures are presented in Table IX.

Table VIII. Microscopic Observation of Samples from GS-VI and VII Drainage

Distance from GS-VI Pool in feet	Morphological Biotypes
0	Rod shaped bacteria; single.
10	Rod shaped bacteria; single, short and long filaments.
20	Rod shaped bacteria; single, short and long filaments.
30	Rod shaped bacteria; single, short and long filaments.
40	Rod shaped bacteria; single, short and long filaments.
50	Rod shaped bacteria; single. Filamentous green alga Zygogonium.
60	Rod shaped bacteria; single bacteria; circular, irregular with a refractile body in each cell. (May be Pluto).
70	Rod shaped bacteria; single, short filaments, chains. Circular cell (Pluto or bacterium). Filamentous green alga Zygogonium.
80	Bacterial rods; single. Filamentous green alga Zygogonium.
90	Bacterial rods; single. Filamentous green alga Zygogonium
100	Bacterial rods; single.
125	Bacterial rods; single. Filamentous alga Zygogonium. Protozoan Amoeba.
150	Bacterial rods; single. Filamentous alga Zygogonium. Filamentous blue-green algae.
300	Bacterial rods; single. Filamentous green alga Zygogonium. Filamentous green alga Mougeotia. Diatom Eunotia exigua. Filamentous blue-green algae.

Only sulfur-oxidizing chemosynthetic autotrophic bacteria were obtained from this drainage. No iron-oxidizing bacteria grew in the enrichment medium. The sulfur-oxidizing bacteria of cultures 8-3-65-5 through 8-3-65-11 were typical thiobacilli. They were small, motile, Gram-negative rods, one end of which was often attached to sulfur. The sulfur-oxidizing bacteria in cultures 8-3-65-2 through 8-3-65-4, which grew at 60°C, were entirely different morphologically. The cells were irregular spheres. They were not motile. Each cell possessed a refractile body. The organisms were Gram-negative. The cells attached to sulfur, but unlike the rod shaped thiobacilli, there did not appear to be a preferred position for attachment. These cells appeared similar to those bacteria isolated from hot springs in Israel and described by Kahan (1961).

GS-VI, VII sulfur and iron enrichment media were inoculated with the samples obtained from the GS-VI, VII drainage. The media were examined for the presence of bacteria after two weeks incubation (Table X). Both sulfur and iron-oxidizing chemosynthetic bacteria were present in this drainage. The sulfur-oxidizing bacteria appeared to have a greater tolerance to higher temperature as they were found in the drainage at temperatures higher than 60°C. The iron-oxidizing bacterial population was found at temperatures of 53°C and below. The sulfur-oxidizing bacteria of cultures 8-24-65-4 through 8-24-65-24 were the typical thiobacilli, and closely resembled the sulfur-oxidizing bacteria of the GS-I drainage. The iron-oxidizing bacteria were small, motile Gram-negative rods. The sulfur-oxidizing cultures, 8-24-65-2 and -3, growing at 60°C, were of the same

Table IX. The presence of bacteria as determined using GS-I enrichment cultures.

Sample Number	Feet from Lip of Pool	Field Temp. Range C^{o}	Lab Incub. Temp. C^{o}	Growth on S Medium	Growth on Fe Medium
8-3-65-1	0	71-73.5	60	-	-
8-3-65-2	10	66-69.5	60	+	-
8-3-65-3	20	61.5-65	60	+	-
8-3-65-4	30	56-60.5	60	+	-
8-3-65-5	40	50-53.5	50	+	-
8-3-65-6	50	43.5-49	50	+	-
8-3-65-7	60	39-44.5	45	+	-
8-3-65-8	70	36-43	45	+	-
8-3-65-9	80	34-42.5	37	+	-
8-3-65-10	90	33-41.5	37	+	-
8-3-65-11	100	27-38.5	35	+	-

Table X. The presence of bacteria as determined using GS-VI, VII enrichment cultures.

Sample Number	Feet from Lip of Pool	Field Temp. Range C°	Lab Incub. Temp. C°	Growth on S Medium	Growth on Fe Medium
8-24-65-1	0	67-72	60	-	-
8-24-65-2	10	57-66	60	+	-
8-24-65-3	20	53.5-61.5	60	+	-
8-24-65-4	30	49-59	50	+	-
8-24-65-5	40	43-53	50	+	+
8-24-65-6	50	39-49.7	45	+	-
8-24-65-7	60	36-46	45	+	+
8-24-65-8	70	33-46.5	45	+	-
8-24-65-9	80	27-43.5	37	+	+
8-24-65-10	90	28-42	37	+	+
8-24-65-11	100	34-41	37	+	+
8-24-65-12	125	31.5-54	35	+	+
8-24-65-13	150	29.5-41	35	+	+
8-24-65-14	175	27-33	Room temp.	+	+
8-24-65-15	200	24.5-29.5	"	+	+
8-24-65-16	225	29.5-34	"	+	+
8-24-65-17	250	26.5-31.5	"	+	+
8-24-65-18	275	24-30.5	"	+	+
8-24-65-19	300	22-28	"	+	+
8-24-65-20	325	20-26.5	"	+	+
8-24-65-21	350	20.5-36	"	+	+

(Continued on next page)

(Table X Continued)

Sample Number	Feet from Lip of Pool	Field Temp. Range C°	Lab Incub. Temp. C°	Growth on S Medium	Growth on Fe Medium
8-24-65-22	375	19-34.5	Room temp.	+	+
8-24-65-23	400	18.5-34	"	+	+
8-24-65-24	425	17-33	"	+	+

irregular morphological form described for the sulfur-oxidizing bacteria which developed at 60°C in the GS-I drainage.

Twenty strains of sulfur-oxidizing bacteria were isolated from the enrichment cultures of GS-I samples. Thirty strains of sulfur-oxidizing bacteria and 7 strains of iron-oxidizing bacteria were obtained from the enrichment cultures of GS-VI, VII samples. The characteristics of the sulfur-oxidizing bacteria are presented in Table XI, and the characteristics of iron-oxidizing bacteria are presented in Table XII.

The sulfur-oxidizing chemosynthetic bacteria from the drainages of GS-I and GS-VI, VII were all strict autotrophs which brought about a drop in pH by oxidation of sulfur or thiosulfate, and did not grow on an organic medium. They were all, with the exception of strain 8-3-65-2(1), "typical" thiobacilli and were probably strains of Thiobacillus thiooxidans or T. thioparus. This was determined by comparing the isolated strains with descriptions of these species in Bergey's Manual of Determinative Bacteriology (Breed, Murray and Smith, 1957). The strains which decreased the pH of thiosulfate medium to pH 2 or below, were similar to the T. thiooxidans description, and those which did not decrease the pH below pH 2 may have been of the T. thioparus type. Some of the strains from the GS-VI, VII drainage caused a slight increase in the pH of the medium when grown in thiosulfate medium. This may be due to the accumulation of tetrathionate in the medium as a result of energy metabolism by the bacteria.

