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Title THE INFLUENCE OF (2-CHLOROETHYL)-TRIMETHYLAMMONIUM CHLORIDE ON THE RIBONUCLEIC ACID CONTENT OF WHEAT (TRITICUM AESTIVUM) LEAVES

Abstract approved Redacted for Privacy
(Major professor)

A study was made on the effect of CCC (2-chloroethyl)-trimethyl-ammonium chloride), a plant growth retardant, on the RNA (ribonucleic acid) content of wheat plants. It was found that the RNA content of wheat leaves generally decreased with increasing levels of CCC up to $1x10^{-5}$M. Thereafter, the RNA content again increased until the threshold of toxicity to wheat for this compound was reached. Very low levels, $1x10^{-8}$M., of CCC tended to slightly increase the RNA content of the same tissue.

There was a qualitative difference in the RNA of treated plants as compared to the non-treated check. This qualitative difference was demonstrated by an alteration of the maximum absorbing wave length in the ultra-violet region of the spectrum. The non-treated material had the greatest optical density at 256.1 mμ, treatments of $1x10^{-8}$M. and $1x10^{-7}$M. CCC had a maximum optical density at 257 mμ,

while the treatments of $1x10^{-6}$M. to $1x10^{-4}$M. CCC had an absorption peak at 257.75 mμ.

The radio tracer data suggested there was less RNA-C^{14} present in the leaves of the plants treated at $1x10^{-5}$M. CCC compared to those of the non-treated in the period of thirty to thirty-three hours after the initial exposure to $C^{14}O_2$. Thereafter, the labeled RNA appeared in nearly equal amounts in both tissues. The RNA-C^{14} level of the non-treated plants was almost constant in all of the harvests after the thirty-six hour period, whereas that of the treated material was quite variable. This data is interpreted as evidence of a cyclic synthesis and degradation of RNA, the periodicity of the cycle is a multiple of eight hours (the period between harvests). The CCC treated material also appeared to have a cycle. However, this cycle was out of phase with that of the normal plant. The cyclic nature of the RNA content was also determined spectrophotometrically. It was also apparent that the rate of RNA degradation was greatest in the plants treated with CCC.

The RNA content data presented was similar to that presented by other workers studying the effect of 2,4-D on the RNA content of corn mesocotyl tissue. It is reported elsewhere that CCC applications increase the auxin content. When the data presented here were considered with that of the 2,4-D and other CCC work, the hypothesis that CCC increases the diffusible auxin content of treated tissue is supported.

THE INFLUENCE OF (2-CHLOROETHYL)-TRIMETHYLAMMONIUM CHLORIDE ON THE RIBONUCLEIC ACID CONTENT OF WHEAT (*TRITICUM AESTIVUM*) LEAVES

by

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INTRODUCTION

In recent years there has developed an extraordinary interest in plant growth regulation. A host of chemical compounds are now in commercial use as agents to control various facets of plant growth. The most common of the plant growth regulators are the herbicides used selectively to kill vegetation. In addition to inducing the actual death of a plant, growth regulators are used to prevent sprouting of stored tubers or root crops, defoliation or dessication of crops such as cotton and legumes, to aid the harvest operations, for the induction of fruit set or to cause fruit thinning, initiate the flower and root, and for the regulation of fruit and plant size.

In this study, some of the physiological influences on plants of the growth retardant CCC (2-chloroethyltrimethylammonium chloride) are examined. Agricultural workers became interested in CCC when it was demonstrated to dwarf various species of crop plants. One possible use for this compound which is being explored by agricultural workers is for the reduction of lodging in long stemmed cereal varieties. It has found commercial acceptance in the dwarfing of poinsettias. This same compound has been observed to modify several plant responses to environment such as increasing resistance to high and low temperatures and tolerance to drought. Reports also indicate higher tolerance to salt content,

decreased desirability of treated plants to certain insects and increased tolerance to some pathogens.

Because there is no apparent relationship between the various observed influences of CCC upon plants, it might be assumed that a fundamental metabolic process is affected. Therefore, an attempt was made to examine the effect of CCC on the content of ribonucleic acids in wheat leaves.

REVIEW OF THE LITERATURE

2-chloroethylammonium chloride (CCC) is an analogue of choline and is classed as a member of the quaternary ammonium compounds. This family has long been known for its biological activity which was first reported in the early twentieth century. Wirwillie and Mitchell (43) studied other quanternary ammonium compounds and reported their growth retarding properties. In 1954, CCC was studied in animal physiology as an inhibitor of the acetyl cholinesterase system (11). It was not until work by Tolbert, Wittwer and co-workers was reported in 1960 that very much interest was shown in CCC by agricultural scientists.

Tolbert, Wittwer and their colleagues (22, 38, 39, 40, 46) reported that CCC caused wheat, poinsettias and chrysanthemums to be dwarfed. Treated wheat plants were not only stunted, but possessed a much darker green color and thicker foliage (3). No reduction in yield or root growth resulted; however, in some cases CCC altered the time of flowering (45, 46). This compound also increases the plant's tolerance to some harsh environmental conditions such as drought (14), soils of high salt or fertilizer content (27, 30), low temperatures (2), high temperatures (37), and decreases water and phosphate uptake and translocation (12). It also may prevent insect attack (1).

Phosfon D (tributyl-2,4-dichlorobenzylphosphonium chloride)

and Amo 1618 (2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine carboxylate methochloride) cause the same responses on plants as the factors that have been measured and it is assumed would show similar responses in the non-measured criteria. Although CCC does not equal either Amo 1618 or Phosfon in the effect on some species it generally has a wider activity spectrum (9). There is evidence that these materials also have growth inhibiting properties with respect to some bacteria (36).

The actual mode of action of these compounds has not yet been determined. Tolbert (38, 39) suggests that CCC is specific for a particular enzyme site and that the trimethylammonium group is responsible for the activity. Tolbert (38) further proposes that there is a two-point attachment involved, the ethyl group being the optimum length for the facilitation of the two-point attachment and the trimethylammonium group being mandatory.

The nature of the enzyme system is still in doubt; however, it is thought by most of the workers that the enzyme system must be involved with the gibberellic acid growth scheme (10, 24, 38, 44, 45, 46). Fockhart (24) proposes that CCC and gibberellic acid do not compete for the same enzyme site due to the lack of molecular similarity; however, he proposes that CCC upsets the equilibrium of one reaction associated with the gibberellic acid promoting scheme. Amo 1618 is believed to be a mitotic poison acting in the same area as the endogenous gibberellins (10). CCC

inhibits the growth stimulation of IAA (indoleacetic acid) in Avena coleoptiles; however, the inhibition is overcome by the addition of exogenous gibberellin (46). Kurashi and Muir (20, 21) report that CCC reduces the diffusible auxin content in pea plants by one-sixth; this agrees with some unpublished observations of Gould and Baldwin. As a result of these observations, Kurashi and Muir propose that CCC is actually an anti-auxin. If one subscribes to the proposal of Kefford and Goldacre (15), it is really of little importance whether a material is an anti-gibberellin, an anti-auxin or an anti-kinen, as these three groups of plant growth regulators are intimately interdependent and in a delicate balance for any given stage of the plant's development. It is interesting to note these two views as there have been reports of a CCC-like material isolated from plant tissue (28, 29) and from a plant pathogen (Tilletia sp.) (31). The symptoms of Tilletia sp. infection are much the same as those of CCC treatments. These data which indicate there are endogenous quaternary ammonium compounds, suggest that CCC may exert its influence by altering the effective concentration of a natural compound.

Due to the fact that the plant growth regulators are active at very low concentrations and that they affect a multitude of systems, one is led to believe that these materials are actually a triggering mechanism for some system that is common to all cells involved. Bonner and co-workers (6) suggest that growth regulators

are actually the messengers directing gene activity.

Bonner's group has shown the existence of a histone sheath about DNA (deoxyribonucleic acid) and cites evidence that this histone sheath is removed during times of high plant growth activity such as flowering, at which times there is also a high concentration of auxin. The histone sheath is believed to act as a physical barrier to the RNA nucleotides, thus preventing the DNA molecules from acting as a template to synthesize messenger RNA (ribonucleic acid). Bonner (6) suggests that the plant growth regulators induce the removal of the histone sheath from the DNA molecule.

There has been other evidence presented which supports the hypothesis for a direct relationship between the growth regulators and the nucleic acids. The application of auxin increases the rate of phosphorus incorporation into the nucleic acid fraction (5). However, this incorporation was not followed in the nucleic acids per se but rather the synthesized nucleotides. With the application of low levels of 2,4-D (2,4-dichlorophenoxyacetic acid), a herbicide with auxin-like activity, the RNA content of the treated tissue was observed to increase and to be altered in the base ratios (18, 42). West (42) has shown that it is the RNA of the microsomal fraction which increases. However, this is not a reflection of gene activation according to the results of Brenner et al. (7).

Etiolated buds which have a higher auxin content than normal

buds, also have a lower RNA content (41). However, in the etiolated buds the DNA content has been shown to increase. This suggests a greater number of cells per unit weight.

The maintenance of a normal content of IAA, which in this case can be replaced by 2,4-D, tends to prevent the tissue from aging (4) presumably by preventing the degradation of protein and RNA. It has been shown that the RNA content of plant tissue increases until maturity, at which time the RNA content decreases (16, 26), while RNAase activity increases after tissue maturity has been maintained (16).

It is now a commercial practice to prevent the aging of some harvested crops by the application of a kinen material. It is probably that the kinen interacts with an indogenous auxin to prevent senility, in contrast to the applications of auxin-like compounds where the endogenous kinens are involved. The addition of both kinens and auxins to Nicotiana induces the formation of tumors (33). This indicates there is an alteration of the RNA system, or perhaps an abnormality formed within the DNA molecule. The former appears to be more plausible.

Since the ribonucleic acid fraction of the plant will be a major concern in this study, the literature on RNA is reviewed on the role of RNA in cellular metabolism. The function of this material is two-fold, the first being a transfer agent for the coded information of the gene to the site of protein synthesis

and the second providing the site for protein synthesis.

There are three types of RNA, messenger RNA (mRNA), soluble or amino acid acceptor RNA (sRNA) and ribosomal RNA; the latter forms the bulk of the RNA present (9, 13, 34).

It is now generally accepted that DNA is the material responsible for information storage and the continuity of the species from generation to generation. DNA molecules are long chains of four bases joined by sugar and phosphate ester linkages. Two complimentary strands of DNA come together and with the aid of hydrogen bonds form a double helix. In the double helix adenine must hydrogen bond with thymine and guanine must bond to cytosine.

It is not yet understood how the RNA derives its configuration from DNA. It is postulated that the mRNA becomes associated with the DNA and by means of a "replicase" (23) gains the complimentary configuration with respect to template DNA. It is not known whether the DNA molecule disassociates into two single strands; however, it is apparent that RNA copies only a portion of one strand.

