ENDOGENOUS LEVELS OF INDOLE-3-ACETIC ACID
IN SYNCHRONOUSLY GROWN CHLORELLA
PYRENOIDOSA

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The purpose of this study was to determine the endogenous levels of IAA throughout the life cycle of *Chlorella pyrenoidosa* (TX-7-11-05), and to show a correlation between onset of cell division and IAA levels.

The materials and methods section presents the method of synchronization of test material by photoinduction and the cultures conditions under which the alga was grown. Also presented in this section is the method of preparation of the cellular extract, the fluorometric assay technique and construction of a standard "quench curve."

The results include the proof of induction of synchrony on the test material and a presentation of hormone levels at 3 stages of the life cycle. The IAA levels were found to increase gradually from the autospore to the adolescent stage and more rapidly when approaching the ripened adult stage. The levels of IAA were found to be approximately 3 times higher just prior to division compared to the autospore stage. A statistical analysis using a randomized block design showed significant differences in IAA levels for the different sampling times at the 0.005 per cent level.
It was concluded that there was a significant increase in IAA levels throughout the life cycle of this alga under the conditions of the test, but this increase could not be determined as the direct cause of cell division.

Further research would be valuable to determine the effect of various environmental factors upon endogenous IAA levels and how these factors affect cell function through regulation of IAA levels in the cell.
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THESIS

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ENDOGENOUS LEVELS OF INDOLE-3-ACETIC ACID IN
SYNCHRONOUSLY GROWN CHLORELLA PYRENOIDOSA

Since the discovery of auxin in algae by Van der Weij (18), much work has been done to elucidate the presence, quality, and role of this group of compounds. The earliest work was done with marine forms because of the initial isolation of auxin from these forms, and because of the large quantity of material needed to detect the extremely small quantities present.

Van der Weij analyzed the cell sap of young Valonia macrophysa and found between $1.2 \times 10^{-4}$ and $2.1 \times 10^{-4}$ μg of auxin per ml and 20 times that amount in cell wall material. Using the standard *Avena* curvature test, Du Buy and Olson (9), in 1937, found the highest concentration of auxin in *Fucus vesiculosus* to be present in the sperm and egg cells with the lowest concentration in the fruiting tips and thallus. In 1938, Van Overbeek (19), determined the auxin content of the green alga *Bryopsis* to be $8 \times 10^{-2}$ μg of indole acetic acid (IAA) per gm fresh weight. He determined that the particular auxin involved was IAA by diffusion coefficients and molecular weight determinations of the extracted auxin. In 1940, he studied *Macrocystis pyrifera* and found $5.0 \times 10^{-4}$ μg of IAA per gm fresh weight in the terminal blades and $1.5 \times 10^{-4}$ μg in the bladder and stipe (19). Van Overbeek
studied other marine forms and reported $5.0 \times 10^{-4}$ µg per gm in *Fucus evanescens* and $0.5 \times 10^{-4}$ µg per gm in *Desmarestia aculeata* (20).

All previous work became subject to question when Thimann and Skoog (17), in 1940, first showed that the yield of auxin extracted with ether from *Lemna* tissue was a function of time. They also discovered that the yield of auxin could be increased by the addition of enzyme preparations such as chemotrypsin. This suggested to Thimann and Skoog that some of the auxin was in some way bound to protein. While the work on the auxin content of marine forms continued, the interest in the effects of exogenous applications of IAA on freshwater forms increased. Yin (21), one of the first to investigate the effects of exogenous applications of IAA on *Chlorella vulgaris*, reported an increased average cell size in the presence of 10 to 40 µg IAA per ml after 12 days, but no effect on the rate of cell division.

In 1937, Leonian and Lilly (11), working with the effect of IAA on *C. minata*, *C. pyrenoidosa*, *Cystococcus cohaerans*, *Oocystis magellani*, and *Scenedesmus flavescent*, found only concentrations of IAA exceeding 33.3 µg per ml stimulated cell growth. Pratt (13) found 50 µg per ml of IAA was the optimum level for increased cell division in *Chlorella*.

