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ABSTRACT

ON THE MECHANISM OF HORMONE CONTROLLED ENZYME
FORMATION IN BARLEY ALEURONE LAYERS

By

Tuan-hua David Ho

Ribonucleic acid containing polyadenylic acid (poly A-RNA) is present in barley aleurone layers. The poly A-RNA is polydisperse in size and the 3'-OH end of the molecule is occupied by the poly A segment which is about 80 to 200 nucleotides long. The poly A-RNA becomes labeled with radioactive precursors of RNA during the incubation of isolated aleurone layers with or without gibberellic acid (GA_3). However, the rate of synthesis of poly A-RNA is enhanced by GA_3 . This enhancement begins within 3-4 hours after addition of the hormone and reaches a maximum, which is about 50-60% over the control, 10-12 hours after the addition of the hormone.

Cordycepin, 3'-deoxyadenosine, inhibits synthesis of total RNA as well as poly A-RNA in barley aleurone layers. However, cordycepin inhibits the hormone-controlled formation of α -amylase only if it is added 12 hours or less after GA_3 . The rapid accumulation of

1. The first step in the process of identifying a problem is to recognize that a problem exists. This is often done by comparing current performance with a desired state or goal. For example, a manager might notice that sales are declining or that customer satisfaction is low. Once a problem is identified, the next step is to define it clearly and specifically. This involves determining the scope of the problem, its causes, and its effects. A clear definition of the problem is essential for developing an effective solution.

2. The second step in the process is to analyze the problem. This involves gathering information about the problem and its context. This information can be obtained through various methods, such as interviews, surveys, and data analysis. The goal of this step is to understand the underlying causes of the problem and to identify the factors that are contributing to it. This information is then used to develop a plan of action.

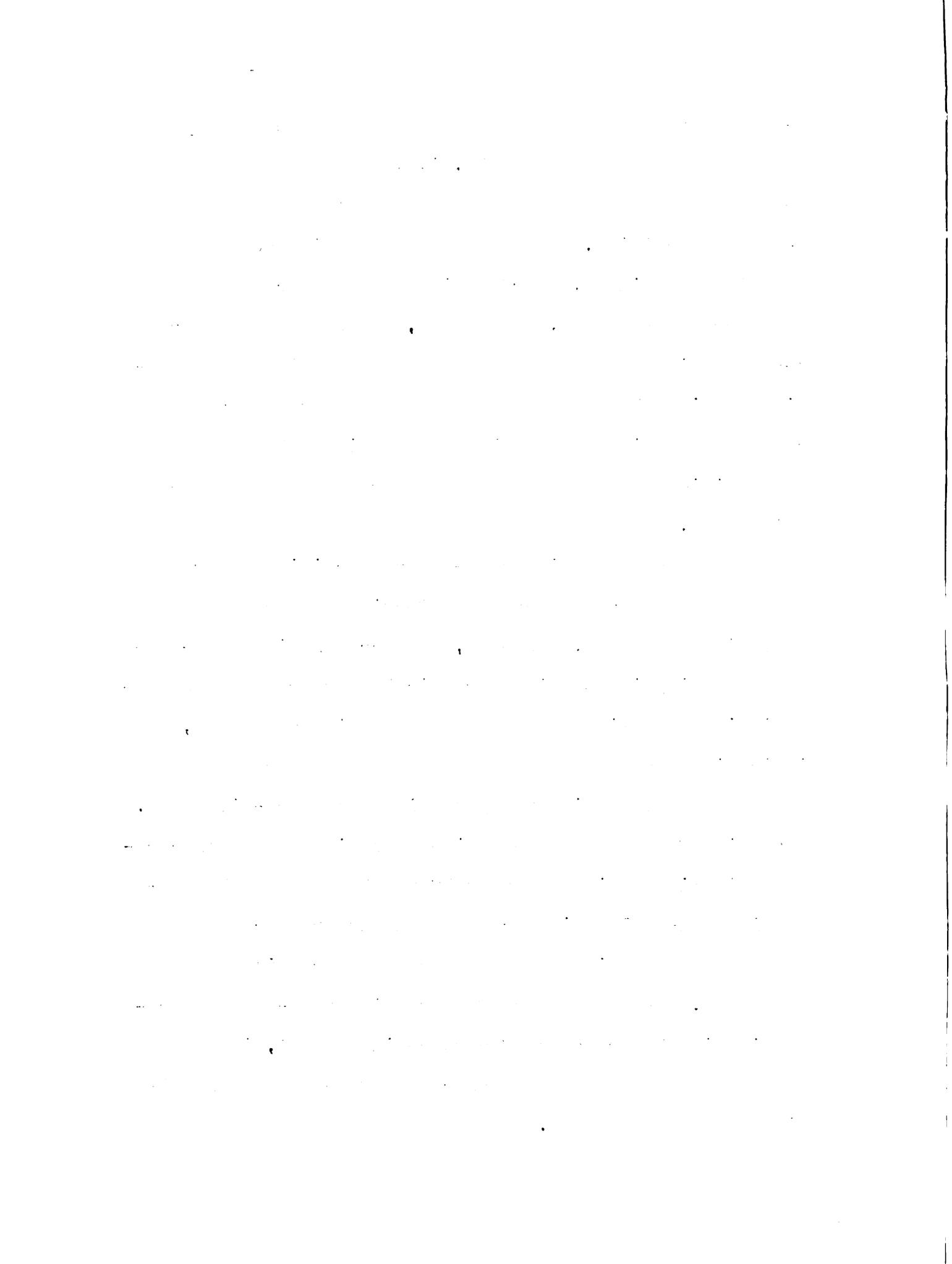
3. The third step in the process is to develop a solution. This involves identifying the most effective and efficient way to address the problem. This is often done by brainstorming ideas and evaluating them against various criteria, such as cost, time, and effectiveness. Once a solution has been identified, the next step is to implement it. This involves putting the solution into action and monitoring its progress.

