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GROWTH AND PHYSIOLOGY OF A SOIL ORGANISM
WHICH IS RESISTANT TO THE TOXIC
EFFECTS OF SODIUM SELENITE

By

John C. Mickelson

Submitted to the Graduate Faculty
of
South Dakota State College
of Agricultural and Mechanic Arts
in Partial Fulfillment of the Requirement for
the Degree of Master of Science

March, 1957

119597

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GROWTH AND PHYSIOLOGY OF A SOIL ORGANISM
WHICH IS RESISTANT TO THE TOXIC
EFFECTS OF SODIUM SELENITE

This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser *J*

Head of the Major Department *J*

ACKNOWLEDGMENT

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J. C. M.

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INTRODUCTION

The occurrence of selenium in soils of the United States and its connection with a disease of livestock originally called alkali disease, now called selenium poisoning, is well established (11). The states of North Dakota and South Dakota have both reported areas of land on which the disease occurs (11, 16).

In 1954, soil samples (from areas in South Dakota in which there is selenium poisoning) were brought to the South Dakota State College Experiment Station for analysis. From one of these samples, a bacterium was found which was noted as being resistant to the toxicity of selenium and also as being capable of reducing sodium selenite to the elemental form. In the process of this reduction, a foul odor described as resembling that of garlic or of rotten radishes was noted and at that time attributed to either hydrogen selenide or dimethyl selenide, both compounds having odors which resemble that of garlic.

Little work was done in attempting to identify this organism except to attempt growth on silica gel, eosin methylene blue agar and soil patties.

The eosin methylene blue agar provided a suitable nutrient base, but the organism being incapable of fermenting lactose, gave none of the generally observed reactions on this medium. A very limited toxicity study using this medium was conducted using various concentrations of sele-

nium in the form of sodium selenite. Growth was observed on all plates, however the highest concentration of selenium used was only 30 parts per million.

The silica gel medium proved to be of no value as growth was very slight. Reasons for this will be brought out later in this paper.

The soil patties gave poor growth, but further studies were carried on using culture media containing soil inasmuch as the organism was isolated from the soil. The results obtained from these studies were of limited value because of the limited growth obtained.

LITERATURE REVIEW

During the past few years, metabolic studies of the effect of selenium on animals, yeasts, bacteria and algae have been on the increase. These life forms are all poisoned to some extent by selenium.

The idea that some forms of life are resistant to the effects of selenium is not a new one. One of the enrichment media used in the isolation of Salmonella typhosa contains selenium (selenite broth). The selenium is in the form of sodium selenite at a concentration of 0.1 per cent. At this concentration, S. typhosa is unaffected by the selenite while Escherichia coli is inhibited for the first 8 to 12 hours (8). This procedure was developed by Guth in 1916 using an agar medium (5). Leifson, in 1936, extended this work and found the broth enrichment medium more suitable (8).

Studies on yeast cells, Saccharomyces cerevisiae, have shown a markedly greater poisoning effect. The selenate ion would inhibit the growth of these cells completely at a concentration of 60 parts per million while the selenite ion was slightly less toxic (1). The selenite ion was reduced to elemental selenium in these experiments, and was thought to be less toxic for that reason. It was also shown by these investigators that arsenate and arsenite neither enhanced nor depressed the growth of the yeast cells.

Studies on laboratory animals have shown that arsenic will effect a partial reversal of selenium toxicity (13,12). It has also been shown that methionine or sulfate will effect a partial reversal (4), while Klug and co-workers found methionine ineffective in protecting rats against selenium toxicity (7).

Studies on the alga Chlorella vulgaris showed that selenate decreased both the growth rate and final population density of cultures (14). Relatively low concentrations of the selenate could completely inhibit this organism. Sulfate partially reversed this toxicity and when it was added to the medium before inoculation, the selenium was unable to penetrate the cells. Poisoning was prevented by sulfate when present in a particular definite ratio to the selenate.

In later work, a comparison was made of growth with L-methionine and with seleno-methionine (15). The toxicity

shown by this selenium compound was competitively reversible, it being thought that L-methionine competed for the site of entrance into the cell. Growth with this selenium compound was very characteristic. The cells became large and distended and failed to divide. This behavior was compared to that of growth in the presence of an antibiotic capable of interfering with cell division.