After a 25-day incubation period, Pratt observed a 20-fold increase in cell numbers due to IAA. Also working with *Chlorella*, Brannon and Bartsch (8) found no effect of
IAA concentration on cell size in a sugar-free medium, but the number of cells increased up to 30-fold. Since their studies, similar contradictions are exceedingly prevalent in the literature that has accumulated.

In 1946, Algeus (3) performed an extensive study on the Chlorophyta and noted wide variations in response to IAA by several species of Scenedesmus. In a mineral medium containing 0.1 μg per ml of IAA, S. obliquus exhibited a 3-fold increase in cell number without any increase in average cell size. Other species were either unaffected or inhibited.

Roborgh and Thomas (14) supported the earlier work of Yin by reporting an increase in cell size rather than cell number in C. vulgaris grown in a medium containing 1.0 μg per ml of IAA.

Bentley (5) was the first to clearly elucidate the naturally occurring auxins extracted from C. pyrenoidosa and other algae. She used solvent extraction and paper chromatography to separate and identify auxin isolates from 3 separate fractions, and found a compound (s) which moved with an Rf similar to IAA, and gave characteristic indole color reactions.

Many of the experiments using exogenous applications have failed to provide consistent results because of the use of ethanol as a solvent for IAA. In 1958, Bach and Fellig (14) showed that ethanol served as a carbon source, and that large increments of growth could be accounted for by just the addition of ethanol. Also, many investigators expressed their
results in wet weight, and due to the wide fluctuations of water content of algal cells, dry weight would yield a more meaningful measurement. Much of the early experimentation is difficult to interpret because of the wide range of experimental conditions that were used. An example of this is the work of Albaum et al. (2), who found that the pH affected the rate of entry of IAA into *Nitella* cells. Light intensity, temperature and constituents of the media were other conditions that varied in past experiments.

It seems that no studies have been performed to measure endogenous levels of IAA throughout the life cycle of an alga. All of the previous studies have only noted the presence of IAA, or have quantitatively expressed the amounts, as measured by biological assays which are subject to a great deal of variation due to the inherent nature of biological material and environmental variables during the growth of the test material. A measurement of endogenous levels would not only give an idea of the actual hormone operating levels at stages from young autospore to ripened adult, but correlated with the life cycle, it could produce an insight into the function of IAA in algal cells.

The purpose of this study was to quantify the endogenous levels of IAA throughout the life cycle of *C. pyrenoidosa* (T x 7-11-05), grown in synchronous cultures.
Materials and Methods

Organism and Growth Conditions

Synchronous stock cultures of Chlorella pyrenoidosa (TX-7-11-05) were prepared by inoculating 100 ml of medium in a 500 ml Erlenmeyer flask, with cells transferred from a Knops medium agar slant. These cultures were exposed to an alternating light:dark regime of 15:9 hr duration for 3 cycles, after which synchronization was achieved. The stock cultures were kept in synchronization by daily dilution with medium back to 90 per cent transmission, measured at 600 mu. The medium used for all cultures was Bristol’s as modified by Bold (7).

Forty 500 ml Erlenmeyer flasks were filled with 100 ml of medium and stoppered with foam rubber plugs. The medium was sterilized at 120 C and 17 pounds per square inch of pressure for 15 min. Each flask was inoculated with 4 ml of stock culture, which was shown to be the optimum inoculation size to insure synchrony throughout the experiment and still provide a large yield for extraction.