Table XI. Characteristics of Sulfur-Oxidizing Bacteria

Strain Designation	Spring Sample	Sample Distance from spring ft.	Sample Field Temp. C°	Lab. Temp. for isolation and tests C°	Cell Morphology	Cell size length μ width μ	Gram Reaction	Motility	Energy Sources				Effect on pH by growth using $S_2O_3^=$		using S^o		Temperature Range using S^o					Colonies on $S_2O_3^=$ agar precip. S^o	Colony Type
									organic*	$S_2O_3^=$	S^o	Fe^{++}	begin	after 14 da.	begin	after 12 da.	Room temp.	37	45	50	60		
8-3-65-2(1)	GS-I	10	66-69.5	60	irreg.sphere	1.6	neg	-	-	-	+	-	6.587	5.120	4.509	2.457	N.D.	-	+	+	+	N.D.	C
8-3-65-6(2)	"	50	43.5-49.7	50	rod	2.6-0.8	neg	+	-	+	+	-	6.595	2.780	4.417	1.933	"	++	++	++	-	+	A
8-3-65-6(3)	"	"	"	"	"	2.6-0.7	"	+	-	+	+	-	6.621	2.070	4.360	2.005	"	++	++	++	-	+	A
8-3-65-7(1)	"	60	39-44.5	45	"	2.2-0.5	"	+	-	+	+	-	6.763	1.860	4.650	1.360	"	++	++	++	-	+	A
8-3-65-9(1)	"	80	34-42.5	37	"	1.9-0.6	"	+	-	+	+	-	6.740	3.740	4.477	1.570	"	++	++	+	-	+	B
8-3-65-9(2)	"	"	"	"	"	2.4-0.6	"	+	-	+	+	-	6.690	1.740	4.760	1.561	"	++	++	++	-	+	A
8-3-65-9(3)	"	"	"	"	"	1.7-0.5	"	+	-	+	+	-	6.740	1.730	4.579	1.541	"	++	++	++	-	+	A
8-3-65-9(4)	"	"	"	"	"	1.8-0.6	"	+	-	+	+	-	6.755	1.780	4.629	1.685	"	++	++	++	-	+	A
8-3-65-9(5)	"	"	"	"	"	1.9-0.8	"	+	-	+	+	-	6.740	1.745	4.590	1.665	"	++	++	++	-	+	A
8-3-65-9(6)	"	"	"	"	"	2.1-0.8	"	+	-	+	+	-	6.660	1.930	4.551	2.140	"	++	++	-	-	+	A
8-3-65-10(1)	"	90	33-41.5	"	"	2.2-0.7	"	+	-	+	+	-	6.738	1.780	4.499	1.562	"	++	++	++	-	+	A
8-3-65-10(2)	"	"	"	"	"	2.0-0.6	"	+	-	+	+	-	6.713	1.755	4.760	1.581	"	++	++	++	-	+	A
8-3-65-10(3)	"	"	"	"	"	2.6-0.7	"	+	-	+	+	-	6.709	1.720	4.535	1.567	"	++	++	++	-	+	A
8-3-65-10(4)	"	"	"	"	"	2.3-0.7	"	+	-	+	+	-	6.739	1.755	4.581	1.682	"	++	++	++	-	+	A
8-3-65-10(5)	"	"	"	"	"	2.3-0.8	"	+	-	+	+	-	6.750	1.710	4.570	1.690	"	++	++	++	-	+	A
8-3-65-10(6)	"	"	"	"	"	2.2-0.6	"	+	-	+	+	-	6.730	1.785	4.720	1.595	"	++	++	++	-	+	A
8-3-65-10(8)	"	"	"	"	"	2.0-0.6	"	+	-	+	+	-	6.700	1.965	4.650	1.885	"	++	++	+	-	-	B
8-3-65-11(1)	"	100	27-38.5	"	"	1.9-0.6	"	+	-	+	+	-	6.750	3.855	4.730	1.380	"	++	++	+	-	+	B
8-3-65-11(2)	"	"	"	"	"	2.1-0.6	"	+	-	+	+	-	6.765	3.795	4.761	1.475	"	++	++	+	-	+	B
8-3-65-11(5)	"	"	"	"	"	2.6-0.7	"	+	-	+	+	-	6.750	1.955	4.650	1.837	"	++	++	-	-	+	B
8-24-65-4(1)	GS-VI, VII	30	49-59	50	"	2.0-0.6	"	+	-	+	+	-	6.525	1.750	4.433	1.490	+	++	++	++	-	+	A
8-24-65-6(1)	"	50	39-49.7	45	"	2.6-0.7	"	+	-	+	+	-	6.490	1.770	4.445	1.405	+	++	++	++	-	+	A
8-24-65-6(2)	"	"	"	"	"	2.4-0.7	"	+	-	+	+	-	6.515	1.750	4.562	1.350	+	++	++	++	-	+	A
8-24-65-7(1)	"	60	36-46	45	"	2.0-0.7	"	+	-	+	+	-	6.440	1.782	4.590	1.375	+	++	++	++	-	+	A
8-24-65-7(2)	"	"	"	"	"	2.0-0.8	"	+	-	+	+	-	6.513	1.740	4.573	1.440	+	++	++	++	-	+	A
8-24-65-8(1)	"	70	33-46.5	45	"	2.0-0.8	"	+	-	+	+	-	6.523	1.725	4.510	1.460	+	++	++	++	-	+	A
8-24-65-8(2)	"	"	"	"	"	2.4-0.8	"	+	-	+	+	-	6.575	1.720	4.565	1.485	+	++	++	++	-	+	A
8-24-65-9(1)	"	80	27-43.5	37	"	2.0-0.7	"	+	-	+	+	-	6.446	7.200	4.678	1.395	+	++	++	++	-	+	A
8-24-65-9(2)	"	"	"	"	"	2.3-0.7	"	-	-	+	+	-	6.459	7.140	4.655	1.455	+	++	++	++	-	-	B
8-24-65-10(1)	"	90	28-42	"	"	2.2-0.8	"	+	-	+	+	-	6.455	3.990	4.640	1.590	+	++	++	++	-	+	A
8-24-65-11(1)	"	100	34-41	"	"	2.0-0.6	"	+	-	+	+	-	6.433	7.110	4.580	1.527	++	++	++	-	-	+	A
8-24-65-11(2)	"	"	"	"	"	2.2-0.6	"	+	-	+	+	-	6.415	3.917	4.605	1.595	+	++	++	++	-	+	A
8-24-65-12(1)	"	125	31.5-54	"	"	2.0-0.6	"	+	-	+	+	-	6.360	6.985	4.520	1.510	+	++	++	++	-	+	A
8-24-65-12(2)	"	"	"	"	"	2.1-0.6	"	+	-	+	+	-	6.360	7.125	4.480	1.600	+	++	++	++	-	+	A
8-24-65-13(1)	"	150	29.5-41	"	"	2.1-0.7	"	+	-	+	+	-	6.322	7.110	4.570	1.510	+	++	++	++	-	+	A
8-24-65-13(2)	"	"	"	"	"	2.0-0.6	"	+	-	+	+	-	6.325	3.975	4.490	1.640	+	++	++	++	-	+	A
8-24-65-14(1)	"	175	27-33	room temp.	"	1.8-0.7	"	+	-	+	+	-	6.571	4.217	4.565	1.675	+	++	++	++	-	+	A
8-24-65-14(2)	"	"	"	"	"	2.3-0.8	"	+	-	+	+	-	6.437	4.129	4.760	1.689	+	++	++	++	-	+	A
8-24-65-15(1)	"	200	24.5-29.5	"	"	2.3-0.7	"	+	-	+	+	-	6.460	4.145	4.570	1.940	++	++	++	++	-	+	A
8-24-65-15(2)	"	"	"	"	"	1.9-0.6	"	+	-	+	+	-	6.470	4.567	4.489	2.069	+	+	-	-	-	+	B
8-24-65-16(1)	"	225	29.5-34	"	"	2.0-0.6	"	+	-	+	+	-	6.560	3.845	4.555	2.055	+	++	++	++	-	+	A
8-24-65-17(1)	"	250	26.5-31.5	"	"	1.8-0.6	"	+	-	+	+	-	6.540	4.210	4.530	1.931	+	++	++	-	-	+	A
8-24-65-18(1)	"	275	24-30.5	"	"	2.0-0.6	"	+	-	+	+	-	6.555	4.300	4.485	2.000	+	++	++	-	-	+	A
8-24-65-19(1)	"	300	22-28	"	"	1.9-0.6	"	+	-	+	+	-	6.500	4.097	4.405	2.257	+	++	++	++	-	+	A
8-24-65-19(2)	"	"	"	"	"	1.8-0.6	"	+	-	+	+	-	6.537	4.347	4.533	1.845	+	+	+	-	-	+	F
8-24-65-20(2)	"	325	20-26.5	"	"	2.5-0.6	"	+	-	+	+	-	6.509	4.189	4.527	1.875	++	++	++	-	-	+	B
8-24-65-21(1)	"	350	20.5-36	"	"	2.2-0.6	"	+	-	+	+	-	6.345	4.021	4.525	1.973	++	++	++	++	-	+	A
8-24-65-22(1)	"	375	19-34.5	"	"	1.9-0.7	"	+	-	+	+	-	6.300	4.130	4.580	1.590	++	++	++	++	-	+	A
8-24-65-23(2)	"	400	18.5-34	"	"	2.2-0.6	"	+	-	+	+	-	6.340	4.255	4.555	1.881	+	++	++	++	-	+	A
8-24-65-24(1)	"	425	17.0-33	"	"	2.1-0.6	"	+	-	+	+	-	6.305	7.360	4.515	1.920	++	++	++	++	-	+	A

- No growth or reaction + Growth or reaction ++ Good growth N.D. Not determined A Lens, some with slightly irregular margin, <0.5 mm diameter, translucent, smooth. B Flat, circular, translucent, 0.5 mm diameter, peaked center, smooth. C Lens, <1 mm diameter, translucent, smooth. F Flat, circular, translucent, 3 mm diameter, smooth. * Trypticase - glucose - extract agar (BBL) used as an organic medium.

Table XII. Characteristics of Iron Oxidizing Bacteria

Strain Designation	Sample Distance from GS-VI ft.	Sample field temp. C°	Lab temp. for isolation & all tests except temp. range	Cell Morphology	cell size length width μ	Gram Reaction	Motility	*Energy Sources				Effect on pH by growth using Fe^{++}		using S^{o}		Temperature range using S^{o} of Fe				Colony type
								organic	$S_2O_3^{=}$	S^{o}	Fe^{++}	begin	after 21 days	begin	after 12 days	Room temp.	37	45	50	
8-24-65-9(1F)	80	27-43.5	37	rod	1.8-0.6	neg.	+	-	-	+	+	2.995	2.887	4.565	5.667	+	+	-	-	D
8-24-65-10(1F)	90	28-42	"	"	1.7-0.7	"	+	-	-	+	+	2.980	2.923	4.650	6.060	+	+	-	-	E
8-24-65-10(2F)	"	"	"	"	1.6-0.6	"	+	-	-	+	+	2.987	3.009	4.697	6.069	+	+	-	-	D
8-24-65-11(1F)	100	34-41	"	"	1.6-0.6	"	+	-	-	+	+	2.990	3.009	4.490	5.650	+	+	-	-	D
8-24-65-19(1F)	300	22-28	room temp.	"	1.6-0.5	"	+	-	-	+	+	2.973	2.919	4.665	4.440	+	+	-	-	D
8-24-65-22(1F)	375	19-34.5	"	"	1.6-0.6	"	+	-	-	+	+	2.989	2.921	4.533	4.353	+	+	-	-	D
8-24-65-23(1F)	400	18.5-34	"	"	1.5-0.5	"	+	-	-	+	+	2.970	2.923	4.647	4.773	+	+	-	-	D

Explanation of symbols used in table:

- \- No growth or reaction
- \+ Growth or reaction
- D Lens, ⌀0.5 mm diameter, smooth, translucent with slight reddish coloration
- E Lens, ⌀0.5 mm diameter, smooth, translucent with slight reddish coloration, form depression in agar.
- * Trypticase - glucose-extract agar (BBL) used as an organic medium.

The strains were incubated at different temperatures which were within the temperature range of respective thermal spring drainages from which they were isolated. Sulfur was used as an energy source. The strains of type "A" colonies demonstrated good growth at room temperature, 37°C, 45°C and 50°C with the exception of the strains 8-3-65-9(6), 8-24-65-11(1), 8-24-65-17(1) and 8-24-65-18(1) which did not grow at 50°C. The strains of type "B" and "F" colonies demonstrated only slight or no growth at 50°C except for strain 8-24-65-9(2) which grew well at 50°C. Only strain 8-3-65-2(1) was able to grow at 60°C, but it was not able to grow at 65°C.

The "B" and "F" type colonies, except strains 8-3-65-10(8) and 8-3-65-11 (5), showed pH reactions on thiosulfate which place them in the T. thioparus group. However, many of the type "A" colony strains also would fit into this group.

The strain 8-3-65-2(1) was a very unusual type of sulfur oxidizing bacterium. The spherical shape of this organism (Figure 8) has not been noted for sulfur-oxidizing chemosynthetic bacteria. The cells at times

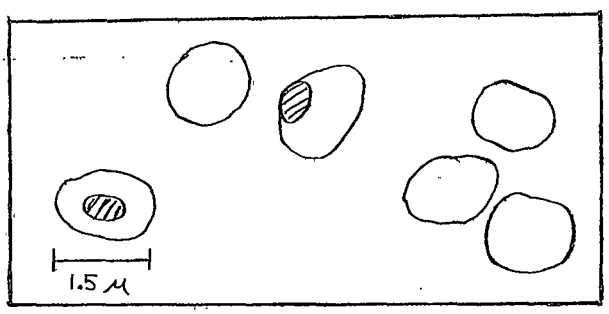


Figure 8. Cells of strain 8-3-65-2(1)

seemed to form groups suggesting that a matrix may be holding them together. The refractile body within the cells was stained green by the Bartholomew and Mittwer spore stain method (Society of American Bacteriologists, 1957).

The resistance of strain 8-3-65-2(1) to 80°C was determined and compared with the resistance to 80°C of a typical thiobacillus, strain 8-3-65-6(2) (Table XIII). Cells of strain 8-3-65-2(1) grew and decreased the pH after 90 minutes of heating at 80°C. Strain 8-3-65-6(2) could only tolerate heating at 80°C between 10 and 20 minutes. The resistance to heat by strain 8-3-65-2(1), coupled with the microscopic observation of a refractile body in each cell which stained with a spore stain, is evidence of the ability of this organism to form spores.