EXPERIMENTAL PROCEDURE

Part I - Stock Cultures

The organism was isolated from a selenium soil. A culture was obtained from the South Dakota State College Experiment Station on an eosin methylene blue slant. Several nutrient agar plates were inoculated from this slant and allowed to incubate at 37 degrees Centigrade for 48 hours. Following this, several colonies were examined microscopically and a small gram negative, non-spore forming rod was found in all cases. Growth was quite slow and sparse on all plates, indicating that an optimum temperature range should be found. Transfers were made to one nutrient agar slant and five nutrient agar plates. All inocula were obtained from one single well-isolated colony. The agar slant was allowed to grow out at room temperature and the five plates were incubated at 15 degrees Centigrade, room temperature (approximately 22 degrees Centigrade), 32 degrees Centigrade, 37 degrees Centigrade and 45 degrees Centigrade. There was no growth at 45 degrees Centigrade, very sparse

growth at 37 degrees Centigrade, good growth at 32 degrees Centigrade and at room temperature, and sparse growth at 15 degrees Centigrade. The plates which grew sparsely were allowed to grow out at room temperature. The 45 degrees Centigrade plate was sterile, the 37 degrees Centigrade plate finally developed few colonies and the 15 degrees Centigrade plate grew out well. It is obvious this organism is very sensitive to high temperatures.

Ten nutrient agar slants were inoculated and allowed to grow out fully at room temperature. They were sealed with paraffin to prevent contamination and dehydration and used for stock cultures for all experiments.

Part II - Characterization of the Organism

The morphology of the bacillus was studied under various conditions. For morphological study, nutrient agar strokes and nutrient broth were used, the cultures being incubated at room temperature. The cells were found to be short rods, between 0.5 micron and 1.0 micron in length, gram negative, nonflagellated and nonmotile. There is no capsule. They grow singly rather than in pairs or chains and do not form endospores (10).

Growth on an agar stroke is abundant, glistening and filiform. Colonies are circular to slightly irregular with smooth surfaces and entire edges.

Growth in gelatine was at the surface only, suggesting a preference for aerobic growth. Liquefaction was stratiform. A similar growth was shown in nutrient broth with formation of a ring at the surface and a heavy sediment. In 24 hours at room temperature, litmus milk had increased in alkalinity. After 72 hours, the litmus had been reduced and the casein hydrolyzed. Cultures in broth containing KNO_3 were consistently positive to tests for the nitrite ion (10).

The following classification was given to the organism in accordance with Bergey's Manual of Determinative Bacteriology (2).

Class: Schizomycetes Nageli

Order I: Eubacteriales Buchanan

Sub-Order I: Eubacteriineae Breed, Murray
and Hitchens

Family IX: Achromobacteriaceae Breed

Genus II: Achromobacter Bergey et al.

There is no species already described which resembles this organism completely.

The following biochemical characteristics were determined:

Good growth in 1 per cent peptone

Citrate utilized as sole carbon source

H_2S not produced

Indole not produced

Acetyl-methyl-carbinol not produced

Methyl red negative

Nitrites produced from nitrates

Strictly aerobic in common culture media

Starch not hydrolyzed

Stratiform liquefaction of gelatine

Acid but no gas produced from glucose and maltose but lactose, sucrose, mannitol, dulcitol, xylose, galactose, salicin, glycerol, levulose, ribose, cellobiose, arabinose, inulin and ethanol were not fermented.

The media containing carbohydrates which were not utilized turned strongly basic. Fermentation of glucose was not established until pH curves were run because of the large amounts of amino acids freed in the breakdown of peptone. Maltose was fermented more rapidly and acidity was apparent when using phenol red as an indicator.

An attempt was made to grow the organism in a defined medium similar to Koser's citrate medium (in which the organism is capable of growing), but using other carbon sources. Three carbohydrates were decided upon, maltose, dextrose and lactose, two of which the organism can use and one of which it can not. Escherichia coli was used for a comparison since it is capable of growing in this type of medium and can use all three carbohydrates. The media were as follows:

KH_2PO_4 - - - - - 0.15 gram

Na_2HPO_4 - - - - - 1.65 grams
 NH_4Cl - - - - - 0.20 gram
 Carbohydrate - - - - 1.00 gram
 Distilled water - - - 100 milliliters
 Final pH - - - - - 7.6

The carbohydrates were added individually to 100 ml. amounts of the above medium in 250 ml. Ehrlenmeyer flasks and sterilized by filtering through Seitz filters. These were then left in the sterile side arm flasks which were capped with sterile aluminum foil. They were inoculated with 1.0 ml. each of a suspension of bacteria obtained by washing the growth from a 48 hour agar slant with 10 ml. of sterile distilled water. A flask of nutrient broth was similarly inoculated and was observed for growth.