The cultures were grown in a Sherer Gro Lab at 40°C and exposed to 1500 100 ft-c during the light period unless otherwise noted.
All flasks were agitated at 80 cycles per min on an Eberbach reciprocal shaker modified with an enlarged wooden platform to enable the use of forty 500 ml flasks. The cultures were allowed to grow through 2 complete light:dark cycles, and were then subjected to a low light treatment at the end of the final dark period, consisting of illumination at $350 \pm 100$ ft-c. The low light treatment lasted 6 hr and 15 min, which was determined to be the length to incipient cell division under these conditions. Three sampling periods were chosen: low light 0 hr (LL0), low light 3 hr (LL3), and low light 6:15 hr (LL6:15), with all cells being harvested at each sampling time. Since 2 complete runs were made, it was necessary to grow 6 separate batches to provide the necessary cell material.

Two flasks from each sampling period were saved for dry weight determinations. The contents were centrifuged at 3000 rpm for 10 min and washed in distilled water 3 times. The packed cells were filtered through a 0.45 u Millipore filter that had been rinsed for 3 hrs in 3 changes of distilled water and dried overnight in a desiccator. The filter with cells was placed in a previously oven-dried and tared weighing dish, and placed in a Boekel oven at 95 C for 16 hr. This drying time was determined for this particular algal material by weighing at regular intervals until no further weight reduction occurred. After the drying period, the
dish was cooled in a desiccator to room temperature and weighed on a Mettler balance, model H6.

Preparation of Indole Acetic Acid Extract

At each sampling time, the contents of flasks were poured into a 4-liter Erlenmeyer flask and centrifuged in a Sorvall RC-2B centrifuge at 12,500 rpm utilizing a Sorvall KSB continuous-flow accessory. A chamber temperature of 3°C was maintained, with a medium exit rate of approximately 180 ml per min. A constant check on the supernatant was kept to insure that cell loss to the supernatant was minimum.

The cells were immediately removed and immersed in boiling spectroanalyzed methanol contained in a small mortar. The centrifuge tubes were thoroughly rinsed with methanol to insure complete removal of the cells. The solvent extraction continued for 45 min until loss due to evaporation had reduced the sample to a volume of about 30 ml. The crude extract was poured into a rotary evaporation flask and attached to a Rino vacuum evaporator for complete methanol removal. The evaporation flask was immersed in a water bath maintained at 40°C until only the water fraction remained. The aqueous remainder was saturated with NH₄SO₄ and filtered through a 0.45 μm Millipore filter to remove the precipitated protein and cellular fragments. The filtrate was adjusted to pH 2.5 with concentrated H₂SO₄, and extracted with 3 equal volumes of peroxide-free ether.
Since the ethereal fraction still contained what appeared to be a carotenoid pigment, the solvent partition procedure of Bentley's (6) was performed to prevent interference with the subsequent fluorometric assay. This was accomplished by raising the pH of the ethereal fraction to 9.0 by the addition of a sufficient amount of 0.5 M NaHCO$_3$, and shaking in a separatory funnel with the presumptive carotenoid pigment retained in the top ethereal layer and the IAA remaining in the lower bicarbonate layer. This process was repeated 3 times in a separatory funnel with equal volumes of peroxide-free ether. The clear ethereal fraction was evaporated to dryness at 30°C in a water bath. The IAA-containing residue was taken into solution by thoroughly rinsing the flask with spectroanalyzed methanol and this sample was saved for later fluorometric analysis.

An estimated 95 per cent of the IAA was recovered by this extraction method. This was determined by comparison of IAA content values for algal extracts with known amounts of IAA added to those in which no IAA had been added.

Fluorometric Assay of IAA and "Quench Curve" Construction

A Turner Model 110 Fluorometer was used for the fluorometric determination of IAA according to the method of Stoessl and Venis (16). This fluorometric assay measures the indole-α-pyrrole product which is formed by the action of
trifluoroacetic acid-acetic anhydride on the IAA initially present. The fluorescent product formed is directly proportional to the amount of IAA initially present.

The primary filter system consisted of a 47-B and 2A filter, with the 2A placed closest to the T-5 blue-light lamp. This provided an excitation wavelength of 435 μm. The secondary filter system consisted of a number 8 and 65A filter which provided a maximum emission wavelength of 505 μm. Figure 1 shows that these passage peaks satisfactorily approach the excitation and emission peaks of the fluorescing indole-α-pyrone product.

