METABOLISM OF METHYGLYOXAL BY SCENEDESMUS QUADRICAUDA

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THESIS

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Many researchers have combined their efforts in order to elucidate the pathways of carbohydrate metabolism. These efforts established the fact that pyruvic acid and lactic acid occupied important positions in the carbohydrate pathway.

Dakin and Dudley (5,6), in 1913, discovered the enzyme, glyoxalase, which is capable of converting methylglyoxal, a compound similar to pyruvic acid, to lactic acid. This suggested that the enzyme and methylglyoxal could be intermediates in the glycolytic pathway. They postulated that glyoxalase was a two-enzyme complex because they found that both D-lactate and L-lactate were formed when the enzyme acted on methylglyoxal.

Lohman, as cited by Racker (25), discovered that the tripeptide, glutathione, was required as a cosubstrate in this reaction. While Jowett and Quastel (18), in 1933, agreed with Dakin and Dudley that glyoxalase was a two-enzyme complex, it was Racker, in 1951, who used spectrophotometric studies to prove this fact. Racker named the two enzymes glyoxalase I and glyoxalase II. Glyoxalase I (lactoyl-glutathione lyase) catalyzed the formation of the methylglyoxal-glutathione complex, whereas, glyoxalase II (hydroxyacyl-glutathione hydrolase) catalyzed the breakdown
of the methylglyoxal-glutathione complex and the subsequent production of lactic acid from methylglyoxal.

Glyoxalase was found in many biologically active systems by Dakin and Dudley (6). Human liver, heart, kidney, and muscle tissue were found to contain glyoxalase. Additionally, extracts of sheep, cow, dog, cat and rabbit liver showed glyoxalase activity. Yeast and bacterial extracts also showed the presence of glyoxalase (28).

Dakin and Dudley (7) found little glyoxalase activity in the pancreas and also suggested the presence of a powerful "anti-glyoxalase" compound present in this organ.

Szent-Gyorgyi, Egyud, and McLaughlin (30) found the substrates of glyoxalase, ketoaldehydes, present in all living systems tested except cancerous tissue. Egyud and Szent-Gyorgyi (12) demonstrated that methylglyoxal, in a 0.001M concentration, inhibited cell division in Escherichia coli. Szent-Gyorgyi, Hegyeli and McLaughlin (25) called glyoxalase, "promine", a growth stimulator, and methylglyoxal, "retine", a growth retarder. In all systems tested, they found methylglyoxal, in a 0.001M concentration, was inhibitory to cell proliferation. They suggested that glyoxalase was at least secondarily related to cell division. Methylglyoxal, by acting on glyoxalase as a substrate inhibitor, could inhibit cell division.

Infrared and ultraviolet spectrophotometric studies performed by Egyud (11) showed that retine was a compound
which contained an alpha-ketoaldehyde moiety. Methylglyoxal was an alpha-ketoaldehyde. He said that retine was either methylglyoxal or a derivative of methylglyoxal.

Krebs, as cited by Downes (10), demonstrated that glyoxalase and methylglyoxal were probably not integral parts of the glycolytic pathway.

Most of the experiments with methylglyoxal and glyoxalase interactions have been done on life-forms other than plants, but Strzinek and Vance (unpublished) studied the effects of methylglyoxal on green and blue-green algae. They found the green alga, Scenedesmus quadricauda, exhibited increased growth, compared with controls, when treated with 0.001 molar methylglyoxal. The growth of the blue-green alga, Anabaena flos-aquae, did not appear to be affected by methylglyoxal. These results were surprising and suggested further studies of the enzyme system were necessary.

Reeves and Ajl (26) reported an enzyme capable of detoxifying compounds related to methylglyoxal. They proposed a glyoxalase-based enzyme system capable of converting hydroxypyruvic acid to 1,3-diphosphoglyceric acid. They postulated an enzyme system which could detoxify methylglyoxal by converting it to hydroxypyruvic acid. The resultant hydroxypyruvic acid could then be converted to 1,3-diphosphoglyceric acid and incorporated into either the glycolytic or tricarboxylic acid pathway.
Carl Monder (22,23), discovered an enzyme, in beef liver extracts, which converted methylglyoxal directly to pyruvic acid. This was a NAD-requiring alpha-ketoaldehyde dehydrogenase.

