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The Effects of Light Quality and Culture Age on Protein Synthesis in Oscillatoria Species

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THE EFFECTS OF LIGHT QUALITY AND CULTURE AGE ON PROTEIN
SYNTHESIS IN OSCILLATORIA SPECIES

by

Jon Chesnut

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
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TABLE OF CONTENTS

	PAGE
INTRODUCTION	1
REVIEW OF LITERATURE	2
GROWTH OF ALGAE	2
AMINO ACIDS OF ALGAE	4
LIGHT INTENSITY AND QUALITY	6
MATERIALS AND METHODS	11
ALGAE	11
PROTEIN EXTRACTION AND HYDROLYSIS	14
CHROMATOGRAPHIC TECHNIQUE	15
¹⁴ C ₂ PHOTOSYNTHESIS EXPERIMENTS	16
DETERMINATION OF AMINO ACID ACTIVITY	16
OBSERVATIONS AND RESULTS	18
GROWTH OF ALGAE	18
AMINO ACIDS OF <u>OSCILLATORIA</u>	18
DISTRIBUTION OF ¹⁴ C AMONG THE AMINO ACIDS	21
DISCUSSION AND CONCLUSIONS	26
SUMMARY	29
LITERATURE CITED	30

INTRODUCTION

Since the advent of chromatographic techniques and radioactive labeling, there has been an increase in investigations concerning metabolic and sub-cellular activities in blue-green algae. Researchers have traced the pathways of carbon in photosynthesis while growing plants in varying wave lengths of light. Experiments in this area have dealt with various algae such as Chlorella, Microcystis, Chlamydomonas, Scenedesmus, and Phormidium.

Previous investigators such as Hess and Tolbert (1967) have extracted the free amino acids with alcohol. The proteins were not analyzed. The final pathway of free amino acids is the proteins they eventually form, therefore it is important to include a study of carbon fixation by analyzing the amino acids in hydrolyzed protein.

This thesis will consider the following: The bound amino acid composition of Oscillatoria by paper partition chromatography. The analysis of labeled hydrolyzed protein after the algae have been grown under red, blue or white light and the effect of culture age on protein synthesis. The method employed for an efficient culturing method for Oscillatoria.

REVIEW OF LITERATURE

GROWTH OF ALGAE

In studying algae, care must be taken to achieve and maintain cultures that are as bacteria-free as possible. The presence of microorganisms may affect the final results of metabolic and nutritive studies. Fogg (1965) stated that the initial objective is usually to isolate the organism in axenic culture, which is commonly a difficult task for algae and is perhaps achieved far less often than claimed. Even rigorous tests for the presence of bacteria may fail to demonstrate those with unusual nutritional requirements. Zobell (quoted by Fogg, 1965) established that bacterial development in algal cultures is proportional to the surface area of the vessel containing the culture.

Parsons and Strickland (quoted by Fogg, 1965) demonstrated a definite uptake of organic substances by a natural phytoplankton population. They were interested in the particulate carbon production by heterotrophic processes in a marine environment. It was not stated whether bacteria played a significant role in the utilization of the radioactive tracer.

Since difficulties are encountered in the isolation and purification of Cyanophyta, Krauss (1966) irradiated sixteen blue-green species with a gamma emitting cobalt-60 source. After varying doses and time rates, a sample of algae was streaked out on trypticase soy agar to test for bacterial growth while another

sample was sub-cultured into fresh medium to observe algal growth. Four hours irradiation with 260,000 rads was sufficient in producing a bacteria-free culture in two species of Oscillatoria: *brevis* and *tenuis*. No observable algal damage was recorded after irradiation.

The initial isolation technique described by Smith (1951) involved an agar plate upon which the algae were grown. Agar concentration was two per cent. The water used for dissolving the agar contained twenty-five per cent (by weight) nutrient substances. Smith recommended isolation of algal cultures by plating if: (1) The cells are too small to be picked up under the microscope. (2) The creeping organisms such as many blue-green algae which attach themselves to solid surfaces and may be injured by pipetting. However, they can be transferred with bits of agar. (3) Some algae grow easily on agar which develop either poorly or not at all in liquid media.

The agar plates were inoculated by spreading, and then placed in a cool, well lighted room. The peripheral, motile filaments were then transferred to sterile liquid media.

With studies on lag phases and inocula sizes, Gerloff (quoted by Fogg, 1965) stated that only large inocula of planktonic blue-green algae will survive.