The iron-oxidizing chemosynthetic bacterial strains isolated from the GS-VI, VII drainage were able to oxidize sulfur in addition to ferrous iron. They were not able to oxidize thiosulfate, nevertheless they may be placed in the group of bacteria, Thiobacillus ferrooxidans.

Their growth on ferrous iron medium did little to the pH of the medium when grown in stationary cultures. However, when the ferrous iron cultures were aerated, there was a decrease in pH.

Growth of the iron-oxidizing strains of bacteria on sulfur brought about an unexpected change of pH. The strains 8-24-65-9(1Fe), -10(1Fe), - 10 (½ Fe), and -11(1Fe) increased the pH when grown at 37°C. The increase of pH probably represented an accumulation of some end product of the oxidation of elemental sulfur and has not been previously noted.

Table XIII. Resistance of strains 8-3-65-2(1) and 8-3-65-6(2) to the temperature of 80°C.

Time at 80°C minutes	8-3-65-2(1) growth	pH after 7 days incubation	8-3-65-6(2) growth	pH after 7 days incubation	Uninoculated controls incubated at 50°C growth	pH after 7 days incubation	Uninoculated control incubated at 60° C growth	pH after 7 da. incuba.
0	+	2.401	+	1.947	-	2.891	-	2.915
10	N.D.	-	+	2.401				
20	"	-	-	2.832				
30	"	-	-	2.820				
40	+	2.461	-	2.847				
50	+	2.529	N.D.					
60	+	2.507	-	2.849				
70	+	2.575	N.D.					
80	+	2.669	N.D.					
90	+	2.660	N.D.					

N. D. - Not determined.

The strains 8-24-65-19(1Fe) and -22(1Fe) brought about a slight decrease in pH. These strains had been incubated at room temperature. Strain 8-24-65-23(1Fe) failed to grow in this experiment.

The temperature range of growth for these isolates was limited to room temperature and 37°C when either ferrous iron or sulfur was used as an energy source.

Geochemical Role of Bacteria

Table XIV shows the effect on the water chemistry by the enrichment culture of sulfur-oxidizing bacteria, 8-24-65-5, and the enrichment culture of iron-oxidizing bacteria, 8-24-65-5Fe, when grown at 50°C in the presence of GS-VI, VII drainage bed material.

There was an almost equal pH drop in the flask with the sulfur-oxidizing bacterial culture (flask 1), the uninoculated control with energy source (flask 2), and the uninoculated control without any energy source (flask 5). However, the total acidity of the inoculated flask was much greater than the two control flasks. The calcium, magnesium, potassium, and sodium showed very small or no changes during the growth period. The chloride decreased in the inoculated flask. The aluminum, sulfate, and silica all markedly increased in the inoculated flask (1) compared to the control flasks (2 and 5). The phosphate also showed an increase in the inoculated flask.

The iron-oxidizing culture, 8-24-65-5Fe, brought about a marked decrease of pH in flask 3 compared with the very slight decrease which occurred in its corresponding uninoculated control flask, 4. There was

Table XIV. Effect of chemoautotrophic bacteria growing at 50°C in the presence of GS-VI, VII drainage bed material.

Flask	1	1	2	2	3	3	4	4	5	5
Sample	Initial	Final	Initial	Final	Initial	Final	Init.	Final	Init.	Final
Inoculum	8-24-65-5, S	Oxidizers	Uninoculated Control		8-24-65-5, Fe	Oxidizers	Uninoculated Control		Uninoculated Control	
Energy Source	S^o		S^o		Fe^{++}		Fe^{++}		None	
pH	2.747	1.870	2.920	2.135	2.721	1.941	2.761	2.660	2.95	2.09
titratable acidity, meq	3.72	21.77	3.44	13.56	21.87	25.79	20.53	21.58	2.96	14.80
Ca^{++} meq/L	0.05	0.07	0.03	0.02	1.67	0.47	1.75	1.77	0.03	0.02
Mg^{++} mg/L	0	0	0	0	0	0	0	0	0	0
K^+ meq/L	0.537	0.581	0.56	0.58	0.55	0.026	0.545	0.563	0.53	0.54
Na^+ meq/L	0.425	0.425	0.38	0.38	0.43	0.43	0.43	0.47	0.43	0.42
Al^{+++} meq/L	0.257	1.71	0.372	0.855	1.80	1.94	1.11	2.33	0.502	1.37
Cl^- meq/L	0.422	0.042	0.673	0.495	N.D.	N.D.	N.D.	N.D.	0.842	0.842
$SO_4^=$ meq/L	8.54	30.00	8.12	19.08	30.49	34.06	27.08	31.76	6.08	21.01
$PO_4^{\equiv}$ meq/L	1.34	1.74	1.53	1.50	0.33	0.20	0.42	0.13	1.43	1.54
SiO_2 mg/L	12.1	51.0	8.0	28.0	N.D.	N.D.	N.D.	N.D.	7.6	36.0

0 Not detectable by method used.

N.D. Not determined.

also a higher total acidity in the inoculated flask. The initial level of titratable acidity was much higher in flasks 3 and 4 than in flasks 1, 2, and 5 because of the ferrous iron present in the flasks 3 and 4. The presence of iron led to some difficulties. It was not possible to determine chloride and silica content with the methods used in our laboratory because of iron interference. Generally, the changes due to iron-oxidizing bacteria were very slight in the reaction vessel. Calcium, phosphate and potassium levels decreased during the growth period. Sulfate increased slightly. Magnesium and sodium showed no change. The aluminum level increased slightly but it also increased in the uninoculated control.

The effect of sulfur and iron-oxidizing bacterial cultures from the GS-VI, VII drainage upon the water chemistry was also determined at room temperature (Table XV). One of the reaction vessels, flask 5, was prepared by adding 25 ml of 1 N H_2SO_4, after the initial sample was removed, to simulate the acid produced by bacterial oxidation of sulfur. This quantity of acid had been established in a preliminary experiment.

Culture 8-24-65-20 (flask 1), the sulfur-oxidizing bacteria, decreased the pH of the medium over one unit while the uninoculated control (flask 2) showed a slight increase in pH. The titratable acidity increased 25.79 meq/L and the control had a slight decrease in titratable acidity. The aluminum, sulfate, silica and phosphate showed definite increases in concentration in the inoculated flask, which was significantly greater than the uninoculated control. Potassium concentration increased slightly in the inoculated flask. Chloride showed a decrease

in concentration in the inoculated flask which was similar to the chloride change at 50°C (Table XIV). No real changes occurred in the magnesium and sodium concentrations. The uninoculated control at room temperature, in contrast to the uninoculated controls at 50°C (Table XIV), showed little change over the incubation period.

Flask 5, to which the sulfuric acid had been added, showed changes of nearly the same magnitude and direction noted for the inoculated flask, 1.

The culture of iron-oxidizing bacteria (flask 3), 8-24-65-20-Fe, decreased the pH and increased the titratable acidity with only small changes occurring in the uninoculated control (flask 4). There were very small increases in the concentrations of calcium and phosphate in the inoculated flask. There were also small increases in the amounts of potassium and aluminum in both the inoculated and uninoculated flasks. There were no significant changes in the sodium and magnesium concentrations in either flask. The sulfate ion concentration increased in both the inoculated and uninoculated flasks. The inoculated flask showed the greater increase.

The effect of GS-I sulfur enrichment cultures on the water chemistry when grown at 50°C and 37°C in the presence of GS-I drainage bed material was also determined. Only sulfur-oxidizing cultures were used as no iron-oxidizing bacteria were obtained from the GS-I drainage.

Table XVI shows the effect of culture 8-3-65-5 on the water chemistry at 50°C. The changes occurring in the inoculated flask, 1, were generally

Table XV. Effect of chemoautotrophic bacteria growing at room temperature in the presence of GS-VI, VII drainage bed material.

Flask	1	1	2	2	3	3	4	4	5	5
Sample	Initial	Final	Initial	Final	Initial	Final	Init.	Final	Init.	Final
Inoculum	8-24-65-20,S	Oxidizers	Uninoculated Control		8-24-65-20 Fe	Iron Oxidizer	Uninoculated Control		Uninoculated Control +25 meq/L H_2SO_4	
Energy Source	S^o		S^o		Fe^{++}		Fe^{++}		None	
pH	2.940	1.840	2.960	3.000	2.899	2.312	2.901	2.862	2.97	1.78
titratable acidity meq	3.24	29.03	3.24	3.06	23.68	32.28	22.44	24.26	3.25	30.56
Ca^{++} meq/L	0.32	0.14	0.25	0.11	2.0	2.12	1.96	1.98	0.03	0.02
Mg^{++} mg/L	0	0	0	0	0	0	0	0	0	0
K^+ meq/L	0.47	0.55	0.44	0.45	0.44	0.47	0.45	0.47	0.54	0.52
Na^+ meq/L	0.43	0.42	0.42	0.44	0.45	0.44	0.46	0.44	0.44	0.37
Al^{+++} meq/L	0.22	0.966	0.20	0.339	1.63	3.88	1.53	2.74	0.676	1.46
Cl^- meq/L	0.669	0.357	0.448	0.422	N.D.	N.D.	N.D.	N.D.	0.521	0.388
$SO_4^=$ meq/L	6.29	29.48	5.99	6.29	23.95	30.46	24.79	28.22	6.75	33.33
$PO_4^=$ meq/L	1.49	1.64	1.48	1.48	0.41	0.77	0.23	0.15	1.58	1.79
SiO_2 mg/L	5.1	14.9	5.6	8.7	N.D.	N.D.	N.D.	N.D.	6.3	21.0

0 - Not dectable by method used.
N.D. - Not determined.

quite small except for the decreased pH, increased titratable acidity, sulfate and silica concentrations. The chloride concentration decreased in the inoculated flask. Small increases occurred in potassium and aluminum concentration. The uninoculated control with sulfur (flask 2) and the uninoculated control without sulfur (flask 3) also had a decrease of pH, an increase in titratable acidity, sulfate and silica concentrations. Although titratable acidity, sulfate, and silica concentrations increased, these concentrations were much less than in the inoculated flask. The remainder of the constituents showed very slight or no change in concentration.

Table XVII shows the effect of the sulfur-oxidizing bacterial culture, 8-3-65-9, at 37°C on the water chemistry. In this experiment one reaction vessel (flask 3) was also set up which contained GS-I basal medium and drainage bed material in which 25 ml of 1 N H_2SO_4 was added after the initial sample was removed.

The pH, titratable acidity, and sulfate concentration showed the most change in both the inoculated flask and the uninoculated control with acid. The change was of the same magnitude and direction in both flasks. The aluminum and silica concentrations showed a slight increase in the flasks 1 and 3, but the chloride, in contrast to the inoculated flask at 50°C, showed a slight increase in both flasks 1 and 3. The remainder of the constituents showed little change. The uninoculated control with sulfur (flask 2) had little change in any of the chemical concentrations in contrast to the same uninoculated controls at 50°C, which did have

Table XVI. Effect of chemoautotrophic bacteria at 50°C on drainage bed material of the GS-I thermal spring drainage.