Results:

The organism was not capable of growth in a defined medium using an inorganic nitrogen source and the above carbohydrates as carbon sources. Escherichia coli grew well in all flasks. It was thought that a small amount of peptone added to the medium might give the organisms a start. The same procedure was repeated with the addition of .01 per cent peptone, resulting in a very faint turbidity. A comparison to Koser's citrate broth indicated the inability to use these carbohydrates without an organic nitrogen source. Again in this trial, E. coli grew abundantly.

Part III - Carbohydrate Studies

Growth of this organism in carbohydrate media containing phenol red as a pH indicator resembles that of the micrococci. In the presence of a carbohydrate which the organism can not use, the pH of the medium increases due to released amino acids and ammonia. If the carbohydrate is used, the protein is spared and the metabolic by-products decrease the pH. These by-products are generally CO₂ and a variety of organic acids. The micrococci do not break the carbohydrates down to detectable CO₂ but do produce small quantities of organic acids from them. This sparing action by the carbohydrates does not seem to work as well with this organism as with the micrococci. The organism apparently continues to use the protein portion of the medium while the carbohydrate is being used. The phenol red indicator has a pH range of pH 6.8 to pH 8.5; is red in the basic range and yellow in the acid range. The micrococci will show a definite yellow color if the carbohydrate is used, but this organism first turns the indicator a darker red showing that the peptone is being used and then the medium reverts to its original color. Thus it would seem that phenol red broth is of little value in studying sugar utilization by this organism (10). However, if the pH is determined at definite intervals it can be shown that this organism is capable of using some carbohydrates as a supplementary energy source

in the presence of peptone.

In order to determine the pH at definite intervals, the various carbohydrate media were prepared in 250 ml. Erlenmeyer flasks. Nutrient broth was the base in all cases and the carbohydrate was added in a concentration of 1.0 per cent. A volume of 100 ml. was used so that repeated samples could be taken. The pH was determined in all cases before inoculation and then at 8-hour intervals for 64 hours.

The carbohydrates used in the experiment were: maltose, dextrose, mannitol, xylose, sucrose, lactose and galactose. For a control, an eighth flask was used containing no carbohydrate. The media were sterilized by autoclaving the carbohydrates separately at 15# pressure (121 degrees Centigrade) for 15 minutes and adding sterile nutrient broth just before inoculation. The pH was determined using a Beckman line type pH meter.

Figures I and II show the results of these readings. The control showed the expected rise in pH, increasing from pH 6.5 to pH 8.5. It appeared that mannitol was not used as the pH change in its presence was similar to that in the control. There was a short lag in the maltose flask followed by a rapid drop in pH. There was some utilization of peptone following this, causing a subsequent rise in pH. Dextrose was used at a slower rate. After the initial rise in pH, the organism used just enough of the carbohydrate to cause a

gradual decrease in pH. It is very unlikely that any of the other carbohydrates were used even in small amounts.

It is a unique thing that maltose should be used at a more rapid rate than the dextrose. It might be explained by phosphorylation when the maltose molecule is hydrolyzed. This would give one molecule of glucose-phosphate and one molecule of glucose from one molecule of maltose. This could account for a more rapid intake of at least half the maltose molecule. This could also well account for an organism's using maltose but being incapable of using dextrose.

Part IV - Toxicity Level

This experiment was designed to find the concentration of Na_2SeO_3 needed to completely inhibit the growth of the organism. Escherichia coli and Saccharomyces cerevisiae were also tested for purposes of comparison.

Sodium selenite was added to nutrient broth to make concentrations as listed in Table I. It was found that heating Na_2SeO_3 in the presence of organic compounds, especially carbohydrates, reduced the selenite to elemental selenium which appeared as a red precipitate. Because of this, the Na_2SeO_3 was autoclaved separately. Stock solutions for making the various concentrations of Na_2SeO_3 were prepared as follows:

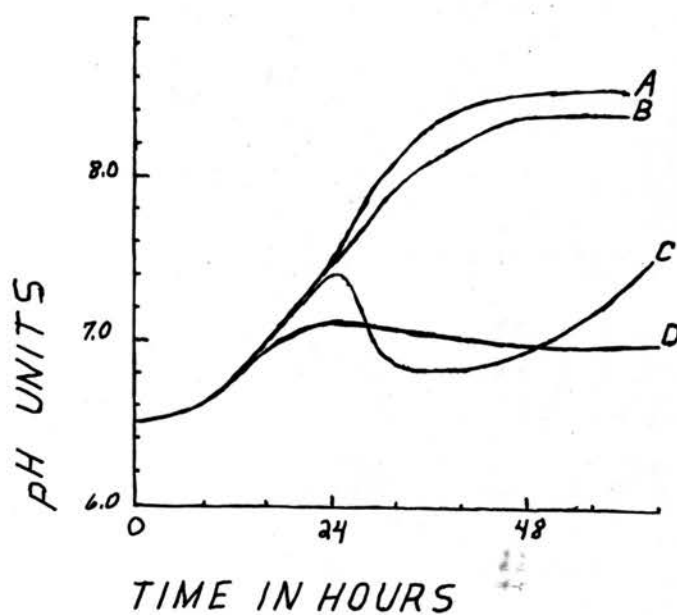


Fig. I. pH changes in carbohydrate utilization (Set I)