AMINO ACIDS OF ALGAE

The first investigators of amino acid composition of plant material were La Cour and Drew (1947). They described the free amino acids of Trillium root-tips and tradescantia anthers. The tissue was applied to filter paper strips in the form of a smear. Phenol saturated with water was the solvent used on a one-way chromatogram. On developing with ninhydrin, two or more colored bands appeared indicating the presence of two or more colored positive amino acids.

Dent, Stepka, and Steward (1947) analyzed the free amino acids of potato tuber. Extracted was a protein-free solution. The solution was evaporated and the residue redissolved in water. The amino acids were run for thirty hours in phenol saturated with water, and then run thirty hours in a collidine (2-4-6 trimethyl pyridine)-lutidine (2-4 dimethyl pyridine) mixture (1:1 by volume). The two-way chromatogram developed with ninhydrin revealed twenty-four spots. They concluded that there were twenty-one amino acids as normal constituents of the alcohol-soluble nitrogen of potato tuber tissue.

Fowden (1951) investigated the composition of the bulk proteins of Chlorella, using the techniques of paper partition chromatography. Protein extraction was based on the borate-ether-ethanol method. Separation of amino acids was on one-dimensional chromatograms using three solvent mixtures. Eighteen amino acids were detected, with arginine and lysine comprising twenty-five

per cent of the total protein.

A later study by the same author examined the effect of age on the bulk protein composition of Chlorella vulgaris (Fowden, 1952). Growth conditions and protein extraction procedures were identical with his earlier paper. He concluded that culture age had little effect in the protein composition, although the percentage of protein within the cells steadily decreased. There was however, an increase in the histidine content with increasing age of the culture, and slight increases in alanine, lysine, and arginine. The investigations were concerned with the bound amino acids which account for about eight-five per cent of the total cell nitrogen. The concentrations of soluble nitrogen compounds, particularly the free amino acids, were not studied.

Fowden (1954) made a comparison of algal proteins obtained from Chlorella, Anabaena, Navicula, and Tribonema, which are members of four separate algal classes. The proportions of protein, carbohydrate, and fat present in a single algal cell are variable and depend on the conditions of growth and age of the culture. Quantitative determinations of the amino acids showed that a close similarity exists for the proteins of all four species. For Anabaena, a blue-green alga, tyrosine and cysteine levels were very low and arginine very high.

Williams and Burris (1952) studied a partial amino acid composition of Nostoc muscorum, Calothrix parietina, and Diplocystis aeruginosa. Entire algal cultures were hydrolyzed with

six normal hydrochloric acid for twenty-four hours. Amino acids were separated by column chromatography and analyzed by a photometric ninhydrin method. The levels of methionine and tyrosine were low. Remaining amino acids were present in approximately equal quantities.

Wassink and Rageth (1953) reported on the paper chromatographic analysis of the photosynthetically active phycoerythrin, in Oscillatoria. The results showed sixteen amino compounds of which thirteen were known amino acids and three were unknown. Arginine was reported absent, but otherwise the analysis of the Oscillatoria phycoerythrin did not reveal important differences from the bulk proteins of the green algal Chlorella.

LIGHT INTENSITY AND QUALITY

Horvath and Szasz (1965) investigated the effect of light intensity in metabolic pathways in photosynthesis of Phaseolus vulgaris; although photosynthesis begins with a single primary reaction, a number of photosynthetic pathways lead to various products. They found that an increasing light intensity proportionally increased the relative carbohydrate contents of the plant. Also, with increasing light intensity, the relative soluble nitrogen contents decreased but the protein nitrogen remained constant.

Benson, et al. (1949) were among the first investigators to use paper chromatography and radioautography to study the path of carbon in photosynthesis. They identified the free amino acids,

carboxylic acids, and phosphate esters of Scenedesmus and Chlorella. Amino acids were extracted with eighty per cent ethanol and chromatographed on Whatman number one filter paper. In Scenedesmus they found the following amino acids, listed in order of decreasing intensity of ninhydrin color on the chromatogram: glutamic acid, alanine, serine, arginine, valine, aspartic acid, leucines, phenylalanine, tyrosine, lysine, threonine, glycine, and proline. After ten minutes of photosynthesis by Scenedesmus in $^{14}\text{CO}_2$ the radioactive amino acids included aspartic acid, alanine, asparagine, serine, and phenylalanine.

Norris, Norris and Calvin (1954) studied the rates and products of short-term photosynthesis using $^{14}\text{CO}_2$ and paper chromatography. The four blue-green algae they studied were two species of Nostoc, Phormidium, and Synechococcus. There was uniformity of labeling in the ethanol-soluble (free amino acids) compounds which became radioactive in all the plants studied. An unidentified compound below alanine on the chromatogram became radioactive in two of the blue-green algae, but in the other plants was present only in trace amounts. Aspartic acid was radioactive in all blue-green fractions. Alanine was next, followed by glutamic acid. Activity was absent in threonine with only traces in glycine and serine.