At a given time, a required portion of the DNA stored information becomes available and the corresponding mRNA is synthesized. Bonner (6), as already mentioned, suggests that the timing is carried out by the selective removal of a histone sheath which encircles the DNA molecule.

Ribosomal RNA is also synthesized in the nucleus -- it is presumed that it is also a DNA dependent RNA. However, after synthesis it migrates to the nucleolus where it apparently directs the synthesis of a protein material and then conjugates with this same protein to form the ribosomes (6). The ribosomes then migrate to the endoplasmic reticulum.

The recently synthesized mRNA migrates from the nucleus to the ribosomes where the mRNA forms an apparent loose association with the ribosomal RNA. The latter is reported to play a passive role in the subsequent protein synthesis (23). It is at this site that the amino acid acceptor, or sRNA, joins with the activated amino acids (23). The exact amino acid attached to the sRNA depends on the configuration of the sRNA involved (34). The sRNA, with the attached amino acids, then becomes associated with the mRNA resting on the ribosomes. It is here that protein synthesis is believed to occur. Immediately after the polypeptide chain is synthesized, the mRNA degrades and the bases return to the purine-pyrimidine pool (23).

Ribosomal RNA makes up about 80% of the RNA fraction. It is therefore difficult to imagine that it plays only a passive role. Canellakis (8) reports there is a relationship between the total RNA content of an organism and its growth rate, more rapid growth occurs with a higher RNA content, perhaps higher RNA content infers to more numbers of ribosomes in the cell,

which in turn provide more sites for enzyme synthesis. This results in a higher concentration of enzymes or in a rapid turnover rate of transmitting information from DNA; thus growth processes are facilitated.

In examining the literature, it seems quite evident a relationship exists between the growth regulating chemicals, both natural and synthetic, and the nucleic acid systems. It is still unknown what the exact role of IAA is in nucleic acid metabolism. However, one might assume that the naturally occurring analogue of CCC modifies the action of IAA in this sphere.

STUDIES ON THE EFFECT OF CCC ON THE RIBONUCLEIC ACIDS OF WHEAT PLANTS

(a) The Effect of CCC on the Total RNA Content

A study was carried out in which the total RNA content of wheat leaves, subjected to various levels of CCC, was estimated spectrophotometrically. The concentrations of CCC used in this study ranged from the maximum concentration where visible effects were not evident ($1x10^{-8}$M.) to the threshold of CCC toxicity for the test species ($1x10^{-3}$M.). It was decided that a logarithmic rather than an arithmetic progression of rates would be employed so that a greater range of concentrations could be examined.

Materials and Methods. The test material used throughout was Druchamp wheat obtained from the cereal breeding project of Oregon State University. The wheat was sown in "Pyrex" baking dishes containing one-half strength Hoagland's, or White's solution and the various molar concentrations of CCC. The tops of the wheat plants were harvested ten days after seeding, when the material was in the two leaf stage, harvested material was slowly dried in an oven with a temperature of 40-45°C.

The dried leaves were powdered in a Wiley Mill and then passed through a 40-mesh screen. The dried powder was then stored in a refrigerator until used.

The method for estimating RNA generally followed that outlined by Smillie and Krotkov (35) and was only modified in that the RNA was estimated directly by ultra-violet absorption, rather than estimating the ribose content. This modification had no effect on the reliability of the method. The defatted material, referred to as residue C by Smillie and Krotkov, was used as the basis of expressing RNA content in this experiment. It was found that extreme difficulty was encountered in obtaining a uniform yield of the defatted material from the dried sample; thus, RNA expressed on a total dry weight base may have led to erroneous conclusions. It was assumed that the lipid materials and the acid soluble phosphates which were removed occurred in equal quantities in the treated and non-treated material. This assumption is supported by the recovery data, which although highly variable, did not show any indication of a pattern of variation.

Weighed portions of the defatted material were placed in 50 milliliter beakers, to which 25 milliliters of 0.3 normal potassium hydroxide was added. The beakers were then covered with aluminum foil to prevent an increase of potassium hydroxide normality by evaporation, and then placed in a low temperature oven (35-40°C) for sixteen hours to hydrolyze the RNA fraction.

At the end of the sixteen hour hydrolysis period, the beakers were placed in an ice bath. When the material had reached a temperature equilibrium with the ice bath, magnesium chloride was added to

bring the solution to 0.001 molar with respect to the magnesium chloride. The pH was then adjusted to about 2.0 with the addition of perchloric acid. One volume of 95% ethanol was then added to each beaker and the material again left to equilibriate the temperature with the ice bath. The material was centrifuged at 5,000 times gravity for ten minutes, the supernant saved and the pellet washed with 20 milliliters of 1% perchloric acid, which was again centrifuged at 5,000 times gravity for ten minutes. The supernatants of these two steps were bulked.

The combined supernatants were then adjusted to pH 8 with dilute potassium hydroxide and then placed in an ice bath for at least one-half hour to precipitate the potassium perchlorate which was removed by centrifugation. The crude RNA solution was then stored in the refrigerator until the ion exchange columuns were prepared.

Dowex 1- x 8, 50 - 100 mesh, was washed in hot, one normal hydrochloric acid, rinsed with distilled water until a negative test for hydrogen ions was obtained with litmus paper and then placed in 50 milliliter funnels to a depth of 5.0 centimeters. The bed volume of these columns was 0.3125 cubic centimeters. The crude RNA preparation was then added to the respective columns, the columns washed with 20 milliliters of 0.01 normal sodium chloride and then eluted with 25 milliliters of a solution containing 5.6 grams of sodium chloride, 20 milliliters of concentrated

hydrochloric acid and 240 milliliters of distilled water. The elutant was received in 25 milliliter volumetric flasks which insured a constant volume between samples at the end of the eluting process.

It was found that greater than 80% of the RNA was eluted with 25 milliliters of eluting solution. It was decided to sacrifice some RNA in order that the total volume might be kept at a low level (see Figure 1).

The RNA preparation prior to elution through the Dowex-1 column, had a very broad absorption peak with the maximum at a wave length of 270 millimicrons, and an absorption minimum at 252 millimicrons. This crude preparation contained a material which absorbed very strongly below 252 millimicrons. The material which passed through the Dowex-1 column had only one absorption peak, that being near 260 millimicrons (see Figure 2).

The estimation of the RNA was made by measuring the absorption of the solution at 260 millimicrons with a spectrophotometer and comparing it to a purified sample of liver RNA obtained from Nutritional Biochemicals Inc. The quantitative measurements were made using the formula:

$$\frac{\text{optical density sample} \times \text{concentration}}{\text{optical density standard}} = \frac{\text{sample concentration}}{1}$$

A linear relationship was found between the optical density and the concentration of RNA (see Figure 3). The optical density

Figure 1.

The recovery of RNA from the Dowex-1 column. More than 80% of the RNA is eluted with 25 milliliters of the HCl-NaCl-water solution.

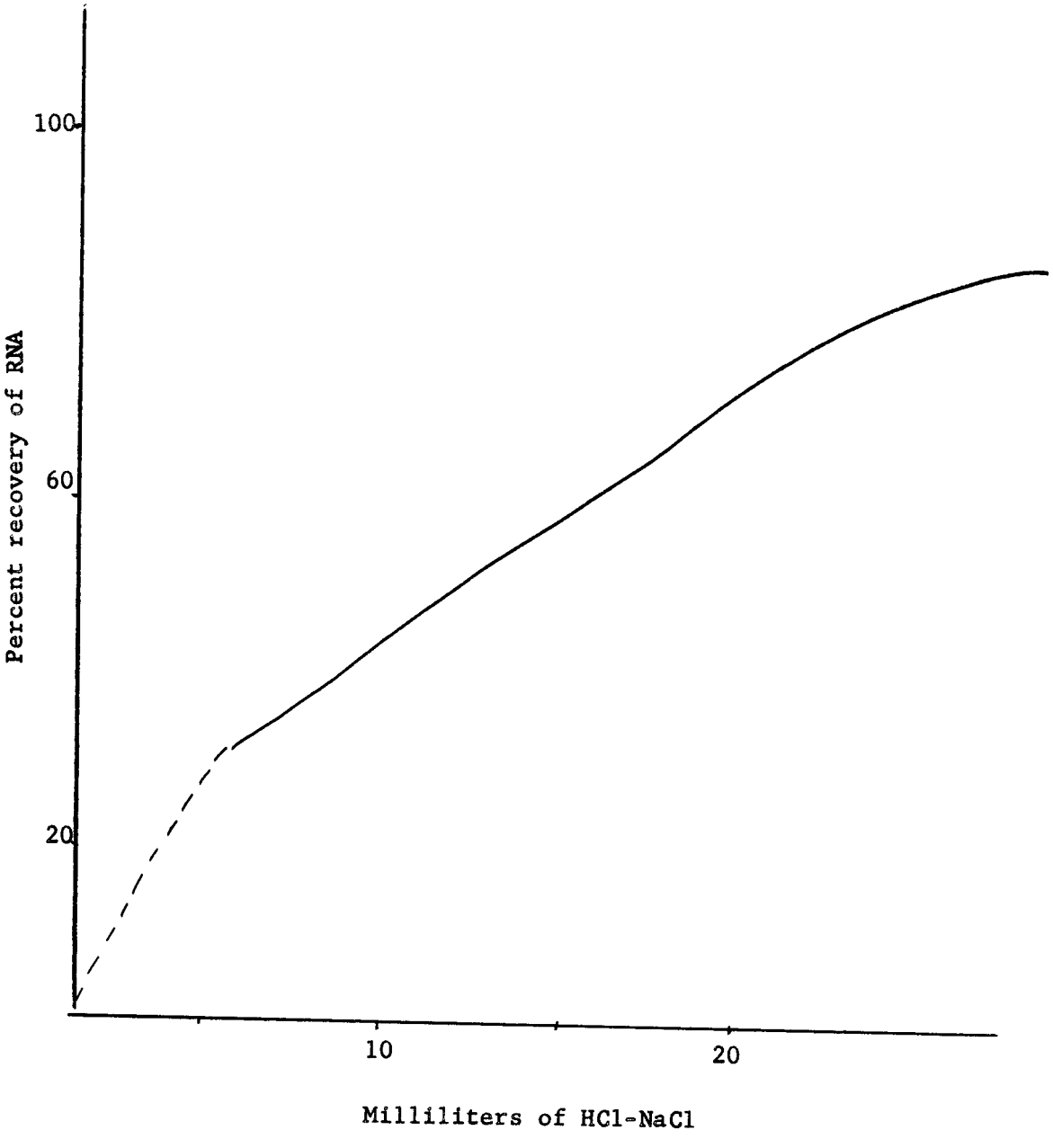
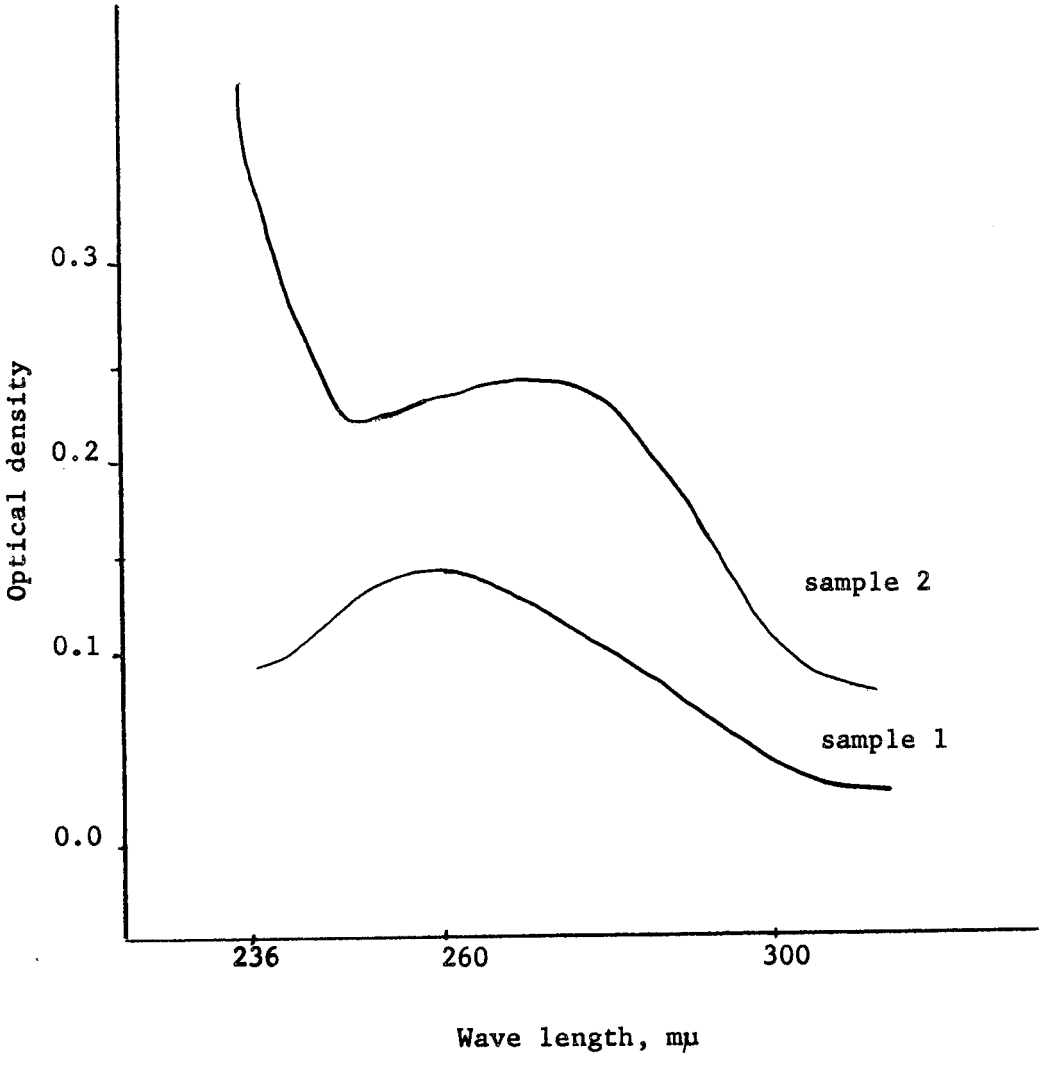