- A. No carbohydrate
- B. Mannitol
- C. Maltose
- D. Dextrose

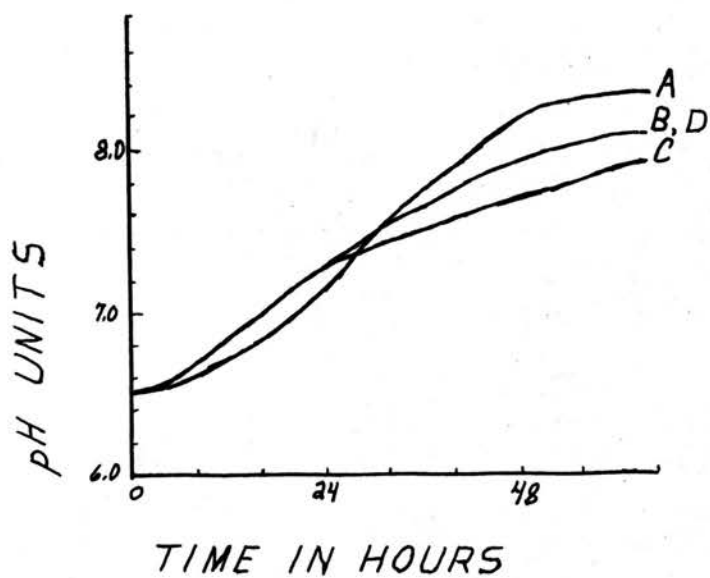


Fig. II. pH changes in carbohydrate utilization (Set II)

- A. Xylose
- B. Lactose
- C. Sucrose
- D. Galactose

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Solution I

Na₂SeO₃ - - - - - 0.015 gram
 Distilled water - - - - - 150 ml.

Solution II

Na₂SeO₃ - - - - - 0.150 gram
 Distilled water - - - - - 150 ml.

Solution III

Na₂SeO₃ - - - - - 1.500 grams
 Distilled water - - - - - 150 ml.

Solution IV

Na₂SeO₃ - - - - - 1.000 gram
 Distilled water - - - - - 10 ml.

Solution V

Nutrient Broth (Difco) - 8 grams
 Distilled water - - - - - 100 ml.

Solution VI

Distilled water - - - - - 500 ml.

These solutions could be autoclaved without harm, and were measured into sterile test tubes according to the quantities shown in Table I. The entire series was prepared in triplicate. The cultures to be used for inoculation were prepared by washing the cells from a nutrient agar slant with 10 ml. distilled water. The tubes were inoculated with one drop each of this suspension. *

Results:

Saccharomyces cerevisiae:

TABLE I

Composition of Media for Toxicity Studies

Per cent Na_2SeO_3	SOLUTIONS					
	ml. I	ml. II	ml. III	ml. IV	ml. V	ml. VI
0	-	-	-	-	1	9
0.001	1	-	-	-	1	8
0.002	2	-	-	-	1	7
0.003	3	-	-	-	1	6
0.004	4	-	-	-	1	5
0.005	5	-	-	-	1	4
0.006	6	-	-	-	1	3
0.007	7	-	-	-	1	2
0.008	8	-	-	-	1	1
0.009	9	-	-	-	1	-
0.01	-	1	-	-	1	8
0.02	-	2	-	-	1	7
0.03	-	3	-	-	1	6
0.04	-	4	-	-	1	5
0.05	-	5	-	-	1	4
0.06	-	6	-	-	1	3
0.07	-	7	-	-	1	2
0.08	-	8	-	-	1	1
0.09	-	9	-	-	1	-
0.1	-	-	1	-	1	8
1.0	-	-	-	1	1	8

The medium used in this experiment does not contain added carbohydrates nor was the pH at a proper level for good growth of yeasts. As a result, this organism grew very slowly. However, final growth was heavy where it occurred not inhibited. Total growth decreased as the concentration of Na_2SeO_3 increased and the highest concentration containing observable growth was .07 per cent. There was little if any odor of dimethyl selenide in any of the tubes. A red-colored precipitate was noted in tubes with growth in them. The composition of this sediment was partly the yeast cells and some small coarse particles as seen under a microscope. The nature of these particles was not determined, but the brick-red color is comparable to that of elemental selenium.