Cayle and Emerson (1957) analyzed the effect of light quality on photosynthesis by Chlorella. Cells were preilluminated for five minutes before a thirty second exposure to carbon-14

bicarbonate under red light. Identical trials were performed using blue light. Both light qualities were isolated from the light of a 400 watt mercury-cadium lamp by means of filters. The red beam had an emission band at 644mu, but was contaminated with a small amount of radiation of longer wave lengths. The blue light had a wave length of 436mu. Extraction of amino acids and phosphate esters was in seventy per cent alcohol after which the extract was fractionated by paper chromatography to separate the amino acids. The activity incorporated into the combined amino acids was not different for cultures grown in red or blue light. The specific activity (counts per minute per milligram of material) of the amino acids was almost twice as great after photosynthesis in blue light as it is after photosynthesis in red.

The greater specific activity of the amino acids, following photosynthesis in blue light, together with the equal counts per minute in the amino acid fraction for both red and blue light, implies that the amount of amino acids in the isolated fraction must have been smaller after photosynthesis in blue than after photosynthesis in red light.

In an attempt to determine the influence of light quality on tobacco leaves, Tregunna, Krotkov, and Nelson (1962)_a grew tobacco plants in a chamber illuminated with white, red or blue light in the presence of $^{14}\text{CO}_2$. No effect of wave length was observed in the distribution of absorbed carbon dioxide between the ethanol-soluble and insoluble fractions. Red light, compared

to white, increased the activity of glycine, but had no effect on serine. Blue light brought about a decrease in glycine and a slight decrease in serine.

Hauschild, Nelson, and Krotkov (1962)_b studied the effect of light quality on green algae, Chlorella and Scenedesmus, a blue-green algal, Microcystis, and on a photosynthetic bacterium, Chromatium. The organisms were placed in ^{14}C -bicarbonate and illuminated with red, blue or white light. After thirty minutes, the distribution of ^{14}C among the photosynthetic products was analyzed by paper chromatography and autoradiography. In Chlorella blue light caused an increase of carbon-14 in aspartic and glutamic acid. The quality of light had little effect on the distribution of carbon-14 in Microcystis. However, activity in glutamic acid was consistently increased in aspartic acid. Phosphate esters accounted for eighty to ninety per cent of the total activity. No effect of light quality was observed in Chromatium.

Hauschild et al., (1962)_c also experimented with Chlorella vulgaris. The algal was grown in synchronous culture, pre-treated in darkness for 45 to 225 minutes and illuminated in the presence of ^{14}C -bicarbonate with red and blue light. The total amino acid and organic acid fraction contained approximately thirty per cent of the total carbon-14 fixed after five minutes of photosynthesis under both conditions of light. In thirty minute experiments only ten per cent was incorporated into this fraction in red, and fifteen per cent in the presence of blue light.

The effect of light quality was more pronounced after thirty minutes of photosynthesis, when more compounds became radioactive. Their results also demonstrated that light quality has a marked effect on the distribution of carbon-14 among the components of the amino acid plus organic acid fraction. The total increase of radioactivity in aspartic, glutamic and fumaric acids was observed when cultures were grown in blue light.

Hess and Tolbert (1967) reported on the products of $^{14}\text{CO}_2$ fixation by Chlamydomonas and Chlorella grown in blue and red light. After five minutes of preillumination in a designated light a $\text{NaH}^{14}\text{CO}_3$ solution was added and samples removed at one, three, and ten minute intervals. Aliquots were extracted in methanol and the components separated by paper chromatography. Algae grown for ten days in blue or red light produced about the same products regardless of whether $^{14}\text{CO}_2$ fixation was measured in white, blue or red light. For both algae, the total percentage of carbon-14 incorporation into alanine was somewhat greater with algae grown in red light than white light and much greater than with algae grown in blue light.

MATERIALS AND METHODS

ALGAE

Cultures of Oscillatoria were obtained from General Biological Supply House, Chicago, Illinois, and cultured in a modified "Chu" (Smith, 1951) nutrient solution.