100
60
20
Percent recovery of RNA
10
20
Milliliters of HCl-NaCl

Figure 2.

The effect of the Dowex-1 column on the absorption spectrum of the RNA sample.

Sample No. 1 has been eluted from the Dowex-1 column with a HCl - NaCl - H_2O solution, sample number 2 is the crude preparation.


Optical density
0.3
0.2
0.1
0.0
sample 2
sample 1
236
260
300
Wave length, mμ

measurements were made with a Beckman Model DU spectrophotometer and with a Hitachi Perkin-Elmer model 139 spectrophotometer.

Results. The total RNA content of wheat leaves is altered with treatments of CCC. The extent and manner of the modifications vary with the particular concentrations of CCC to which the plants were exposed. These data are presented in Table 1.

At the lowest concentration of CCC used in these studies ($1x10^{-8}$M.), there is an apparent slight increase in the total RNA content as compared to the untreated check. The levels of CCC which exceed $1x10^{-8}$M. tend to decrease the RNA content until a minimum RNA content is expressed in the plants grown in the $1x10^{-5}$M. CCC solution. High levels of CCC, such as $1x10^{-4}$M. and $1x10^{-3}$M., tend to increase the level of RNA in the plant, the latter being greater than that of the non-treated material. These data are expressed graphically in Figure 4.

Table 1

Molar concentration of CCC	Micrograms of RNA per mg. of defatted tissue
0.00	67.2
$1x10^{-8}$	75.5
$1x10^{-7}$	63.9
$1x10^{-6}$	58.3
$1x10^{-5}$	55.1
$1x10^{-4}$	57.7
$1x10^{-3}$	70.6

Figure 3

The curve relating optical density and RNA concentration in test solution.

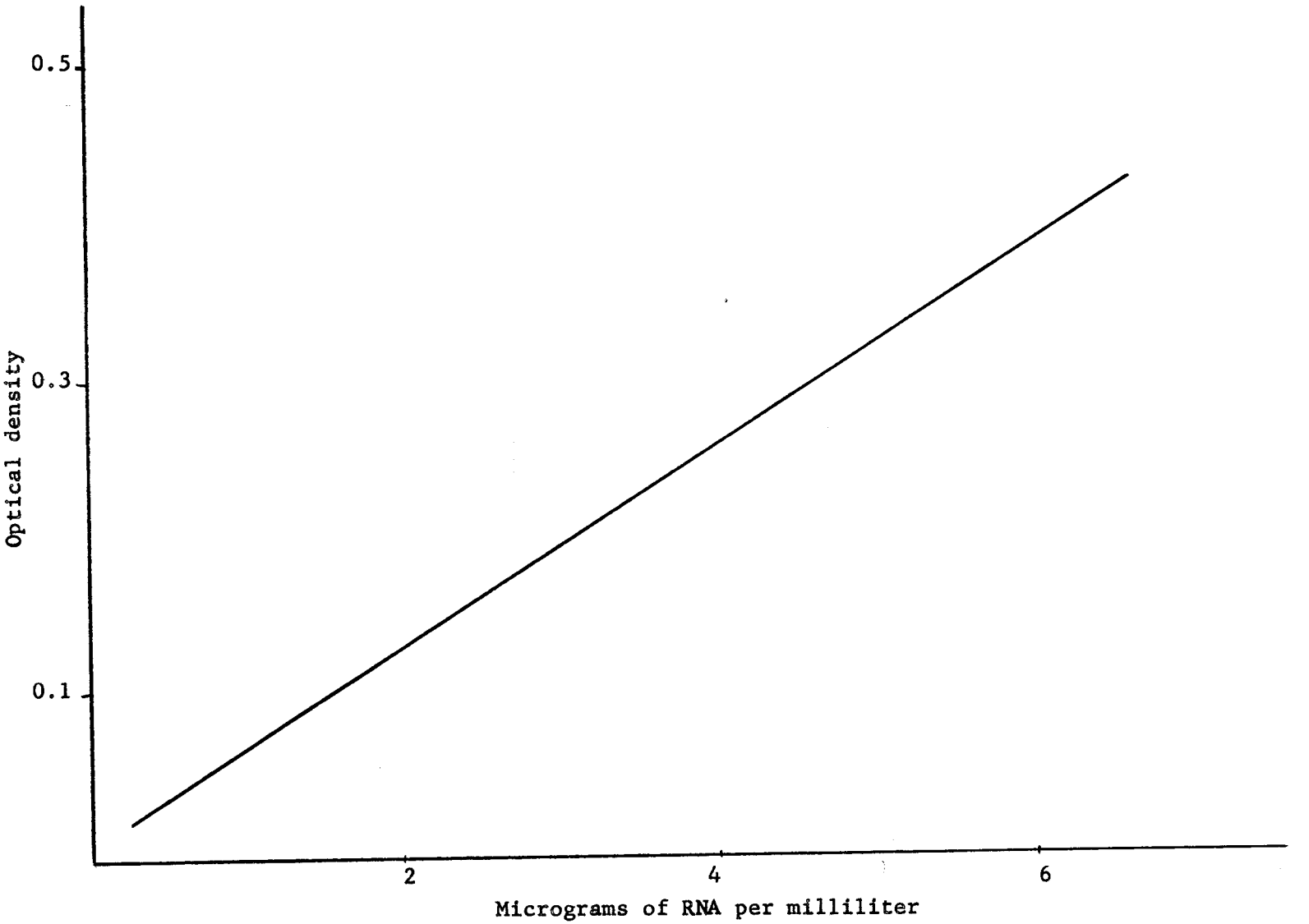
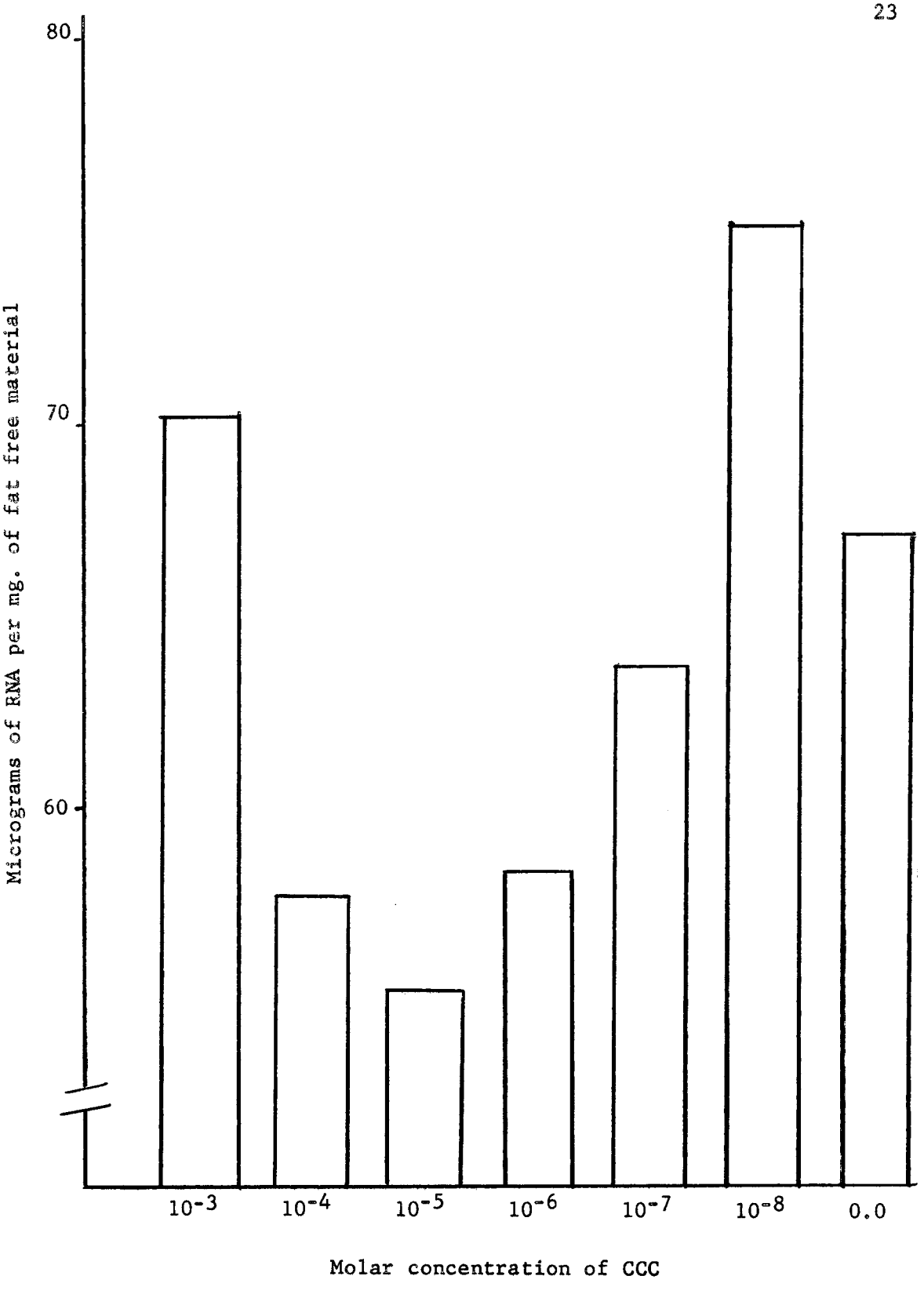