4. The fourth step in the process is to evaluate the solution. This involves assessing the effectiveness of the solution and determining whether it has solved the problem. This is often done by comparing current performance with the desired state or goal. If the solution is found to be ineffective, the process may need to be repeated.

5. The fifth and final step in the process is to prevent the problem from recurring. This involves identifying the factors that led to the problem in the first place and taking steps to address them. This can be done through various methods, such as training, process changes, and improved communication. By preventing the problem from recurring, the organization can ensure that it remains effective and efficient in the long run.

α -amylase after 12 hours of GA_3 is due to the de novo synthesis of the enzyme molecule, i.e. accumulation of an α -amylase precursor does not precede the appearance of α -amylase activity, as examined by ^{13}C -amino acid density labeling experiment. Cordycepin has no effect on the turnover of α -amylase. Therefore, it is suggested that α -amylase is translated from stable mRNA which is synthesized during the first 12 hours of GA_3 treatment and the control mechanism of α -amylase synthesis 12 hours after the addition of GA_3 appears to be strictly post-transcriptional.

The accumulation of α -amylase activity after 12 hours of GA_3 treatment can be effectively decreased by abscisic acid (ABA). However, the accumulation of α -amylase activity is sustained or quickly restored when cordycepin is added simultaneously or some time after ABA, indicating that the response of aleurone layers to ABA depends on the continuous synthesis of a short-lived RNA. Analysis of the newly synthesized proteins by gel electrophoresis with sodium dodecylsulfate showed that the synthesis of α -amylase is decreased in the presence of ABA while the synthesis of most of the other proteins remains unchanged. From the rate of resumption of α -amylase production in the presence of cordycepin and ABA, it appears that ABA does not have a measurable effect on the stability of α -amylase mRNA.



ON THE MECHANISM OF HORMONE CONTROLLED ENZYME
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2. The second part of the document outlines the various methods used to collect and analyze data. These methods include interviews, surveys, and focus groups. Each method has its own strengths and weaknesses, and it is important to choose the most appropriate method for the specific research objectives.

3. The third part of the document describes the process of data analysis. This involves identifying patterns and trends in the data, and then interpreting these findings in the context of the research objectives. It is important to be objective and to avoid drawing conclusions that are not supported by the data.

4. The final part of the document discusses the importance of reporting the results of the research. This involves writing a clear and concise report that summarizes the findings and provides recommendations for future action. The report should be written in a way that is easy to understand and that is accessible to all relevant parties.

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PART I
ON THE MECHANISM OF HORMONE CONTROLLED ENZYME
FORMATION IN BARLEY ALEURONE LAYERS

INTRODUCTION

Mobilization of endosperm reserves during the germination of cereal grains supplies nutrients for the growth of the embryo. This is accomplished by several hydrolases, including α -amylase (E.C.3.2.1.1.) and protease (E.C.3.4), which are synthesized in the aleurone tissue that surrounds the endosperm. Aleurone tissue of barley consists of three layers of homogeneous non-dividing triploid cells. These cells respond to gibberellic acid (GA_3), which is formed in the embryo during the early stage of seed germination, by a series of morphological and biochemical changes (66,68). The most prominent among these changes is the increase in α -amylase and protease activities after an eight and ten hour lag period (10,29). The GA_3 enhanced activities of α -amylase, protease, and ribonuclease (E.C.3.1.4) have been found to be due to the de novo synthesis of the enzyme proteins (3,17,29), and most of the α -amylase and protease are secreted into endosperm after their synthesis (68). Besides the synthesis and secretion of α -amylase, protease, and ribonuclease, GA_3 also enhanced the secretion and to a less extent the synthesis of α -1,3-glucanase (E.C.3.2.1.6) (34) and the release of acid

phosphatase (E.C.3.1.3.2) from the cell wall (2).

α -Amylase becomes the most predominant protein (40%) synthesized in barley aleurone layer after several hours of GA_3 treatment (66). Therefore, it is usually used as a marker in studies of the mechanism of the hormone controlled formation of enzyme in this system. Inhibitors of RNA synthesis, such as actinomycin D (10,21), 6-methylpurine (10), or cordycepin (3'-deoxyadenosine) (this study) can block the GA_3 enhanced α -amylase synthesis. Incorporation into salt-soluble RNA is enhanced by GA_3 (8). Zwar and Jacobsen (69) demonstrated that the incorporation of radioactive nucleotides into polydisperse RNA was increased in the presence of GA_3 , and it was reported recently that GA_3 enhanced the synthesis of rapidly labeled RNA species in barley aleurone layer (62). These observations tend to support a previous suggestion that the synthesis of α -amylase may depend on the GA_3 mediated synthesis of their mRNA (10).

