EFFECTS OF EQUAL MOLAR CONCENTRATION OF ETHIONINE AND METHIONINE UPON THE GROWTH OF SCENEDESMUS QUADRICAUDA AND GLOEOCAPSA SP

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EFFECTS OF EQUAL MOLAR CONCENTRATION OF ETHIONINE AND METHIONINE UPON THE GROWTH OF *SCENEDESMUS QUADRICAUDA* AND *GLOEOCAPSA* SP

An Abstract

Presented to

the Graduate Council of

Austin Peay State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Douglas Anderson Hite

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ABSTRACT

A study of the effects of ethionine and equal molar concentrations of ethionine-methionine upon the growth of the green alga, Scenedesmus quadricauda, and the blue-green alga, Gloeocapsa sp. was conducted.

The growth was monitored by four parameters of growth: 1) absorbance, 2) chlorophyll-a content, 3) cell counts, and 4) exponential growth rate. Also the growth curves of each alga were studied to determine the effect the various treatments may have upon the growth phases.

The results indicate: 1) all concentrations of ethionine tested caused some degree of inhibition of growth, 2) on an equal molar basis, Gloeocapsa was more sensitive to the inhibitory effects of ethionine than was Scenedesmus quadricauda, 3) the effect of ethionine upon the growth curve of Gloeocapsa was that it prevents the cells from entering an exponential phase of growth, while the cells of Scenedesmus do enter into exponential growth, but the rate of growth was reduced, and 4) most concentrations of ethionine-methionine reversed the inhibitory effects of similar concentrations of ethionine.

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August, 1976

To the Graduate Council:

I am submitting herewith a Thesis written by Douglas Anderson Hite entitled "Effects of Equal Molar Concentrations of Ethionine and Methionine Upon the Growth of Scenedesmus quadricauda and Gloeocapsa sp." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Biology.

Major Professor

We have read this thesis and recommend its acceptance:

Second Committee Member

Third Committee Member

Accepted for the Council:

Dean of the Graduate School

ACKNOWLEDGEMENTS

I would like to thank Dr. Benjamin Stone, Dr. Diane Findley and Dr. Davis Findley for their advice and assistance, and my wife for her patience and understanding.

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CHAPTER I

INTRODUCTION

Various studies have been made on the effects of ethionine, an analogue of methionine, upon biological systems.

Dyer (1938) first showed the inhibitory effects of ethionine in rats as a weight loss. Kerridge (1959) indicated that flagellated bacteria were inhibited by ethionine. Ethionine has been shown to inhibit protein synthesis in Avena coleoptiles (Cleland, 1960), in rat liver (Villia et al, 1963), and in chick embryos (Ruddick, 1972).

Ethionine inhibits invertase synthesis in sugar beets (Stone et al, 1970), severely inhibits the elongation of hypocotyls and roots in lettuce seedlings (Merrel, 1970), and inhibits the incorporation of ^{3}H leucine into the protein of lettuce seedlings (Harker, 1971).

Glaser and Mager (1972) and Kisilevsky et al (1973) indicated that protein synthesis in rats was inhibited by ethionine.

Aaronson and Ardois (1971) suggested that ethionine repressed cell multiplication of the blue green algae, Synechococcus and Anabaena, of the euglenoid, Euglena, and of the golden brown algae Ochromonas. Ochiai-Yanagi et al (1973) showed that the light independent phase of growth in Chlorella was strongly suppressed by ethionine.

The reversal of ethionine induced growth inhibition by methionine was first observed with Escherichia coli, Harris and Kohn (1941). Boll (1960) indicated that methionine would reverse the effects of ethionine in excised tomato roots, while Merrel (1970) suggested similar results in lettuce seedlings.

The purpose of this investigation is to study: (1) the effect of ethionine upon the growth of the green alga Scenedesmus quadricauda and the blue green alga Gloeocapsa and (2) the action of various equal molar combinations of ethionine and methionine upon the growth of Scenedesmus and Gloeocapsa.

CHAPTER II

REVIEW OF LITERATURE

Ethionine is the s-ethyl homologue of methionine and is termed an antimetabolite in that its inhibitory effect may be reversed by methionine in some biological systems. Dyer (1938) first noted the inhibitory effects of ethionine as a weight loss in rats. The majority of the early workers used bacteria and rats as test organisms. Harris and Kohn (1941) showed that the growth of Escherichia coli is inhibited by ethionine; also, ethionine completely inhibited the growth and cell division of Escherichia coli as denoted by Volcani and Sarid (1956) and Smith and Salmon (1965). Cheng and Hartman (1968) indicated that ethionine reduced the growth, protein synthesis and RNA synthesis in Escherichia coli. Other bacteria studied for the effect of ethionine are Lactobacillus, in which partial inhibition was evident (Camien, et al 1950) and Saccharomyces, where complete inhibition of growth and division was shown (Loveless et al, 1954).

Schmidt et al (1956) indicated that growth was inhibited in rats by ethionine. This is supported by the works of Stekol et al (1963), Glaser et al (1972), Kisilevsky et al (1973), and Endo et al (1975).

Mills (1958) denoted that 2000 mg/ml ethionine would totally inhibit Influenza B. The fungus Torula monosa is partially inhibited

by 3260 mg/ml ethionine (Halvorson, et al 1952), while the yeast Candida sloofii is completely inhibited by 1000 mg/ml ethionine (Mendonca and Travassos, 1972). Urey (1971) showed that conidiation of the mold Neurospora crassa is partially inhibited by 50 mg/ml ethionine. Aaronson and Ardois (1971) indicated that growth of the euglenoid, Euglena gracilis, and the golden-brown alga, Ochromonas danica, was completely inhibited by 100 mg/ml ethionine while the blue-green algae Synechococcus cedrorum and Anabaena cylindrica are totally inhibited by 10 mg/ml ethionine.

Schrank (1956) specified that cell elongation in excised sections of Avena coleoptiles is inhibited by ethionine, while Cleland (1960) denoted the 83 mg/ml ethionine would inhibit cell elongation in Avena coleoptiles. Boll (1960) indicated that ethionine inhibits growth in excised tomato roots. Invertase synthesis in sugar beets is inhibited by ethionine, (Stone, et al 1970), and Binion (1971) showed that chlorophyll synthesis in Early Alaska peas are reduced drastically by ethionine. Chlorophyll concentration was found to be lowered in Phaseolus bean chloroplasts after treatment with ethionine (Wrischer, 1973). Elongation of hypocotyls and roots of lettuce seedlings have been shown to be severely inhibited by ethionine (Merrel, 1970) and Harker (1971) showed that the incorporation of ^{3}H-leucine into protein is inhibited by ethionine.

Marzluf (1969) specified that ethionine produced a phenocopy of the eyeless mutant in Drosophila melanogaster, while Hackney (1972) indicated that ethionine exerts an inhibitory effect upon metamorphosis in wild type Drosophila and in vestigial flies. Also, ethionine inhibited the population of wild type flies.

Equal molar concentrations of methionine with ethionine was first shown to reverse the inhibitory effects of ethionine Harris and Kohn (1941). Methionine has been shown to reverse the effect of ethionine upon cell elongation in excised sections of Avena coleoptiles (Schrank, 1956), (Cleland, 1960) and (Norris, et al 1962).

Stekol et al (1950) indicated that the inhibitory effect of ethionine upon the growth of rats was alleviated by methionine, and the study by Simpson et al (1950) shows similar results with rats.

The inhibitory effect of ethionine upon bacteria is reversed by ethionine as cited in work by Gibons (1962) and Norris et al (1973).

Other biological systems where methionine has been shown to reverse the inhibitory effects of ethionine are with excised tomato roots (Boll, 1960), lettuce seedlings (Merrel, 1970), yeast (Mendona et al 1972), and algae (Hochberg and Rahat, 1971) and (Ochiai-Yangia, 1973).

The mode of action for ethionine which results in inhibition of growth may be classified in four categories; (1) ethylation of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA), or metabolites

of low molecular weight or both, which may behave abnormally due to altered structure or due to competition with methylated compounds (Stekol et al,1960) and (Stekol et al, 1950); (2) ethionine incorporation into proteins which may cause them to lose biological activity (Farber, 1963) and Spizek et al (1967); (3) adenosine triphosphate (ATP) trapping mechanism, where S-adenosylethionine is accumulated faster than ATP is synthesized which depressed the production of nicotinamide adenine dinucleotide (NAD), proteins and nucleic acids (Schmidt, et al 1956), (Stekol et al, 1960), (Villa-Trevino et al, 1962) and (Stekol et al, 1950), and (4) inhibition of enzyme formation (Halverson et al, 1952).

CHAPTER III

MATERIALS AND METHODS

Stocks and Experimental Cultures

The colonial green alga, Scenedesmus quadricauda, was obtained from the Indiana Algal Culture Collection as Number 76. A supply of seeding cells for the stock cultures was maintained on nutrient agar slants and incubated in a Percival incubator at 25°C under a light intensity of 300 foot-candles.

The source of the blue-green alga Gloeocapsa was the Carolina Biological Supply Co. A supply of seeding cells for the stock cultures were maintained in liquid EPA defined media (Environmental Protection Agency, 1971) and incubated in a Percival incubator at 25°C under 300 foot-candles of light.

Stock cultures were maintained at temperature conditions of 24 $\pm$ 2°C and under 350 foot-candles of continuous light. They were aerated with air to discourage clumping and to supply CO_2. Evaporation was reduced by bubbling the air through a water filter which contained charcoal. Experimental cultures were grown at 24 $\pm$ 2°C and under 300 foot-candles of fluorescent light. These cultures were oscillated 95 times per minute in a Precision Scientific incubator to prevent clumping, to distribute cells evenly and to aid in carbon dioxide distribution.

Approximately half of the Gloeocapsa test flasks were oscillated 95 times per minute with a wrist-action shaker to increase the test culture capacity and also to prevent clumping, to distribute cells and to aid in carbon dioxide distribution.