0.5
0.3
0.1
Optical density
2
4
6
Micrograms of RNA per milliliter

Figure 4

Graph illustrating the alteration of total RNA content with applications of CCC


80
70
60
Micrograms of RNA per mg. of fat free material
10-3
10-4
10-5
10-6
10-7
10-8
0.0
Molar concentration of CCC

There is a great deal of variation between replications in the data, the causes of which will be discussed below. However, the differences between treatments are quite consistent in relative values and are significant at the 5% level.

The question arose as to whether or not the increase of RNA content continued at higher levels of CCC. In Table 2 the results of four experiments show that there is an apparent increase of RNA content up to the toxic concentration of CCC. This increase is not, however, significant at the 5% level (see Figure 5).

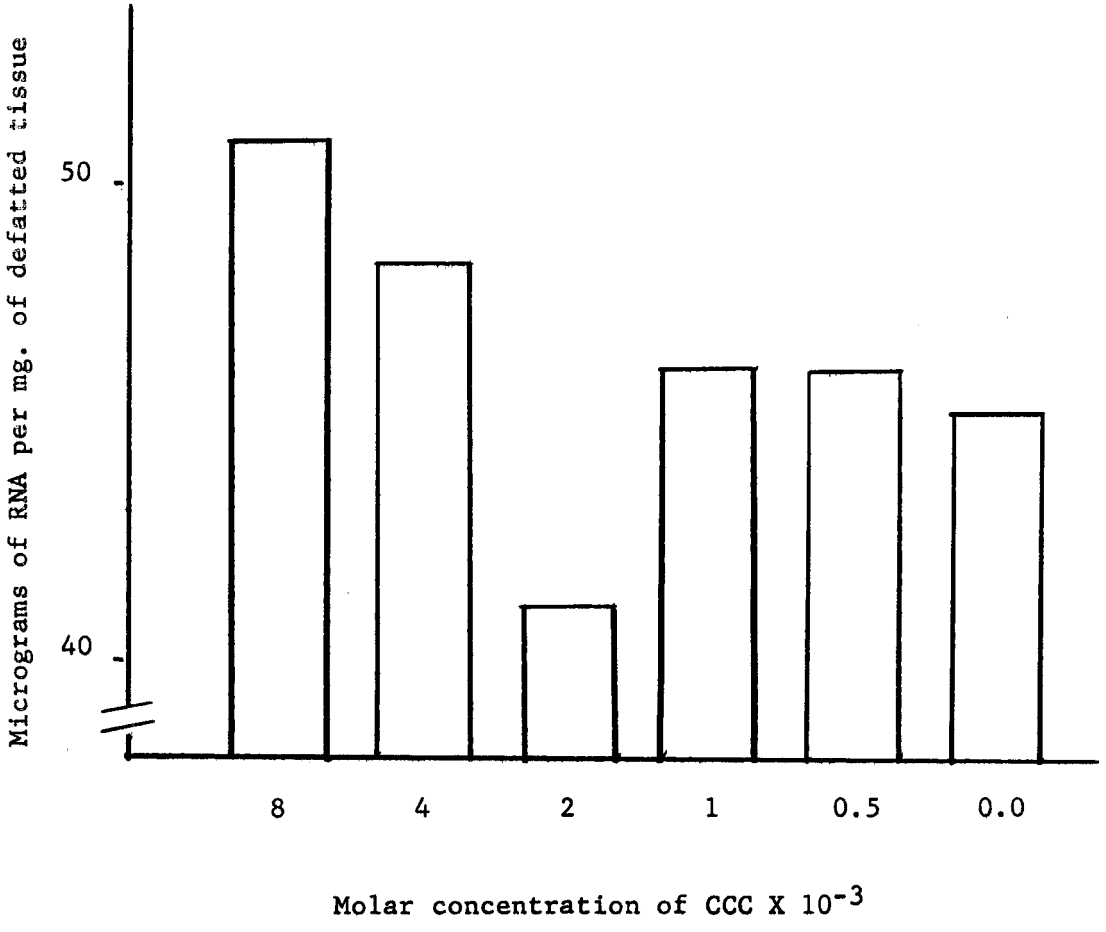
Table 2

Molar concentration of CCC	Micrograms of RNA per mg. of defatted tissue
1x0.00	45.4
$0.5x10^{-3}$	46.0
$1.0x10^{-3}$	46.0
$2.0x10^{-3}$	40.7
$4.0x10^{-3}$	47.4
$8.0x10^{-3}$	50.8

It was not possible to harvest enough material from the $1.0x10^{-2}$M. treatment to estimate the RNA content. There was no germination at higher concentrations of CCC.

Figure 5

A bar graph showing the tendency of the RNA content to increase when the plants are grown in a nutrient solution containing high levels of CCC.


Micrograms of RNA per mg. of defatted tissue
50
40
8
4
2
1
0.5
0.0
Molar concentration of CCC X 10^{-3}

(b) The Effect of CCC on the Absorption Spectrum RNA

The question was raised by the change of total RNA content whether the alteration of RNA was qualitative as well as quantitative. To examine this question experimentally, the absorption at different wave lengths, near 260 mμ, was scrutinized to check the wave length which exhibited the maximum optical density.

Materials and Methods. The isolation of the fraction was carried out as described previously. The measurements of optical density were made with a Hitachi Perkin-Elmer model 139 spectrophotometer. To prevent erroneous readings due to degradation of the RNA, samples were scanned from both ends of the spectrum and the results compared.

Results. A definite shift was found in the wave length exhibiting the maximum absorption with treatments of CCC (see Table 3 and Figure 6). The shift of absorption maxima was quite striking. With a very low level of CCC ($1x10^{-8}$ - $1x10^{-7}$M.) the maximum absorption wave length was increased from 256.1 mμ to 257.0 mμ. The higher levels of CCC, $1x10^{-6}$ to $1x10^{-4}$M., tended to further increase the wave length to near 257.75 mμ, while again at the higher concentration of $1x10^{-3}$M. CCC, there appears to be a reduction in the wave length, although it did not approach the wave length with maximum optical density displayed by the non-treated tissue.

Figure 6

The effect of various levels of CCC on the absorption spectrum of RNA. Soluble liver RNA (Nutritional Biochemicals Inc.) exhibits a maximum absorption at 260 mμ.

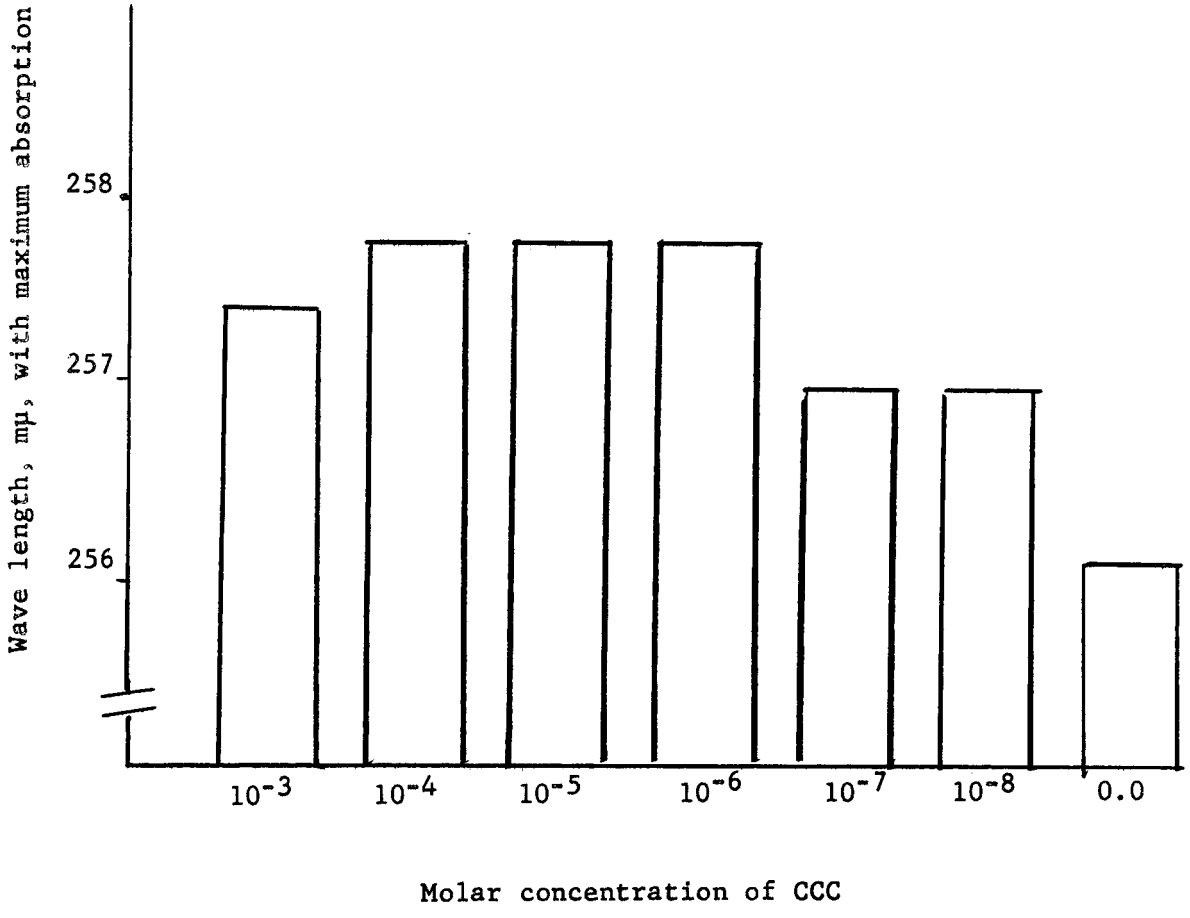

Wave length, mμ, with maximum absorption
258
257
256
10-3
10-4
10-5
10-6
10-7
10-8
0.0
Molar concentration of CCC

Figure 7

A demonstration of the negligible effect that the shift of the maximum absorption wave length has on the estimation of the total RNA. The marks on the curve represent 256, 258 and 260 mμ.
This particular curve is absorption spectrum of the RNA extracted from plants treated with $1x10^{-5}$M. CCC.