TABLE 1

NUTRIENT SOLUTION FOR CULTIVATING ALGAE

Nutrient	Grams per 500 milliliters
$\text{Ca}(\text{NO}_3) \cdot 4\text{H}_2\text{O}$	11.60
K_2HPO_4	0.50
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	1.25
$\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$	2.20
Na_2CO_3	1.00
ferric citrate	0.175
citric acid	0.175

Stock solutions were made in 500 milliliter volumes with distilled water. All solutions were sterilized except the citrates which were made by adding the correct amount of nutrient to a sterile flask containing the correct amount of sterile distilled water. Ten milliliters of each stock solution were combined and the volume increased to one liter with sterile distilled water. The final pH was 8.4, since according to Smith (1951) only the algae which live in acid bogs and peatwaters are adaptable to a

pH considerably below 7. Samples of culture media were removed and plated on nutrient agar to determine if bacteria were present. By means of a serial dilution, bacteria were only detected in the one milliliter (undiluted) portion of the thirty day-old cultures. This level varied between zero to three colonies. Fourteen day-old cultures contained no detectable bacteria.

Oscillatoria was plated on a gelatinous medium according to the procedure of Smith (1951). Agar concentration was two and one-half per cent. Before the preparation of the medium, the agar was washed with glass distilled water. The agar solution and stock nutrient solutions were autoclaved separately since some substances may weaken the power of gelation when heated together. Care was exercised in autoclaving the agar since according to Smith, overcooked agar is a poor substratum for optimum growth. Concentrations of the agar media was three parts water to one part nutrient solution. Small amounts of Oscillatoria were transferred with a sterile loop to the gelatinous medium in petri dishes, and set aside on a cool surface under fluorescent lighting. In eight to twelve days a thin filamentous mat of Oscillatoria covered the entire surface of the plate. A small mass of filaments were transferred into sterile 250 milliliter flasks containing 150 milliliters of nutrient solution.

Flasks of varying sizes from 125 to 2800 milliliters were compared for their suitability as culture vessels. The 250 milliliter size was the best in achieving a thin filamentous mat grow-

ing across the bottom and sides to the water line.

The inocula were taken from the periphery of the mat of cells. The flasks were new and plugs of non-absorbent cotton were used.

The growing cultures were maintained next to a North window with a room temperature of twenty-five degrees centigrade. Continuous illumination by two fifteen watt fluorescent bulbs in a twenty-four degree cold room resulted in equal to slower growth rates, as compared to cultures maintained in the laboratory. Bubbling carbon dioxide into the culture was not required since its addition did not increase the rate of culture growth. After ten to twelve days of growth, the algae were subcultured into fresh nutrient solution.

Algae were also cultured under red and blue light. A bio-growth chamber, model 31675 (Wilkins-Anderson Company, Chicago, Illinois) was used for experiments utilizing a monochromatic light. The plexiglass doors were covered with black paper and cardboard hoods were placed over the fan intake and exhaust so as to prevent any room light from entering the chamber. Red light was obtained from three Sylvania twenty-five watt photographic darkroom bulbs. The emission band of the red light was between 640 and 700 μ as measured by a Winsco spectrometer model 125 (Walbash Instrument Corporation, Walbash, Indiana). Blue light (Hess and Tolbert, 1967) was provided by a fifteen watt blue fluorescent light (Sylvania F15T-B). The light was filtered through 3.5 centimeters of

a copper sulfate solution (30g/l), to which was added one gram of "marina blue" tint and dye (Rit commercial dye). This combination passed an emission band between 420 and 510 mu. Temperature inside the growth chamber varied between twenty-seven and twenty-eight degrees centigrade. Sample distance from the blue light was one-half the distance of the sample from the red light, so as to adjust for differences in light intensity. A Honeywell Pentax exposure meter was used in determining sample distance from the light source.

PROTEIN EXTRACTION AND HYDROLYSIS

The procedure for extraction of protein was based on the ether-ethanol method of Lugg (1939). The harvested Oscillatoria cells were twice washed with distilled water and suspended in thirty milliliters of water. The cells were broken with a sonifier cell disrupter model W-140-C (Heat Systems, New York) at a 40 watt output for a period of three minutes. An equal volume of ice-cold ethanol-ether mixture (four volumes ethanol to one volume ether) was slowly added to the original solution. The mixture was stored for one hour at three degrees centigrade, and then centrifuged for thirty minutes at approximately 500g. The residue was discarded and the supernatant adjusted to a pH of 4.5 with dilute acetic acid. The solution was then warmed to 70 degrees centigrade in an oven to flocculate the protein. By cooling the solution overnight all the protein was precipitated out. The residue was washed with acetic acid (pH 4.5), hot ethanol

(twice), dilute citric acid, hot ethanol (twice), and ether.

The proteins were hydrolyzed by sealing them in ampules containing two milliliters each of six normal hydrochloric acid and glacial acetic acid, and then heated to 105 degrees centigrade for 24 hours. If any humin formation occurred, it was filtered off with paper or charcoal. The acid solution was evaporated and the residue dissolved in five milliliters of distilled water.