The culture medium for all cultures was defined as follows (Environmental Protection Agency, Algal Assay Procedure, Bottle Test, 1971):

Macronutrients	(mg/l)	Micronutrients	(mg/l)
$NaNO_3$	25.500	H_3BO_3	185.520
K_2HPO	1.044	$MnCl_2 \cdot 4H_2O$	415.587
$MgCl_2 \cdot 6H_2O$	12.170	$ZnCl_2$	32.709
$MgSO_4$	7.179	$CoCl_2 \cdot 6H_2O$	1.429
$CaCl_2 \cdot 2H_2O$	4.410	$CuCl_2 \cdot 2H_2O$	0.114
$NaHCO_3$	15.000	$Na_2MoO_4 \cdot 2H_2O$	7.260
		$FeCl_3 \cdot 2H_2O$	159.972
		$Na_2EDTA \cdot 2H_2O$	300.000

One ml per liter of chloroform was added to the stock flasks containing media and to the experimental flasks before autoclaving to lower the chance for contamination.

Chemicals used in this study were obtained from the following source: DL-ethionine and L-methionine were obtained from the Sigma Chemical Company, St. Louis, Missouri.

A constant stock of physiologically viable cells was maintained by the semi-continuous culture method (Bassham and Calvin, 1957), (Fogg, 1971) and (Ukeles, 1973). This method involves the maintenance of a culture in the log phase of its growth by diluting the culture with fresh media at specified time intervals.

In this study the culture was maintained in logarithmic phase by diluting with one half or three-fourths the volume every seven to eight days.

Stock culture solutions were prepared in the following fashion: one liter of media was inoculated with 1 ml of chloroform, then the flask was stoppered with a cotton plug and autoclaved. After the media cooled, 480 ml were seeded with a 20 ml suspension of 8.1×10^6 cells/ml density; a teflon coated, magnetic stirring bar was added and the flask was stoppered with a cotton plug.

The experimental cultures were set up by adding 44 ml (24 ml for Gloeocapsa) of sterile media to a 250 ml flask, then aseptically adding a 10 ml inoculum of an algal suspension with 3.5 to 3.7×10^5 cells/ml (20 ml for Gloeocapsa), followed by 6 ml of the test chemical, then the flasks were stoppered with a polyfoam plug. Controls were similarly prepared, but minus the test chemicals. The control volume was adjusted with 6 ml of sterile media. The experimental flasks also contained 25-30 4mm glass beads to aid in agitation of the cells and was plugged with a polyfoam plug.

Biomass Determinations

The complete growth curve was investigated since the growth of Scenedesmus quadricauda normally shows a lag, exponential and stationary phase. Therefore, to evaluate the entire effect of a chemical upon growth, all aspects of growth need to be studied. This has been emphasized by Shrift (1954) in antimetabolite experiments.

Growth of the stock culture was observed every 24 hours, and experimental culture every 12 hours by cell counts and absorbance determinations. Cell counts were made with a Sedgwick-Rafter counting chamber according to the method outlined by Ingram and Palmer (1952). The absorbance of the culture was determined at 650 nanometers (NM) with a red filter, using a model 240 Gilford photospectrometer.

The exponential growth rate of the cells was found by taking the logarithm base two of the absorbance for two time periods and then solving the following equation (Sorokin, 1973):

$$R = \frac{\log_2 X_2 - \log_2 X_1}{t_2 - t_1}$$

where: R = growth rate

X_1, X_2 = numerical values for cell mass at the beginning and end of a specified interval of time

t_1, t_2 = corresponding times for X_1 and X_2

To determine when the cells were in exponential growth, three interpretations of the growth measurements were made. First, from the absorbance, the growth rate was determined (Sorokin, 1973); second, a plot of the change (Δ) in the log base two of the absorbance was made; and third, a semi-log plot of the cell count was used (Guillard, 1973).

The time period from which the exponential growth rate was calculated was the transition point (Blankley, 1973) from the lag to the log phase, and from the log to the stationary. This time interval ranged from the 19th hour to the 64th hour with the Scenedesmus control culture and from the 0 hour to the 121st hour in the Gloeocapsa control culture. Other time intervals varied according to the effects of the test chemical.

In the daily monitoring of the growth of cells in the experimental cultures, absorbance was used as the main parameter of growth.

Sorokin (1973) indicated that the use of a dilution curve constructed from absorbance measurements can make turbidity readings self-sufficient and as reliable as the determination of growth rate by any other index of growth.

A serial dilution curve was constructed by taking a dense suspension of algae and diluting by half, by the addition at each dilution to the measured volume of suspension, an equal volume of the medium. The population density of one flask per each duplicate set of

cultures was measured by taking the cell count with a Sedgwick-Rafter counting chamber following the method described by Ingram and Palmer (1952). Cell counts of Scenedesmus were made with a Unitron microscope model MSA3243295, and counts of Gloeocapsa were done with a Zeiss standard WL research microscope at 200 X with ND 6 filter. All cell counts were subjected to statistical analysis of significance (Chi square) at the 95 per cent confidence level.

Interpretation of inhibition was expressed as specific effects on the growth curve. These interpretations were based upon measurements of biomass taken at the lag and stationary phase. Growth determinations were ended after the cells in the control culture reached the stationary phase.

Comparisons of experimental cultures were made with the control culture based on the effects upon: (1) the length of the lag phase or induction phase where no increase in cell numbers occurs; (2) final biomass determinations by absorbance, the increase in cell numbers and chlorophyll-a content; and (3) the growth rate in the exponential phase where cell multiplication is rapid and numbers increase in geometric progression.

Chlorophyll-a was extracted from the cells at the termination of growth by the method of Talling and Driver (1963) and Parsons and Strickland (1963). With this method the cells are filtered on a Millipore HA 0.45 mm pore size filter, and suspended in 10 ml of 90 per

cent acetone to which 0.1 gm of Na_2CO_2 was added. Then the cells on the filter are ground in a glass homogenizer (Pyrex) and allowed to stand for twenty minutes. Chlorophyll-a was determined spectrophotometrically using the supernatant and applying the following equation:

$$\text{mg chlorophyll-a per sample} = (11.6 \times D665 - 1.3 \times D645) \times \frac{v}{l}$$

Where: v = volume of sample in ml

l = length of spectrophotometric cell in cm

CHAPTER IV

RESULTS AND DISCUSSION

Effects of Various Combinations of Ethionine and Methionine Upon the Growth of Scenedesmus quadricauda

Ochiai-Yanagi et al (1973) indicated that 3 mM ethionine when added at the beginning of incubation, most strongly suppressed chlorophyll formation in Chlorella protothecoides. Ethionine together with methionine completely reversed the suppressive effects of ethionine (Ochiai-Yanagi et al, 1973). Aaronson and Ardois (1971) denoted that 0.6 mM ethionine resulted in complete inhibition of growth in Euglena and Ochromonas. The following experiment was performed to determine what effect ethionine has on the growth of Scenedesmus, and whether methionine in equal molar concentration with ethionine would reverse any inhibitory effects.

The total increase in cell number (cells/ml) for Scenedesmus in various concentrations of ethionine and methionine is shown in Table I. All concentrations of ethionine severely inhibited the total increase in cell numbers after 117.0 hours of growth with 0.5 and 10.0 mM ethionine showing similar degrees of inhibition. The addition of methionine in equal molar concentration with ethionine resulted in partial reversal as compared to the control except at 0.5 mM. Statis-

Table I

Effects of Various Concentrations of Ethionine and Ethionine-Methionine Upon the Increase in Cell Numbers of *Scenedesmus quadricauda*

Treatment (mM)		Percent of Control
Ethionine	0.5	17.7
Ethionine-Methionine	0.5	8.7
Ethionine	1.0	28.3
Ethionine-Methionine	1.0	44.0
Ethionine	2.0	27.5
Ethionine-Methionine	2.0	30.2
Ethionine	10.0	18.0
Ethionine-Methionine	10.0	37.5

The cell numbers for the control after 116.0 hours of growth was 9.3×10^4 cells/ml. The data represents two experiments with replicates for each treatment.

tical analysis of cell number measurements indicated that there is a significant difference between the treatment and the control at the 5 per cent level of significance using the students t-test (Bailey, 1959).

The data in Table II indicates that the exponential growth rate is severely inhibited by 10.0 mM ethionine, with 0.5 mM showing the least effect upon exponential growth. The reversing effect of ethionine-methionine is denoted by the high degree of reversal of the ethionine inhibition. There is no significant difference between the effects of 1.0 and 2.0 mM ethionine upon the exponential growth rate.

Tables III and IV represent the total increase in absorbance and the chlorophyll-a content respectively. Both show that as the concentration of ethionine increases, the inhibitory effect increases. This is also apparent with the reversal effect of ethionine, except at 1.0 mM ethionine-methionine.

To obtain an overall view of the effect that various concentrations of ethionine and ethionine-methionine have upon the growth of Scenedesmus, an average per cent growth of the control is determined. This is accomplished by taking the mean value of each index of growth, as compared to the control, and calculating a single mean value for each concentration and expressing this as a percentage. Figure 1 illustrates a comparison of the average per cent growth of the control, for the ethionine concentrations. When all the parameters of growth are compiled, the result shows a definite relationship between the

Table II

Effects of Various Concentrations of Ethionine and Ethionine-Methionine Upon the Exponential Growth Rate of *Scenedesmus quadricauda*

Treatment (mM)		Percent of Control
Ethionine	0.5	68.6
Ethionine-Methionine	0.5	71.5
Ethionine	1.0	44.6
Ethionine-Methionine	1.0	77.4
Ethionine	2.0	47.8
Ethionine-Methionine	2.0	51.6
Ethionine	10.0	26.7
Ethionine-Methionine	10.0	62.3

The exponential growth rate of the control after the exponential time period of 55 hours, was 0.6945. The data represents two experiments with replicates for each treatment.