Flask	1	1	2	2	3	3
Sample	Initial	Final	Initial	Final	Initial	Final
Inoculum	8-3-65-5,S	Oxidizer	Uninoculated		Uninoculated	
Energy Source	S^o		Control S^o		Control None	
pH	2.732	1.679	2.821	2.275	2.86	2.17
Titratable Acidity, meq	4.58	41.54	4.58	11.36	4.01	12.99
Ca^{++} meq/L	0.09	0.09	0.09	0.09	0.12	0.09
Mg^{++} mg/L	0	0	0	0	0	0
K^{+} meq/L	0.74	0.82	0.77	0.77	0.73	0.73
Na^{+} meq/L	5.3	5.4	5.3	5.1	5.4	5.5
Al^{+++} meq/L	0.035	0.222	0.055	0.055	0.080	0.153
Cl^{-} meq/L	4.89	0.65	5.20	4.96	5.06	5.13
$SO_4^{=}$ meq/L	12.00	50.00	9.50	18.03	8.38	19.61
$PO_4^{=}$ meq/L	2.36	2.40	2.44	2.48	2.24	2.11
SiO_2 mg/L	3.7	16.0	4.0	13.0	1.25	4.20

0 - Not detectable by method used.

Table XVII. Effect of chemoautotrophic bacteria growing at 37°C in the presence of GS-I drainage bed material.

Flask	1	1	2	2	3	3
Sample	Initial	Final	Initial	Final	Initial	Final
Inoculum	8-3-65-9,S	Oxidizer	Uninoculated Control		Uninoculated Control +25 meq H_2SO_4	
Energy Source	S^o		S^o		None	
pH	2.740	1.810	2.870	2.850	2.87	1.78
titratable acidity, meq	4.78	30.08	4.58	4.58	3.82	30.66
Ca^{++}meq/L	0.12	0.14	0.12	0.12	0.10	0.09
Mg^{++}mg/L	0	0	0	0	0	0
K^{+}meq/L	0.77	0.83	0.74	0.77	0.74	0.74
Na^{+}meq/L	4.5	4.8	4.6	4.7	5.3	5.2
Al^{+++} meq/L	0.032	0.222	0.146	0.174	0.093	0.102
Cl^{-}meq/L	4.83	5.32	4.87	5.09	4.93	5.06
$SO_4^{=}$meq/L	12.08	36.45	11.70	10.66	11.00	35.41
$PO_4^{\equiv}$meq/L	2.30	2.56	2.30	2.24	2.24	2.26
SiO_2mg/L	1.0	3.0	1.4	2.4	1.1	2.2

0 - Not detectable by method used.

changes in pH, titratable acidity, sulfate, and silica concentrations.

Bacterial Activity of the Acid Water Drainages

The activity of the thiobacilli in the drainages of GS-I and GS-VI, VII pools was determined by comparing the uptake of gases (O_2 plus CO_2) by the plug samples. Samples of the drainage water were collected at the same site from which a plug sample was obtained. This was done so that a comparison could be made between biological activity of the drainage channel material and the flowing water. The water was treated in the same manner described for the plug sample.

Figures 9 and 10 show the uptake of gases by the plug samples and water, respectively, obtained at a location 40 feet down the drainage of the GS-VI pool. The field temperature range at this location was from 43° to 53°C, and the manometric study was made at 50°C. The plug samples had a rate of gas uptake of about 50μl of gas/hr. The water sample from the same location showed no gas uptake. The sterile control and endogenous control for the plug sample each had an uptake of gas of 13μl gas/hr. and 12μl gas/hr. respectively. The sterile and endogenous controls for the water samples each had a negative uptake of gas, or actually an evolution of gas. The net rate of gas uptake, the gross uptake of gas by the sample less the uptake of gas by the sterile control, by the plug samples was 37μl of gas/hr.

Other plug and water samples were collected 325 feet down the drainage from the GS-VI pool. The temperature at this location varied

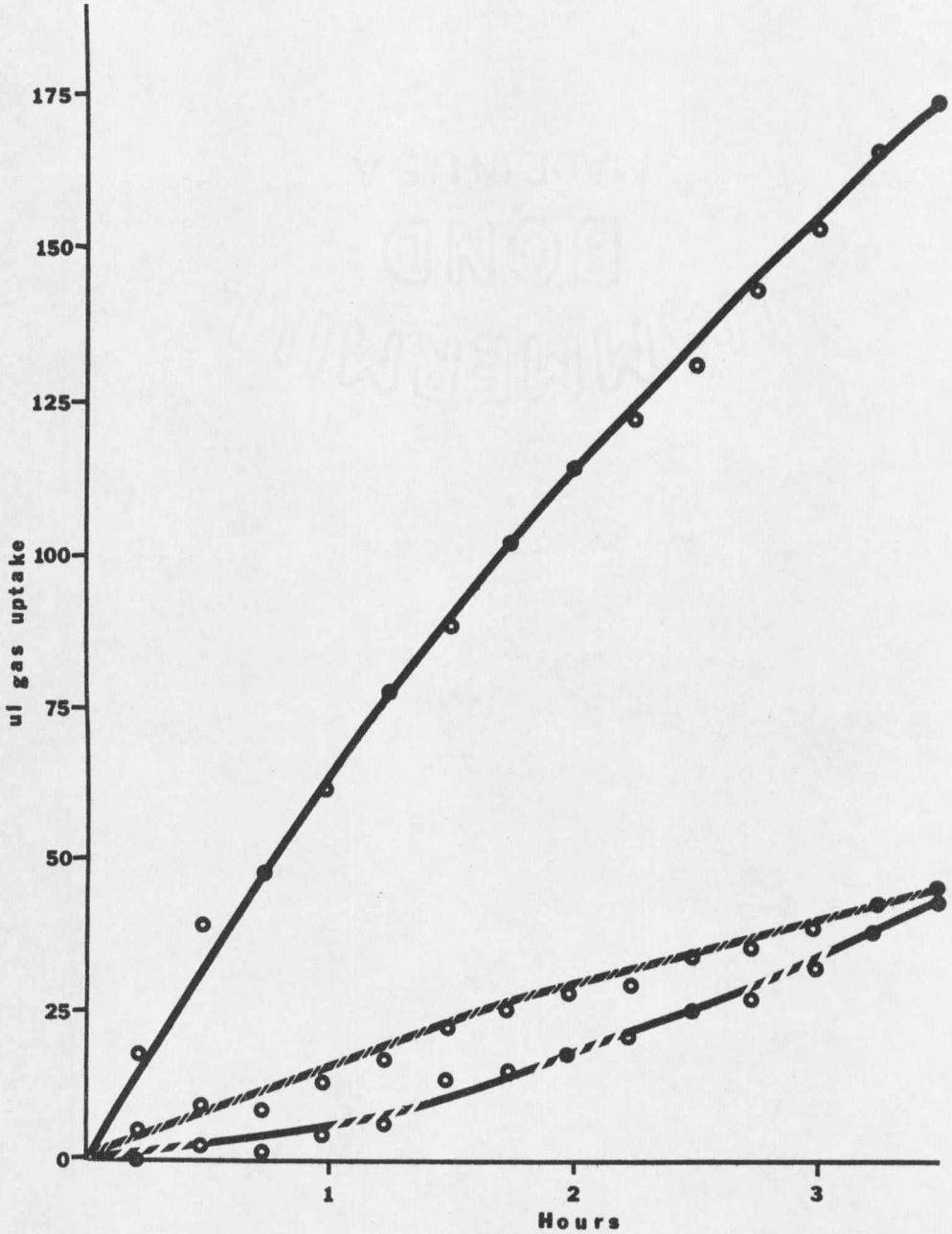


Figure 9. Gas uptake at 50°C by plug sample 40 feet from GS-VI pool. Sample plus thiosulfate ▬▬▬, sample without thiosulfate ▰▰▰▰, and sterile sample plus thiosulfate ▰▰▰.

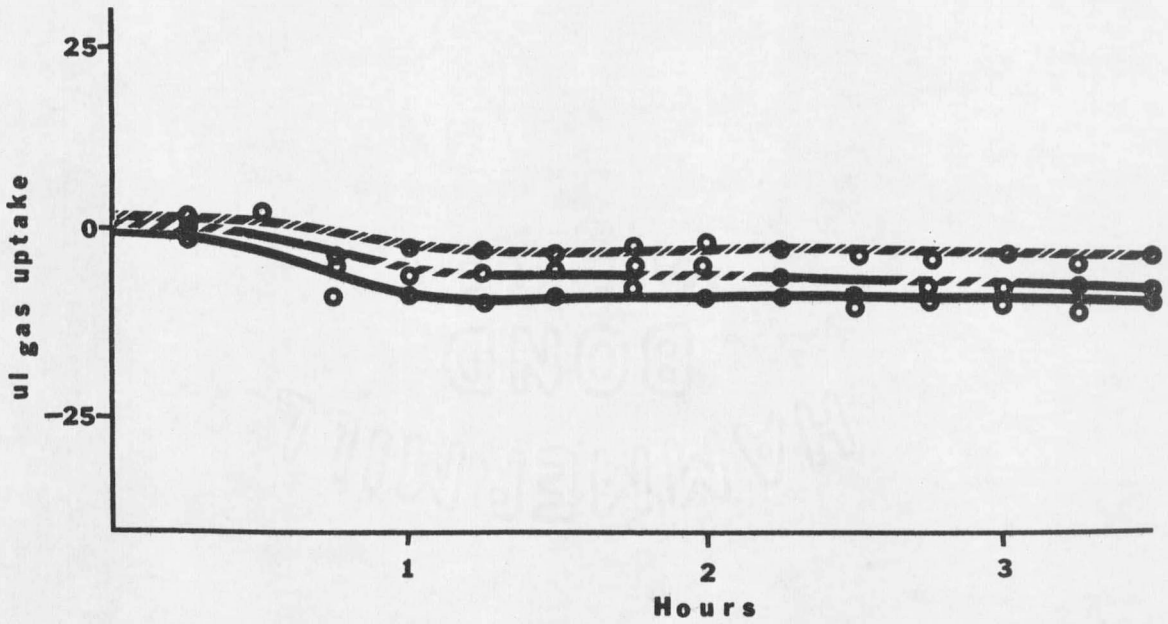


Figure 10. Gas uptake at 50°C by water sample 40 feet from GS-VI pool. Sample plus thiosulfate ▬▬▬, sample without thiosulfate ▬▬▬, and sterile sample plus thiosulfate ▬▬▬.

between 20° and 26.5°C. The gas uptake activity of these samples was determined at 35°C in the laboratory. Figure 11 shows the gas uptake rate by the plug sample. The gas uptake rate of 39μl of gas/hr. for the plug samples was less than that at 50°C. The gas uptake rate by the sterile and endogenous controls was 2μl of gas/hr and 4μl of gas/hr, respectively. These results were also less than those at 50°C. Thus, the net uptake of air by this plug sample was 37μl of gas/hr, which is exactly the same as the uptake at 50°C. The gross uptake rate of gas by the water sample (Figure 12) was only 3μl of gas/hr. Both controls had a gas uptake of 1μl of gas/hr. The endogenous control was only run for 2½ hours when a leak developed in the system allowing water from the bath to enter the flask. The net rate of gas uptake by the water sample was then 2μl of gas/hr.