If this enzyme was present in *S. quadricauda*, it would account for the production of an available carbon source, pyruvic acid, from methylglyoxal, in the algal system.

Possible explanations for the increased growth of *S. quadricauda* when treated with methylglyoxal included: substrate induction of glyoxalase and the subsequent behavior of this enzyme in a very unusual manner, or, the induction of some other enzyme in the system. If another enzyme were present, it would have to be capable of converting methylglyoxal to some usable metabolite such as pyruvic acid. The purpose of this study was to investigate the metabolic pathways of methylglyoxal, in *S. quadricauda*. 
MATERIALS AND METHODS

Organisms and Instrumentation

*Scenedesmus quadricauda* (Indiana University Culture Collection # 614) and *Anabaena flos-aquae* (Indiana University Culture Collection # 1444) were used as test organisms in the experiments. *S. quadricauda*, a green alga, was cultured in Bristol's medium, as modified by Bold (3). The algal cultures were grown in a Percival growth chamber under continuous light (Cool-Ray fluorescent bulbs) of 275-300 foot-candles. One milliliter of algal stock was inoculated into forty-nine milliliters of Bristol's medium. The optical density, at 705 μm, was adjusted to 0.22 by the addition of additional algal stock solution, if needed. Enough methylglyoxal was added to the test flasks to bring the methylglyoxal concentration to 0.001 molar. Optical densities, used in charting the algal growth curve, were measured on a Coleman 124 Twin-Beam Spectrophotometer.

Batch cultures of *S. quadricauda*, for enzyme studies, were grown in a twenty-two liter, spherical flask under the same conditions described above, except, filtered air, at the rate of 4 liters per minute, was passed through the batch cultures for agitation. Sterile cotton filters were
used in the air conduits to filter out any air-borne contaminants. Algal cultures were isolated on Bristol's agar plates. Samples from these isolated colonies were used to inoculate the experimental medium. This procedure reduced the bacterial contamination of the algal cultures. The medium was sterilized at 120 C and 17 pounds per square inch of pressure for 20 minutes.

The blue-green alga, *A. flos-aquae*, was cultured in #11 medium, as described by Hughes (16). Optical density measurements were taken at 687 mu. Except for the above, the blue-green alga was treated in the same manner as the green alga. After the alga had been stimulated (7 days after inoculation), 0.001 M methylglyoxal was again added to the system. The algal culture was then observed and growth characteristics monitored by the methods described above.

The blue-green alga was not affected by the treatment with 0.001 molar methylglyoxal. Consequently, *S. quadricauda* (the green alga) was used for further experimentation to elucidate the cause for its unexpected behavior when treated with methylglyoxal.

**Preparation of Cellular Extracts**

*Scenedesmus quadricauda* was cultured in 0.001 molar methylglyoxal until the third day of the logarithmic growth. The control cells were grown in the same manner without methylglyoxal. Both the test and control cells were harvested
at the same time (6-7 days after inoculation) by centrifuga-
tion on an International (Model HN) centrifuge at 800
X g for ten minutes. Approximately five grams (wet weight)
of the cells were placed in a cold (-15 C) mortar. One-
half milliliter of Tris buffer (pH=9.6) was added to a
mortar along with one-half milliliter of cold (-15 C)
acetone. The cells were ground with a pestle for ten
minutes. The bottom of the mortar was then immersed in an
acetone-dry ice bath until the mixture began to freeze.
The mortar was then removed from the acetone-dry ice bath,
one-half milliliter of cold acetone added, and grinding
continued. This freezing, addition of cold acetone, and
grinding procedure continued for two hours or until micro-
scopical examination revealed approximately thirty per cent
of the cells had been lysed. The mixture was then centri-
fuged at 800 X g for five minutes. The supernatant was
designated "cellular extract." Tests were run on this
supernatant immediately after the extraction procedure
was completed.