Escherichia coli:

This organism grew at all selenite concentrations tested. There was a slight odor of dimethyl selenide and a corresponding brick-red sediment in tubes containing less than 0.09 per cent selenite. In tubes over this concentration, the red color diffused throughout the medium and increased in intensity as the concentration of Na_2SeO_3 increased. Microscopic examination of the growth in this medium showed rather coarse cells, slightly filamentous and somewhat thinner than normal. No other abnormality was noted. Growth was slightly retarded in the higher concentrations.

Test organism:

Like E. coli, this organism grew in all concentrations.

The odor produced by it in the selenite medium is very characteristic at low concentrations of the Na_2SeO_3 . At higher concentrations, the strength of the odor is extremely nauseous. The red color was present here also, with a diffusion through the medium at higher concentrations of the Na_2SeO_3 . This organism grew out as fast in the selenite medium as did E. coli. This was the reason for assuming a slowing down of E. coli in selenite media. In broth without selenium present, E. coli grows out rapidly, far surpassing this organism in growth rate. Microscopic examination of the cells showed long filaments and normal sized single cells as well. No septa were discernible in the long strands. There were many cells of normal length present and there was no thickening of the cells such as has been reported in algae poisoned by seleno-methionine. These filaments were present in all tubes of selenite media and not in the controls with no selenium so there was little chance of their being contaminants.

It was considered possible either that resistance to selenium might not persist on serial transfer, or else that selenium dependence might develop. These possibilities were explored in the following simple experiment.

Twenty tubes of nutrient broth and twenty tubes of broth with Na_2SeO_3 at a concentration of 0.1 per cent were made up in the same manner as already described. These tubes

were set up in pairs of one tube of nutrient broth with one tube of selenite broth. The first tube in this experiment was inoculated from the tube in the previous experiment which contained 0.1 per cent of selenite. After three days, the second tube of selenite and the first tube of nutrient broth were inoculated from the first tube in this series. After another three days, the third selenite tube and the second tube of nutrient broth were inoculated from the second tube of selenite. These steps were repeated at three day intervals until all the tubes had been inoculated. The colon bacillus and the yeast were not studied in this manner.

Results:

Each set of tubes was examined carefully. All tubes showed normal growth and even the last tube of broth after twenty transfers in selenite medium was normal. It seems as though a dual purpose was accomplished by the reduction of Na_2SeO_3 to dimethyl selenide and elemental selenium. It acts as a detoxification process as elemental selenium is nontoxic and the dimethyl selenide is given off as a volatile gas. Also, the organism could well use the small amounts of oxygen released in the reduction process in much the same way as it uses KNO_3 .

Part V - Effect of Na_2SeO_3 on General Metabolism

Since Na_2SeO_3 affected the morphology of the organism somewhat, it could conceivably affect the metabolism as well. It has previously been shown by means of curves plotting pH levels against time that carbohydrate breakdown is closely correlated with peptone utilization. If Na_2SeO_3 affects either the carbohydrate or the peptone metabolism, it should become apparent on this same type of curve. While a change on this curve could not be attributed to any specific change in metabolism, it would indicate whether or not the selenite were actually poisoning the organism.

The media used in this study were nutrient broth, nutrient broth containing 0.1 per cent Na_2SeO_3 , and two sets of the above containing 1.0 per cent dextrose and 1.0 per cent maltose respectively. Since both Na_2SeO_3 and maltose are thermolabile, special care had to be taken so they would not be damaged. Stock solutions similar to those of Table I could have been used but the use of a Seitz filter seemed more simple. The media were prepared in 100 ml. amounts and filtered through a Seitz filter into 250 ml. side arm flasks previously sterilized by autoclaving. These were capped, after filtering, with sterile aluminum foil. After the initial pH of the media had been determined, they were inoculated with 1.0 ml. of bacterial suspension as described below and the pH was measured at 8-hour intervals for 72 hours.

The inoculum was prepared by washing the growth from an agar slant into sterile distilled water and diluting this suspension to 20 ml. Figures III and IV show the pH curves resulting from this study.

Results:

Figure III shows curves which follow closely those of Figure I of the carbohydrate study. Figure IV shows the curves as affected by Na_2SeO_3 . Curve A shows a definite decrease in rate of change and in maximum pH from peptone metabolism. This same reduction in rate of change and in final values is found in the carbohydrate curves. Overall, it would appear that the rate of growth was the factor most affected. From the turbidity of the media after 72 hours, it appeared that the total growth was apparently unaffected.