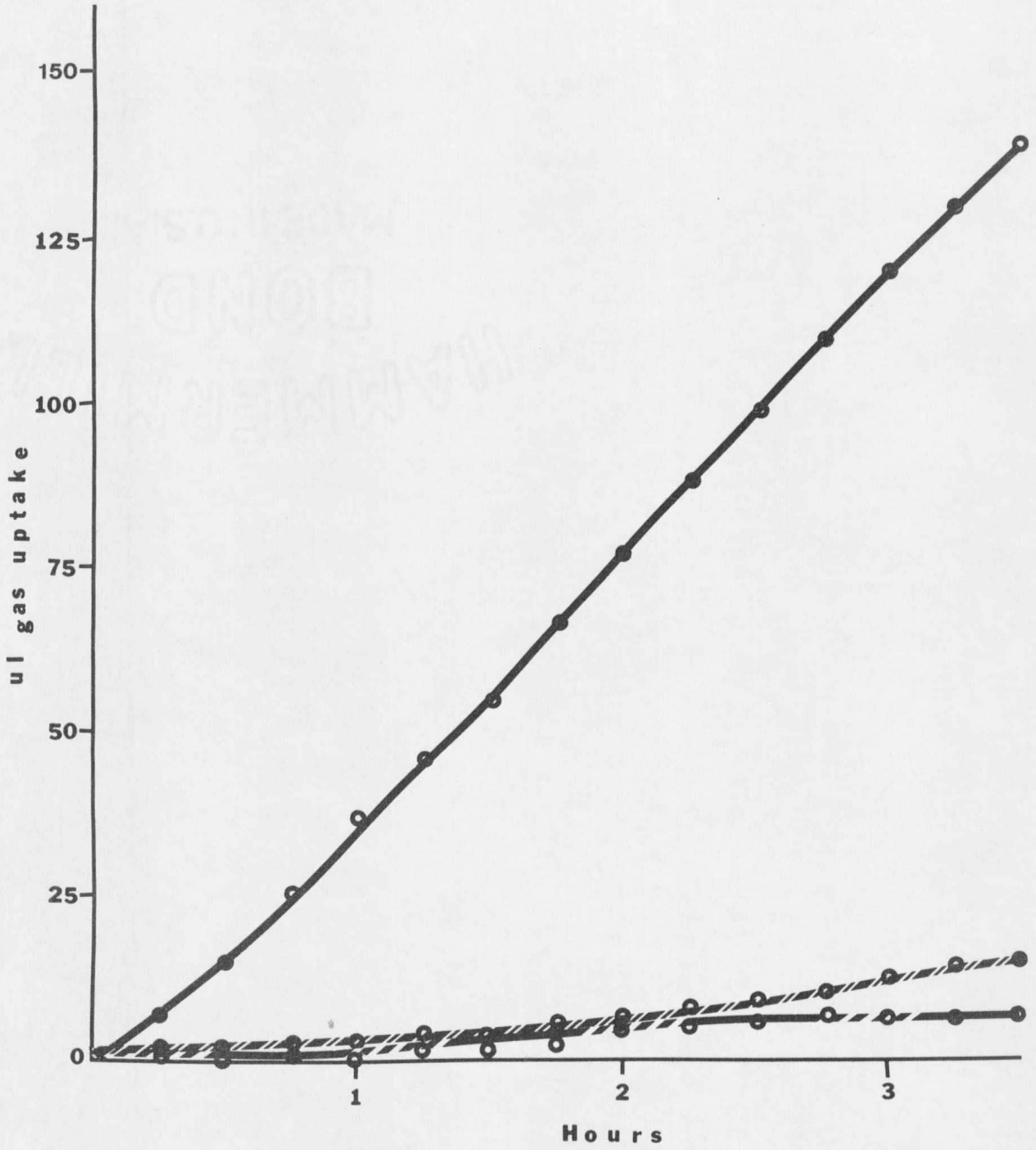


Figure 11. Gas uptake at 35° C by plug sample 325 feet from GS-VI pool. Sample plus thiosulfate ▬▬▬, sample without thiosulfate ▰▰▰, and sterile sample plus thiosulfate ▰▰▰.

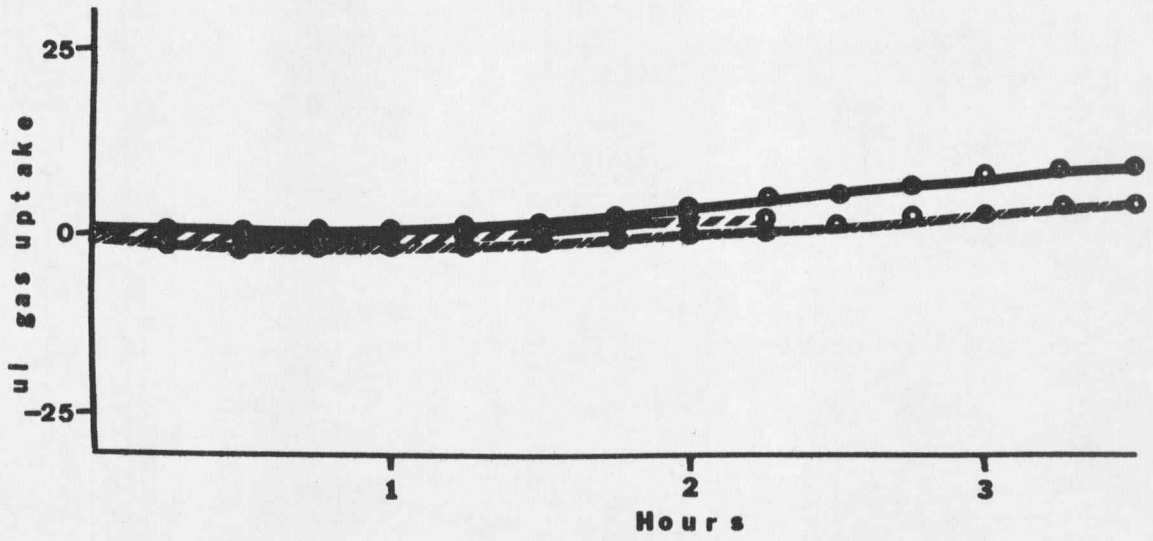


Figure 12. Gas uptake at 35°C by water sample 325 feet from GS-VI pool. Sample plus thiosulfate ▬▬▬, sample without thiosulfate ▬▬▬, and sterile sample plus thiosulfate ▬▬▬.

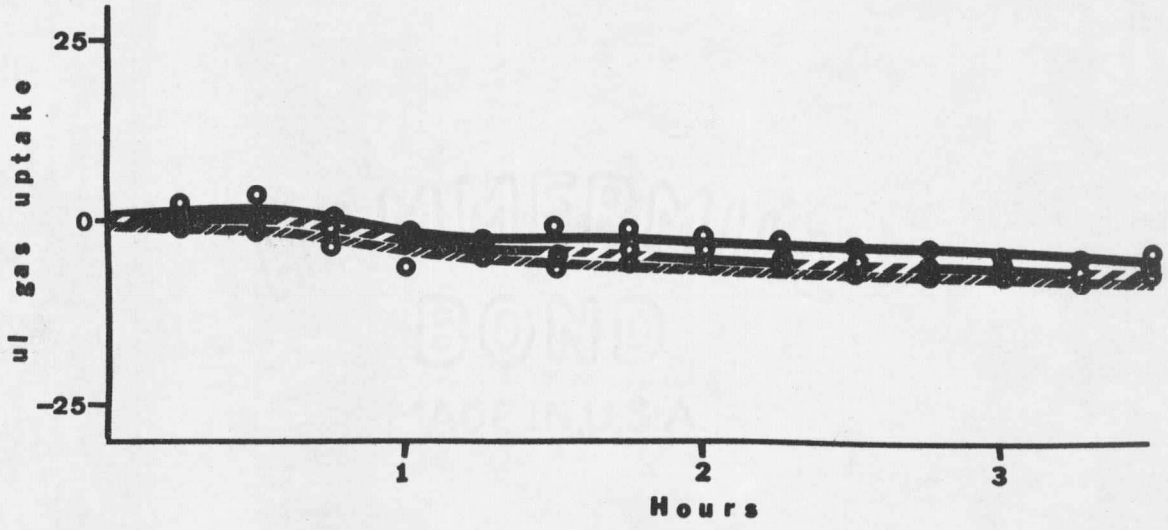


Figure 13. Gas uptake at 50°C by plug sample 40 feet from GS-I pool. Sample plus thiosulfate ▬▬▬, sample without thiosulfate ▬▬▬, and sterile sample plus thiosulfate ▬▬▬.

Plug and water samples were collected at a location 40 feet down the drainage from the GS-I pool. The temperature range at this location was between 50° and 53.5°C. The manometric study was made at 50°C. The plug sample of drainage channel material showed no uptake of gas during a 3½ hour period (Figure 13), in fact, there was a small gas evolution of about 2µl of gas/hr. The same results occurred for the sterile and endogenous controls. The water samples from this location likewise show no gas uptake (Figure 14) but a slow rate, 4µl of gas/hr., of gas evolution was noted. A similar rate of gas uptake rate occurred in the sterile and endogenous controls.

Figures 15 and 16 indicate that some gas uptake had occurred with the plug sample, but not the water sample, collected from the channel 80 feet from the GS-I pool. This location had a temperature range between 34° to 42.5°C and the manometric study for this series of samples was run at 35°C. The plug sample gross rate of uptake was 19µl of gas/hr., the rate for the endogenous control was 7µl of gas/hr., and the rate of the sterile control was about 1µl of gas/hr. The net uptake by the plug samples was 18µl of gas/hr.

The rate of gas uptake by the water samples was negative, indicating gas evolution. After the first 45 minutes the evolution rate for the sample and the sterile and endogenous controls was less than 1µl of gas/hr.