Protein Assay

Protein determinations were made using the Lowry method
(20). One part of cellular extract was combined with one
part 0.5 N NaOH. One-hundred milliliters of two per cent
Na2CO3 was placed in a 250 ml Erlenmeyer flask. To this
Na2CO3 were added, in order, 1.0 ml of 1.0 per cent CuSO4
and 1.0 ml of 2.7 per cent sodium tartrate. The resulting mixture was referred to as the carbonate reagent. One milliliter of the cellular extract: NaOH was added to 5.0 ml of the carbonate reagent and allowed to stand at room temperature for ten minutes. After ten minutes, a 0.5 ml aliquot of one part Folin Phenol Reagent (Fisher Chemical Co.) and one part distilled water was added to the tube containing the carbonate reagent plus the cellular extract. The mixture was allowed to stand at room temperature for thirty minutes. A positive protein test was indicated if the color of the mixture changed from yellow to blue. The amount of protein per milliliter of cellular extract was then determined for a standard curve made with beef albumen (Fig. 1).

**Fig. 1**—Standard protein curve (Lowry Method used for assay of ug protein per milliliter of cellular extract. Bovine albumen was used as the protein-source for the curve.)
The spectrophotometer was standardized against a blank which was treated the same way as described for the test sample, except, in the blank, one milliliter of distilled water was substituted for the cellular extract.

**Methylglyoxal Assay**

Determinations of methylglyoxal (Sigma Chemical Co.) concentrations were made by the method of Ariyama (1), as modified by McKinney and Gocke (21). A 0.5 ml aliquot of methylglyoxal was added to 2.5 ml distilled water to give a final volume of 3.0 ml. Then, in order, were added 0.1 ml of arsenophosphotungstic acid reagent prepared by the method of Ariyama, 0.1 ml of 1.0 molar NaCN, and 0.3 ml of 1 molar Na$_2$CO$_3$. The solution was allowed to stand at room temperature for sixty minutes. The resulting blue mixture had a maximum absorption between 705 and 710 μm. The color was stable at room temperature for 200 minutes. Cooling and heating the mixture did not affect color development. After one hour, the optical density of the solution was determined and a standard reference curve prepared (Fig. 2). The standard curve was prepared by the method of Neiswanger (24). A blank was prepared using 3.0 ml of distilled water instead of the enzyme extract.
Analysis for Glyoxalase Activity

The analysis for glyoxalase activity was done by the procedure of Ariyama (1), as modified by McKinney and Gocke (21). This procedure determines glyoxalase activity by examination of the disappearance of methylglyoxal.

The reaction mixture consisted of the following: 0.5 ml of the enzyme extract; 0.05 mg reduced glutathione
(Nutritional Biochemicals Corp., Cleveland, Ohio) in 2.0 ml distilled water; 0.5 ml of 0.12 per cent methylglyoxal and 0.3 ml of 1.3 per cent NaHCO₃. This mixture was incubated under nitrogen (99.99 per cent pure nitrogen) for thirty minutes. After incubation, 0.5 ml of the incubation mixture was added to 8.5 ml of 0.083 N H₂SO₄ followed by 1.0 ml of 3.0 per cent sodium tungstate. The resulting solution was centrifuged at 5°C and at 5,000 X g for ten minutes.

A 0.5 ml aliquot of the supernatant, from the incubation mixture, was added to 2.5 ml distilled water. Then, in order, were added 0.1 ml of arsenophosphotungstic acid reagent prepared by the method of Ariyama, 0.1 ml of 1M NaCN, and 0.3 ml of 1M Na₂CO₃. This solution was allowed to stand at room temperature for sixty minutes. The blue color which developed indicated the presence of methylglyoxal. The more intense the color, the greater the concentration of methylglyoxal in the sample. After the color had developed, the optical density was read on a Coleman 124 Twin-Beam Spectrophotometer at 705 μm. This optical density was designated "O.D. at thirty minutes time".