During the lag period before the production of hydrolases, there is an extensive proliferation of cellular membranes, principally endoplasmic reticulum (ER) (32,33), and the formation of membrane bound polyribosomes (13). Concurrently the incorporation of choline and of ^{32}P into membrane phospholipids is also enhanced by GA_3 starting at about 4 hours after the addition of GA_3 (38). Furthermore, two enzymes, phosphorylcholine cytidyl transferase (E.C.2.7.7.15) and phosphorylcholine glyceride transferase

(E.C.2.7.8.2), which are involved in the synthesis of lecithin (phosphatidylcholine), are activated within minutes of GA₃ addition (4,31). All these events seem to indicate that membrane proliferation is a major GA₃ effect before the rapid increase of α-amylase activity. α-Amylase is a secretory protein and there is ample evidence for the participation of rough ER in the synthesis and exportation of secreted proteins in eucaryotic cells (53, 60). Thus, a possible post-transcriptional model to account for the GA₃ enhanced synthesis of α-amylase has been proposed by Johnson and Kende (31). They reasoned that α-amylase specific mRNA be present and turning-over in the aleurone cell before GA₃ treatment and the synthesis of α-amylase may solely depend on the availability of proper membrane for the attachment of polysome that carry the α-amylase specific mRNA (31).

It is now well established that mRNA (with the exception of histone mRNA) in eucaryotic cells contains a covalently linked polyadenylic acid (poly A) segment (5,22). The occurrence of poly A-RNA in higher plants has been reported in mung bean (24), rice (41), corn (61), barley (25,30), Vicia faba (56), soybean (57), and pea (67). Since poly A can be hybridized with immobilized poly U or oligo dT, mRNA together with a certain fractions of heterogeneous nuclear RNA can be easily isolated from the other RNA species. There is recent evidence

which indicates that up to 40% of the mRNA in certain mammalian cells does not contain a poly A segment (45). However, it has been reported that in higher plant essentially all the translatable messages are poly A containing RNA species (67).

In order to elucidate the mechanism of hormonal control of enzyme formation in the barley aleurone cell, that up to 40% of the mRNA in certain mammalian cells does not contain a poly A segment, a detailed study of the relationship between the metabolism of RNA, especially that of mRNA, and the formation of α -amylase becomes necessary. The approaches used in this study are :

- 1) to determine the effect of hormones on the synthesis of poly A-RNA.
- 2) to check the stability of α -amylase mRNA by monitoring the synthesis of α -amylase in the presence of specific transcription inhibitors.

MATERIALS AND METHODS

Sources of Seed and Chemicals

Barley seeds (Hordeum vulgare L.cv. Himalaya, 1969 crop) were supplied by Department of Agronomy, Washington State University, Pullman, Wash. in 1972 and stored in the cold room since then. GA₃, ABA (mixture of equal amounts of cis-trans and trans-trans isomers, all concentrations mentioned in this study refer to that of cis-trans isomer only), cordycepin, and azocasein were obtained from Sigma Chemical Co., St. Louis, Mo. Diethyl pyrocarbonate was obtained from Calbiochem., La Jolla, Calif. Potato starch for the α -amylase assay was obtained from Nutritional Biochemical Co., Cleveland, Ohio. ¹³C-Labeled amino acids mixture (hydrolyzate of alal proteins) was obtained from Merck Inc. Radioactive labeled compounds were obtained from New England Nuclear, Boston, Mass., Amersham/Searle Co., Arlington Heights, Ill. NCS tissue solubilizer was obtained from Amersham/Searle Co. and a mixture of 9 parts of full strength of solubilizer and one part of water was used. Poly A and poly U were supplied by Sigma Chem. Co. and Milts Laboratory. Oligo dT cellulose (T-2 and T-3) was obtained from Collaborative Research, Waltham, Mass. All the other reagents used in

this study reagent grade.

Preparation of Aleurone Layers

The method described by Chrispeels and Varner (10) was followed. The embryos of dry barley seeds were mechanically separated from the endosperm by cutting with a dissecting knife. The embryo-less half of the seeds (half seeds) were surface sterilized by sodium hypochlorite (5 fold dilution of commercial bleach) for 20 min. After a thorough rinse with sterilized deionized water, the half seeds were imbibed on a sand plate moistened with sterilized water in a glass petri dish. The aleurone layer can be readily peeled from the endosperm with two spatulas after three days of water imbibition at room temperature. Ten, twenty, or fifty aleurone layers were put in a 25 ml, 50 ml, or 125 ml foil capped sterilized Erlenmeyer flask containing 2 ml, 4 ml, or 60 ml of 2 mM acetate buffer, pH 5.0, respectively. The concentration of GA₃ in this study was 1 μM, and one drop of chloramphenicol (500 ug/ml) was usually added to every 2 ml of buffer in order to prevent bacterial contamination. All solutions used were sterilized either by autoclaving or by filtration through a Millipore filter (VC, pore size: 0.13 μ). The flasks containing aleurone layers were shaken in a reciprocal metabolic shaker (120 oscillations/min) at 25 °C.