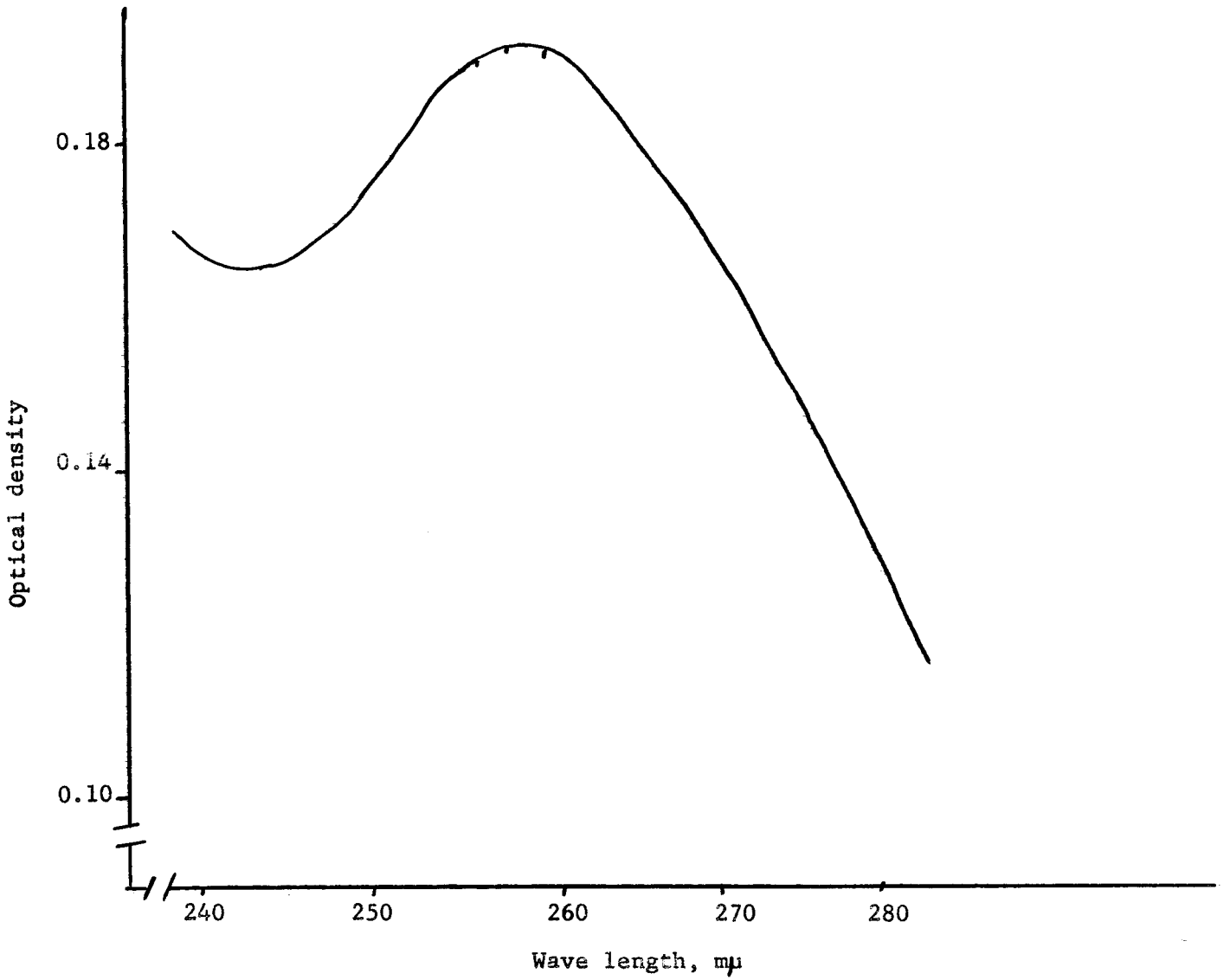

0.18
0.14
0.10
Optical density
240
250
260
270
280
Wave length, mμ

Table 3

Molar concentration of CCC	Average wave length exhibiting maximum absorption
1x0.0	256.1
$1x10^{-8}$	257.0
$1x10^{-7}$	257.0
$1x10^{-6}$	257.75
$1x10^{-5}$	257.75
$1x10^{-4}$	257.75
$1x10^{-3}$	257.3

The alteration of the wave length exhibiting maximum absorption would not be responsible for the differences that were observed in the total RNA content. This effect is assumed to be negligible as the optical density at 256 mμ to 260 mμ is not greatly different (see Figure 7). If the estimation of RNA at 260 mμ. instead of at the wave length exhibiting maximum optical density was a significant factor, it would in fact accentuate the differences that have been reported.

(c) The Effect of CCC on the Synthesis and Degradation of RNA

It is evident that a decrease of total RNA is the result of either a decrease in RNA synthesis or an increase in the degradation of RNA. In an attempt to elucidate this question, Druchamp wheat plants were grown in an atmosphere containing radio-active carbon dioxide (labeled with C^{14}).

Materials and Methods. The wheat plants were germinated and grown to the one leaf stage in "Pyrex" dishes containing Hoagland's nutrient solution as described in section (a). When the plants were in the one leaf stage, they were taken from the "Pyrex" dishes, the roots washed thoroughly and placed in 50 milliliter erlenmeyer flasks containing Hoagland's nutrient solution with either CCC present at $1x10^{-5}$M. or without. The plants were placed in the erlenmeyer flasks so that the roots were immersed in the nutrient solution and the foliage was entirely above the neck of the flask. This was accomplished by wrapping the seed of the germinated wheat plant in absorbant cotton, which was very satisfactory in keeping the plant in position. The plants were then left to grow to the early two leaf stage at which time they were placed in a ten gallon glass aquarium. The aquarium was equipped with a two-holed glass plate lid which formed an airtight seal on the rim of the aquarium with the aid of a stop-cock

grease. The sides of the aquarium were covered with aluminum foil to prevent any stray light from entering and causing C^{14} fixation by photosynthesis.

The aquarium was equipped with an external air cirulating system so that the unused $C^{14}O_2$ could be trapped in potassium hydroxide prior to opening the system for harvesting (see Figure 8).

When the plants were in the full two leaf stage, a blanket was placed over the glass lid to exclude all light from the plants, thus preventing any fixation of $C^{14}O_2$. One hundred microcuries of barium carbonate-C^{14} was placed in a suction flask, which was a part of the closed air circulating system. $C^{14}O_2$ was released by the addition of 10% perchloric acid to the suction flask. The air was circulated for four hours with the circulating pump; however, to insure complete dispersion of the $C^{14}O_2$, the blanket was left in place for sixteen hours.

Upon removal of the blanket, the plants were subjected to a twelve hour light period, eight hours of darkness followed by a nine hour period of light.

The excess $C^{14}O_2$ was trapped in a solution of potassium hydroxide by flushing the air from the aquarium and bubbling the exhaust through the basic solution.

The plants were harvested at 20, 33, 36, 38, 40, 48, 56, 64 and 72 hours after the initial exposure to light in the radio-active atmosphere. Immediately upon harvesting, the plants were immersed

in boiling water so that all enzymatic activity would cease. After killing, the tissue was dried at 40°C in an oven.

The dried material was ground in a mortar and pestle until a fine powder had been obtained. Weighed portions of the dried material were placed on planchets and counted on a Phillips model PW 4032 counter and scaler.

The defatted pellet was obtained in the manner that has been described above. Weighed portions of this material was checked for C^{14} content.

The defatted pellet was placed in 0.3 normal potassium hydroxide for sixteen hours to hydrolize out the RNA fraction. The RNA fraction was then purified with the use of the Dowex-1 column, and then one milliliter of the elutant was placed on planchets, dried and examined for radiation. The presence of the RNA in the elutant was confirmed spectrophotometrically.

Results. The detection of C^{14} in the RNA fraction was very difficult. It was found that greater than twenty-four hours exposure to 500 μc was required before RNA labeled with C^{14} could be isolated. The radio activity of RNA in the treated material is much less than the non-treated up to thirty-six hours after the initial exposure. Thereafter, there is an apparent reincorporation of the labeled products by the purine and pyrimidine salvage scheme. This is indicated as both the treated and non-treated tissue have about the same level of C^{14} present (see Figure 9).