A blank was prepared that contained 3.0 ml of distilled water instead of the enzyme extract. The blank was treated in the same way as the enzyme mixture except that immediately after the addition of 0.5 ml of enzyme extract to 0.5 ml of methylglyoxal, a 0.5 ml aliquot of this solution was added to 8.5 ml of 0.083 N H₂SO₄ and precipitated with
3 per cent sodium tungstate. This inhibited any enzymic reaction that could have taken place. This sample was treated identically to the test reaction mixture and the optical density recorded at "zero time" was indicative of the amount of methylglyoxal initially present. The optical density at "zero time" minus the "O.D. at thirty minutes time" showed the amount of methylglyoxal converted to lactate in thirty minutes. Enzyme activity was expressed as micromoles (u mole) of methylglyoxal converted to lactate in thirty minutes per microgram protein (u mole methylglyoxal converted/ 30 min/ protein).

Method for Isolation of Glyoxalase I

The attempted isolation of glyoxalase I (E.C.4.4.1.5) was by the method of Davis and Williams (8). An acetone powder was obtained (7 grams) by the method described above. The acetone powder was extracted with 180 ml of 66 µM phosphate buffer (pH=7.0) for two hours at room temperature. It was then centrifuged at 4 C and 14,500 X g for ten minutes. The resulting pellet was then re-extracted with 200 ml of the phosphate buffer for thirty minutes and centrifuged again. One-hundred milliliters of n-butanol were added to the extract and the mixture was agitated at 37 C for ten minutes. The mixture was then centrifuged at 800 X g for ten minutes. This centrifugation separated the n-butanol fraction from the aqueous fraction. The aqueous layer was removed by suction through a pipette
inserted through the bulky, green precipitate which was suspended in the n-butanol layer. The acetone fractionation was carried out in the following manner. To each 100 ml of extract (aqueous layer), 70 ml of cold acetone (-10 C) was added. The mixture was cooled in an ice-rack salt bath and constantly stirred. The precipitate which formed was removed by centrifugation at 14,500 X g for ten minutes. An aliquot of the resulting supernatant was taken and combined with an equal amount of cold acetone. This mixture was centrifuged as above. The precipitate that formed from this centrifugation was dissolved in a minimal amount of distilled water and centrifuged at 4,000 X g for ten minutes. The resulting supernatant was assayed for glyoxalase I activity.

Glyoxalase I activity was ascertained by the following process. Into a cuvette of 1 cm light path was placed, in order, 2.74 ml of 0.1M imidazole-HCL buffer (pH=6.8), 0.05 ml of 16.0 mM MgSO$_4$, 0.05 ml of 0.85 mM reduced glutathione, and 0.10 ml of 1M methylglyoxal. The mixture was allowed to stand for ten minutes and then 0.10 ml of the enzyme extract was added to the cuvette. The change in optical density at 240 mu was recorded and the initial slope of the line (for the first two minutes of the reaction) was proportional to the amount of S-lactoylglutathione formed during this period. This gave a measurement of the glyoxalase I activity.
Methods of Methylglyoxal and Glucose Assay

Growth curves of *S. quadricauda*, treated with 0.001M methylglyoxal, 0.001M glucose, and 0.0005M glucose were compared with each other. Optical density studies and cell counts (using a Petroff-Hauser bacteria counter) were made to measure any differences in growth in the algal cultures.

Methylglyoxal concentrations were measured at twelve-hour intervals by the colorimetric method of Ariyama (1). Glucose concentrations were measured at the same times using the anthrone reagent of Semple's method (27). The anthrone reagent consisted of the following: Two-hundred milligrams of anthrone combined with one-hundred milliliters of ninety-five per cent sulfuric acid. The reaction mixture contained the following: 2.0 ml of the algal medium and 4.0 ml of the anthrone reagent. The reaction components were mixed by swirling and heated in a Lab-Line Instruments (Model 3000-2) water bath for six minutes at 80°C. Immediately after removal from the water bath, the samples were placed in a freezer at -20°C for ten minutes. The optical density, at 625 nm, was recorded. A blank was prepared from anthrone reagent and distilled water. The color remained stable up to 30 minutes if the reaction mixture was stored at 3°C. A standard curve was obtained using varying concentrations of glucose and anthrone reagent (Fig. 3).
Fig. 3-- Standard glucose curve using anthrone reagent.