CHROMATOGRAPHIC TECHNIQUE

This was carried out in a Precision Scientific chromatography cabinet (Precision Scientific, Chicago, Illinois). The interior dimensions were: 25 inches long, 19 inches wide, and 27 inches high. Amino acid separations were achieved on Whatman number four paper. The two-way chromatograms (25 centimeter flow) and one-way (30 centimeter flow) were performed in a descending technique.

The solvents were the same used by Smith (1960). Butanol-acetic acid was followed by phenol-ammonia in two-way chromatograms. Butanol-acetic acid was the one-way solvent.

Standard solutions of all amino acids (1-2 mg/ml in 10 per cent iso propanol) were chromatographed and localized with ninhydrin (0.3 per cent in acetone). All chromatograms were dipped in the location reagent and developed at room temperature.

Ten microliters of sample were used in all runs with spot diameter of four milliliters. All samples were air dried on the filter paper.

$^{14}\text{CO}_2$ PHOTOSYNTHESIS EXPERIMENTS

The $\text{NaH}^{14}\text{CO}_3$ was obtained from New England Nuclear. The radioactivity was one millicurie; the weight 4.6 milligrams; the specific activity 18.5 millicuries per millimole. To the bicarbonate was added 4 milliliters of distilled water.

The method of introduction of the algae to carbon-14 was similar to Hauschild, Nelson, and Krotkov (1962). Fourteen and thirty day-old cultures were used for each of the three wave lengths of light. The culture was first pretreated in darkness for three hours, followed by exposure to the particular wave length for five minutes without the radioactive carbonate. The culture received 0.3 milliliters of the $\text{NaH}^{14}\text{CO}_3$ solution. At the end of thirty minutes, the algae were dumped into boiling water to stop photosynthesis. The cells were filtered, washed with distilled water and suspended in 30 milliliters of water for extraction.

DETERMINATION OF AMINO ACID ACTIVITY

All determinations were made in a model 480 gas-flow detector mounted on a model M-5 semi-automatic sample changer (Nuclear Chicago Corporation). The detector was connected to a Nuclear Chicago manual lab scaler model 8775. The high voltage was 1500 volts and the sensitivity 10 millivolts. The gas was 90 per cent argon and 10 per cent methane with a flow rate into the detecting chamber of 4 psi.

Determinations were made along the entire sample flow for radioactivity. The paper was cut into pieces so as to fit into

the planchets for counting. The strip was cut so as to include the entire ninhydrin positive area. For each culture, six chromatograms were run. Each planchet was counted three times.

OBSERVATIONS AND RESULTS

GROWTH OF ALGAE

When cultures were grown in a twenty-four degree cold room with continuous illumination by two fifteen watt fluorescent bulbs, algal growth was slow, as compared to growth obtained in the laboratory. The better of the two methods were obtained with sunlight and room temperature of twenty-five degrees centigrade, with the culture being gently shaken twice daily. Noticeable growth in this case began sooner as compared to the cold room culture.

Visible increases in algal mass while cultured in red or blue light was slow at first and not until approximately one week was there any noticeable increase. After this initial period, normal growth, as compared to cultures grown in white light, began.

AMINO ACIDS OF OSCILLATORIA

The R_f values of the amino acid standards (table 2) with two exceptions, were not significantly different from the values obtained by Smith (1960). The long solvent flow produced good separation of amino acids. Two-way chromatograms achieved better separation than one-way, however larger sample sizes were used in order to be detected. Tryptophane and threonine were the only amino acids whose R_f values differed significantly from Smith. This difference is probably due to a larger flow, plus smaller

solvent volumes.

Identical conditions were employed in all chromatographic runs and the R_f values remained the same. A vinyl sheet was placed over the combined standards and locations of various components traced onto the plastic. In subsequent runs, a quick check of unknown spots was possible. Final confirmation of unknown areas proved the vinyl overlay effective for identification.

TABLE 2

 R_f VALUES FOR AMINO ACID STANDARDS

Amino acid	R_f experimental	R_f Smith
Cysteine	8	8
Cystine	6	5
Lysine	13	12
Histidine	14	11
Asparagine	15	12
Arginine · HCL	16	15
Aspartic acid	20	23
Glycine	25	23
Glutamic acid	26	28
Serine	27	22
Proline	33	34
Threonine	35	26
Alanine	41	30
Tyrosine	47	45
Tryptophane	57	50
Methionine	60	50
Valine	65	51
Phenylalanine	69	60
Isoleucine	74	67
Leucine	75	70

Except for arginine and tyrosine, all amino acids known to be essential for biological material were detectable in Oscillatoria. Cysteine and methionine were detected only in very