Table III

Effects of Various Concentrations of Ethionine and Ethionine-Methionine Upon Total Increase of Absorbance for *Scenedesmus quadricauda*

Treatment (mM)		Percent of Control
Ethionine	0.5	62.9
Ethionine-Methionine	0.5	56.1
Ethionine	1.0	38.4
Ethionine-Methionine	1.0	28.0
Ethionine	2.0	40.3
Ethionine-Methionine	2.0	39.9
Ethionine	10.0	20.3
Ethionine-Methionine	10.0	47.3

The total increase in absorbance of the control after 116.0 hours of growth is 0.313. The data represents two experiments with replicates for each treatment.

Table IV

Effects of Various Concentrations of Ethionine and Ethionine-Methionine Upon Chlorophyll-a Content in *Scenedesmus quadricauda*

Treatment (mM)		Percent of Control
Ethionine	0.5	68.4
Ethionine-Methionine	0.5	57.7
Ethionine	1.0	38.0
Ethionine-Methionine	1.0	60.7
Ethionine	2.0	22.3
Ethionine-Methionine	2.0	47.3
Ethionine	10.0	17.4
Ethionine-Methionine	10.0	57.9

The chlorophyll-a content of the control after 116.0 hours of growth is 5.698 ug/10 ml. The data represents two experiments with replicates for each treatment.

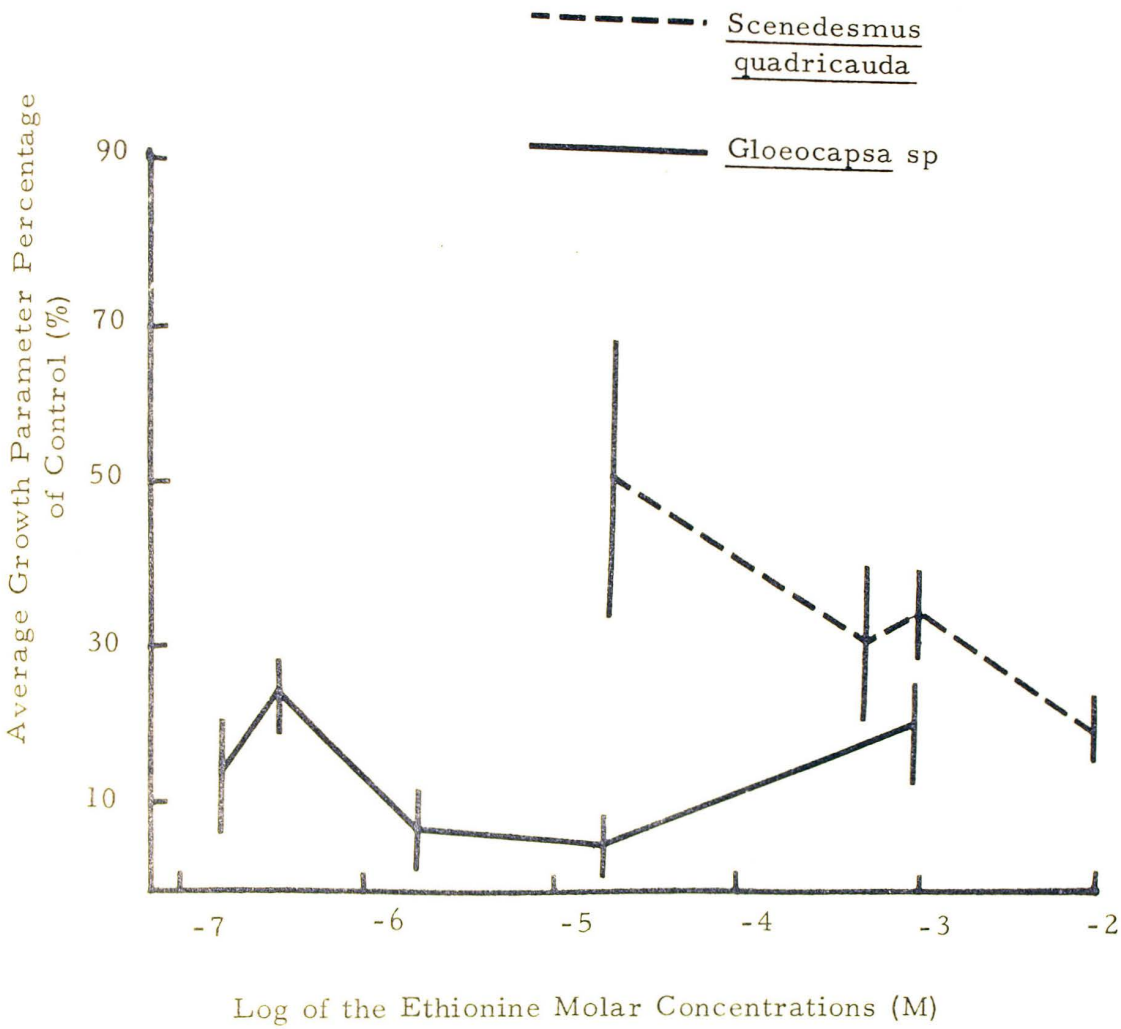

Figure 1. The average percent growth of the control for Scenedesmus quadricauda and Gloeocapsa sp at various concentrations of ethionine. This data represents an average of the measurements obtained from four parameters of growth. Bars represent standard deviations.

concentration of ethionine and the per cent of inhibition. As the concentration of ethionine increases the per cent of inhibition increases or per cent of control decreases. This data supports the idea that ethionine inhibits growth and is in agreement with studies done by Boll (1960) with sections of Avena coleoptiles, Kerridge (1959) with the bacteria Salmonella; Aaronson and Ardois (1971) with Chlorella.

Figure 2 through 5 illustrate the growth curve of Scenedesmus quadricauda with various combinations for ethionine and methionine. The absorbance is used to monitor growth and the change in the logarithm base two of the absorbance plus ten ($\Delta \log_2 A + 10$) is calculated to give a more explicate view of the growth phases (Sorokin, 1972).

The effect of 0.5 mM ethionine and 0.5 mM ethionine-methionine are shown in Figure 2. The illustration indicates that at this concentration ethionine does not affect the length of the log phase, but does reduce the rate of increase of the log phase. The most notable effect is upon the slope of the exponential phase, which is 68.5 per cent of the control (Table II). This shows that 0.5 mM ethionine is inhibiting exponential cell division which results in a lowering in the total biomass as denoted after 117.0 hours of growth (Figure 2). At 0.5 mM methionine, (Figure 2) in equal concentrations with ethionine, does show some reversing characteristics in the lag phase. This is also evident in the log phase to a lesser amount where ethionine-methionine is 71.5 per cent of the control (Table II). Yet this reversing quality

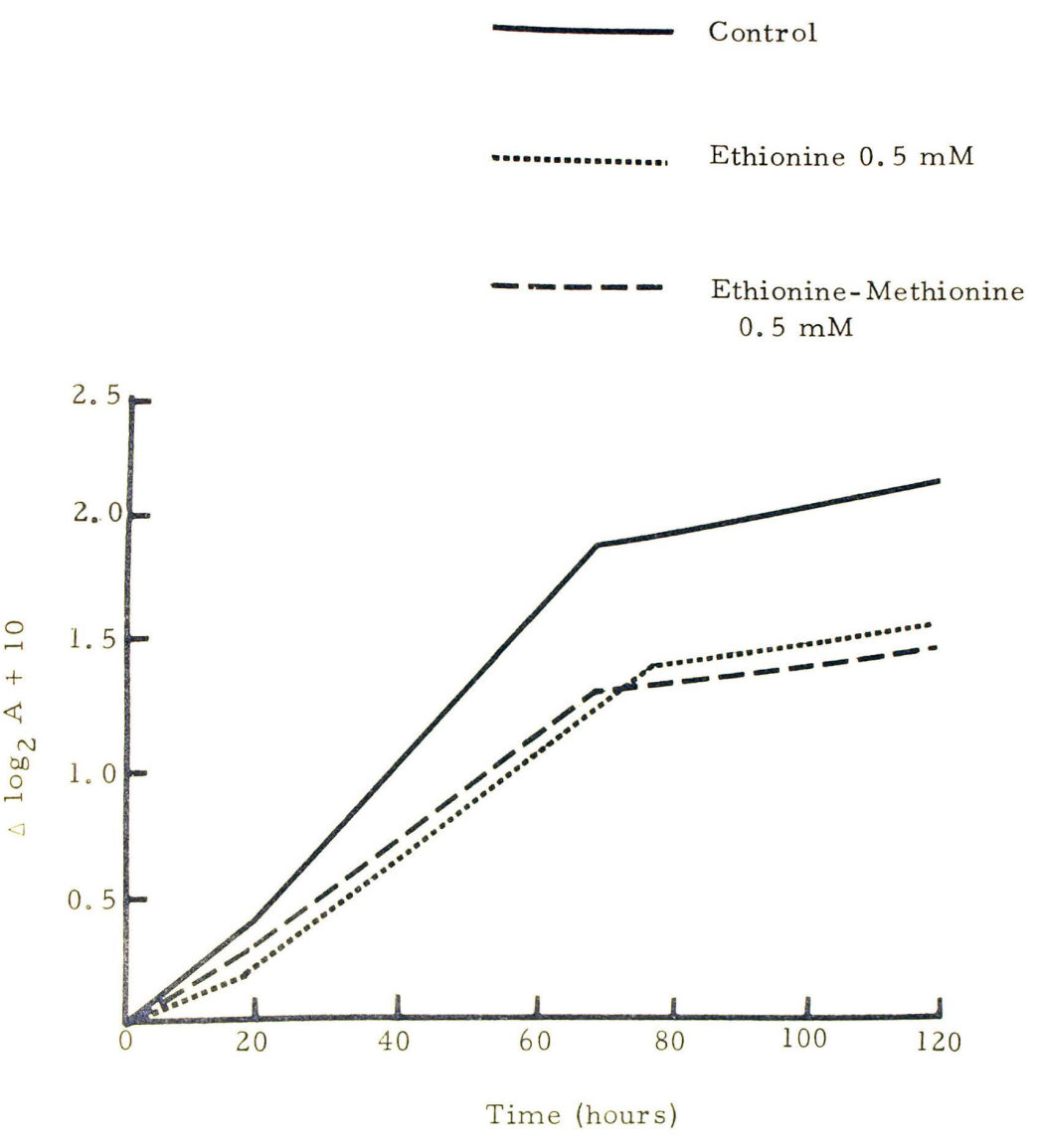

Figure 2. Growth curves of Scenedesmus for the control, 0.5 mM ethionine and 0.5 mM ethionine-methionine. The data represents two experiment of replicate solutions.

is not found in the stationary phase where after 117.0 hours of growth the increase in biomass, as measured by absorbance and chlorophyll-a content, is lower than the 0.5 mM ethionine treatment.