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that proper record-keeping is essential for transparency and accountability, particularly in financial matters. This section outlines the various methods and tools used to collect and store data, ensuring that all information is readily accessible and secure.

2. The second part of the document focuses on the analysis and interpretation of the collected data. It describes the process of identifying trends, patterns, and anomalies, which are crucial for making informed decisions. This section also covers the use of statistical methods and software tools to facilitate data analysis, providing a clear and concise summary of the findings.

3. The third part of the document addresses the challenges and limitations of the current data management system. It highlights the need for continuous improvement and the implementation of new technologies to enhance efficiency and accuracy. This section also discusses the importance of training and education for staff involved in data management, ensuring they are equipped with the necessary skills to handle the data effectively.

4. The final part of the document provides a conclusion and recommendations for future actions. It summarizes the key findings and offers practical suggestions for improving the data management process. This section also includes a list of references and a bibliography, providing a comprehensive overview of the research and resources used in the document.

Extraction and Assays of Enzymes

In all the experiments dealing with α -amylase and protease assays, duplicate 10 layer samples were used. After incubation, the medium was decanted and the layers were rinsed with 3 ml deionized water. The medium and rinse solution were combined (5 ml total). The layers were homogenized with 5 ml water in a mortar and pestle. Because α -amylase is fairly stable, the enzyme preparation can be stored at 2-5 °C, but not frozen, for several hours without significant loss of activity. On the other hand, protease is very unstable and it was assayed immediately after extraction. The α -amylase assay was a modification of that of Varner and Mense (65). The starch solution was prepared by boiling 150 mg of potato starch in 100 ml of 50 mM KH_2PO_4 , pH 4.2; 10 mM CaCl_2 for 1 min. After centrifugation at 12,000g for 15 min, the upper two thirds of the supernatant was used. The iodine solution was prepared by adding 0.3 ml iodine stock solution (600 mg and 6 g KI/100 ml water) to 100 ml 0.01 N HCl. The assay mixture contains 5 to 100 μl of enzyme, 0.5 ml starch solution and 1.0 ml deionized water. After incubation at room temperature (22 °C) from 1 to 5 min the reaction was stopped by adding 1.0 ml iodine solution and the absorbance at 620 nm was measured in a Coleman Junior II A spectrophotometer. The starch solution was diluted with water in order to have an A_{620} close to 1.0 after reaction with iodine.

The amount of enzyme and the length of incubation can be varied in order to give an $A_{620 \text{ nm}}$ between 0.55 to 0.75. The unit of α -amylase was defined as a change of one absorbance unit per minute. Alpha-amylase activity is proportional to enzyme concentration under these assay conditions (Figure 1). Protease was assayed in 20 mM sodium acetate, pH 5.0; 10 mM β -mercaptoethanol. The mixture was incubated at 30 °C for one hour and the reaction was stopped by adding 0.5 ml 50% trichloroacetic acid (TCA). After being cooled in an ice bucket for 10 min, the mixture was centrifuged at 12,000g for 10 min. The supernatant was decanted and absorbance at 330 nm was measured. The enzyme unit for protease was defined as one tenth of an absorbance unit per hour. The protease assay also is proportional to enzyme concentration under the assay conditions used (Figure 2).

Total RNA Extraction

The aleurone layers were ground with 100 mM Tris-HCl buffer pH 7.6 containing 1% SDS and 0.1% diethyl pyrocarbonate (1 ml buffer per 10 layers) in a prechilled mortar. An equal volume of phenol (redistilled and saturated with deionized water); chloroform (1;1) mixture was added to the homogenate and the whole mixture was stirred vigorously at cold for at least 10 min. After centrifugation at 12,000g for 10 min, the aqueous phase was

Figure 1.--Validation of α -Amylase Assay.

The concentration of α -Amylase in the medium of a +GA₃ sample was assigned as "100". Serial dilutions were then made and the α -amylase activity in each diluted sample was assayed.