Part VI - Growth on Agar Containing Na_2SeO_3

In general, the various morphological and physiological types of organisms often show unique colony characteristics on nutrient agar. These characters will appear as color, smooth or rough colonies because of capsule formation, colonies that spread due to motility of the organisms, stringy colonies due to chains of organisms, and in various other ways. It was thought that abnormal morphology such as seen with selenite broth might modify the form of the colonies which would appear on selenite agar.

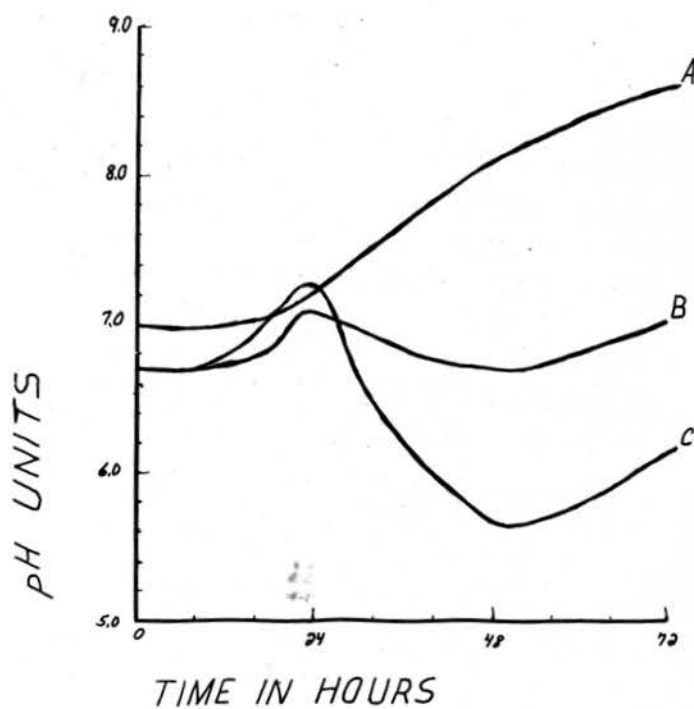


Fig. III. The effect of selenite on pH changes in cultures (Set I, controls)

- A. Nutrient broth
- B. Nutrient broth with dextrose
- C. Nutrient broth with maltose

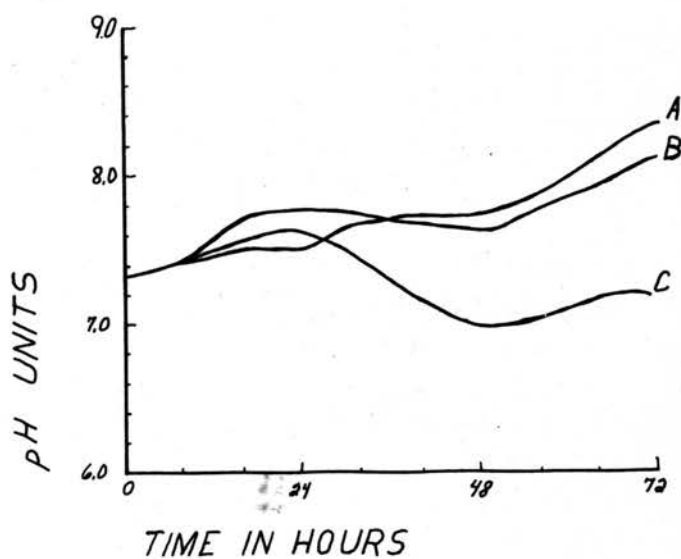


Fig. IV. The effect of selenite on pH changes in cultures (Set II, with selenite)

- A. Nutrient broth with 0.1 per cent Na_2SeO_3
- B. Nutrient broth with dextrose and 0.1 per cent Na_2SeO_3
- C. Nutrient broth with maltose and 0.1 per cent Na_2SeO_3

Three stock solutions were prepared as follows:

Solution I:

Na_2SeO_3 - - - - - 0.150 gram

Distilled water - - - - - 75 ml.

Solution II:

Nutrient agar (Difco) - - 6.9 grams

Distilled water - - - - - 150 ml.

Solution III:

Distilled water - - - - - 75 ml.

These solutions were sterilized by autoclaving, along with six aluminum capped Erlenmeyer flasks numbered I through VI. Flasks number II and V contained 0.5 gram of dextrose each and flasks III and VI contained 0.5 gram of maltose each. These six flasks were prepared as shown in Table II.