At the ethionine concentration of 1.0 mM (Figure 3) the reduction of the growth rate is again prevalent, and to a greater extent than at 0.5 mM ethionine. The exponential growth rate is significantly lower (Table II), and the length of the log phase is prolonged. The total increase in biomass is also less than the control and less than the 0.5 mM ethionine treatment.

One mM methionine in equal concentration with ethionine reverses the effect of 1.0 mM ethionine, but not completely to the control level (Figure 3). The exponential growth rate with equal concentrations of ethionine and methionine is 77.3 per cent of the control, while the ethionine treatment is 44.6 per cent of the control (Table II). This approximately doubling of the exponential growth rate is seen in Figure 3.

Figure 4 illustrates the effect of 2.0 mM ethionine and equal concentrations of ethionine and methionine. The amount of inhibition of the exponential growth rate indicated at 1.0 mM is almost identical to the results obtained at 2.0 mM, with there being no significant difference between the two values as shown by the students t-test (Bailey, 1959). The growth of Scenedesmus under the influence of 1.0 and 2.0 mM ethionine seems to follow a similar pattern.

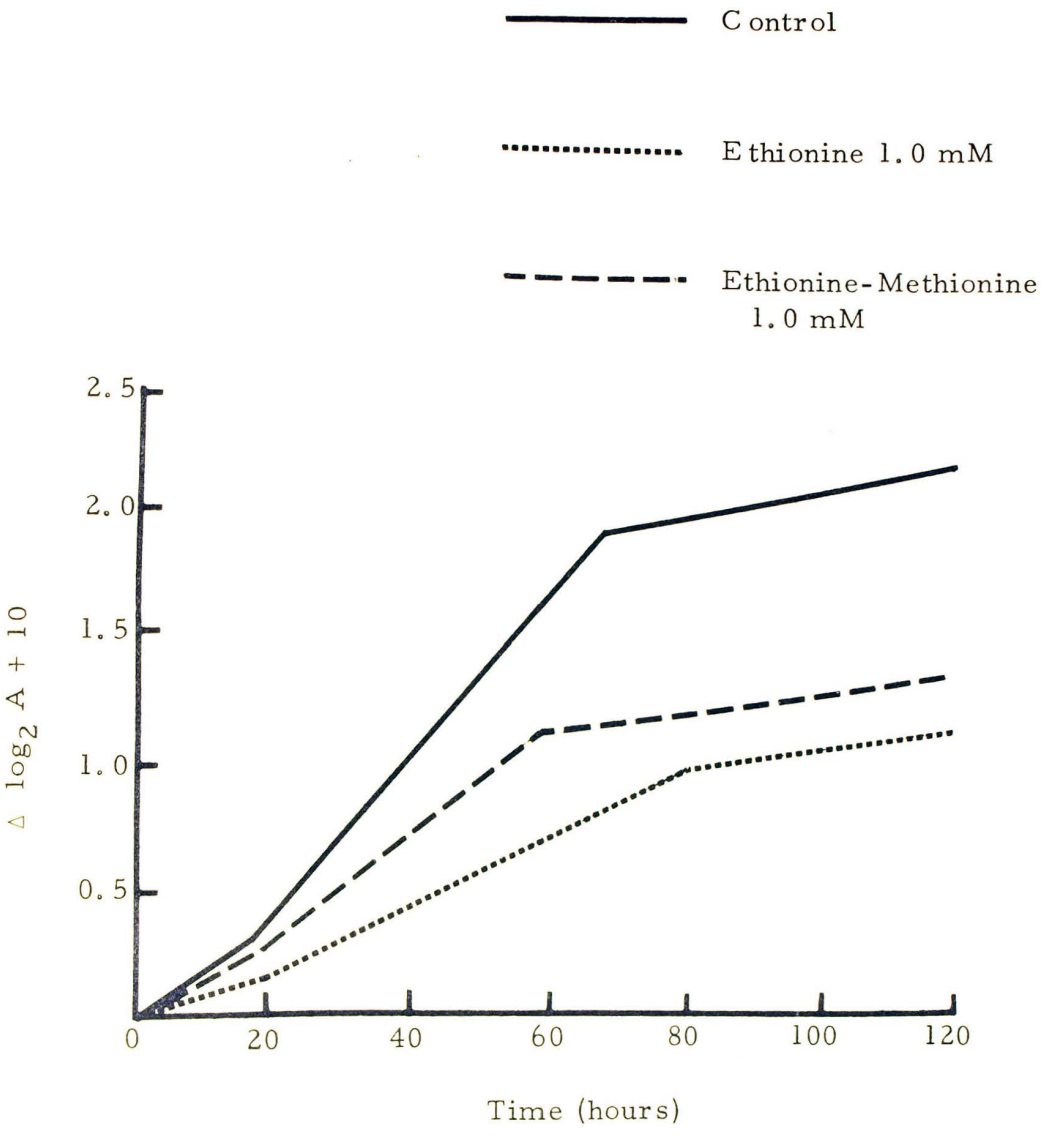

Figure 3. Growth curves of Scenedesmus for the control, 1.0 mM ethionine, and 1.0 mM ethionine-methionine. The data represents two experiments of replicate solutions.

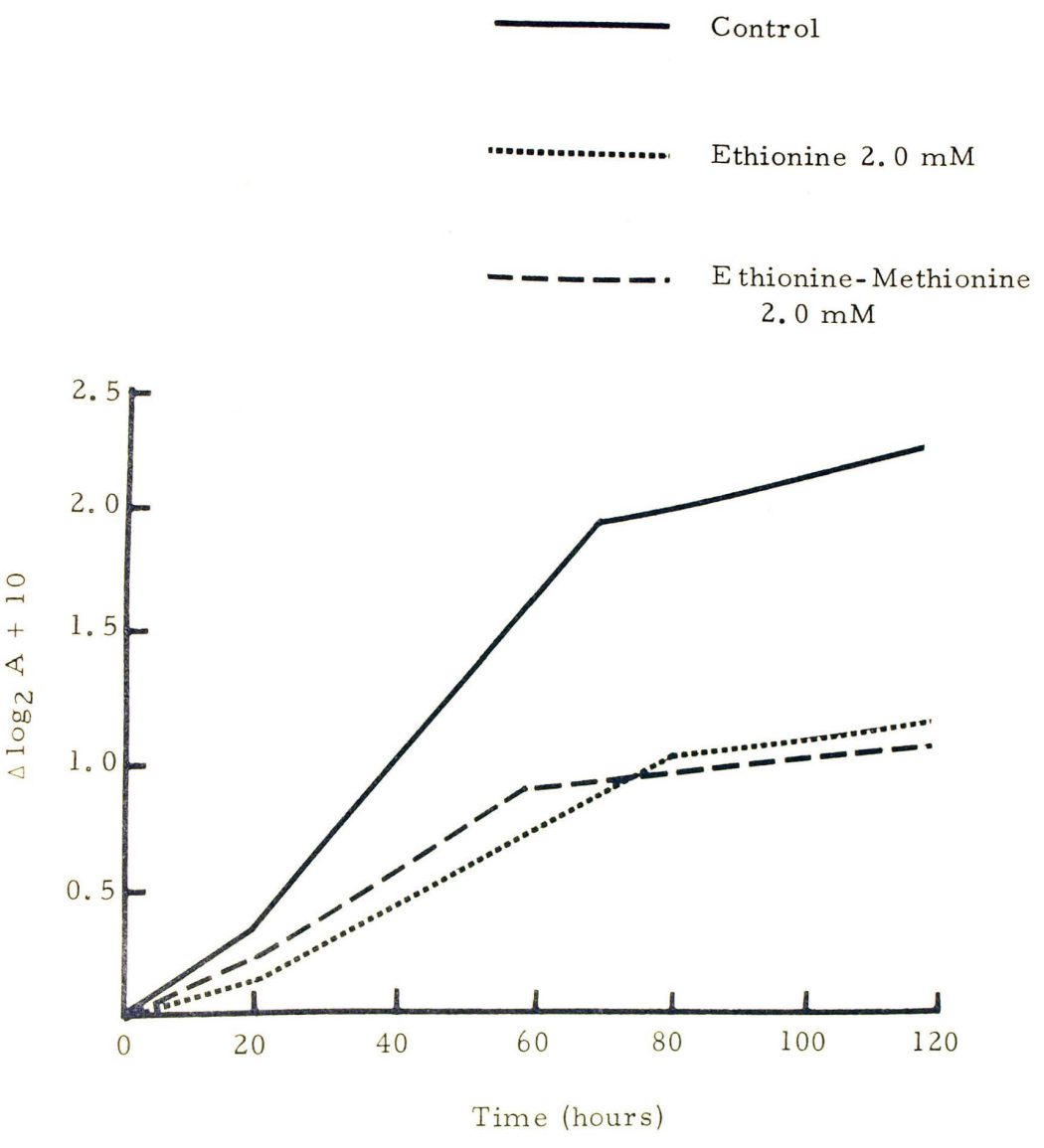

Figure 4. Growth curves of Scenedesmus for the control, 2.0 mM ethionine, and 2.0 mM ethionine-methionine. The data represents two experiments of replicate solutions.

The most inhibitory effect upon the growth of Scenedesmus, as indicated by its growth curve, is with 10.0 mM ethionine (Figure 5). The slope of the log phase is much lower than the control. Exponential growth rate is inhibited by more than half that of the control, and the total increase in biomass, as seen in Figure 5, and by the other biomass indicators (Tables I, III, IV) is substantially suppressed.