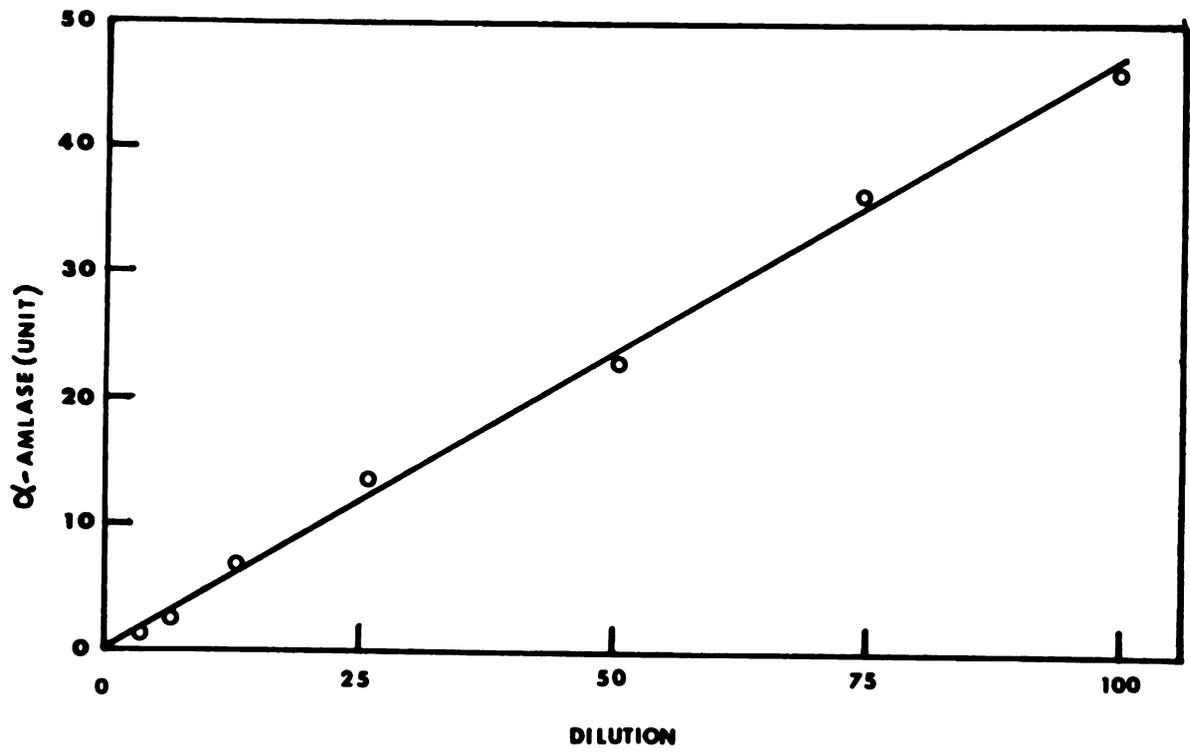
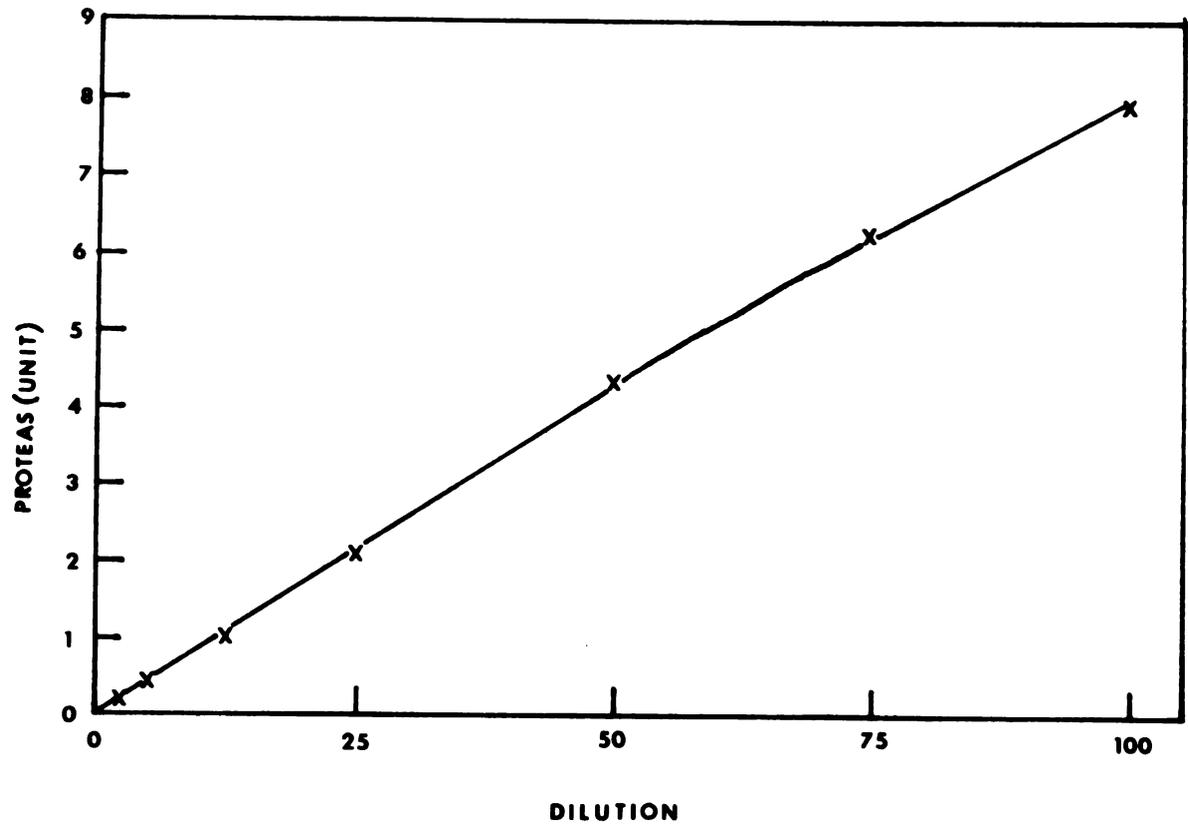


Figure 2.--Validation of Protease Assay.