The points which diverge radically from the curve on Figures 15 and 16 for both plug and water samples at 2¼, 2½ and 2 3/4 hours probably

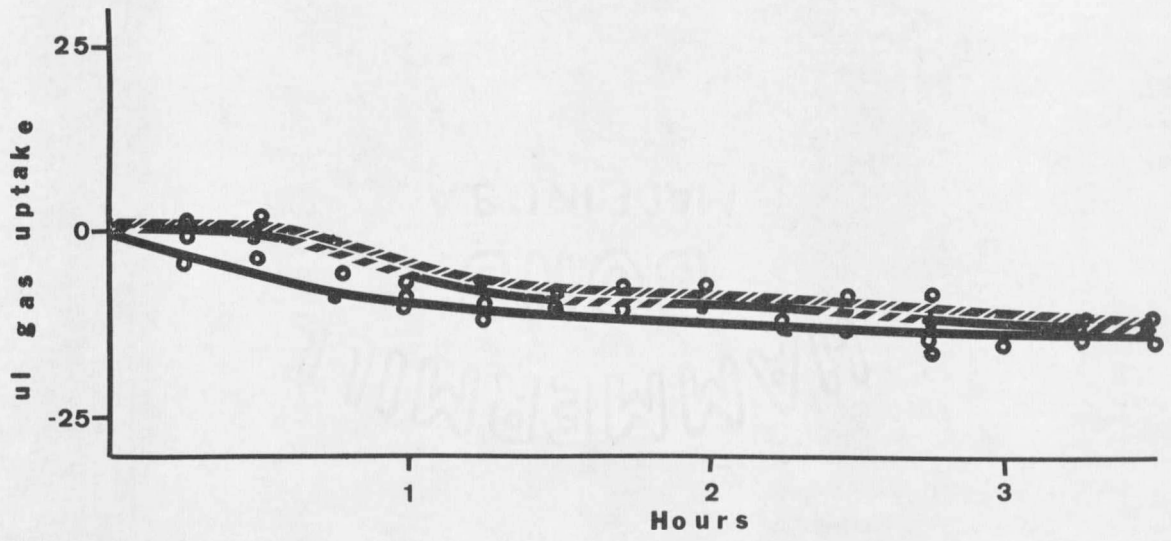


Figure 14. Gas uptake at 50°C by water sample 40 feet from GS-I pool. Sample plus thiosulfate ▬▬, sample without thiosulfate ▬▬, and sterile sample plus thiosulfate ▬▬.

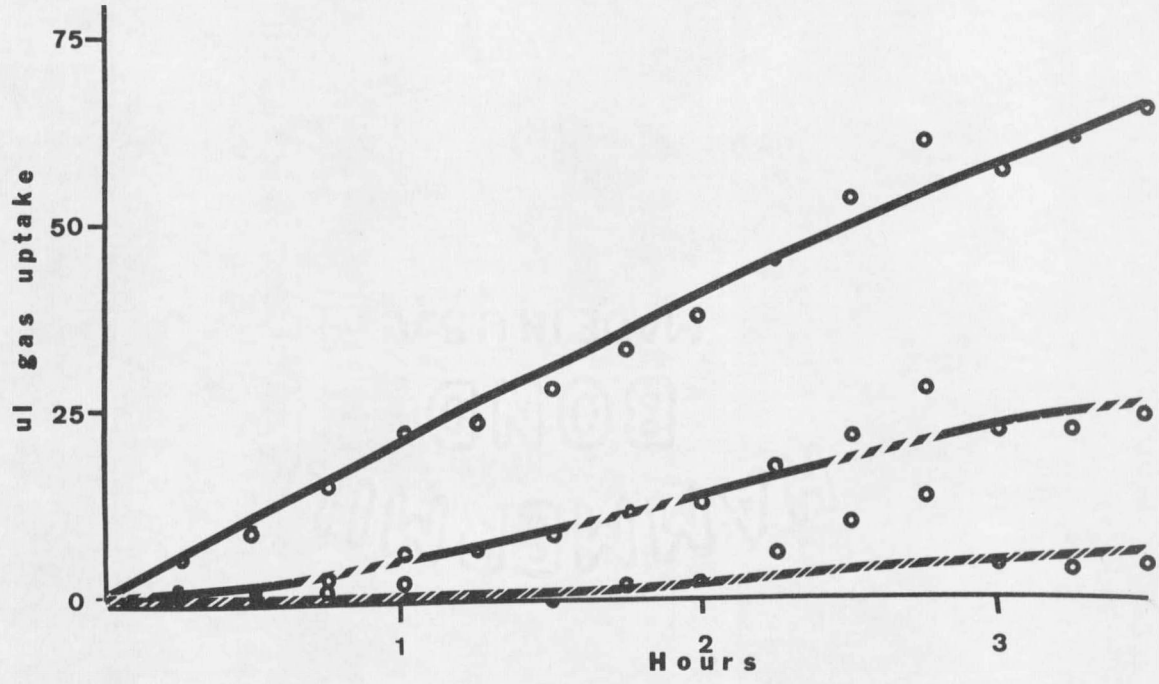


Figure 15. Gas uptake at 35°C by plug sample 80 feet from GS-I pool. Sample plus thiosulfate ▬▬▬, sample without thiosulfate ▬▬▬, and sterile sample plus thiosulfate ▬▬▬.

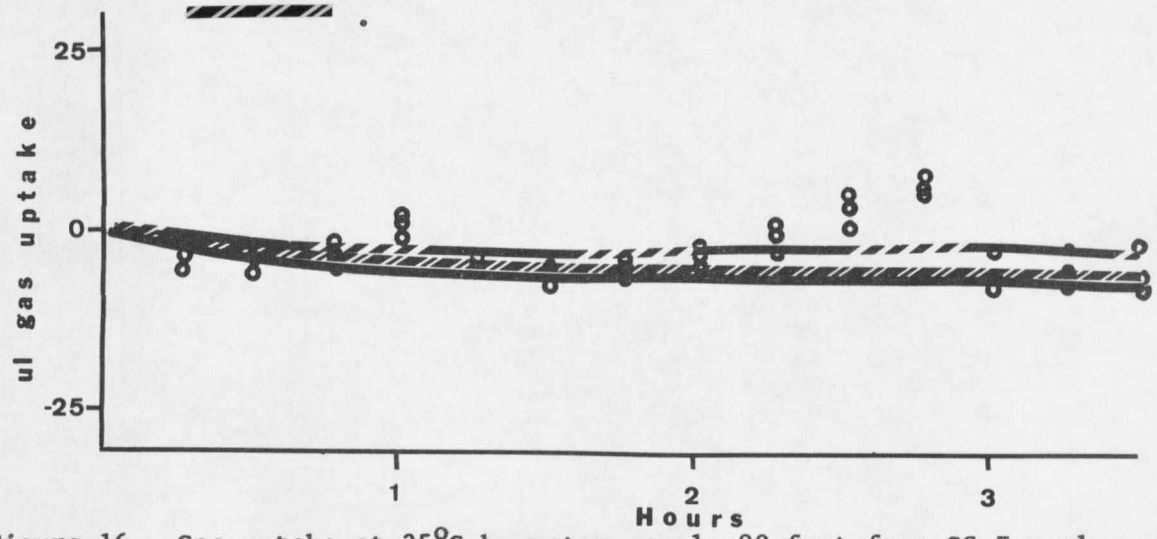


Figure 16. Gas uptake at 35°C by water sample 80 feet from GS-I pool. Sample plus thiosulfate ▬▬▬, sample without thiosulfate ▬▬▬, and sterile sample plus thiosulfate ▬▬▬.

resulted from a temperature induced factor. This phenomenon occurred in 6 manometric reaction vessels simultaneously.

DISCUSSION

The chemical analyses done by Allen and Day (1935) for particular springs in the Geyser Spring group may be compared with some of the results obtained during this study (Table XVIII). These agree well with the exception of the temperature values for station 8 and the fluoride values for station 3. From the table, the temperature values for station 8 in this study show a wide range of fluctuation. The temperature of the spring water was observed to change during the course of this study. Thus, it is believed that the discrepancy is in the nature of the spring itself and is reflected by comparison of the data. It is believed that the fluoride value for station 3, obtained during this study, probably represents the true value. A value of 1.89 ppm, similar to Allen and Day's value, was obtained for a sample from station 3 which was not distilled. The aluminum content of this water interferes with the fluoride test and decreases the value of fluoride if it is not removed. The higher value obtained in this study was for a distilled sample.

The comparative values do indicate that there has been little or no change in the water chemistry of the compared thermal springs during a 30 year period. The small differences in results may only reflect differences in technique.

In addition, the physical characteristics of the Geyser Springs Group, which are completely recognizable from Allen and Day's (1935) description, provide more evidence for the stability of this thermal area.

The thermal waters of the Geyser Springs Group alter the chemical

Table XVIII. Comparison of Chemical Analyses.

Station	Temp C°		$SO_4^=$ meq/L		Cl^- meq/L		F^- mg/L	
	Allen & Day	Present Study	Allen & Day	Present Study	Allen & Day	Present Study	Allen & Day	Present Study
3	75.8	70-73	9.94	8.76	3.63	3.94	1.6	3.84
8	84.2	31-69	3.33	4.28	0.25	0.38		
9	86.6	85-91	3.25	3.99	0.11	0.46		
17	93.5	94	1.98	1.82	18.17	17.10		

composition of the Geyser Creek as it proceeds through the thermal area. The change may be noted by comparing the results in Tables II and III for stations 10, 12, 15, 16, and 1. Station 10 was located prior to the entrance of Geyser Creek into the thermal area. Stations 12, 15, and 16 were sampling stations located on Geyser Creek as it passed through the thermal area. Station 1 was also located on Geyser Creek. It was located at the lower end of the thermal basin and the water sampled here represented the major contribution of this thermal area. The pH of the creek at station 10 was 6.19 but at station 12 the pH was 2.88. The acidification of the stream was the result of the drainage of the GS-VI, VII pools entering the creek. However, at station 15 the pH was 7.52. Geyser Creek was neutralized by the drainages of the pool on the upper bench. The pH of the stream emerging from the thermal area (station 1) was 7.13.

Stations 10 and 1 show very little difference in calcium, magnesium, and aluminum concentrations. The aluminum content had a sharp increase at station 12 because of the influx of the GS-VI, VII water. The aluminum concentration at station 15 was again very small, since the addition of alkaline water, prior to this station, resulted in the loss of aluminum. The potassium, total iron, and phosphate concentrations increase at station 1. The sodium, chloride, sulfate, and silica concentrations increased greatly in the water (station 1) after the creek flowed through the thermal area. At station 10 the following concentrations were found (Table III): sodium, 0.51 meq/L; chloride, 0.04 meq/L; sulfate, 0.39; and silica 97.2 mg/L. At station 1 the following increase in concentrations

were found: sodium, 14.66 meq/L; chloride, 11.43 meq/L; sulfate, 3.08 meq/L; and silica, 234.6 mg/L. Thus, the total contribution of the thermal area had little effect on pH but greatly increased the silica concentration and the above noted ions.

The primary purpose of this investigation was to study changes in water chemistry on a more limited scale than those which occurred along Geyser Creek. The two acid water drainages, GS-I and GS-VI, VII, previously described, were selected. The possible relationships between the chemolithotrophic bacteria and the changes in water chemistry in these drainages was also explored.