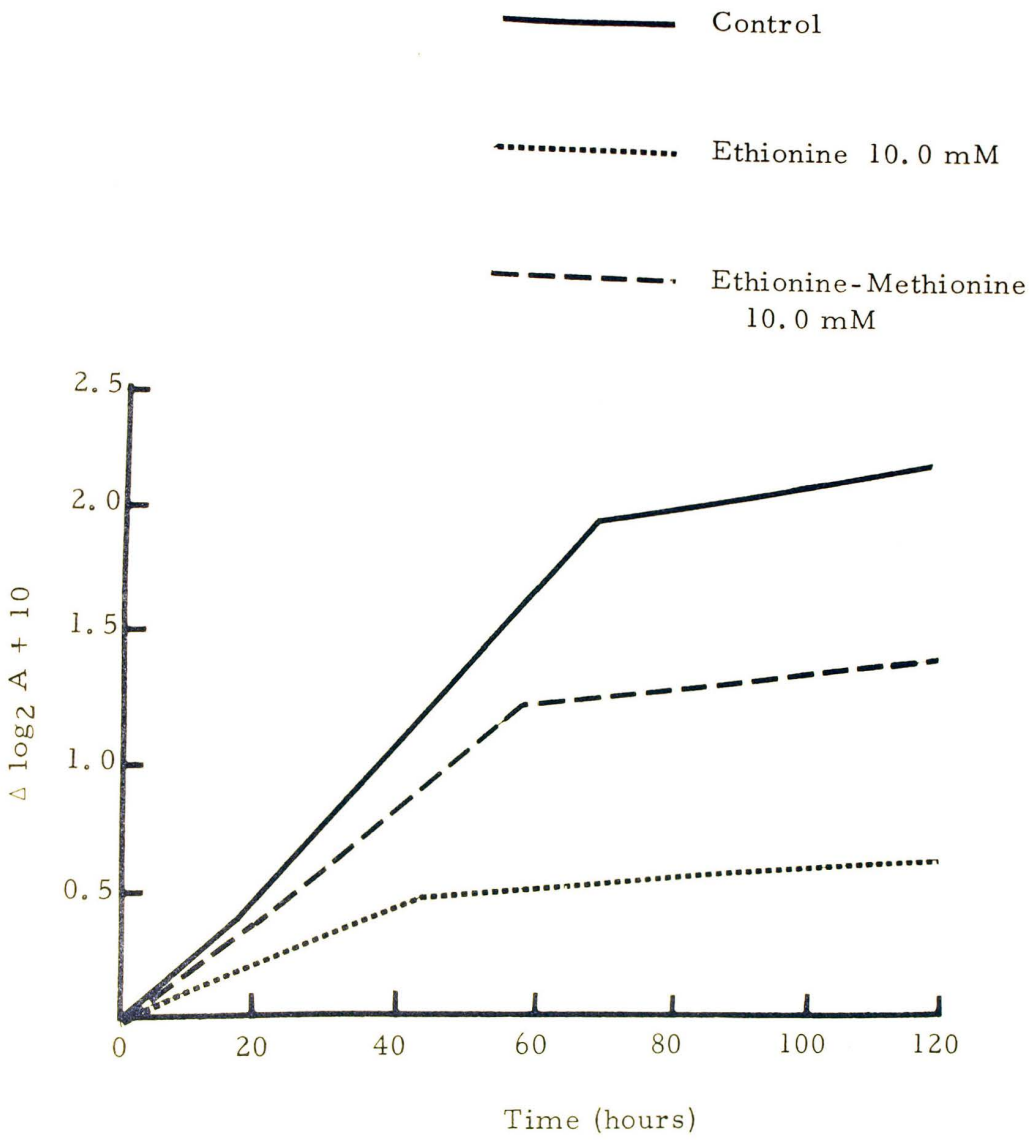

Figure 5. Growth curves of Scenedesmus for the control, 10.0 mM ethionine and 10.0 mM ethionine-methionine. The data represents two experiments of replicate solutions.

Effects of Various Combinations of Ethionine and Methionine on the Growth of Gloeocapsa sp.

Aaronson and Ardois (1971) showed that 0.06 mM ethionine completely inhibited the growth of the blue-green algae Synechococcus cedrorum and Anabaena cylindrica, while the lowest concentration that indicated inhibition was 0.006 mM. In the study by Aaronson and Ardois (1971), the only parameter of growth was cell counts. The purpose of this phase of investigation is to study various concentrations of ethionine and ethionine-methionine upon the growth of the blue-green alga, Gloeocapsa sp. The indices of growth used with Scenedesmus will again be used, and the results will be compared with the results observed in the green alga, Scenedesmus quadricauda.

The total increase in cell numbers (based upon cell counts) is shown in Table V. All concentrations of ethionine inhibited increases in the cell numbers. Equal concentrations of ethionine-methionine reversed the inhibitory effects of ethionine. The most severe inhibition with ethionine occurs at 0.5 mM, and the least amount of inhibition is attained at 1.0 mM. Reversal effects of ethionine-methionine are similar at each concentration.

Statistical analysis indicates a significant difference between the control and ethionine treatments and between the control and ethionine-methionine treatments for the increase in cell numbers. Comparisons of 0.003 and 0.006 mM ethionine denotes no significant difference in the effect of ethionine upon the total increase in cell numbers. Also, at the concentrations of 0.06 and 0.05 mM

Table V

Effects of Various Concentrations of Ethionine and Ethionine-Methionine Upon the Increase in Cell Numbers of Gloeocapsa sp.

Treatment (mM)		Percent of Control
Ethionine	0.003	20.0
Ethionine-Methionine	0.003	47.2
Ethionine	0.006	19.3
Ethionine-Methionine	0.006	45.0
Ethionine	0.06	9.3
Ethionine-Methionine	0.06	53.4
Ethionine	0.50	10.0
Ethionine-Methionine	0.50	55.3
Ethionine	1.00	31.2
Ethionine-Methionine	1.00	41.8

Control population density is 9.3×10^5 cells/ml after 231.0 hours of growth. The data represents one experiment with replicates for each treatment.

ethionine, there is no significant difference in the effects upon the cell numbers.

Table VI shows the effects of ethionine and equal molar concentrations of ethionine-methionine upon the exponential growth rate. All concentrations of ethionine inhibited the exponential growth rate with 0.006, 0.06 and 0.50 exhibiting the greatest degree of inhibition. The students t-test (Bailey, 1959) indicated that there is a significant difference between the control and ethionine treatments, and between the control and equal concentrations of ethionine-methionine. At the ethionine concentrations of 0.006, 0.06 and 0.50 mM, no apparent exponential growth rate can be found. Ethionine-methionine, at all concentrations, tends to reverse the effects of ethionine.

The total increase in absorbance, Table VII, and the chlorophyll-a content, Table VIII, both denote an increase in inhibition as the concentration of ethionine increases, except at 1.0 mM. The t-test (Bailey, 1959) indicates that there is a significant difference between the ethionine treatments, the control, and the ethionine-methionine treatments and control. The effects of equal concentrations of ethionine and methionine upon total increase in absorbance and chlorophyll-a content are similar with reversal being approximately half of the control.

Figures 6 through 10 show the effect of various concentrations of ethionine and ethionine-methionine upon the growth curve of

Table VI

Effects of Various Concentrations of Ethionine and Ethionine-Methionine Upon the Exponential Growth Rate of *Gloeocapsa* sp.

Treatment (mM)		Percent of Control
Ethionine	0.003	30.9
Ethionine-Methionine	0.003	64.1
Ethionine	0.006	0.0
Ethionine-Methionine	0.006	66.1
Ethionine	0.06	0.0
Ethionine-Methionine	0.06	59.6
Ethionine	0.50	0.0
Ethionine-Methionine	0.50	56.1
Ethionine	1.00	19.8
Ethionine-Methionine	1.00	55.6

Control exponential growth rate, after the exponential period of 121 hours was, 0.220. The data represents one experiment with replicates for each treatment.

Table VII

Effects of Various Concentrations of Ethionine and Ethionine-Methionine Upon the Total Increase in Absorbance of *Gloeocapsa* sp.

Treatment (mM)		Percent of Control
Ethionine	0.003	24.0
Ethionine-Methionine	0.003	65.0
Ethionine	0.006	16.6
Ethionine-Methionine	0.006	53.7
Ethionine	0.060	10.8
Ethionine-Methionine	0.060	59.5
Ethionine	0.50	1.6
Ethionine-Methionine	0.50	62.5
Ethionine	1.00	22.1
Ethionine-Methionine	1.00	50.4

Control absorbance is 0.383 nm after 231.0 hours of growth. The data represents one experiment with replicates for each treatment.

Table VIII

Effects of Various Concentrations of Ethionine and Ethionine-Methionine Upon the Chlorophyll-a Content of Gloeocapsa sp.

Treatment (mM)		Percent of Control
Ethionine	0.003	29.1
Ethionine-Methionine	0.003	55.8
Ethionine	0.006	22.2
Ethionine-Methionine	0.006	46.5
Ethionine	0.06	13.6
Ethionine-Methionine	0.06	57.2
Ethionine	0.50	5.5
Ethionine-Methionine	0.50	61.2
Ethionine	1.00	13.0
Ethionine-Methionine	1.00	57.2

The chlorophyll-a content of the control after 231.0 hours of growth is 3.2467 ug/10 ml. The data represents one experiment of replicate treatment.