Three Petri dishes were poured from each flask (about 15 ml. in each dish). When the agar had solidified, two dishes of each type were inoculated as described below. The third dish was kept as a sterility control. For inoculating the plates, a tube of nutrient broth was inoculated from an agar slant and incubated for four hours at room temperature. One drop of this culture was placed on the surface of each medium to be inoculated and was spread over the surface of the agar by means of a sterile bent glass rod.

Results:

After 24 hours incubation, at room temperature, each

plate was examined. All six plates showed normal growth. The only detectable difference was a slightly heavier growth on the media containing maltose. After 48 hours, the colonies on the selenite plates showed a slight orange color. When the plates were last examined, after 72 hours' incubation, the odor of dimethyl selenide had diffused through the laboratory. The colonies on selenite media had a brick-red color in all cases, the medium itself being clear. The brick-red color being in the colony and not in the surrounding medium could well show that the reduction of Na_2SeO_3 is an intracellular process rather than being brought about by metabolic end-products that have reducing powers. It has already been pointed out that Na_2SeO_3 is easily reduced.

It was thought desirable to compare the bacterium under investigation with Escherichia coli and Salmonella typhimurium with respect to colony formation on selenite agar. For this purpose six flasks of media were prepared as before (Table II). Brain heart infusion agar (Difco) (6, 3) was also used with and without selenium but without additional carbohydrate. These plates were inoculated and incubated as before.

Results:

Plates I through VI are photographs of the Petri dishes containing the maltose media, taken after five days incubation at room temperature. The photographs were made on Eastman Ectachrome Type F film with an Exakta 35 milli-

TABLE II
Composition of Selenite Agar

AMOUNTS ADDED TO EACH FLASK

Flasks	Solution I	Solution II	Solution III	Dextrose	Maltose
1	-	25 ml.	25 ml.	-	-
2	-	25 ml.	25 ml.	0.5 gram	-
3	-	25 ml.	25 ml.	-	0.5 gram
4	25 ml.	25 ml.	-	-	-
5	25 ml.	25 ml.	-	0.5 gram	-
6	25 ml.	25 ml.	-	-	0.5 gram

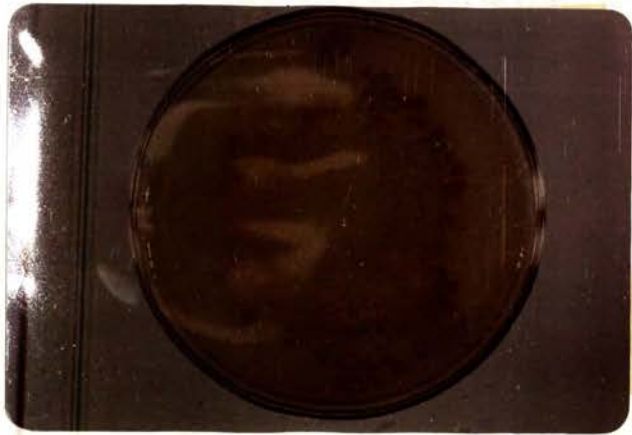


Plate I. Escherichia coli



Plate II. Escherichia coli on Na_2SeO_3

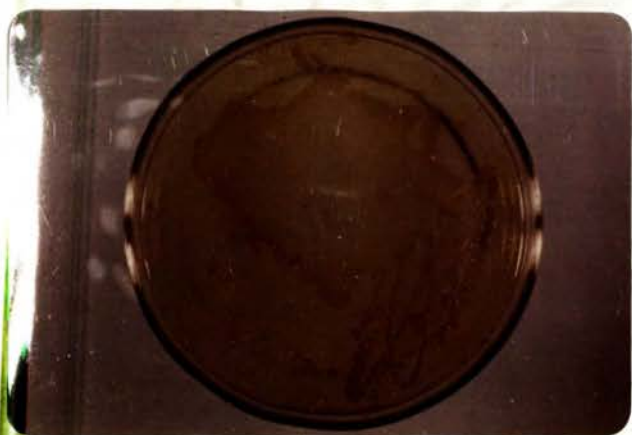
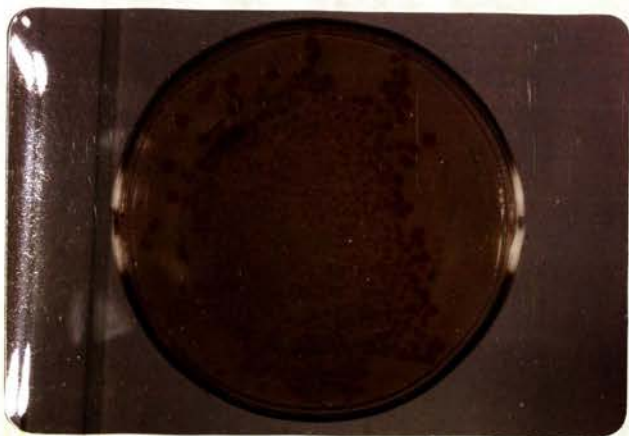