Figure 8

A diagram of the apparatus used to incorporate Carbon 14 into the wheat plants by photosynthesis -

(a) light source (incandescent & fluorescent)

(b) plate glass lid

(c) air pump

(d) aquarium

(e) $C^{14}O_2$ generator

(f) valves for directing air flow

(g) KOH solution for trapping the excess $C^{14}O_2$

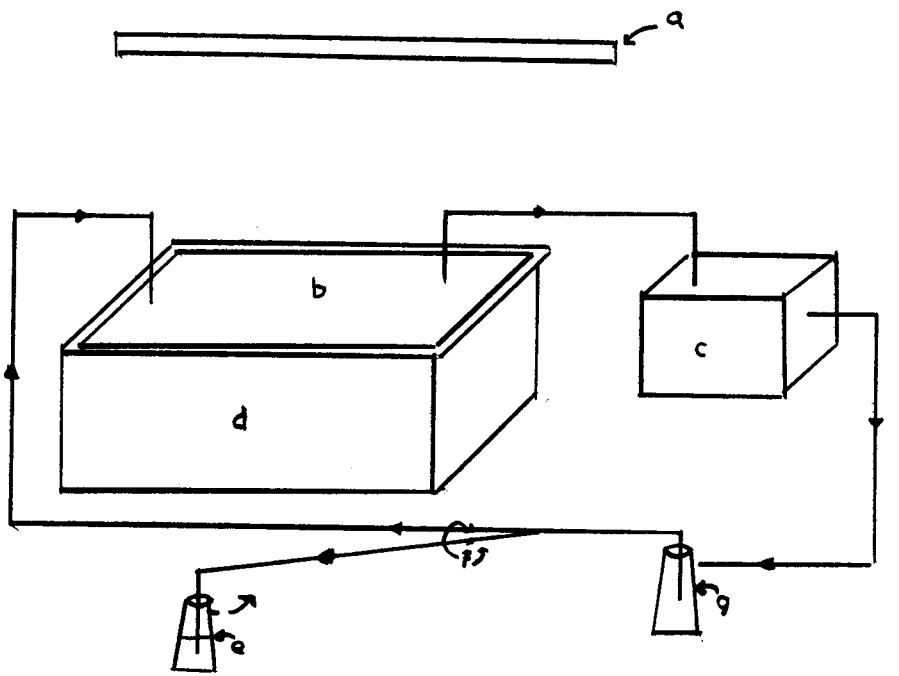
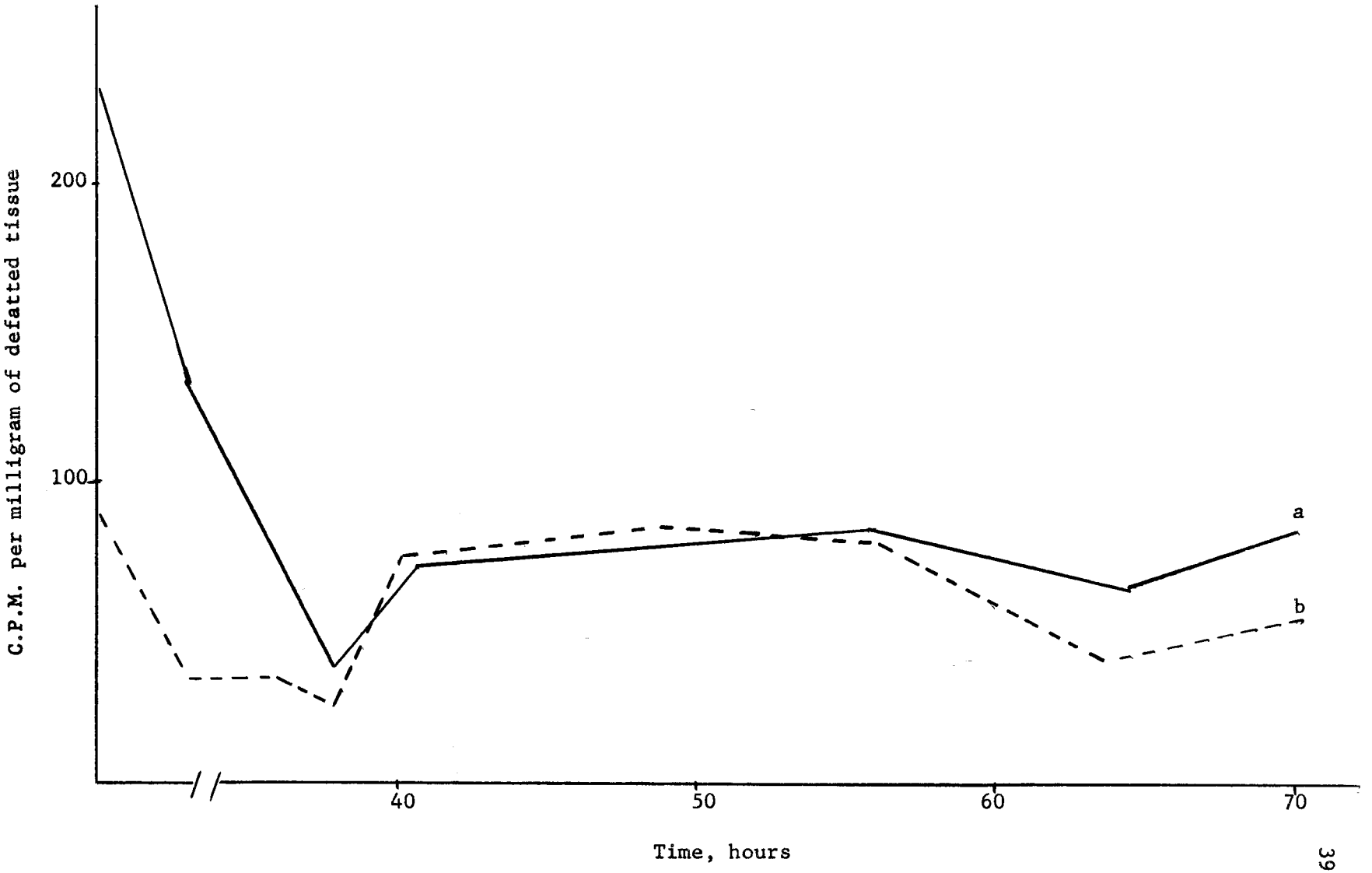

a
b
c
d

Figure 9

The degree of incorporation of C^{14} into RNA, per unit weight of defatted plant tissue, at different periods of time from the initial exposure to $C^{12}O_2$.

Curve a represents the check, curve b $1x10^{-5}$M. CCC


C.P.M. per milligram of defatted tissue
200
100
a
b
40
50
60
70
Time, hours

Results. The detection of C^{14} in the RNA fraction was very difficult. It was found that greater than twenty-four hours exposure to 500 μc was required before RNA labeled with C^{14} could be isolated. The radio activity of RNA in the treated material is much less than the non-treated up to thirty-six hours after the initial exposure. Thereafter, there is an apparent reincorporation of the labeled products by the purine and pyrimidine salvage scheme. This is indicated as both the treated and non-treated tissue have about the same level of C^{14} present (see Figure 9).

When the specific activity is considered, the dats is somewhat confusing due to the variable results. It is apparent that the specific activity of the RNA from treated tissue is less than that of the non-treated (see Table 5).

Table 5

Time (hours after initial exposure to $C^{14}O_2$)	cpm/mg. of RNA	
	Check	CCC
30	27	201
33	171	1221
36	458	70
38	261	52
40	147	172
48	137	105
56	146	170
64	136	77
72	143	135

It would appear from the specific activity data that there is a more rapid degradation of the treated tissue's RNA when compared with the non-treated and that the lower total RNA content that is experienced by treated tissue may be the result of a more rapid degradation. It may be noted in Table 6 that at 30 and 33 hours after initial exposure to the $C^{14}O_2$, the radio activity of the CCC treated RNA was greatly above that of the non-treated, whereas at the end of thirty-six hours, the reverse situation seems to hold.

When the above data, or related data which appear in the appendix, are expressed graphically, it suggests that the RNA metabolism is cyclic and that this cycle is disrupted with CCC applications. This same cycle of RNA synthesis and degradation is also evident with the spectrophotometric data which is expressed in the following table:

Table 6

Time (hours after initial exposure to light in $C^{14}O_2$ atmosphere)	RNA/mb. defatted pellet	
	Check	CCC
30	285.6	38.2
33	114.6	46.8
36	42.6	164.1
38	37.1	94.0
40	155.8	134.0
48	129.5	219.2
56	112.9	80.9
64	108.7	146.2
72	181.8	98.7

When one compares the incorporation of C^{14} into the RNA to the activity of the defatted pellet from whence the RNA was extracted, the cyclic effect is apparent to a limited degree. However, the rapid incorporation and degradation of the RNA in the CCC treated samples is evident (see Table 7).

Table 7

Time (hours after initial exposure)	cpm/mg. RNA / cpm/mg. fat free material	
	Check	CCC
30	0.012	0.081
33	0.031	0.874
36	0.110	0.046
38	0.092	0.024
40	0 033	0.120
48	0.054	0.045
56	0.055	0.067
64	0.076	0.062
72	0.088	0.062

When one examines the incorporation of C^{14} into the total plant with the defatted material a cyclic phenomena is observed for the CCC treated material apparently out of phase with the normal cycle (see Table 8).

Table 8

Time (hours after initial exposure)	cpm/mg. dry tissue		cpm/mg. defatted tissue	
	Check	CCC	Check	CCC
30	2504	2668	2333	2480
33	1724	1684	5561	1397
36	2387	1324	4173	1513
38	1645	1324	2836	2164
40	2005	2124	4414	1435
48	3011	1515	2526	2344
56	1233	1983	2660	2535
64	1490	2336	1780	1252
72	2067	2088	1620	2161

In a preliminary trial, the exposure to $C^{14}O_2$ was not intensive enough to provide any radio activity in the RNA fraction, yet the material examined six and one-half hours after the initial exposure to $C^{14}O_2$ again exhibited the cyclic metabolic effect in the total dried plant and the defatted tissue. The trial showed very clearly the rapid loss of the C^{14} from the defatted material; however, this is not necessarily related to the loss of RNA by degradation.

It is apparent from this experimental series that the reduction of RNA in leaves of CCC treated wheat leaves is a reflection of a decrease in total RNA being maintained by the system. There is more evidence that the lack of total RNA is the result of a more rapid degradation process. This series of experiments also suggests that CCC treatments interrupt the timing mechanism for a natural

rhythm of synthesis and degradation within the plant. The synthesis and degradation scheme is not evident. However, it appears that there is a carbon pool which is an integral part of the system and that this carbon pool is labile under conditions of the experiment.

DISCUSSION AND CONCLUSIONS

(a) The Effect of CCC on the Total RNA Content

The results of this experiment have shown that CCC has a definite influence on the RNA metabolism of wheat plants.

The reduction of total RNA content of the plants seems to be dependent on the concentration of CCC in the nutrient solution. The maximum reduction of RNA occurs at a concentration of $1x10^{-5}$M. CCC. At CCC concentrations exceeding $1x10^{-5}$M. the RNA content again increases up to $8x10^{-3}$M., which was the highest concentration tested which permitted growth.

This experiment did not examine which RNA fraction was influenced. It has been assumed that primarily the ribosomal RNA is involved.

It is tempting to think that the reduction of total RNA would result in a reduction of protein synthesis, thus the metabolism of the treated plant would be reduced, due to a decrease in enzyme activity. This could result in a miniature plant which is identical in all respects, except quantity of constituents, to the normal plant.

Woodstock and Skoog (47) have evidence which shows a direct relationship between the growth rate and the RNA content of corn roots. The effect of CCC is not this simple, however, as evidence

presented suggests that the quality of the RNA is also influenced.