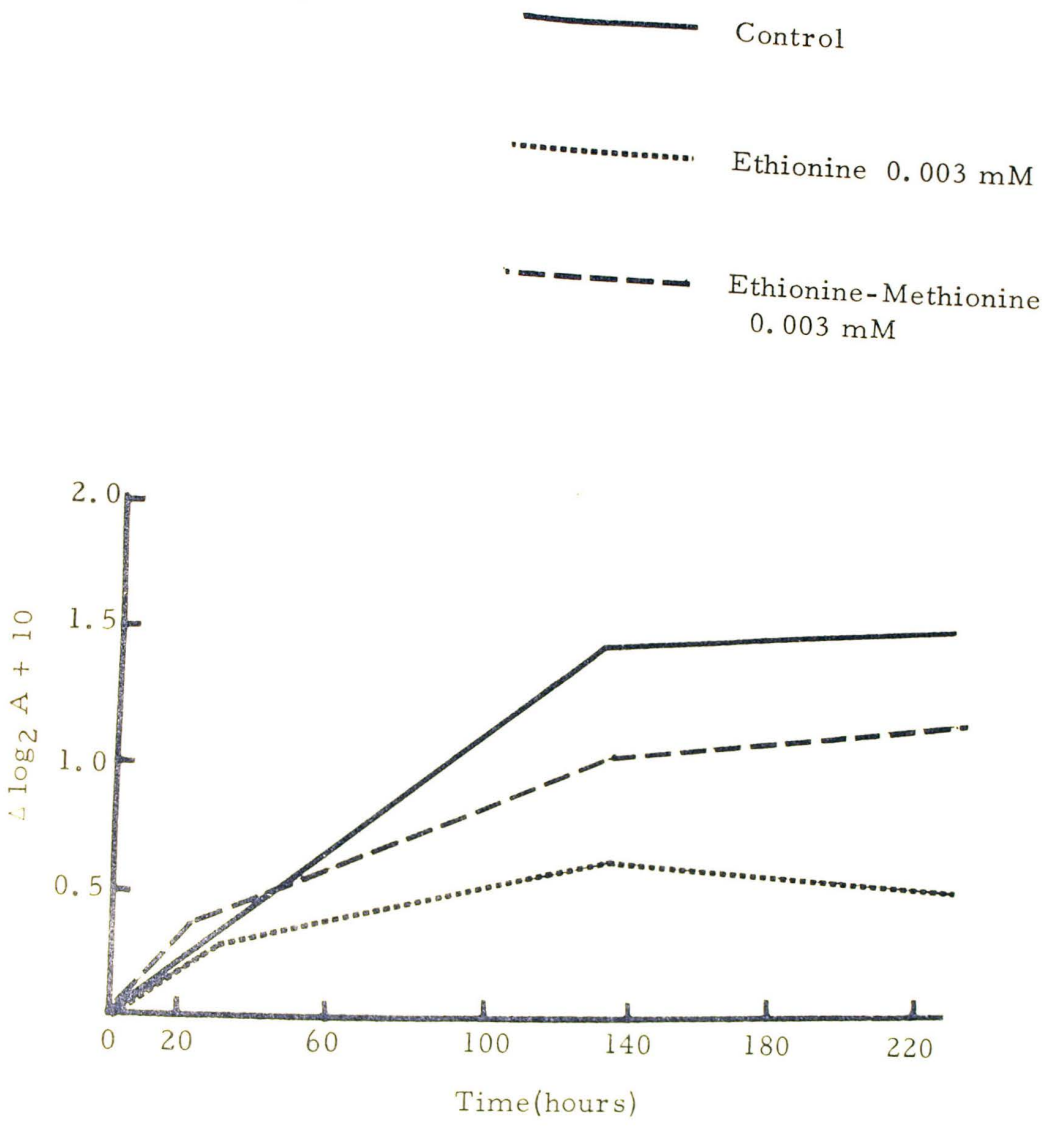

Figure 6. Growth curves of Gloeocapsa sp. for the control, 0.003 mM ethionine, and 0.003 mM ethionine-methionine. The data represents one experiment of replicate solutions.

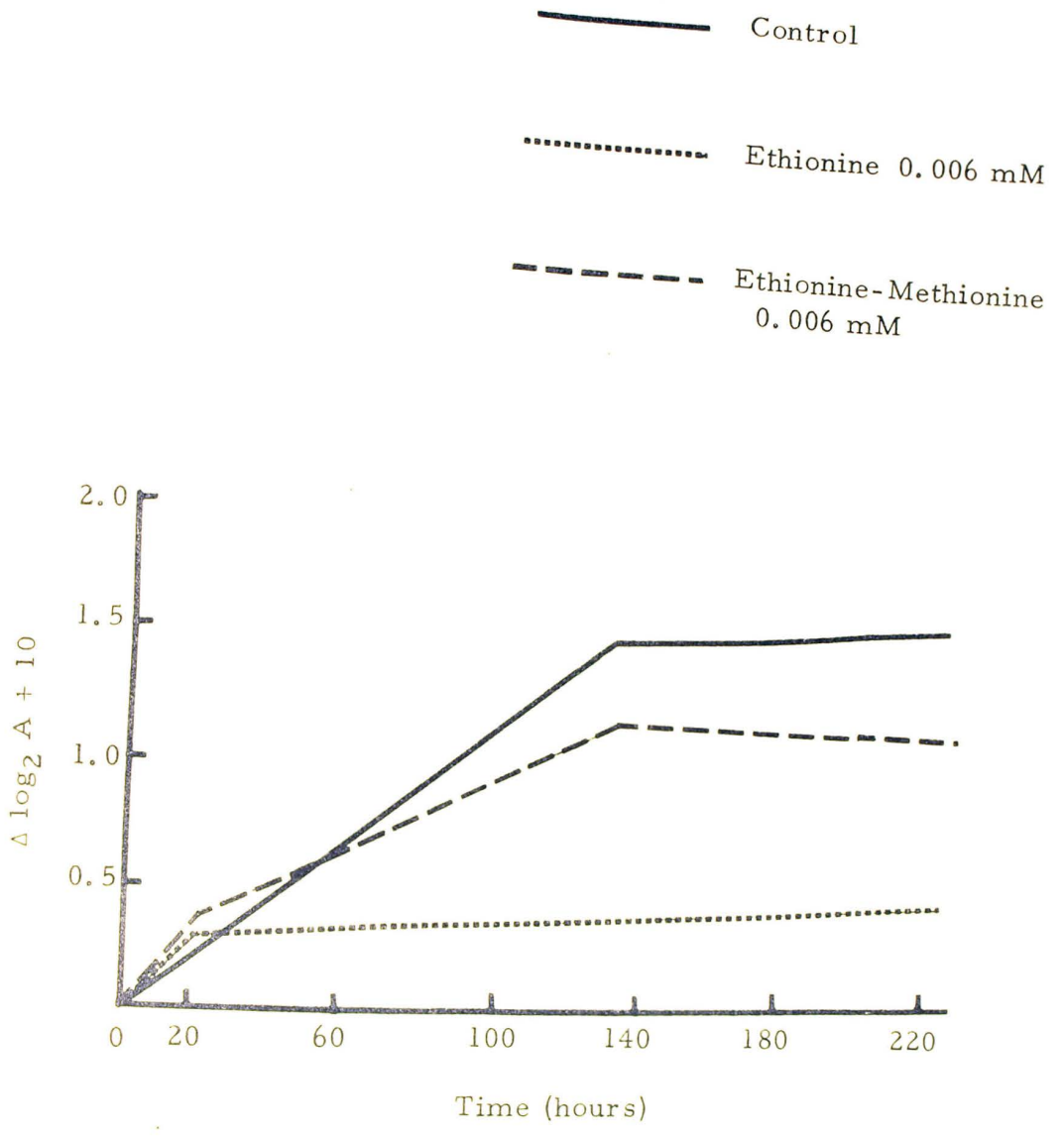

Figure 7. Growth curves of Gloeocapsa sp. for the control, 0.006 ethionine and 0.006 ethionine-methionine. The data represents one experiment of replicate solutions.

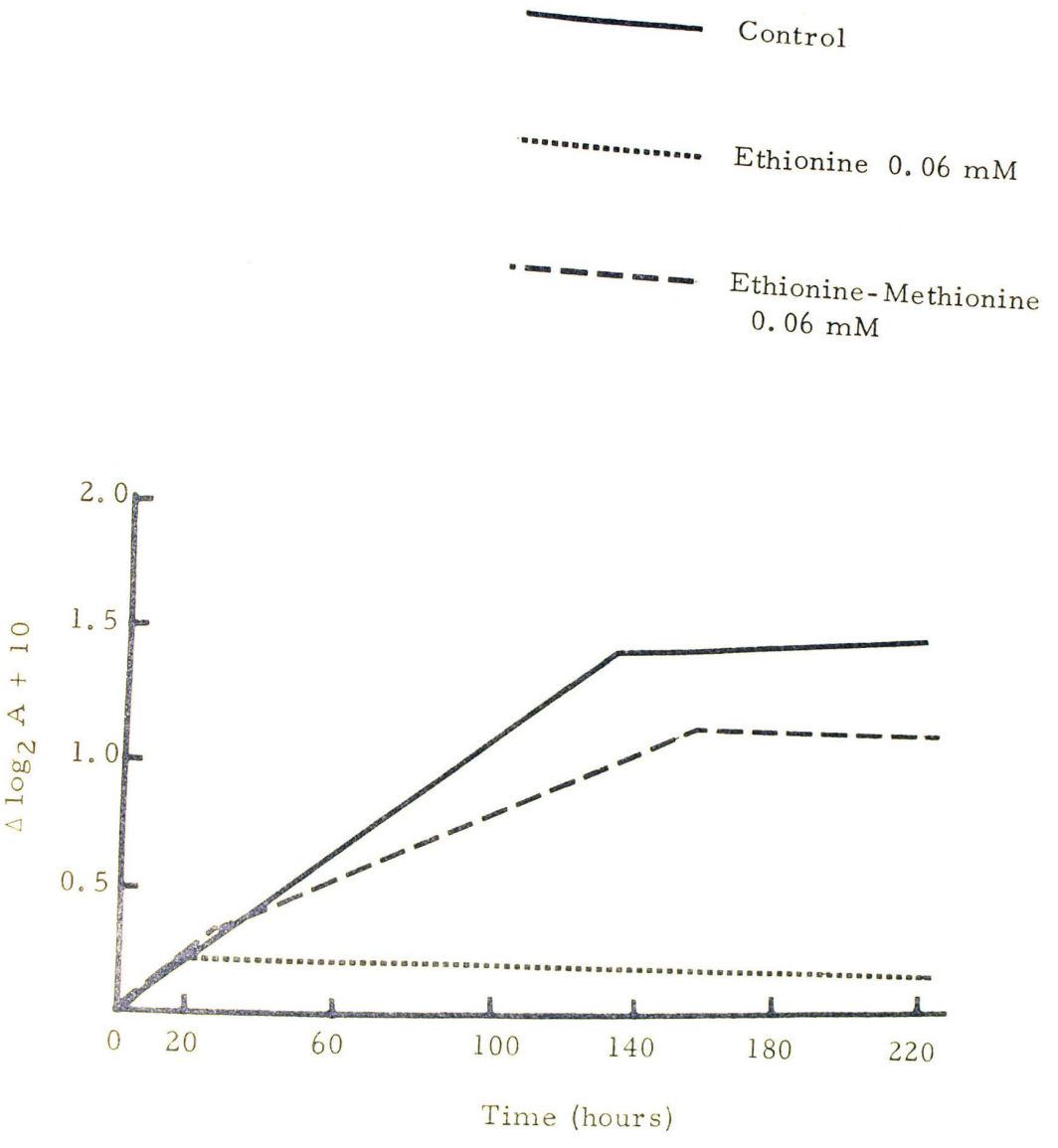

Figure 8. Growth curves of Gloeocapsa sp. for the control, 0.06 mM ethionine, and 0.06 mM ethionine-methionine. The data represents one experiment of replicate solutions.