The concentration of protease in the medium of a +GA₃ sample was assigned as "100". Serial dilutions were made and then the protease activity in each diluted sample was assayed.



decanted and the non-aqueous phase was reextracted by stirring with 100 mM Tris-HCl, pH 9.0 (0.8 ml per 10 layers), and the two phases were again separated by centrifugation. All aqueous phases were combined and further extracted with phenol:chloroform mixture. Total nucleic acid was precipitated by adding one tenth volume of 1 M NaCl and 2.5 volumes of absolute ethanol and storing overnight at -20 °C. The precipitated nucleic acid was collected by centrifugation (12,000g for 30 min) and then dissolved in either deionized water or buffer as indicated in some experiments. RNA was further purified by DNase digestion. However, barley aleurone cells were found to incorporate little radioactivity from RNA precursors into DNA. Therefore, the DNase digestion step was discontinued.

Isolation of Poly-A RNA

Oligo dT cellulose column (1 x 5 cm) was equilibrated with 10 mM Tris-HCl buffer, pH 7.6, containing 0.5 M KCl (binding buffer). RNA samples were dissolved in binding buffer and applied to the column. After thorough washing with binding buffer and the same concentration of Tris buffer containing 0.1 M KCl, the bound RNA was then eluted with buffer alone. Preparation of the fiberglass filter with immobilized poly-U and the filtration procedure were modified from that of Sheldon et al (59). A 0.15 ml volume of poly-U solution (1 mg/ml

10 mM Tris-HCl, pH 7.5) was added to a fiberglass filter (Whatman GF/C, 2.4 cm diameter) supported on a plastic grid. The filter was then dried at 37 °C and irradiated for 3 min on each side at a distance of 20 cm from a 30 Watt Sylvania germicidal lamp. The filter can be stored in the cold for a month. Just before use each filter was rinsed with 50 ml of deionized water to remove unbound poly-U. The filter was then equilibrated with 10 mM Tris-HCl buffer, pH 7.5, containing 0.12 M NaCl. RNA samples were dissolved in the same buffer and filtration was done under unit gravity. The filter was washed with 20 ml buffer followed by 20 ml 10% TCA, and 10 ml ethanol (95%). After drying, the radioactivity on the filter was measured.

Isolation of Poly-A Segment

Bound RNA eluted from the oligo dT cellulose column was made to 10 mM Tris-HCl, pH 7.6, 0.1 M NaCl, 1 mM MgCl₂. After digestion with pancreatic ribonuclease A (2 ug/ml) and T₁ RNase (10 units/ml) for 30 min, diethylpyocarbonate or SDS was added to inactivate the enzymes. After phenol:chloroform extraction, the RNase resistant fragment was precipitated by ethanol.

Polyacrylamide Gel Electrophoresis for Poly-A

The method described by Laemmli (40) was followed. Ten cm sample gels (10%) with 0.1% SDS were used. One

hundred μ l of RNA solution was applied on each gel. The electrophoresis was run under constant power supply (0.5 Watt/tube) until the tracking dye reached the bottom of the gel. The gel was sliced into 1 mm pieces and each piece was digested with 0.5 ml NCS solubilizer at 50 °C for 2 hours before radioactivity was measured.

Sucrose Density Gradient Centrifugation for RNA

A linear density gradient of 15% to 30% sucrose dissolved in 10 mM Tris-HCl, pH 7.6, 1% SDS, 5 mM EDTA was prepared in a nitrocellulose centrifuge tube. The RNA sample was dissolved in the same buffer without sucrose, heated at 65 °C for 10 min, and cooled rapidly in an ice bucket in order to dissociate the aggregated RNA molecules. One to two hundred μ l of the solution was applied on the top of the gradient. Centrifugation was carried out at 25 °C in a Beckman L-4 ultracentrifuge equipped with SW-50 L rotor at 47,500 rpm for 7 hours. After centrifugation the tube was punctured and 5 drop fractions were collected. The RNA in each fraction was precipitated by TCA and collected by filtration through nitocellulose filters. After washing with 20 ml 10% TCA the filters were dried and subjected to scintillation counting.

Base Composition Analysis of RNA

The RNA sample labeled with ^{32}P was hydrolyzed with 0.3 N KOH at 37 °C for 18 to 20 hours. After neutralization with perchloric acid the hydrolysate was subjected to paper electrophoresis as modified from that of Sebring and Salzman (58). RNA hydrolysate was applied on a 7.5 x 16 in electrophoresis paper and electrophoresis was carried out in pyridine-acetate buffer, pH 3.5, containing 10 mM EDTA at 500 V for about 5 1/2 hours immersed in CCl_4 as coolant. After electrophoresis, the pyridine was evaporated and the paper was dried by autoclaving for 3 min. The ultraviolet light-absorbing region on the paper was cut off and radioactivity was determined by scintillation counting. Alternatively the radioactivity on the paper could be scanned directly by a Packard strip scanner.