It is interesting to note that as the concentration of CCC exceeds $1x10^{-5}$M., there is apparently a different effect on the RNA content. The increase of RNA as the CCC concentration approaches the toxic level is similar to the effect of 2,4-D (2,4-dichlorophenoxyacetic acid) on the RNA of corn mesocotyl tissue (19). Key suggests that low rates of 2,4-D enhance the degradation of RNA, whereas the higher rate of 500 parts per million of 2,4-D (about $2.3x10^{-2}$M.) prevents the degradation process, thus RNA accumulates.

The fact that 2,4-D and CCC both tend to cause a decrease in the RNA content at low concentrations and an increase at higher concentrations may be significant in that it is reported that CCC increases the diffusible auxin content of treated tissue (20, 21). One would expect an application of 2,4-D, a compound with auxin-like activities, to cause the same responses as high levels of endogenous auxin.

Gould and Baldwin (unpublished data) have shown that CCC and IAA in very low ($1x10^{-7}$) equimolar concentrations tend to have a herbicidal action with respect to duckweed (Lemna minor). It could be that the addition of CCC increased the free auxin content to such a level that an addition of small amounts of IAA increased the level of total auxin beyond the toxic threshold.

It is reported that the pathogen Tilletia species cause an accumulation of a CCC-like compound within the host plant (31). It would be interesting to examine and compare the tolerance of cereals infected and non-infected with Tilletia to 2,4-D or other auxin-like herbicides.

The decrease of total RNA with low levels of CCC, with the consequent increase of RNA with higher CCC levels may be the result of increased auxin within the treated plant resulting from the presence of CCC.

(b) The Effect of CCC on the Absorption Spectrum of RNA

The shift of wave length of the absorption maximum that is shown with the various treatments of CCC suggests a qualitative as well as a quantitative effect on the RNA.

The purine bases, under acid conditions, absorb more strongly below 260 mμ, whereas under similar conditions the pyrimidines absorb more strongly above 260 mμ (32). The shift of the absorption maxima from near 256 mμ indicates that perhaps the RNA of treated tissue has a higher concentration of one or both of the pyrimidines.

Kessler and Tishel (17) have reported that drought conditions induce an increase of RNA content as well as a favoring of the presence of quanine plus cytadine as compared to adenine and

uracil. These workers suggest that the change in RNA composition is a protective mechanism for the plants against drought. CCC, as a seed soak of about $3x10^{-2}$M. (30), is capable of increasing the plants hardiness to physiological and probably physical drought. The relative quantities of the respective purines and pyrimidines were not determined in this experiment. However, it would be interesting to make such a study in the future and compare the effect of various chemicals which induce drought escape on the RNA composition with RNA of plants that are capable of withstanding both drought and non-drought conditions.

An alteration in the base ratios of RNA must be predetermined by the DNA template. There is no evidence that CCC induces mutations or in any way decreases the degree of stability of the DNA molecules. It is therefore assumed that the changes of RNA bases may be due to abnormal timing of releasing DNA stored information.

Bonner (6) cites the work of Gifford who has observed with flower induction a rapid increase of auxin content with a concurrent loss of histone protein. The loss of histone protein, as described above, is believed to result in the release of DNA molecules for template action.

This apparent change of RNA bases which results from CCC treatments, supports, in an indirect manner, the hypothesis that CCC causes an increase of endogenous auxin. An increase of auxin

would then, according to Bonner's proposal, stimulate the release of histone protein from the DNA, thus causing a DNA with a different base sequence to act as a template. The RNA that is synthesized on this new template could well have a different composition than that which is normal for that period of growth.

(c) The Effect of CCC on the Synthesis and Degradation of RNA

The incorporation of $C^{14}O_2$ into the plants by photosynthesis and the ultimate labeling of the RNA did not provide concrete evidence of what process the CCC actually alters.

The data indicate that the CCC treated plants tend to have a lower specific activity in the RNA than did the non-treated material, as well as a lower radio activity in the RNA at the 30-36 hour period after the initial exposure to $C^{14}O_2$. After this period, both the treatments had nearly the same degree of radio activity per unit weight of the defatted tissue.

The equalization of the levels of radio activity in the two treatments may be explained by the purine and pyrimidine salvage routes of the nucleic acid metabolism. The degraded RNA would have the four bases in the free pool, they would then be reincorporated into RNA. As the two levels of RNA - C^{14} are essentially the same, it is suggested that the total incorporation of the C^{14} into RNA from the photosynthate is not greatly different in the

CCC treated and non-treated tissues, certainly not as great as the spectrophotometric data suggests.

The total radio activity in the RNA per unit weight of the defatted material suggest very strongly that the RNA - C^{14} content of the total RNA in the non-treated tissue is much higher at the 30-33 hour period than that of the treated material and that the rate of degradation is very rapid. The same data for the treated material suggests that it had a lower total RNA content at 30 hours or it had already degraded. The data after 36 hours suggests that the two levels of radio activity are equal. Therefore, one would assume that the initial incorporation of C^{14} into the RNA was essentially equal.

When the specific activity data are considered, a rather confusing situation is presented. From the 30 hour to the 40 hour period, the specific activity of the RNA in both treatments appears cyclic; however, the data from the non-treated material after 40 hours appears to be rather constant; this situation does not hold for the specific activity of the RNA from treated tissue. The cyclic nature of these data indicate that the RNA is resynthesized by the salvage route in a rhythmic manner; the constant counts per minute of the non-treated RNA after 40 hours suggest that the rhythm has a periodicity of eight hours or a multiple

thereof. The specific activity of the treated RNA also suggests that a rhythm exists. However, this rhythm is out of phase with that in the normal tissue.

The spectrophotometric data that is associated with the radio active study also indicates that there is a rhythem of RNA concentration, and that the CCC treatments cause the cycle to become out of phase. The ratio of the specific activity of the RNA to the specific activity of the defatted material suggests a cyclic effect and also there is a rapid degradation of the RNA in the treated material.

It is interesting that the rhythm effect appeared in the entire plant when it was examined prior to extraction. The differences are too great to be considered an error and this observation can best be interpreted as a reversible reaction in which one of the products or reactants is labile under the conditions of this experiment.

It should be pointed out that the cyclic effect exists in the non-extracted tissue, the defatted tissue, the crude and the purified RNA fractions. There is a progressive lag between the peaks and troughs of the various fractions. That is to say, the maximum radio activity in the unprocessed material precedes that of the defatted material, which in turn precedes that of the RNA fraction.

F. D. H. MacDowall (25) has shown the retardation of a sap exudation rhythm with actinomycin D, a compound that binds guanine,

thus preventing the synthesis of DNA dependent RNA. He proposes that endogenous rhythms are based on the synthesis of DNA dependent RNA. It follows then, that if CCC disrupts RNA metabolism, it could also disrupt an endogenous rhythm.

The results of the spectrophotometric analysis in these experiments were very variable, although the precision of the technique was within plus or minus fifteen percent. During the bulk of this research, no thought was given to a rhythmic alteration of the RNA concentration. Thus, the test material was harvested and dried at the most convenient time of the particular day. It is quite probable that the results would have been more consistent if the test materials of the different replications were harvested at a constant time of day.

This research has raised some questions which should be subjected to more detailed study. The first question is whether or not there is a rhythm of RNA synthesis and degradation. The effect of plant growth regulating substances, such as IAA, gibberelic acid and the kinens, on this proposed rhythm should also be studied; with this information a more complete understanding of the mode of action of CCC would be gained. The proposed periodicity of the RNA concentration could have important applications in the timing of treatments of hormone-like herbicidal sprays to gain maximum effect and selectivity.

More detailed studies should be carried out with the use of radio active tracers. Future studies should use adenine-C^{14} as a marker in order that less C^{14} be in the waste material and perhaps cause contamination. The employment of "Sephadex" would also be useful in the isolation of RNA-C^{14}.

SUMMARY

A series of experiments were carried out to investigate the influence of CCC (2-chloroethyltrimethylammonium chloride) on the total RNA content of wheat plants. Examinations were made spectrophotometrically of the RNA content. In addition, C^{14} was used to study the rate of synthesis and degradation of the RNA. The following results were found.

1. CCC reduces the total RNA content on treated wheat leaves. The decrease of RNA is progressive with treatments as high as $1x10^{-5}$M. CCC. Thereafter the RNA content increases until the toxic threshold is reached.
2. Applications of CCC tend to increase the wave length showing the maximum absorption. This is probably a reflection of an alteration in RNA quality.
3. The isolation of RNA-C^{14} is difficult using $C^{14}O_2$ as a source of C^{14}. There is evidence which suggests that the CCC treatments increase the rate of RNA degradation.
4. The radio active tracer data suggest that there is a rhythmic synthesis and degradation of RNA and that this rhythm is altered with treatments of CCC.
5. The experimental results of this research indirectly support the hypothesis that CCC increases the diffusible auxin content of treated tissue.

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A P P E N D I X

Table A

The total RNA content of wheat leaves treated with various levels of CCC

Figures represent ug. of RNA per mg. of the defatted material

	Molar Concentration of CCC in the Nutrient Solution						
Rep.	.0	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
1	10.7	83.9	75.2	59.0	56.0	57.3	80.7
2	66.3	46.1	43.8	42.3	42.7	32.5	49.6
3	50.0	55.0	56.9	45.3	56.6	55.0	63.2
4	60.4	51.8	47.8	45.1	50.1	47.7	54.0
5	23.3	57.2	30.6	15.9	34.5	33.7	33.4
6	38.7	11.1	23.3	20.0	24.4	20.3	13.6
7	71.7	66.0	76.3	61.0	38.2	49.9	70.2
8	115.5	187.6	136.5	135.0	125.5	155.1	168.8
9	64.9	61.1	52.4	53.9	55.4	45.6	41.4
10	78.0	135.4	96.5	105.9	67.3	79.5	131.4
Ave.	67.2	75.5	63.9	58.3	55.1	57.7	70.6

Analysis of variance significant at the 5% level

Duncan's Multiple Range

10^{-8}	10^{-3}	0.0	10^{-7}	10^{-6}	10^{-4}	10^{-5}
a	ab	abc	abc	bc	bc	c

Table B

The effects of high levels of CCC on the total RNA content of wheat leaves

Figures represent ug. of RNA per mg. of defatted material

Molar Concentration of CCC in the Nutrient Solution

Rep.	0.0	0.5	1.0	2.0	4.0	8.0	($X10^{-3}$)
1	70.0	49.3	80.4	58.0	51.3	51.7	
2	28.6	50.9	27.6	39.3	39.4	58.7	
3	40.7	37.2	37.0	31.6	52.4	50.8	
4	41.7	46.6	39.1	34.2	46.6	42.0	
Ave.	45.4	46.0	46.0	40.8	47.4	50.8	