Bacteria of the genus Thiobacillus were isolated from both of the acid water drainages of the GS-I and GS-VI, VII pools. There did not appear to be any difference between the strains isolated from these drainages (Table XI). These strains had characteristics which would place them in the T. thiooxidans and T. thioparus groups.

Although temperature gradients existed in both drainages, (Figures 5 and 7) most strains grew at temperatures of 50°C and below with the exception of a few strains which showed little or no growth at 50°C and strain 8-3-65-2(1) which grew between 45° and 60°C. The upper temperature limit for the typical thiobacilli of both acid water drainages appeared to be between 50° and 60°C. Only the unusual sulfur-oxidizing bacterium, strain 8-3-65-2(1), was able to grow at 60°C but it was unable to grow at 65°C in the laboratory. The field temperature of the location at which 8-3-65-2(1) was obtained, was between 66° and 69.5°C. If this organism

was actively metabolizing under field conditions, then the upper limit was between 66° and 69.5°C. This was considerably lower than the 80°C temperature limit which was reported by Emoto (1933a) for his T. thermitanus which was found in waters of pH 2.8. It is very doubtful that Emoto's isolate was actually metabolizing at this temperature. In a later paper Emoto (1933b) found that this organism grew best at 28°C and not at 37°C which was a contradiction of his previous observation. His organism did not survive heating at 80°C in the laboratory. Strain 8-3-65-2(1) of this study remained viable after heating at 80°C for 90 minutes, which may have been the result of the presence of an endospore. The isolation of a spore forming Thiobacillus by the Russians, Egorova and Derygina (1963), further indicates that certain thiobacilli have a heat tolerating ability. In alkaline waters the upper temperature limit of life has been reported by Kempner (1963) to be 73°C. Temple (unpublished data) has found the upper limit to be 78.5°C, and Schwabe (1936) reported 80°C.

However, the acid conditions of the GS-I (pH 2.61 at pool) and GS-VI, VII (pH2.81 at GS-VI pool) waters must certainly add additional physical stress to biological processes. It is believed that the acid conditions lower the ability of organisms in these waters to tolerate any higher temperatures than noted in this study.

It is not possible to classify strain 8-3-65-2(1) as a known bacterium. On a physiological basis it could be classified as a Thiobacillus. However, on a morphological basis it is not a Thiobacillus as it is spherical.

The iron-oxidizing bacteria (Table XII) isolated from the drainage of

GS-VI and VII thermal springs, were called Thiobacillus ferrooxidans. Although other names have been given to iron-oxidizing chemosynthetic bacteria, e. g., Ferrobacillus ferrooxidans and Ferrobacillus sulfooxidans, the present trend is to "lump" this group of bacteria under the name, T. ferrooxidans. This bacterium was originally isolated from acid mine water by Temple and Colmer (1951) and has since been reported in acid mine water studies. This study appears to be the first time that this bacterium has been found in acid water of thermal springs. The "iron" bacteria that have been found in the studies of thermal spring waters have been of the Leptothrix or Gallionella type, and they have never been found in acid conditions.

Changes occurred in the chemical composition of the thermal spring water as it proceeded down the drainages of GS-VI, VII (Table V) and GS-I (Table IV). The role of the chemosynthetic bacteria of these drainages in causing these changes was investigated.

The total change (Table V) which occurred in the GS-VI, VII drainage was small over the first 100 feet from the source. This was probably due to the similarity of the GS-VI and GS-VII pools, and the latter pool masked any changes which might have occurred during the first 100 feet. From GS-VII to station 7, 200 feet down the drainage from GS-VII, there was a marked increase in the concentration of sulfate, aluminum, sodium, and titratable acidity. Table XIV shows the effect of chemosynthetic bacteria which were grown at 50°C in the presence of the drainage channel material. The sulfur-oxidizing bacteria also increased the sulfate,

aluminum and silica concentrations and the titratable acidity. There was no increase in the sodium concentration. The uninoculated controls at 50°C also showed the same increases but to a lesser extent. The sulfur-oxidizing bacteria which were grown at room temperature (Table XV) also increased the concentrations of sulfate, aluminum, and silica and the titratable acidity, but did not increase the sodium concentration. However at room temperature to 37°C the uninoculated control did not change the initial concentrations after the incubation period. This indicated that at temperatures to at least 37°C biological transformations are of major importance, but at temperatures of 50°C, and higher biological processes only supplement the transformations cuased by the physical conditions at 50°C.

The notable increase of aluminum was obviously a solubilization phenomenon. The aluminum became more soluble with the increased production of sulfuric acid by the sulfur-oxidizing chemosynthetic bacteria. This was confirmed (Table XV) by the addition of sulfuric acid in the amount which was equivalent to that produced by the bacteria. Table XV shows that the aluminum concentration increased by 0.746 meq/L in the flask with the sulfur-oxidizing bacteria, and it increased by 0.784 meq/L in the chemical control flask with the sulfuric acid supplement. The major increase of aluminum occurred between GS-VII and station 7. Since this zone had an average temperature beginning at 38.5° and decreasing to 26°C (Figure 7), the biological rather than temperature induced increase was active. However, there may have been chemical reactions involved in

the increase of acidity in this zone which have been overlooked.

The sulfur-oxidizing chemosynthetic bacteria could probably be considered to bring about the syngenesis of the mineral bauxite on a small scale. The acidity of the GS-VI, VII drainage was probably increased, for the most part, by the bacterial oxidations of sulfur compounds. This acidity solubilized the aluminum of the kaolinite in the drainage bed. The aluminum entered Geyser Creek, which was acidified by the drainage of GS-VI, VII, and remained in solution until the creek was neutralized by the drainages of the alkaline springs on the upper bench. At the point of neutralization of Geyser Creek, which occurred near station 19, a zone of yellow-tan coloration in the creek bottom appeared. The neutralization of the water caused the formation of bauxite (Theobald, et. al, 1963). The sequence of genesis of bauxite had been described by Ansheles (in Theobald, et. al., 1963): (1) oxidation of pyrite releasing sulfuric acid, (2) decomposition of clays by the acid, releasing aluminum, and (3) precipitation of the aluminum by hydrolysis when the acid, aluminum bearing waters are neutralized. In the drainage of GS-VI, VII the chemosynthetic sulfur-oxidizing bacteria replaced the first step by producing acid during the oxidation of sulfur compounds.

The sulfate, which increased 1.08 meq/L between GS-VII and station 7, may also have been the result of the oxidation of reduced sulfur compounds by the bacteria. The sulfate concentrations increased in the control flasks for the mineral solubilization experiment of GS-VI, VII (Table XIV and XV); the control at room temperature increased only

slightly. A large increase in the inoculated flasks was because of the activity of the sulfur-oxidizing bacteria. However, the reaction vessels did not truly represent the field conditions since the sulfur, added for an energy source, served as the source of sulfate. The presence of reduced sulfur compounds, particularly sulfide, in the GS-VI, VII drainage was presumably the source of sulfate.

The bacterial acidification which occurred in the reaction vessels in the laboratory can account for the increase of all ions except sodium. Sodium did not increase in the laboratory cultures as it did in the water of the GS-VI, VII drainage. This suggests that the sodium may be added by ground water seeping into the drainage, and is not leached from the drainage channel material.

The role of the iron-oxidizing bacteria in the thermal drainage (Tables XIV and XV) of GS-VI, VII was difficult to determine. Growth occurred both at 50°C and at room temperature, however, chemical analysis of the growth medium at both temperatures indicated that changes were small between initial and final samples. The presence of the iron itself appeared to alter the water constituents when compared to the constituents in which sulfur served as the bacterial energy source. (Tables XIV and XV). The iron increased the initial levels of titratable acidity, calcium and aluminum. Since a high concentration of iron did not occur in the field, no attempt will be made to compare these results obtained with the iron-oxidizing system to the field conditions. However, the role of the iron-oxidizing bacteria was probably similar to the role of the sulfur-

oxidizing bacteria since one of the products of iron oxidation is sulfuric acid.

An average of the flow volume values (Table VI), obtained for the GS-VI, VII drainage from July 24, 1965 to November 11, 1965, was 107 ml/sec. This was a flow of 9, 245 L of water from the GS-VI, VII drainage each day during this period. If the increase of aluminum and sulfate, which occurred between GS-VII and station 7 (Table V), is assumed to be a result of the activity of the chemosynthetic bacterial population, then this group of bacteria contributed 65.9 g of aluminum and 479.2 g of sulfate per day to Geyser Creek during this period.

Very little change occurred in the water chemistry in the 80 feet of the GS-I drainage between the pool and station 11 (Table IV). There was a marked increase in titratable acidity, small increases in the concentrations of potassium, sodium, aluminum, and silica, and a drop in pH.

Growth of sulfur-oxidizing chemosynthetic bacteria in the presence of GS-I drainage bed material at 50°C (Table XVI) and at 37°C (Table XVII) caused a large increase of sulfate, an increase in titratable acidity, and a drop in pH. These results were the result of the oxidation of elemental sulfur by the bacteria. Very small increases in the potassium, aluminum, and silica concentrations occurred in those flasks with either bacteria or the acid supplement.

It is interesting that the acidity produced in the GS-I reaction vessels was greater than that produced in the GS-VI, VII reaction vessels. The over-all changes in the constituents analyzed were much less in the

GS-I vessels than in the GS-VI, VII vessels. This may be due to the fact that the material in the GS-I drainage contained no unaltered feldspar, or kaolinite, but the GS-VI, VII drainage contained both unaltered feldspar and kaolinite. This suggests that possible alteration has already occurred in the GS-I drainage and that the increase of acidity has only a small effect in bringing about the solubilization of the constituents of the drainage bed material. In contrast, the GS-VI, VII drainage alteration may still be proceeding.

The activity of the chemosynthetic sulfur-oxidizing bacteria of the GS-VI, VII drainage was compared with that of the GS-I drainage. The sulfur-oxidizing bacterial populations were selected for this comparison, since their presence was common to both drainages. The manometric technique was employed to compare the activity of the populations. Thiosulfate was used as an energy source because it is a common intermediate in sulfur oxidation.

When the activities which occurred in the drainage channel material of GS-VI, VII (Figures 9 and 11) were compared with those which occurred in the drainage water at corresponding locations (Figures 10 and 12) it was evident that the entire activity, as determined by this technique, was associated with the drainage bed material. The sulfur-oxidizing bacteria were probably being washed from the channel and carried by the water, but the dilution of bacteria in the water was so great that their activity was not significant.

The net rate of thiosulfate oxidation by samples taken at 40 feet

and 325 feet from the GS-VI pool was exactly the same. This suggests that the sulfur-oxidizing bacterial population appeared to remain nearly constant for the entire length of the drainage. The wide temperature range for growth of the sulfur-oxidizing bacteria would seem to substantiate this possibility.