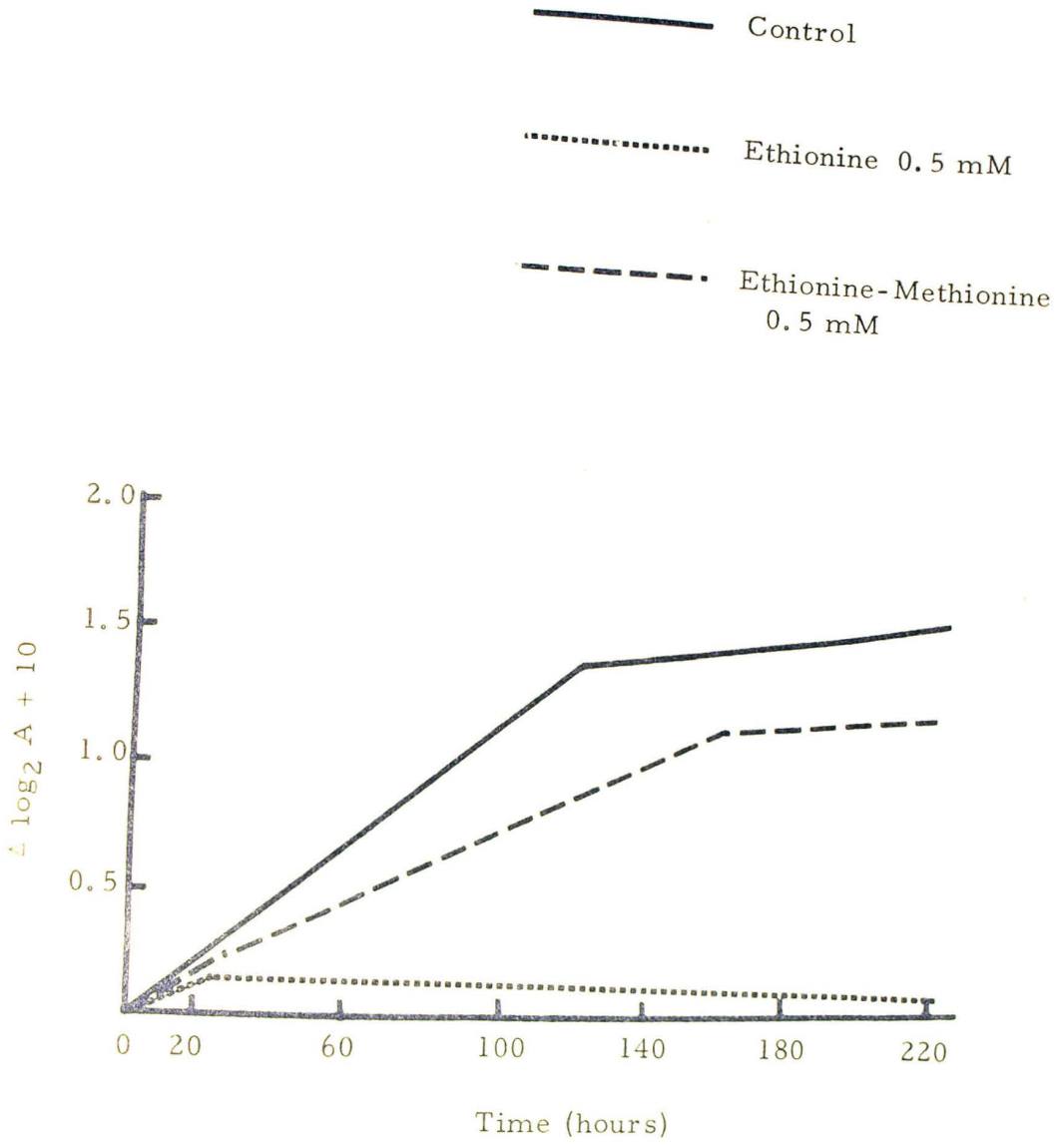

Figure 9. Growth curves of Gloeocapsa sp. for the control, 0.5 mM ethionine and 0.5 mM ethionine-methionine. The data represents one experiment of replicate solutions.

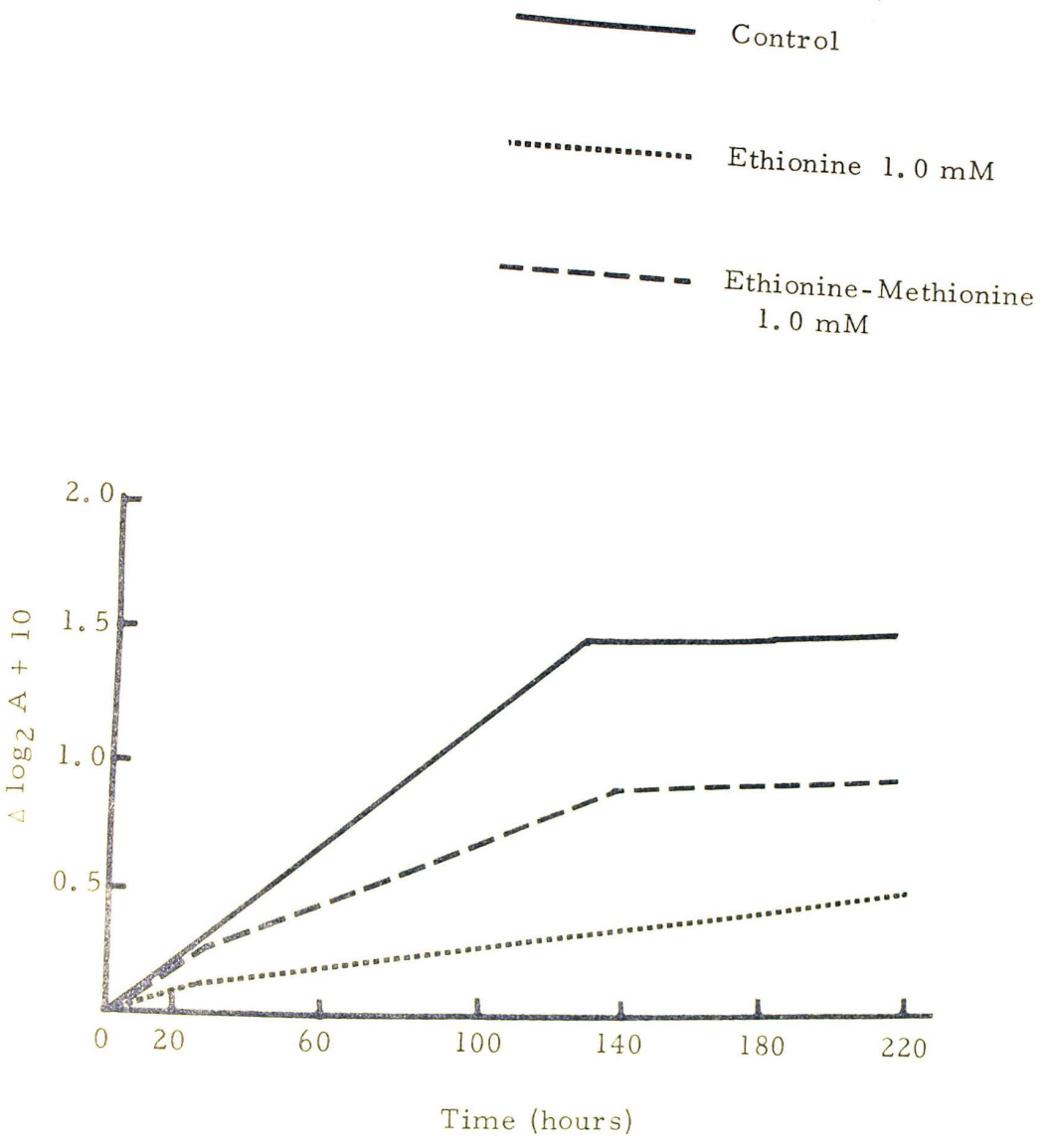

Figure 10. Growth curves of Gloeocapsa sp. for the control, 1.0 mM ethionine, and 1.0 mM ethionine-methionine. The data represents one experiment of replicate solutions.

Gloeocapsa sp. The growth curve is monitored by measuring the absorbance and then calculating the logarithm base two of the absorbance plus ten ($\log_2 A + 10$), (Sorokin, 1973).

The effect of 0.003 mM ethionine upon the growth curve of Gloeocapsa is denoted in Figure 6. The length of the log phase of the ethionine curve is similar to the control, but the slope is not as great. The results of 0.003 mM ethionine-methionine shows that the inhibition of the slope for the log phase is reversed to about half of the control.