Plate III. Salmonella typhimurium



Plate IV. Salmonella typhimurium on Na_2SeO_3



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Plate V. Research organism



Plate VI. Research organism on Na_2SeO_3

meter; shutter speed one-fifth second, lens opening f-8 with a Harrison B2 filter. The dishes were placed on a glass plate four inches above a white mat to prevent the appearance of shadows.

Escherichia coli:

While the colonies, as they appear on Plate II, are smaller and noticeably lighter in color than those of the other two organisms (Plates IV and VI), the reducing powers of the colon bacillus may well be masked by the inhibiting effect of the selenite. Levine, in 1925, wrote of this same effect on E. coli (9). In his study, the organism was inhibited in 0.1 per cent selenite to the point where no reduction took place. However, at lower concentrations, the organism was capable of the reduction. Also, the medium he used, while similar, contained no carbohydrate. In the present study, growth of the organism on nutrient agar with one per cent Na_2SeO_3 and no carbohydrate, the organism was similarly inhibited, showing rough, minute colonies with just a trace of orange color. On brain heart infusion agar, colonies were heavily colored and more nearly normal in shape, size and texture. Microscopic examination showed little or no variation in morphology.

Salmonella typhimurium:

This organism grew equally on all media and produced red colonies on the selenite agar. The growth of the organism was not apparently retarded and it was the first of the

three to show the red color. Microscopic examination failed to show any unusual morphology.

Test organism:

On the selenite media, this organism produced the strong odor previously associated with it. While both E. coli and S. typhimurium did produce it, it was much weaker. The odor produced by the test organism preceded the appearance of the brick-red color. This could indicate the use of two metabolic pathways, or merely that the concentration of the elemental selenium had not increased to the point where it was visible in the colony. The organism grew approximately the same on all media with a somewhat lighter color on the selenite agar containing no carbohydrate. Levine noted in his paper that at 37 degrees Centigrade Na_2SeO_3 was reduced in broth containing dextrose (9). There could well be a connection between Levine's observation and the increased growth on the carbohydrate medium containing selenite.

Microscopic examination of a wet mount showed the same long filaments described before. However, the filaments in this case contained many small, dark granules. These granules could be the elemental selenium causing the red color of the colonies. As before, there were many single cells as well as the long filaments, and these too had the granules in them. Because of the smallness of the granules, the color appeared dark brown and had little of the brick-

red hue. These granules are not distinguishable if the organism has been stained heavily with either methylene blue or Gram's stain.

It was noticed when preparing microscope slides with the test organism that the mass of bacterial cells from the colonies on the various selenite media tended to form long threads which stretched between the transferring needle and the body of the colony. This might be due in part to the presence of the long filaments described, and also might relate to polysaccharide synthesis and capsule formation. The growth on selenite-free media was of ordinary butyrous consistency.

DISCUSSION AND SUMMARY

The organism studied was isolated from a selenaceous soil and classified as belonging to the genus Achromobacter as described in Bergey's Manual of Determinative Bacteriology (2). There was no species listed whose characteristics agree in all respects with those of the organism investigated.

When the pH of the medium was plotted against incubation time, it was found that the organism could ferment two carbohydrates, maltose and dextrose. These fermentations were not noticeable when using phenol red to indicate acid production. When 0.1 per cent Na_2SeO_3 was added to the carbohydrate media, both the rate of change of pH and the final pH were reduced.

The organism was cultivated in media containing various concentrations of Na_2SeO_3 and was found to be capable of multiplication in a concentration of 1.0 per cent Na_2SeO_3 . The reason for this resistance to the toxicity of Na_2SeO_3 was not found but was thought to be due to the organism's ability to reduce the selenite to elemental selenium.

Growth on agar media containing 0.1 per cent Na_2SeO_3 resulted in brick-red colonies. This color was found only in the colony and not in the medium. Long filaments and intracellular granules were seen under the microscope. It is believed that these granules were elemental selenium. A strong garlic-like odor, produced by the growth of the organism on media containing selenite, is thought to have been due to the formation of dimethyl selenide.

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