![Spectral characteristics of filter systems and excitation and fluorescence emission of the indole-α-pyrone product formed by the action of the trifluoroacetic acid-acetic anhydride reagent on IAA.](image-url)
Turner quartz cuvettes (11x75mm) were used for all assays and great care was taken in handling and cleaning to prevent contamination by any fluorescing agent. Readings were taken with empty, clean cuvettes to measure and compensate for an inherent difference in transmittance between cuvettes due to scratches or manufacturing.

Standard solutions were prepared by dissolving a known quantity of IAA in spectroanalyzed methanol, and diluting down to lower concentrations. Five different concentrations were used, encompassing greater than a 100-fold difference from 0.001 μg per gm of solvent to 0.11 μg per gm of solvent.

One gm of each standard solution was added to a 14 x 150 mm disposable test tube, taking care to avoid splashing, with 1 ml of spectroanalyzed methanol serving as a blank. These were evaporated to dryness at 67 °C, and placed in an ice bath. To the chilled tubes, 0.1 ml of ice-cold trifluoroacetic acid-acetic anhydride solution (1+1 by vol) was added. The reaction was allowed to run 15 min, catalyzing the transformation of IAA to the fluorescent indole-α-pyrene product. To stop the reaction, 4.5 ml of ice-cold Na₂CO₃ were added. The tubes were mixed on a vortex mixer and immediately read against the prepared blank to prevent the loss of fluorescence due to photodegradation. Readings were taken at 1x, 3x, 10x, and 30x sensitivity settings. The linear relationship between fluorescent yield versus μg of IAA per gram of methanol is shown in Fig. 2.
The standard curve shown in Fig. 2 indicates the fluorescence of IAA in a pure methanol solution. To accurately estimate the IAA content of an algal extract, it was necessary to determine the amount of "quenching" of the fluorescent indole-α-pyrone by other interfering components. This was accomplished by estimating the apparent IAA content of a Chlorella extract by use of the standard curve shown in Fig. 2. To other aliquots of the same extract were added
known amounts of IAA in methanol with the fluorescence analysis run in the standard way.

Construction of a "quench curve" from the resulting fluorescence values shows the reduction of fluorescence due to quenching components in the extract. (Fig. 3).

![Graph](image)

Fig. 3. "Quenched" calibration curve showing fluorescence of algal extract with known addition of IAA.

The algal extracts for each sampling period were evaporated and analyzed as the standard curve solutions, but the
blank was prepared by addition of the reagent after the Na$_2$CO$_3$ solution. This permitted the blank to compensate for any fluorescence due to naturally fluorescing compounds in the extract.

The extracts for each sampling time were analyzed in triplicate and the resultant values were averaged to give a mean value for IAA content for each extract.
Results

The effect of the alternating light:dark regime on the growth of *Chlorella pyrenoidosa* (Tx-7-11-05) is shown in Fig. 4. Inoculations were made at $L_0$, the beginning of illumination, using 4 mL of stock culture.

![Graph showing synchronous growth of *Chlorella pyrenoidosa* induced by an alternating light:dark regime. Light treatment of 1500 ft-c and low light treatment of 350 ft-c.](image)
Growth in cell size continued throughout the light period and maximum cell size was attained immediately before the dark period. The onset of division began immediately after the light cycle reached completion, with approximately 90 per cent of the cells completing division by 3 hr into the dark phase. The autospore "burst" number appeared to be 5 or 6, assuming that most cells undergo division. No measurement was made to determine the actual number of cells that divided, but visual observation showed that at least 90 per cent contained autospores just prior to division.