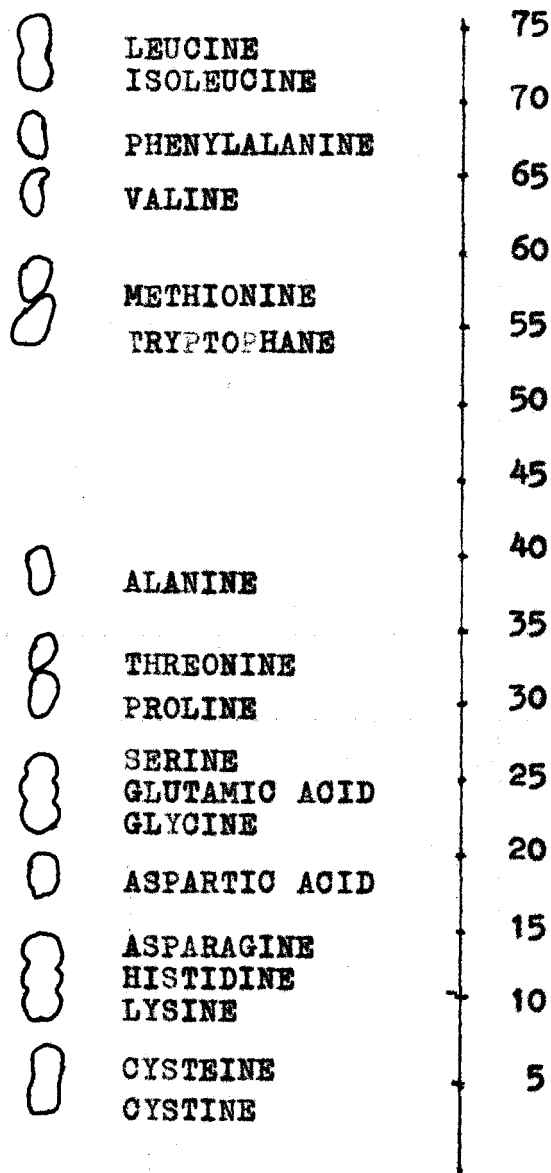


Figure 1. One-way chromatogram of the amino acids of Oscillatoria

small quantities, and only when large volumes of algae were used were they detectable at all.

One-way chromatograms (figure 1) revealed all eighteen amino acids while the two-way chromatogram (figure 2) did not show cysteine or proline. Wassink and Rageth (1953) reported arginine