Figure 7 illustrates the effect of 0.006 mM ethionine and ethionine-methionine upon the growth of Gloeocapsa. At the concentration of 0.006 mM ethionine, the cells seem to undergo a twenty-four hour period of cell division, then they are completely inhibited with only a slight increase in the absorbance after 231 hours of incubation. Equal amounts of ethionine-methionine at a concentration of 0.006 mM provide an increase in the angle of the log phase. Although 0.006 mM ethionine-methionine does reverse the effects of 0.006 mM ethionine, this reversal is not to the level of the control.

At the ethionine concentrations of 0.06 mM (Figure 8), the effects upon growth are similar to the effects produced at 0.006 mM. There is the presence of a log phase, then growth levels off without an increase near the end of the monitoring period as indicated with 0.006 ethionine. Ethionine-methionine again reverses the effects of ethionine over half of the control.

Figure 9 shows the effects of 0.5 mM ethionine. While the results are similar to those obtained at 0.06 mM, period is more severe. Ethionine-methionine at 0.5 mM reverses the effects of 0.5 mM ethionine.

Ethionine at the concentration of 1.0 mM (Figure 10) does not effect the growth of Gloeocapsa as severely as the other concentrations tested. The period of growth following the log phase does not level off but continues to increase, yet this increase in growth is much lower compared to the control. Ethionine-methionine at 1.0 mM reverses the effects of ethionine at the same concentration.

It should be noted that all concentrations of ethionine-methionine reverse the inhibitory effects of ethionine upon Gloeocapsa at similar levels as compared to the control.

Figures 1 and 11, illustrate a comparison between the average per cent growth based upon the control for the green algae Scenedesmus quadricauda and the blue-green algae Gloeocapsa under various equal molar concentrations of ethionine and ethionine-methionine. In Figure 1 the effect of ethionine upon Scenedesmus and Gloeocapsa are shown. Gloeocapsa seems to be more sensitive to ethionine than Scenedesmus, which is in agreement with Aaronson and Ardois (1971). The idea that the blue-green alga is more sensitive is based upon the fact that Gloeocapsa is inhibited more at lower concentrations than is the green alga. Statistical analysis by the students t-test (Bailey, 1959)

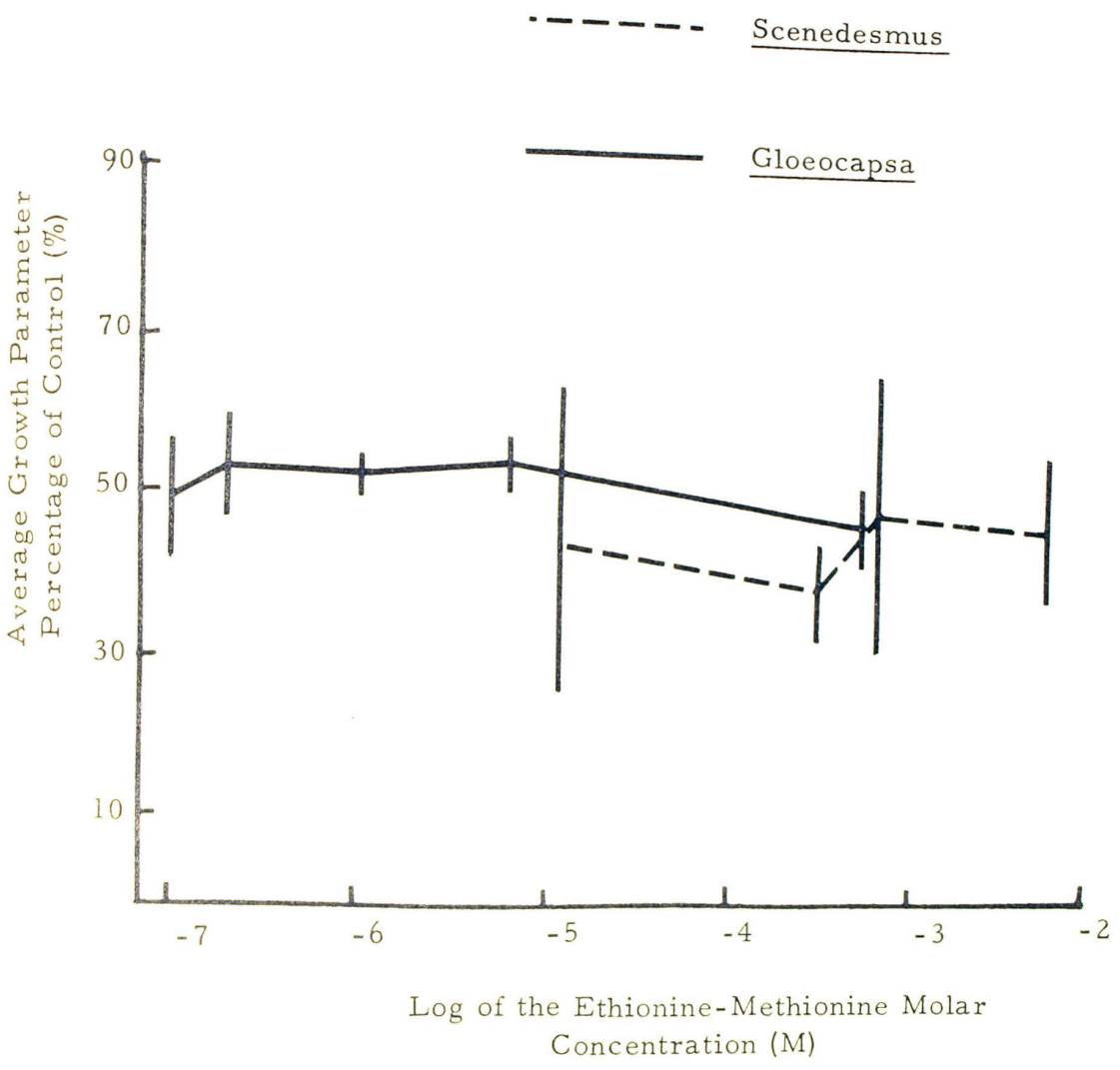

Figure 11. The average percent growth of the control for Scenedesmus quadricauda and Gloeocapsa sp. at various equal molar concentrations of ethionine-methionine. This data represents an average of the measurements obtained from four parameters of growth. Bars represent standard deviations.

indicates that the values obtained for all concentrations of ethionine tested with Gloeocapsa are significantly different from values obtained for Scenedesmus. The same method of statistical analysis shows that there is no significant difference among the values obtained for ethionine treated Gloeocapsa at concentrations of 0.006, 0.06 and 0.5 mM, or between the concentrations 0.003 and 1.0 mM. So the greatest degree of inhibition by ethionine upon Gloeocapsa is in the range 0.006 to 0.5 mM, more likely from 0.06 to .05 mM. The least amount of inhibition by ethionine seems to be at concentrations of 0.003 and 1.0 mM. The concentration tested at which the most severe inhibition of growth occurs to Gloeocapsa (0.5 mM) is also the concentration at which Scenedesmus is least inhibited (Figure 1).

In Figure 11 equal molar concentrations of ethionine-methionine affect both Gloeocapsa and Scenedesmus in similar fashions. Only the Gloeocapsa curve indicates that ethionine-methionine at all concentrations reverses the effects of ethionine. The students t-test (Bailey, 1959) denotes that there is no significant difference at the 95 per cent level between the values obtained for the ethionine-methionine treatments of Gloeocapsa and Scenedesmus.

The results of this investigation show that ethionine at all the concentrations tested will inhibit to some degree the growth of Scenedesmus and Gloeocapsa. In most cases as the concentration of ethionine is increased, the amount of inhibition is increased, except

with the effect observed upon Gloeocapsa at the concentration of 1.0 mM, where inhibition is not as severe as 0.5 mM ethionine.

Gloeocapsa shows a definite tendency to be more sensitive to the inhibitory effects of ethionine than does Scenedesmus. This is true because lower concentrations of ethionine result in greater inhibition of growth in Gloeocapsa than in Scenedesmus.

The reversing effect produced by ethionine-methionine is exerted to a fuller extent with Gloeocapsa than with Scenedesmus. Concentrations of ethionine-methionine, except 0.5 mM, reversed the inhibition by ethionine, although none of the reversals are complete to the level of the control.

Investigations of the growth curves of Gloeocapsa and Scenedesmus indicate that Scenedesmus requires a shorter period of time (117 hours) to complete its growth curve, (from lag phase through early stationary phase) than does Gloeocapsa (230 hours). The effects of ethionine are manifested early in or immediately after the initiation of the lag phase in both algae. The range of time for Gloeocapsa is from 0 to 22 hours into growth and from 0 to 20 hours into the lag phase for Scenedesmus.

This investigation supports the study by Aaronson and Ardois (1971) in that prokaryotic cells, such as the blue-green alga Gloeocapsa, are more sensitive to ethionine than is the eukaryotic green alga Scenedesmus quadricauda. So there may be some safe

and practical use of the carcinogenic amino acid analogue in controlling blue-green alga blooms in fresh water ponds.

SUMMARY

A study of the effects of ethionine, and equal molar concentrations of ethionine-methionine upon the growth of the green alga, Scenedesmus quadricauda and the blue-green alga, Gloeocapsa sp., was conducted.

The growth was monitored by four parameters of growth: 1) absorbance, 2) chlorophyll-a content, 3) cell counts, and 4) exponential growth rate. Also, the growth curves of each alga was studied to determine the effect the various treatments may have upon the growth phases.

The results indicate: 1) all concentrations of ethionine tested caused some degree of inhibition of growth, 2) on an equal molar basis, Gloeocapsa sp. was more sensitive to the inhibitory effects of ethionine than was Scenedesmus quadricauda, 3) the effect of ethionine upon the growth curve of Gloeocapsa was that it prevents the cells from entering an exponential phase of growth, while the cells of Scenedesmus do enter into exponential growth, but the rate of growth was reduced, and 4) most concentrations of ethionine-methionine reversed the inhibitory effects of similar concentrations of ethionine.

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