The sharpness of synchrony was apparent from the rapid rise in cell numbers during the 3 hr following the onset of division and the constant burst number in the 2 successive divisions. Another indicator of the degree of synchronization was the small slope of the line connecting the end of one division and the onset of another. This indicated that very little, if any, cell division occurred out of synchrony, and the small amount indicated is attributable to the error involved in cell counts.

The cell density at all 3 sampling times (LL0, LL3, LL6:15) was approximately $10 \times 10^6$ cells per ml. This was the maximum density where synchrony could be maintained with the maximum possible yield. The onset of incipient cell division required a minimum of 6 hr and 15 min after the beginning of low light treatment.
The results of the fluorometric assays are shown in Fig. 5. Since the extraction method was found to have 95 per cent recovery, to accurately estimate the true IAA content the values determined by the assay were adjusted for 100 per cent recovery. These adjusted values are plotted in Fig. 5.

![Graph showing IAA levels across life cycle stages](image)

**Fig. 5.** The relationship between IAA content (µg/mg-dry wt) and stage of life cycle of *Chlorella Pyrencidosa* (TX-7-11-05).

The IAA levels increased gradually from the autospore stage to the adolescent stage and more rapidly when approaching the ripened adult stage. The percentage increase of the mean line from autospore to adolescent was 36.9 per cent,
and from adolescent to ripened adult 104.6 per cent. The total percentage increase from autospore to adult was 180.3 per cent. This represents about a 2-fold increase in IAA just prior to division. Runs I and II show a great similarity of values considering the extremely small levels measured.

The cell number curve remained constant throughout the low light exposure period until 6 hr and 15 min, when the first cell division was noted.

A randomized block test was run to determine the statistical validity of the data. The results of both runs were added together and mean figures were used for the computation of the block. Table 1 shows that there are no significant differences between blocks.

Table I. Analysis of variance for randomized complete block design showing significant differences in IAA levels.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>Mean Square</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blocks*</td>
<td>(r-1) = 3</td>
<td>1.29</td>
<td>1.43</td>
<td>.35</td>
</tr>
<tr>
<td>Treatment**</td>
<td>(t-1) = 2</td>
<td>512.85</td>
<td>271.42</td>
<td>222.47***</td>
</tr>
<tr>
<td>Error</td>
<td>(r-1)(t-1) = 6</td>
<td>7.30</td>
<td>1.22</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>(rt-1) =11</td>
<td>551.44</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Sensitivity setting 1x, 3x, 10x, 30x

**Sampling time (LL0, LL3, LL6:15)

This means that the differences in IAA levels are not due to error introduced by different sensitivity settings on the
fluorometer. The difference between treatments was found to be significant at the 0.005 per cent level. This is evidence that the change in IAA levels with time is a real change.
Discussion

The use of synchronous cultures made it possible to obtain a large quantity of cells that passed through their life cycle simultaneously. The synchronization of cells presupposes that all, or most of the cells, are in the same physiological state and at the same stage of development. The use of light:dark cycles to produce synchrony was chosen because of the view of Abbo and Pardee (1) that any unnatural interference in cell metabolism, such as a deficiency of an essential nutrient, nutrient shifts or thermal shock, leads to the formation of artifacts in the cells, and to an abnormal type of cell metabolism.

The photoinduction method of synchronization is a reliable and frequently used method, but even with this method, one must realize that the external factor(s) which induce this homogeneity of life stages have themselves specific effects on the life cycle which may have physiological and chemical implications within the cell. This could mean that the process of synchronization may induce unnatural or artificial levels of IAA within the cell but this possibility is offset by the great advantage of synchronous cultures.
If one considers exponential growth during synchronization as a characteristic of "normality," then it is apparent that the cultures fulfill this criterion because of the exponential growth shown in Fig. 4. The criterion most frequently used for the degree of synchronization is simultaneous division of the cells, which is really the only phase in the life cycle of the individual cell that can be observed satisfactorily. Another criterion used is a similar "burst" number in each successive division, which indicates participation of most cells in the division process. As shown in Fig. 4, in two successive divisions the time required for complete division is approximately 3 hrs, and the "burst" number for each division remained constant.