The *S. quadricauda* cells were separated from the medium by centrifugation at 800 X g for ten minutes.

Method for Assay of α-Ketoaldehyde Dehydrogenase Activity

Tests were run to see if the cellular extract contained the enzyme, alpha-ketoaldehyde dehydrogenase, described by Monder (23). Methylglyoxal assays, in this test, were run by the method of Ariyama (1). Pyruvic acid concentrations were determined by the method set forth in Sigma Chemical Co. Technical Bulletin #726-UV (Oct. 1968).
The reaction mixture consisted of the following: 0.5 ml of 1M Tris buffer (pH=8.6), 0.5 ml of 0.12 per cent methylglyoxal, 1.0 ml of 0.01M NAD$^+$, and 1.0 ml of the cellular extract. The change in optical density, at 340 mu, was proportional to the amount of NAD$^+$ converted to NADH+H$^+$. It was also proportional to the amount of pyruvic acid formed from methylglyoxal. A standard curve was made using varying concentrations of pure NAD$^+$ (Sigma Chemical Co.) and is shown in Fig. 4.

Fig. 4-- Standard curve for NAD$^+$ concentrations.
A blank was made following the above directions and using extracts of *S. quadricauda* control cells in the same manner as the cellular extracts of the test cells. The reaction was allowed to run until no further change in optical density (at 340 mu) was observed. The reaction time varied, with different cellular extracts, from two to five minutes.

Pyruvic acid determinations were carried out by the method mentioned above. Two milliliters of the cellular extract were added to four milliliters of 8.0 per cent perchloric acid at 0 C. The mixture was centrifuged for ten minutes at 800 X g. This precipitated the protein and the supernatant was used for the pyruvate determinations. Into 1.0 mg NADH+H⁺ (Sigma Chemical Co.) was placed 2.2 ml 1M Tris buffer (pH=8.6). The vial was inverted several times to dissolve the NADH+H⁺. Into a cuvette of 1 cm light path was added: 2.0 ml of protein-free cellular extract and 0.5 ml of 1.0M Tris buffer (pH=8.6). The cuvette was inverted several times to reduce the acidity before the addition of the NADH+H⁺. Then, to the above mixture was added 0.5 ml of the solution of 1.0M Tris buffer (pH=8.6) and NADH+H⁺ solution. The cuvette was inverted several times to insure adequate mixing. The optical density was taken at 340 mu. Distilled water was used in the reference cuvette. This optical density of the above mixture was called "O.D. max.". The cuvette was removed from the spectrophotometer and 0.05 ml of lactic dehydrogenase
(Sigma Chemical Co.) was added to the cuvette. The solution was mixed by inversion of the cuvette. The change in the optical density readings of this solution was recorded at 340 μm, using distilled water as a reference. The change was measured until a minimum optical density (O.D. min.) was reached. The "O.D. min." was reached within 2-5 minutes after the addition of the lactic dehydrogenase. The "O.D. max." minus the "O.D. min." equalled the change in optical density (Δ O.D.). Pyruvic acid concentrations were calculated from the following equation:

\[ \text{uMoles pyruvate per ml of sample} = \frac{\Delta \text{ O.D.} \times 3}{6.22 \times 0.667} \]

The amount of pyruvic acid originally present in the cellular extract was compared with the amount of pyruvic acid present after the addition of methylglyoxal and NADH+H⁺.

One-half milliliter of 1.6 mM methylglyoxal, 0.5 ml of 1.6 μM NAD⁺, 0.5 ml of 1.6 μM phosphoglyceric acid dehydrogenase (E.C.1.2.1.9.) and 0.5 ml 1M Tris buffer (pH=8.6) was added to the cuvette. A blank was prepared from 1M Tris buffer and the change in NAD⁺ concentration was monitored at 340 μm. Tests for methylglyoxal consumption and pyruvate formation were done by the methods mentioned above.