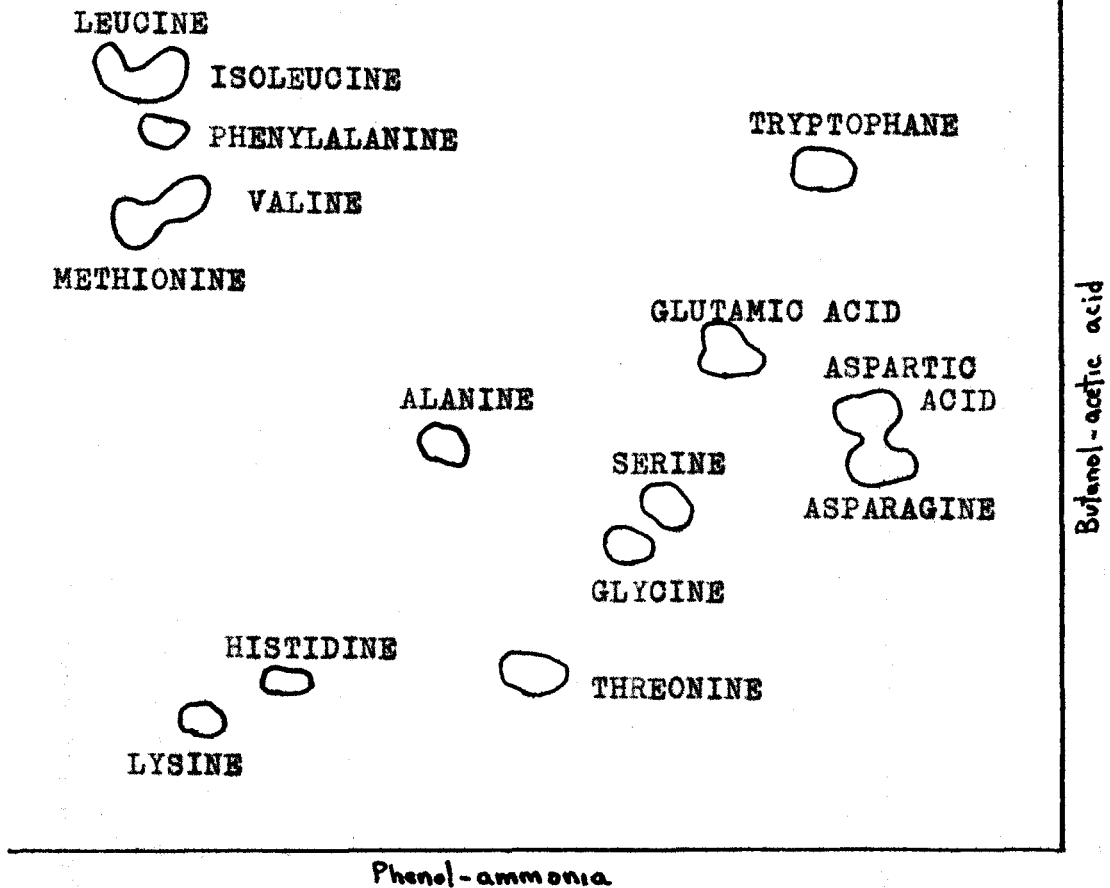


Figure 2. Two-way chromatogram of the amino acids of Oscillatoria species

missing in Oscillatoria phycocyanin.

DISTRIBUTION OF ^{14}C AMONG THE AMINO ACIDS DURING PHOTOSYNTHESIS

For an overall representation, table three compares culture age and total carbon uptake. The volume measured was the same used in subsequent chromatographic analysis. The supernatant count is a sample of the liquid phase after the proteins were precipitated. This was done to determine what per cent if any of the activity was left. There was a difference in total

TABLE 3

DISTRIBUTION OF ^{14}C AMONG TWO FRACTIONS OF OSCILLATORIA
AFTER 30 MINUTES OF PHOTOSYNTHESIS

Culture Age (Days)	Weight of Wet Algae (Grams)	Light Quality	Total Activity Count		Supernatant Count	
			CPM	SA*	CPM	SA*
14	0.31	white	488	1.57	33	0.10
30	0.40	white	107	0.26	18	0.05
14	0.33	red	30	0.09	14	0.04
30	0.42	red	24	0.05	9	0.02
14	0.55	blue	85	0.15	19	0.03
30	0.74	blue	40	0.05	10	0.01

*CPM/milligram material

uptake between fourteen and thirty day-old cultures. The specific activity is an accurate determination of carbon-14 uptake since the wet algal weights vary. Fourteen day-old cultures had an increased total uptake when grown in white or blue light. In white light, there was a seven-fold increase and in blue light, a three-fold increase. The supernatant count followed a similar pattern of greater activity in the fourteen day-old cultures, however the activity is insignificant compared to the total uptake of carbon-14.

The activity of amino acids showed no particular pattern (table 4). Since all activities were counted on one-way chromatograms, the representative amino acids were chosen because of their widely spaced R_f values. Each chromatogram strip was counted from the origin to the end of the solvent flow to determine areas of activity. All activity was localized in the mentioned amino acids except for areas above leucine.

Red light had a greater percentage of labeled amino acids than blue light. Red light increased the activity of asparagine, alanine, and tryptophane; with a decrease in glutamic acid. Leucine was increased in the fourteen day-old culture.

Blue light caused a decrease of activity in alanine. Leucine was the most active amino acid with an increase of approximately thirty per cent over leucine from cultures grown in white light. There was measurable activity in phenylalanine in the fourteen day-old culture grown in blue light. Tryptophane

TABLE 4

PER CENT DISTRIBUTION OF ^{14}C AMONG AMINO ACIDS DURING PHOTOSYNTHESIS

Culture Age	Aspar- agine	Aspartic Acid	Glutamic Acid	Alanine	Trypto- phane	Phenyl- alanine	Leucine	Unknown Above Leucine	Total % of Amino Acids	
14	W	0.7	4.4	1.1	0	3.4	0	4.7	56.7	14.3
30	W	13.4	17.1	11.9	8.9	7.4	0	2.2	12.2	60.9
14	R	0	0.8	0	0	20.0	0	30.0	0	50.8
30	R	41.1	17.1	0	17.1	17.1	0	0	0	92.4
14	B	2.0	0	0	0	10.7	13.3	22.1	3.3	58.1
30	B	7.7	15.4	7.7	0	0	0	34.6	6.0	65.4

also increased in activity in the younger culture.

An unknown labeled compound was present in algae grown in white light. It gave a negative ninhydrin reaction, and had an R_f value of approximately seventy-nine to eighty-one. Activity in the same area was absent from algae grown in red light. Blue light incorporated a small amount of activity in the same area, however this could be disregarded since the level was below the levels of the amino acids.