In the past, sharp synchronization has been characterized by completion of cell division in 10 per cent of the time required for the entire life cycle. Since a 24 hr life cycle was used, the maximum allowable time would be 2 hr and 40 min, and by this time approximately 90 per cent of the division was completed.

The low light intensity was chosen to produce the shortest possible time for incipient cell division. Sorokin and Krauss (15), working with the same strain of Chlorella pyrenoidosa over a wide range of temperatures and illumination, found that 40 C and 300 ft-c, 6 hr was the shortest
possible incipient cell division time attainable. In the chamber used for my experiment, the low light illumination varied ± 100 ft-c, with the average illumination at 350 footcandles. The time to incipient cell division was found to be 6 hr and 15 min, which agrees well with Sorokin and Krauss.

The importance of producing the shortest possible generation time was illustrated by the fact that essential processes such as nuclear division, and release of autospores under the lengthening of the generation time, were influenced differently by varied external factors such as temperature and illumination (12). In addition, cells that were completing their life cycle in the shortest possible time were very active physiologically and provide cell material that was operating optimally.

The extraction procedure used was a short-term extraction method, and it must be realized that IAA and many auxin compounds may exist either "free" or "bound" to various compounds. As pointed out previously, Thimann and Skoog (17) found measurable concentrations of auxin after months of low temperature extraction with ether. The extraction procedure used in my experiment gave only a measure of the "free" IAA content which may be but a small fraction of the total IAA compliment. Thimann and Skoog also found increased auxin yields with trypsin treatments, indicating that the
"bound" auxins may be complexed to protein. It must be pointed out that only the ether soluble fraction was analyzed and some IAA may exist in the water fraction, although pure IAA is only slightly soluble in water. Bentley (5) found that the water fraction may contain several important auxin compounds, one of which may be IAA. Fluorometric assays carried out on this fraction earlier in my experiments showed no measurable fluorescence due to IAA, and in later assays this fraction was ignored.

The use of fluorimetry greatly facilitated this study because of the great specificity this method offers. Filter systems were selected to pass only a narrow band of exciting and fluorescing light which prevented the fluorescence of many of the compounds in the extract which may fluoresce naturally. Those compounds that had excitation and emission spectra close to the indole-α-pyrene could be compensated for by the use of an extract blank. In the past, laborious extract purification procedures had to be performed to remove antagonistic substances in the case of the bioassay, and interfering substances in the case of indole color reactions. This fluorometric assay procedure eliminated the need for this. Also, this method offers much greater sensitivity compared to the standard indole color reactions. The sensitivity offered by this method approaches the biological assay and exceeds it in precision.
The trifluoroacetic acid-acetic anhydride reaction provided a very specific means for fluorescing only IAA by the conversion to an indole-4-pyrone. Even indole compounds closely related to IAA were not affected by the reaction because of the rigid structural requirements required for its formation. The filter system and specific fluorescence reaction greatly increased the probability that any fluorescence measured was the result of the presence of IAA.

The "quench" curve in Fig. 3 was constructed because it was suspected that some of the fluorescence was being absorbed or scattered by other components of the extract. From a comparison of the "quench" curve with the standard curve of IAA in methanol, it is apparent that for similar IAA concentrations the algal extract reduces the fluorescent yield 3 of 4-fold. Also, the lowest detectable concentration using the "quench" curve is 0.006 μg per gm of solvent, compared to 0.001 μg per gm of solvent for the "unquenched" curve.

It was extremely difficult to compare the results of my experiment with those of previous workers, because of the different experimental methods employed and the different units of measurement by which the data were reported.

For example, Van der Weij (18), working with Valonia, expressed his results as μg of auxin per ml of extract.
Since his work gave no indication of the total extract volume and the weight of material used, it was impossible to correlate his work with mine.