These differences are not significant at the 5% level

Table C

The effect of CCC on the wave length exhibiting maximum absorption

The figures represent Optical Density X 1000

Molar Concentration of CCC in the Nutrient Solution

Wave length mu.	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	0.0
246	120	97	101	46	93	127	75
248	124	100	105	48	97	133	76
250	128	102	109	48	101	139	79
252	131	105	114	49	105	144	82
253	131	105	115	50	107	146	84
254	132	107	116	52	110	149	85
255	133	108	118	52	110	150	85
256	133	108	120	52	111	152	86
257	133	109	119	51	111	154	87
258	132	107	119	51	112	154	87
259	131	106	119	52	111	154	86
260	130	105	118	52	112	153	85
261	128	104	117	52	111	152	85
262	126	103	116	48	109	150	85
264	122	100	115	50	107	146	83
266	116	96	111	48	104	142	80
268	113	92	106	45	100	136	76
270	105	87	103	43	95	130	73

Table C - part b

The effect of CCC on the wave length exhibiting maximum absorption

The figures represent the Optical Density X 1000

Molar Concentration of CCC in the Nutrient Solution

wave length mu	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-0}	Liver RNA
246.0	205	206	296	276	232	360	231	288
248.0	218	219	307	293	245	382	245	317
250.0	232	232	331	306	257	405	259	340
252.0	243	244	342	325	268	425	271	362
253.0	247	249	349	331	273	434	274	374
254.0	254	255	358	338	278	443	280	383
255.0	259	260	362	345	283	451	285	392
256.0	262	264	368	348	285	458	289	400
256.5	262	265	368	349	287	458	289	402
257.0	263	266	369	350	287	460	288	405
257.5	263	266	370	350	286	459	287	408
258.0	262	266	369	350	285	459	285	407
258.5	262	265	368	340	285	458	285	408
259.0	260	265	368	348	283	458	284	408
260.0	258	265	365	347	282	457	282	407
261.0	255	263	361	344	280	454	280	404
262.0	253	261	359	341	277	450	278	400
263.0	250	258	352	338	274	446	273	396
264.0	243	253	348	332	267	439	266	390
266.0	233	243	332	319	254	422	252	372
268.0	221	233	320	306	243	406	240	358
270.0	209	223	306	299	232	386	229	342

Table D

Precision of Technique

Figures represent the ug. of RNA per mg. of defatted tissue. All samples are from the same plant material.

Sample No.	ug. RNA	Sample No.	ug. RNA
1	89.60	5	91.21
2	85.20	6	106.41
3	101.95	7	118.11
4	114.73	8	103.07

Average 101.28, standard deviation 11.94

Table E

The recovery of RNA from the Dowex-1 (chloride form) column with the NaCl-HCl eluting solution

MLS. of NaCl-HCl	The Optical Density at 260 mu. X 1000
10	108
20	76
30	31
40	14
50	15

Table F

The effect of Phosfon D on the RNA content of wheat leaves. (This information is not included in the thesis body as there was only one replication.)

Treatment	ug. RNA per mg. of defatted tissue
Not treated	112.9
1 X 10^{-6}M CCC	73.9
1 X 10^{-8}M Ph.	106.9
1 X 10^{-6}M Ph.	56.9

Table G - part a

The Specific Activity of Treated and Non-Treated Tissue
Trial #1

Hours since initial exposure to $C^{14}O_2$	Counts per minute per milligram of dried tissue		Counts per minute per milligram of defatted material	
	10^{-5}M CCC	Non-Treated	10^{-5}M CCC	Non-Treated
6.5	1,010	1,678	538	1,010
9.5	1,880	1,188	1,315	835
12.5	1,039	1,397	672	1,261
15.5	818	1,251	689	1,232
18.5	815	867	902	879
21.5	766	1,259	858	1,236
24.5	741	950	894	1,119
27.5	527	1,160	732	1,601
51.5*	568	733	601	852
91.5*	632	489	776	665
123.5*	539	351	607	404
159.5*	326	445	394	465
195.5*	272	536	323	649

*C^{14} was detected in the RNA fraction at these times.

Table G - part b

The Specific Activity of Treated and Non-Treated Tissue
Trial #2

Hours since initial exposure to $C^{14}O_2$	Counts per minute per milligram of dried tissue		Counts per minute per milligram of defatted material	
	$10^{-5}M$ CCC	Non-Treated	$10^{-5}M$ CCC	Non-Treated
45	3,419	3,544	3,521	3,380
50	4,209	3,085	4,457	3,560
56	2,489	2,085	2,683	2,107
68	3,165	2,572	3,089	2,853
74	3,255	5,002	2,507	3,110
98	3,282	4,831	2,287	2,667
122	2,948	3,306	2,253	2,779
146	3,118	4,073	2,573	3,144

Table H

The Specific Activity of Various Fractions of Treated and Non-Treated Tissue

Part a. Counts per minute per mg. of dried tissue

Hours after initial exposure to $C^{14}O_2$	$1x10^{-5}M$ CCC			Non-treated		
	R_1	R_2	Ave.	R_1	R_2	Ave.
30	2,753	2,584	2,668	1,674	3,334	2,504
33	1,711	1,658	1,684	2,040	1,409	1,724
36	1,095	1,553	1,324	2,282	2,492	2,387
38	1,934	558	1,261	971	2,319	1,645
40	1,748	2,500	2,124	2,232	1,778	2,005
48	1,330	1,700	1,515	3,174	2,849	3,011
56	2,309	1,658	1,983	1,184	1,282	1,233
64	1,917	2,755	2,336	841	2,140	1,490
72	1,654	2,522	2,088	1,881	2,253	2,067

Table H

The Specific Activity of Various Fractions of Treated and Non-Treated Tissue

Part b. Counts per minute per mg. of defatted tissue

Hours after initial exposure to $C^{14}O_2$	$1x10^{-5}M$ CCC			Non-treated		
	R_1	R_2	Ave.	R_1	R_2	Ave.
30	3,742	1.219	2,480	1.978	2,688	2,333
33	1,255	1,539	1,397	4,350	6,772	5,561
36	1,573	1,454	1,513	6,897	1,450	4,173
38	3,229	1,099	2,164	1,556	4,117	2,836
40	1,439	1,431	1,435	5,195	3,633	4,414
48	2,249	2,439	2,344	4,085	967	2,526
56	3,071	1,999	2,535	3,287	2,033	2,660
64	1,435	1,070	1,252	1,367	2,193	1,780
72	2,246	2,077	2,161	2,212	1,028	1,620

Table H

The Specific Activity of Various Fractions of Treated and Non-Treated Tissue

Part c. Counts per minute per mg. of RNA*

Hours after initial exposure to $C^{14}O_2$	$1x10^{-5}M$ CCC			Non-treated		
	R_1	R_2	Ave.	R_1	R_2	Ave.
30	266	137	201	37	17	27
33	26	2,417	1,221	257	86	171
36	28	123	70	202	715	458
38	56	48	52	169	354	261
40	203	141	172	255	39	147
48	145	65	105	204	71	137
56	185	155	170	197	95	146
64	108	46	77	214	58	136
72	100	170	135	196	91	143

*The RNA estimated spectrophotometrically.

Table I

The Radioactivity of RNA per Unit Weight of the Defatted Tissue

Counts per minute per mg. of defatted tissue

Hours after initial exposure to $C^{14}O_2$	$1x10^{-5}M$ CCC			Non-treated		
	R_1	R_2	Ave.	R_1	R_2	Ave.
30	97.5	85.4	91.4	7.9	45.2	26.5
33	5.8	62.1	33.9	210.0	64.6	137.3
36	30.0	42.3	36.2	118.0	59.5	88.7
38	32.6	25.1	28.8	40.9	19.5	30.2
40	65.6	81.4	73.5	86.4	55.5	70.9
48	86.1	90.5	88.3	81.9	71.3	76.6
56	69.1	96.3	82.7	130.0	49.1	89.5
64	51.8	35.9	43.8	62.1	64.6	63.2
72	45.2	59.1	52.2	64.5	106.1	85.3

Table J

Incorporation of C^{14} into the Defatted Tissue From the Dried Plant

$$\frac{\text{Counts per minute per mg. defatted tissue}}{\text{Counts per minute per mg. dried total plant}}$$

Hours after initial exposure to $C^{14}O_2$	1×10^{-5}M CCC			Non-treated		
	R_1	R_2	Ave.	R_1	R_2	Ave.
30	1.359	0.472	0.929	1.182	0.806	0.932
33	0.733	0.928	0.830	2.132	4.806	3.226
36	1.436	0.936	1.143	3.022	0.592	1.748
38	1.701	1.869	1.716	1.602	1.775	1.724
40	0.823	0.572	0.676	2.327	2.043	2.201
48	1.691	1.435	1.547	1.287	0.339	0.839
56	1.330	1.206	1.278	2.776	1.586	2.157
64	0.749	0.388	0.536	1.625	1.025	1.195
72	1.358	0.824	1.035	1.176	0.456	0.784

Table K

Incorporation of C^{14} into the RNA Fraction From the Defatted Tissue

$$\frac{\text{Counts per minute per mg. RNA*}}{\text{Counts per minute per mg. defatted tissue}}$$

Hours after initial exposure to $C^{14}O_2$	$1x10^{-5}M$ CCC			Non-treated		
	R_1	R_2	Ave.	R_1	R_2	Ave.
30	0.071	0.112	0.081	0.019	0.006	0.012
33	0.021	1.571	0.874	0.059	0.013	0.031
36	0.018	0.085	0.046	0.029	0.490	0.110
38	0.017	0.044	0.024	0.109	0.086	0.092
40	0.141	0.098	0.120	0.049	0.011	0.033
48	0.064	0.027	0.045	0.050	0.073	0.054
56	0.060	0.077	0.067	0.060	0.047	0.055
64	0.075	0.043	0.062	0.157	0.026	0.076
72	0.045	0.082	0.062	0.089	0.089	0.089

Table L

The Effect of Time on the Total RNA Content of Wheat Leaves

Micrograms of RNA per mg. of defatted tissue

Hours after initial exposure to $C^{14}O_2$	$1x10^{-5}M$ CCC			Non-treated		
	R_1	R_2	Ave.	R_1	R_2	Ave.
30	63.0	135.0	99.0	33.3	538.0	285.6
33	46.8	3.8	25.3	84.3	145.0	114.6
36	197.6	130.7	164.1	72.4	12.8	42.6
38	99.4	88.6	94.0	34.1	40.1	37.1
40	91.7	176.4	134.0	43.8	267.9	155.8
48	96.6	341.9	219.2	78.7	180.4	129.5
56	40.2	121.6	80.9	54.2	187.6	112.9
64	73.9	218.6	146.2	35.8	181.7	108.7
72	56.5	141.0	98.7	57.6	306.1	181.8