In all wave lengths of light, the incorporation of carbon-14 into amino acids were greater in thirty day-old cultures. The greatest difference in total per cent of amino acid activity between the two cultures was observed in algae grown in white light.

DISCUSSION AND CONCLUSIONS

The data in table three compares culture age with distribution of carbon-14. All three cultures of algae, those exposed to red light, those exposed to blue and those exposed to white, gave evidence of less carbon-14 being held by cells after the culture was thirty days old than at fourteen days. Therefore, as the culture age increased, the total incorporation of carbon-14 decreased. Since the algae cannot distinguish between carbon-14 and carbon-12, it is evident that the fourteen day-old Oscillatoria had a higher level of activity than thirty day-old cultures.

Table four breaks down the total activity into various amino acids. This table indicates a higher total percentage of amino acids were isolated from the older cells (30 day-old culture). This turn about in cultures grown in white light was due in part to the presence of a labeled unknown. Present evidence indicates that this unknown is not an amino acid, since it did not react with ninhydrin to produce a colored spot. Although the thirty day-old cultures had a lower total activity, this lower level was located in the amino acids. Thus, there was more labeling in amino acids in older cultures.

In fourteen day-old cultures grown in white light, more than one-half of the activity (table 4) was detected in the unknown area. The thirty day-old culture exposed to white light had a lower percentage of activity in the unknown.

This is best illustrated when one refers to table four. When the difference in activity between the unknowns above leucine of cultures exposed to white light is added to the total per cent of amino acids in the fourteen day-old culture, the amount of amino acids are almost identical for the two culture ages. The difference between 56.7 and 11.2 is 45.5, which when added to the younger culture value of 14.3 gives 59.8. This is only 1.1 per cent difference between the two culture ages. Therefore, the increase of activity in the total amino acids of the older organism was at the expense of the activity of the unknown.

Instead of accumulation of the label in the unknown area of older cultures, the amino acids received the activity. The unidentified area was not analyzed. It may represent 3-phosphoglycerate, which may be a precursor of amino acids in photosynthesis (Awapara, 1968), or the area may be an alpha keto acid.

Awapara is in opposition to the belief that only carbohydrate is produced photosynthetically and that other products are formed from carbohydrates by ordinary biochemical reactions. In the carbon-reduction cycle of photosynthesis, ribulose-1, 5-diphosphate is reduced to 3-phosphoglycerate. Most of the amino acids and dicarboxylic acids are then formed as a branch from 3-phosphoglycerate.

In this investigation, the incorporation of carbon-14 into the unknown and therefore synthesis of the unknown, was

dependent upon the wave length of light to which the organisms were exposed. Red light, as compared to white, increased the total per cent of amino acids for both culture ages. Table three shows that Oscillatoria grown in red light had the lowest total activity count. The label was distributed exclusively among the amino acids with none found in the unknown area. Therefore, metabolic pathways were probably altered by this wavelength so that the unknown area was by-passed or labeled only briefly.

Table four indicates that the difference between the total activity of amino acids of the two culture ages during growth in red light was due mainly to an increased activity of asparagine in the older organism. White, Handler, and Smith (1964) stated that in plants, asparagine serves as a reservoir of ammonia and aspartic acid, formed by the hydrolytic activity of asparaginase. Therefore, red light may enhance this enzymatic pathway, resulting in an increased level of asparagine. Hess and Tolbert (1967) noted an increase in aspartate and alanine in Chlorella grown in red light.

Blue light, as compared to white light, caused a lower total activity (table three). Table four indicates however, that there was a higher total per cent of labeled amino acids. This result is similar to that obtained in red light, being that this wavelength of light stimulates synthesis of amino acids as incorporation of carbon-14 into amino acids is enhanced.

SUMMARY

Oscillatoria cultures were grown in red, white and blue light respectively. Cultures at age fourteen and thirty days were harvested for protein present. Amino acids were extracted and chromatographed by one and two dimensional techniques. Carbon-14 labeling permitted quantitative analysis of amino acids isolated. For each of the cultures, all twenty of the common amino acids were detected except arginine and tyrosine. The wave length of light to which the cultures were exposed proved to have an effect on the bound amino acid composition of the organism. Younger cells incorporated more activity into the total fraction while older cultures incorporated more activity into the amino acids. An unknown was detected in cultures grown in white and blue light.

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APPROVAL SHEET

The thesis submitted by Jon Charles Chesnut has been read and approved by the director of the thesis.

Furthermore, the final copies have been examined by the director and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

14 June 1969
Date

W. C. Laidler
Signature of Advisor