Enzyme inhibition experiments, with 0.001M p-chloromercuribenzoate, were run to see if the enzyme activity
was affected by this inhibitor. Cysteine was used in 0.001M levels to see if it was capable of reversing the inhibition of the enzyme by 0.001M p-chloromercuribenzoate.
RESULTS

Methylglyoxal did not show any effect on the blue-green alga, *Anabaena flos-aquae*, but *Scenedesmus quadricula*, the green alga, had increased in number three days after treatment with 0.001M methylglyoxal (Fig. 5).

![Graph showing the effect of 0.001M methylglyoxal on the growth of *Scenedesmus quadricula*.](image)

*Fig. 5-- Effect of 0.001M methylglyoxal on the growth of *Scenedesmus quadricula*.***
Fig. 6 shows the comparison of optical density values, at 705 μm, versus time for *S. quadricauda*.

Since there was not a linear correlation between cell number per milliliter and optical density in the cultures, Fig. 7 was constructed and used to correlate optical density and cell number per milliliter.
Beer's law did not apply for the algal cultures as the algal cells progressed through various stages of their life cycle. The graph correlating cell counts and change in optical density reconciled these two variables. Cell counts were made to an accuracy of $\pm 5.0 \times 10^5$ cells per milliliter. Since the final difference in cells per milliliter between the treated and control cells was 10 million cells per milliliter, the test was statistically valid.

Fig. 8 shows the difference in cell size, after seven days, between the test and control cells. The control cells were approximately twice as large as the treated cells.
Fig. 8 -- A comparison of size between *S. quadricauda* treated (0.001M methylglyoxal) and control cells. 1300 X.

Fig. 9 -- Effects of 0.0005M and 0.001M glucose, and 0.001M methylglyoxal on the growth of *S. quadricauda*.
Results shown in Fig. 9 indicate that after the alga had begun to metabolize methylglyoxal, methylglyoxal was a more utilizable carbon source than glucose. The alga began to metabolize the glucose after approximately twenty-four hours. Again, the characteristic seventy-two hour "adaptation" period, before the alga began to use methylglyoxal was noted.

The attempted isolation of glyoxalase I (E.C. 4.4.1.5) yielded no glyoxalase activity. The protein yield of the test cellular extract was 250 ug per milliliter.

Pyruvic acid assays, before and after the addition of methylglyoxal to the reaction mixture, showed 5±1 umoles of pyruvate formed per 8±4 umoles methylglyoxal added to the reaction mixture. Spectrophotometric tests, at 340 mu, run to determine changes in NAD⁺ concentrations, showed 3±1 umoles of NAD⁺ were consumed in the above reaction. Activity was found in the cellular extract which precipitated with 50-60 per cent (NH₄)₂SO₄ at 0°C. The 0.5 ml of methylglyoxal (8±4 umoles), when added to the reaction vessel containing 0.5 ml NAD⁺ (8±1 umoles) and 2.0 ml of the cellular extract, was consumed at the rate of 2.5 umoles per 250 ug protein per minute. At the end of two minutes, 5±1 umoles of pyruvate was formed and 3±1 umoles of NAD⁺ was consumed.

The alga which was treated twice with 0.001 M methylglyoxal showed growth stimulation within twenty-four hours.
The second aliquot of methylglyoxal was applied on the seventh day of the experiment.

Methylglyoxal was not converted to pyruvic acid by phosphoglyceric acid dehydrogenase (E.C. 1.2.1.9.). No enzyme activity was observed under the conditions described in the materials and methods section of this paper.
DISCUSSION

Methylglyoxal, reported by Szent-Gyorgyi (29,30,31) to be a growth retarder (retine), was found to enhance growth in \textit{Scenedesmus quadricauda} at 0.001M concentrations. The data obtained showed the treated culture to have twice as many cells per milliliter, after seven days, as the control culture.