However, an entirely different situation was present in the GS-I drainage. The sample taken at 40 feet from the pool, which was at a temperature nearly the same as the 40 foot sample from GS-VI, VII drainage, did not have any detectable activity. A sample at 80 feet from the GS-I pool had a net uptake of 184l of gas/hr, which was considerably less than the 374l of gas/hr that was found at 325 feet in the GS-VI, VII drainage.

There are two possible explanations for the differences of activity which exist in the two drainages. One is the difference in composition of the plug samples. The material of the GS-VI, VII drainage was a finely divided mud, almost clay like, and the material of the GS-I drainage was coarse with fine gravel and pieces of sinter. The surface area of the particles from the GS-I drainage was much less than that of the particles from the GS-VI, VII drainage. Fewer bacteria would be associated with the GS-I sample.

Secondly, the chemical difference between the two drainages may be important. Although the analyses (Table III) of these areas showed that quite different concentrations of components were found in the two drainages, there was no obvious reason for the difference in activity. However,

there may have been some toxic element, or combination of elements, not included in the analyses, which was responsible for the marked decrease in activity.

This information indicates the importance of the consideration of the water chemistry in studies of activity in thermal spring environments. Brock and Brock (1966) stated that temperature was the only variable of thermal springs. Tables IV and V indicated that changes in the concentrations of inorganic constituents, pH and acidity also occurred as the water proceeded down a thermal spring drainage. Changes such as these, besides temperature, must be considered for ecological study of thermal springs.

SUMMARY

The thermal waters of the Geyser Springs Group in Yellowstone National Park are both alkaline and acid, thus, this area is a "mixed" group of thermal springs. The thermal water from the springs drains into Geyser Creek, which flows through the area. The thermal waters are important, not only in increasing the temperature of the creek, but also in increasing the concentrations of sodium, chloride, sulfate, and silica.

Two acid thermal water drainages and their respective springs were selected for extensive study of their chemical properties, physical properties, chemosynthetic bacteria populations, and the role that these bacteria play in influencing the water chemistry.

The two acid water thermal drainages which were selected for study, and designated GS-I and GS-VI, VII, were quite different in chemical composition. The pool at the source of the GS-I drainage was more acid and had a higher concentration of salts than the pool at the source of the GS-VI, VII drainage.

The most obvious changes in the GS-VI, VII drainage were the increase in the concentrations of sulfate and aluminum, increase of the total titratable acidity, and the decrease of pH.

There was a difference in the chemosynthetic autotrophic bacterial populations found in the two drainages. Both sulfur and iron-oxidizing bacteria were found in the GS-VI, VII drainage, but only sulfur-oxidizing bacteria were found in the GS-I drainage. There were two groups of sulfur-oxidizing bacteria, both of which were common to the two drainages. One

group was similar to Thiobacillus thiooxidans, and the second group was similar to Thiobacillus thioparus. Both groups compared well with the description given in Bergey's Manual of Determinative Bacteriology (Breed, Murray, and Smith, 1957). The iron-oxidizing bacteria were considered as strains of Thiobacillus ferrooxidans.

An unusual chemosynthetic autotrophic sulfur-oxidizing bacterium was isolated from the GS-I thermal spring drainage. This bacterium, in contrast to the typical rod shaped thiobacilli, was spherical. It was the only sulfur-oxidizing bacterium isolated which would grow at 60°C. The cells possessed an unusual tolerance to heat and remained viable following 90 minutes of heating at 80°C. Each cell had a refractile body which took a spore stain. This, coupled with its resistance to heating, indicated the ability of these cells to form endospores. The taxonomic position of this bacterium is unknown.

The upper temperature limit for life in these acid water drainages appeared to be between 66° and 69.5°C in the field, and 60°C for growth in the laboratory. Most of the typical thiobacilli, obtained from these drainages, grew well at 50°C but not 60°C, and some strains grew poorly or not at all at 50°C.

The chemosynthetic autotrophic bacteria appeared to be active in bringing about certain changes in the water chemistry of hot spring drainages. The most notable seemed to be the increase in concentrations of salts as a result of the solubilization of minerals by the direct action of the acid which was produced when bacteria oxidize iron and

sulfur compounds. However, sulfate concentration was also increased by the oxidation of reduced sulfur compounds.

The rate of activity of the chemosynthetic sulfur-oxidizing bacteria also appeared to be different in the two drainages. The uptake of gases by sulfur-oxidizing bacteria in drainage channel samples 40 feet and 325 feet below GS-VI had a rate of 37 μl of gas/hr at 50° and 35°C respectively. The GS-I drainage sulfur-oxidizing bacteria had a very different pattern of activity. There was no detectable activity in a sample from the drainage channel material at 40 feet from the pool at 50°C. A drainage sample 80 feet from GS-I pool had an active uptake of gas of 18 μl of gas/hr.

Although the two acid water thermal drainages investigated were in the same thermal area, each showed individual characteristics regarding its chemical composition and the presence and activity of bacterial populations.

APPENDIX

Figure 1. The author using an extension pole with bucket to collect a water sample from station 2.

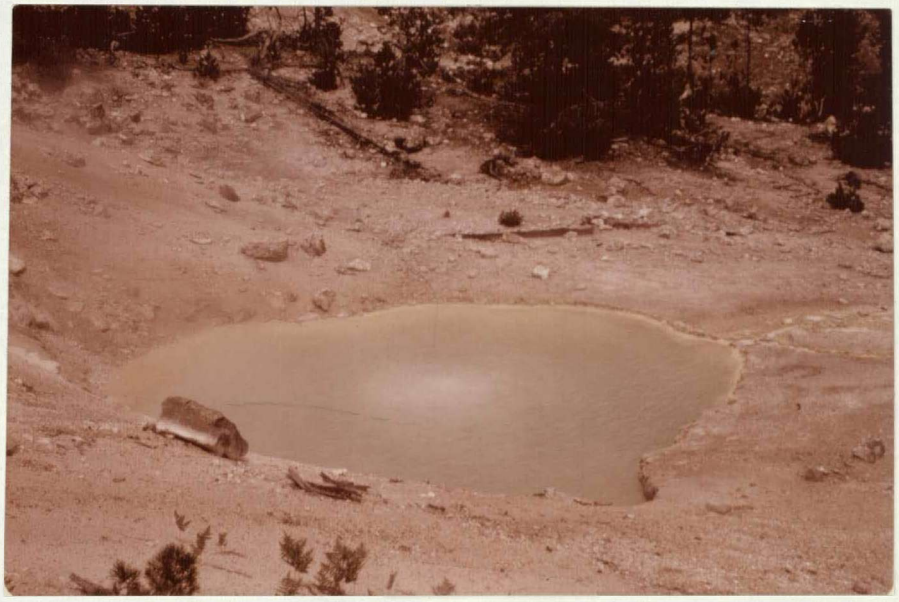


Figure 2. Station 3, the GS-I pool.

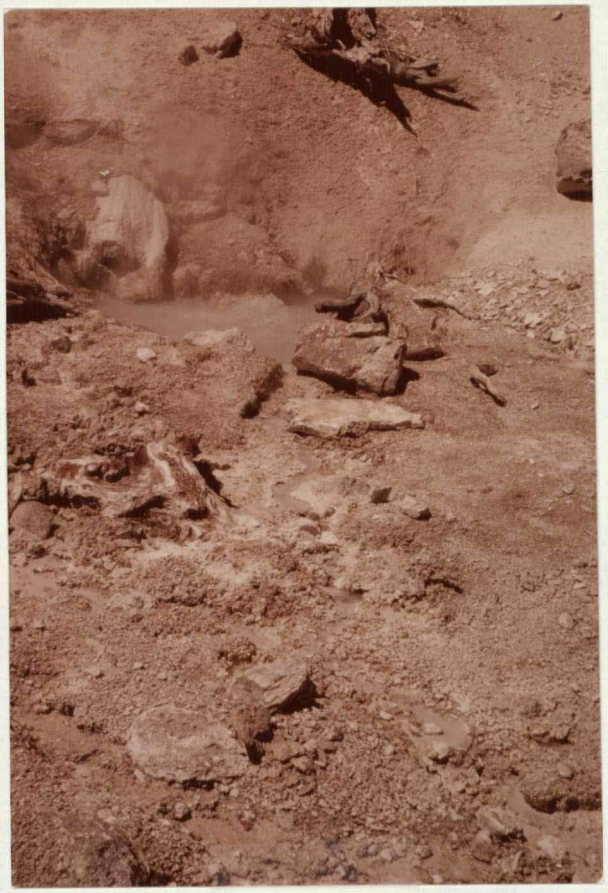


Figure 3. Station 4, the GS-III pool and drainage.

Figure 4. Station 5, the GS-II pool with small sinter cone.

Figure 5. Station 6, the Bone Pool.

Figure 6. The drainage of the GS-VI and GS-VII pools overlooking station 7. The bottles were used for collection of water samples.

Figure 7. Station 8, the GS-VII pool.

Figure 8. Station 9, the GS-VI pool.

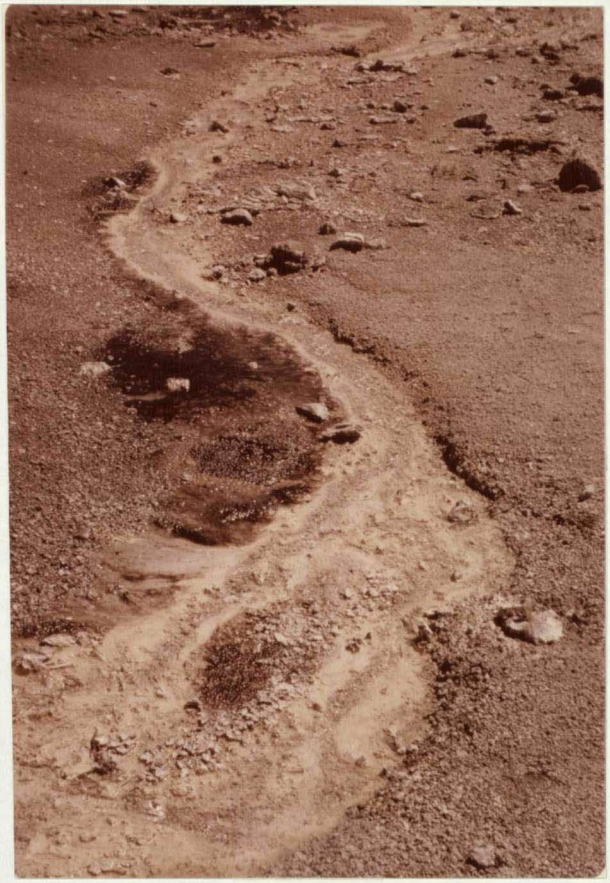


Figure 9. The GS-I pool drainage near station 11.

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