Chemical Labeling of the 3'-OH End of the RNA Molecule

The method derived by Randerath et al (51) was followed. To a solution of about 50 ug of RNA in 100 ul of water was added 20 ul of an aqueous solution containing 2 nmoles of NaIO_4 . The oxidation was allowed to proceed for 2 hours in the dark at room temperature. Then 5 ul of 0.1 N NaB^3H_4 (50 uCi) in 0.1 N KOH was added and the reaction mixture was kept in the dark at room temperature for 2 hours. A drop of 1 M acetic acid was added at the end of 2 hours to convert excess NaB^3H_4 into boric acid

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities.

2. It then outlines the various methods used to collect and analyze data, including surveys, interviews, and focus groups.

3. The next section describes the results of the data collection process, highlighting key findings and trends.

4. Finally, the document concludes with a summary of the overall findings and recommendations for future research.

5. The following table provides a detailed breakdown of the data collected during the study.

6. This table shows the distribution of responses across different categories, allowing for a more granular analysis of the data.

7. The data indicates that a significant portion of respondents are concerned about the impact of climate change on their daily lives.

8. Furthermore, the study found that there is a strong correlation between income level and concern about environmental issues.

9. These findings suggest that targeted interventions may be necessary to address the needs of lower-income populations.

10. The study also identified several key areas for further research, including the role of community organizations in promoting sustainability.

11. Overall, the research provides valuable insights into the complex relationship between social and environmental factors.

12. The following section discusses the implications of these findings for policy and practice.

13. It is clear that a multi-faceted approach is needed to effectively address the challenges posed by climate change.

14. This approach should involve collaboration between government, industry, and civil society.

15. The study also highlights the need for ongoing monitoring and evaluation to ensure that interventions are effective and sustainable.

16. In conclusion, the research underscores the importance of taking a holistic view of the social and environmental challenges we face.

17. By working together, we can create a more resilient and equitable future for all.

18. The following table provides a summary of the key findings and recommendations.

19. This table serves as a quick reference for the most important aspects of the study.

20. The data shows that there is a clear need for action to address the environmental challenges we face.

21. We must act now to protect our planet and ensure a better future for generations to come.

and tritium gas. The last step was done in a well ventilated fume hood. The labeled RNA was collected by ethanol precipitation.

Double Labeling of RNA for Checking the Effect of GA₃ on Poly-A RNA Synthesis

Labeling was carried out by addition of 50 uCi of ³H-adenosine to a sample (20 layers) containing GA₃ and a sample without GA₃, and 2 uCi of ¹⁴C-adenosine to two other samples without GA₃ at specific times. After 2 hours of further incubation, ³H-labeled layers were mixed with ¹⁴C-labeled layers (i.e., ³H-labeled containing GA₃ with ¹⁴C-labeled sample without GA₃; ³H-labeled sample without GA₃ with ¹⁴C-labeled sample without GA₃; see Figure 3) and rinsed extensively with ice cold carrier adenosine solution (10 mM). RNA was then extracted as described previously.

Induction of Nitrate Reductase and Its Assay

Nitrate reductase was induced by incubating aleurone layers with 2 mM sodium acetate buffer pH 5.0, containing 10 mM KNO₃. The intact tissue assay of nitrate reductase developed by Ferrari and Varner (16) was used. Ten induced aleurone layers were rinsed twice with 4 ml of 50 mM KNO₃, then placed in a 25 ml Erlenmeyer flask with 2 ml of 0.1 M potassium phosphate buffer, pH 7.5, 20 mM KNO₃, and 5% ethanol. The flask was deaerated by bubbling

**Figure 3.---Diagram Showing the Double Labeling Procedure
Used to Check the effect of GA₃ on Poly A-
RNA Synthesis.**

2 Hours

50 uCi ^3H -Adenosine \longrightarrow Mix \longrightarrow Poly A-RNA \longrightarrow $^3\text{H}/^{14}\text{C}$

+ GA₃ Sample

2 uCi ^{14}C -Adenosine \longrightarrow

+/-

Control Sample

50 uCi ^3H -Adenosine \longrightarrow

Control Sample

-/-

Mix \longrightarrow Poly A-RNA \longrightarrow $^3\text{H}/^{14}\text{C}$

2 uCi ^{14}C -Adenosine \longrightarrow

Control Sample

$$\text{GA}_3 \text{ Enhancement (\% over Control)} = \frac{^3\text{H}/^{14}\text{C} \text{ ratio of +/- sample}}{^3\text{H}/^{14}\text{C} \text{ ratio of -/- sample}} - 100$$



N₂ gas for 1.5 min and then stoppered tightly. After being shaken at 30 °C in darkness for 30 min, 0.5 ml of the medium was taken out and mixed with 0.3 ml each of 1% sulfanlamide in 3 N HCl and 0.02% N-1-naphtylethylene-diamine dihydrochloride. After standing in darkness for 20 min the absorbance at 540 nm was measured.