No glyoxalase activity was observed in the cellular extracts obtained by the methods of McKinney and Gocke (21) and Williams and Davis (8), respectively. Either these assay procedures did not work on the system in question, or, glyoxalase may not be present in all living systems.

Alpha-ketoaldehyde dehydrogenase, an enzyme reported by Monder (22), is capable of converting methylglyoxal to pyruvic acid. This enzyme was a \textit{NAD}^+ requiring enzyme, and the conversion of methylglyoxal to pyruvic acid did occur under aerobic conditions. The reaction did not occur anaerobically. Para-chloromercuribenzoate (0.001M) inhibited the conversion of methylglyoxal to pyruvic acid. A partial remission of this inhibition could be affected by adding 0.5 ml of 0.001M glutathione to the above mixture containing cellular extract and 0.5 ml of 0.001M \textit{p}-chloromercuribenzoate. This inhibition and reversal of inhibition, described above, indicated that the enzyme contained sulfhydryl bonds.
No methylglyoxal was converted to pyruvate by phosphoglyceric acid dehydrogenase (E.C.1.2.1.9.). This fact lends support to the assumption that the enzyme of this study is an alpha-ketoaldehyde dehydrogenase. The different structural configurations between \( \alpha \)-ketoaldehydes and aliphatic aldehydes, suggest that one enzyme cannot, at the same time, use both types of aldehydes as a substrate.

Many workers have found that glyoxalase concentrations are higher in cancerous tissue than in normal, healthy tissue. This cancerous state could be due to the induction or increased production of alpha-ketoaldehyde dehydrogenase by some unknown stimulus. The alpha-ketoaldehyde dehydrogenase could metabolize the "retine" (methylglyoxal) which previously held the "promine" (glyoxalase) in check. This excess of glyoxalase could then cause the characteristic rapid, uncontrolled growth of cancerous tissue.

The glycolytic pathway and tricarboxylic acid cycle were elucidated to the satisfaction of most biochemists long before this study was initiated. The results of these experiments showed methylglyoxal to be a more readily utilizable carbon source by algae than glucose. This was logical, considering the ease with which methylglyoxal could be converted to pyruvate via an enzyme system such as alpha-ketoaldehyde dehydrogenase. Methylglyoxal needs to be modified only once to produce pyruvate, whereas, glucose needs nine modifications to yield pyruvate. These modifications require more cellular energy than the one-step
conversion of methylglyoxal to pyruvate.

The results that showed an 8:5:3 ratio for methylglyoxal: pyruvate: NAD$^+$, respectively, indicated that methylglyoxal was being converted to pyruvate. The ratios, with a pure enzyme of alpha-ketoaldehyde dehydrogenase, should have been 1:1:1. The cellular extract had many impurities, some of which probably—by consuming and regenerating NAD$^+$ and pyruvate—caused the departure from the 1:1:1 stoichiometric ratio.

Inhibition studies, using protein synthesis inhibitors, would probably be of value in elucidating the aspects of inducibility. Chloramphenicol, and similar protein synthesis inhibitors, would lend themselves to a study of this type.

Kinetic studies, upon purification of the enzyme, would further aid in the elucidation of the nature and reactivity of the proposed alpha-ketoaldehyde dehydrogenase system in *S. quadricauda*. Studies with radioactivity-labeled methylglyoxal would be of value in elucidation of the reaction sequence in the conversion of methylglyoxal to pyruvic acid. Purification procedures, using thin-layer and column chromatographic techniques, are in progress. The results of these experiments, coupled with the other suggested experiments, should help to identify the nature of the enzyme system being studied.
SUMMARY

Methylglyoxal, described by Szent-Gyorgyi et al. (30) as a growth retarder, was found to stimulate growth in Scenedesmus quadricauda.

An enzyme, showing many characteristics of the alpha-ketoaldehyde dehydrogenase reported by Monder (22), was found in S. quadricauda cellular extracts. This enzyme seemed to be induced in the green alga approximately seventy-two hours after the application of 0.001M methylglyoxal. The stimulation of growth apparently was due to the production of pyruvic acid, a carbon source, from methylglyoxal.
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