Most of the previous studies reported results in μg of IAA per gm of fresh weight. If one assumes a conversion coefficient of 10 per cent, one can convert this data to μg of IAA per mg of dry weight. Such an assumption is dangerous and there may be a wide variation in the definition of wet weight by various experimenters, but comparison of this study with previous results is meaningless without similar units of measurements.

In 1940, Van Overbeek (19) reported $8 \times 10^{-6}$ μg of IAA per mg of dry weight for *Bryopsis*. This level was well below the concentration of IAA found at all sampling points during this study. Also, in 1940, Van Overbeek (19) analyzed the terminal blades, the bladder, and the stipe of *Macrocystis pyrifera* and found $5 \times 10^{-7}$ μg of IAA per mg dry weight in the terminal blades and $1.5 \times 10^{-8}$ μg per mg dry weight in the bladder and stipe. Both of the values are substantially smaller than those measured for *Chlorella* in this study.

The reasons for the differences between the previously reported values and the value reported in this experiment may be due to the extraction procedure employed, the great deal of variability of experimental material when using bioassays, or a multitude of other reasons. It is possible that the previous levels reported and the present study are both
correct, and the differences are due to inherent differences between algal groups. The higher levels found here may also be due to the nature of the experimental material used. *Chlorella pyrenoidosa* (TX-7-11-05) is an extremely rapid-growing strain, and at 40°C it is operating at its peak metabolic rate. Under these conditions, increased hormone operating levels would not seem unlikely.

The reason for the increase in IAA throughout the life cycle still remains in question. It is impossible to determine from this data that IAA is involved directly in cell division, but it seems likely that it would be at least indirectly involved considering the hormonal nature of the compound and the rapid rise in IAA toward the end of the life cycle. Also, the studies involving the exogenous application of IAA on algal cells, with the subsequent increase in cell number, lends credence to this theory.

The significance of the higher levels of IAA just prior to division is beyond the scope of this study, but the fact that it does increase lends support to one theory for the action of IAA within the cell. Skoog (14) proposed that the auxin level affects the DNA/RNA ratio and that this in turn influences the relative rates of cell multiplication and enlargement. This was the first suggestion that the action of a hormone is intimately concerned with nucleic acid metabolism. There seems to be little doubt about the
capacity of auxin to enhance RNA synthesis in higher plants at the level of transcription and translation.

Especially important are the findings that higher concentrations of auxins blocked both cell enlargement and nucleic acid accumulation (14). This would lend importance to maintenance of the proper levels of IAA within the cell. The results of this experiment should show the endogenous operating levels for proper function of IAA in Chlorella cells.

Further research would be valuable to determine the effect of various environmental factors upon endogenous IAA levels and to show how these factors affect cell function through regulation of IAA levels in the cell.
Summary

Endogenous levels of indole-3-acetic acid were measured in synchronous cultures of *Chlorella pyrenoidosa* (TX-7-11-05). The cultures were synchronized by alternating light:dark periods of 15:9 hr at a temperature of 40±1°C. After 2 synchronous cycles the cultures were exposed to a low light treatment of 350±100 ft-c. The time to incipient cell division under these conditions was found to be 6 hr and 15 min. Samples were taken at 3 sampling periods during the low light treatment period; low light 0 hr (LL0), low light 3 hr (LL3), and low light 6:15 hr (LL6:15). The algal extracts were analyzed by a fluorometric procedure which measured the indole-α-pyrone product formed by the action of the trifluoroacetic acid-acetic anhydride reagent on IAA.

The IAA levels increased gradually from the autospore stage (LL0) to the adolescent stage (LL3) and more rapidly when approaching the ripened adult stage (LL6:15). The mean percentage increase from autospore to adolescent was 36.9 per cent, and from adolescent to ripened adult 104.6 per cent. The total percentage increase from autospore to adult was 180.3 per cent. Levels of IAA were 2 times higher just prior to division than in the autospore stage.
REFERENCES