Density Labeling of
Proteins with ¹³C-Amino Acids

Twenty aleurone layers were incubated in 2 mM sodium acetate buffer pH 5.0, 10 mM CaCl₂, 1 μM GA₃, 5 mM KBrO₃ containing either casein hydrolyzate (C¹²-amino acids) or ¹³C-labeled hydrolyzate of algal proteins. The concentration of both hydrolyzates was 10 mg/ml, supplemented with 0.5 mg tryptophan (C¹²) per ml. The incubation of enzyme were carried out as described previously. The enzyme preparation was mixed with a ¹⁴C-protein marker and saturated CsCl solution to make a density of 1.3 g/ml. After being centrifuged in a Beckman L-4 centrifuge equipped with either Ti 50 or SW 50 L rotor at 40,000 rpm for 65 hours, the centrifuge tube was punctured and 4 drop (when SW 50 L rotor was used) or 12 drop (when Ti 50 rotor was used) fractions were collected. Refractive index of every eighth fraction was measured with a Bausch and Lomb Abbe-32 refractometer to monitor the density gradient. α-Amylase activity and radioactivity in the fractions were then measured to determine the distribution

of α -amylase and newly synthesized proteins in the gradient.

Liquid Scintillation Counting

Toluene based scintillation fluid was generally used (18.34 g PPO + 0.34 g POPOP/gal. of toluene). For aqueous samples, either Tritosol as described by Fricke (18) (3 g PPO/257 ml Triton X-100, 37 ml ethyl glycol, 106 ml ethanol, and 600 ml xylene) or the combination of NCS solubilizer and toluene scintillation fluid was used. For the latter case, 1 ml of NCS solubilizer was mixed with less than 100 μ l of aqueous sample and incubated at 50 °C for 2 hours before 10 ml of toluene scintillation fluid was added to the sample. A Packard Tri-Carb Model 3385 liquid scintillation counter was used in this study. The counting efficiencies are shown in TABLE 1.

TABLE 1.--Effect of Scintillation Counting

Sample	^3H	^{14}C	$^3\text{H}(^{14}\text{C})$	$^{14}\text{C}(^3\text{H})$	^{14}C Spillover in ^3H channel
NCS Solubilizer and "Toluene only"	49	94	41	58	16
Tritosol	29	88	28	26	69
Nitrocellulose filter and "Toluene only"	20	82	19	23	91
Fiber glass filter and "Toluene only"	30	88	25	53	17

Note: # Numbers indicate the percentages of the ^{14}C counts in the preset $^3\text{H}(^{14}\text{C})$ channel to that in the $^{14}\text{C}(^3\text{H})$ channel.

"Toluene only" is the toluene only scintillation fluid described in the "Materials and Methods".

Millipore HAWP nitrocellulose filter and Millipore AP 2002500 fiberglass filter were used.

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that this is essential for ensuring transparency and accountability in the organization's operations.

2. The second part outlines the various methods and tools used to collect and analyze data. This includes both traditional manual methods and modern digital technologies, highlighting the benefits of automation and data-driven decision-making.

3. The third part focuses on the challenges and risks associated with data management, such as data security, privacy concerns, and the potential for data loss or corruption. It provides strategies to mitigate these risks and ensure the integrity of the information.

4. The fourth part discusses the role of data in strategic planning and performance evaluation. It explains how data can be used to identify trends, measure progress, and make informed decisions that drive the organization's success.

5. The final part concludes by summarizing the key points and emphasizing the ongoing nature of data management. It stresses the need for continuous monitoring, updates, and collaboration across all levels of the organization to maintain effective data practices.

RESULTS

Time Course of the GA₃ Enhanced α -Amylase Production

The GA₃ enhanced α -amylase production has a lag period of about 8 to 10 hours (Figure 4). From 12 to 36 hours after the addition of GA₃ there is a linear accumulation of α -amylase activity indicating a constant rate of net α -amylase formation (Figure 4). After 36 hours of GA₃ treatment the production of α -amylase gradually slows down perhaps due to the senescence of the tissue. Most of the α -amylase (over 80%) is secreted into the bathing medium and this process starts about the same time as rapid accumulation of α -amylase (about 12 hr after GA₃ addition). On the other hand, most of the enzyme in the control tissue (- GA₃) is retained. The GA₃ enhancement of α -amylase production as measured after 24 hr of hormone treatment is 3 to 10 fold if sodium acetate buffer is used. However, the enhancement is greatly improved if sodium succinate buffer is used (see Part II). Usually there is some α -amylase (about 2 units per aleurone layer) present in the freshly peeled aleurone layer, this may represent enzyme already present in the dry seed or that formed during the 3 day imbibition period. The

1. The first step in the process of identifying a problem is to recognize that a problem exists. This is often done by comparing current performance with a desired state or goal. For example, a manager might notice that sales are declining or that customer satisfaction is low. Once a problem is identified, the next step is to define it clearly and specifically. This involves determining the scope of the problem, its causes, and its effects. A clear definition of the problem is essential for developing an effective solution.

2. The second step in the process is to analyze the problem. This involves gathering information about the problem and its context. This information can be obtained through various methods, such as interviews, surveys, and data analysis. The goal of this step is to understand the underlying causes of the problem and to identify the factors that are contributing to it. This information is then used to develop a plan of action.

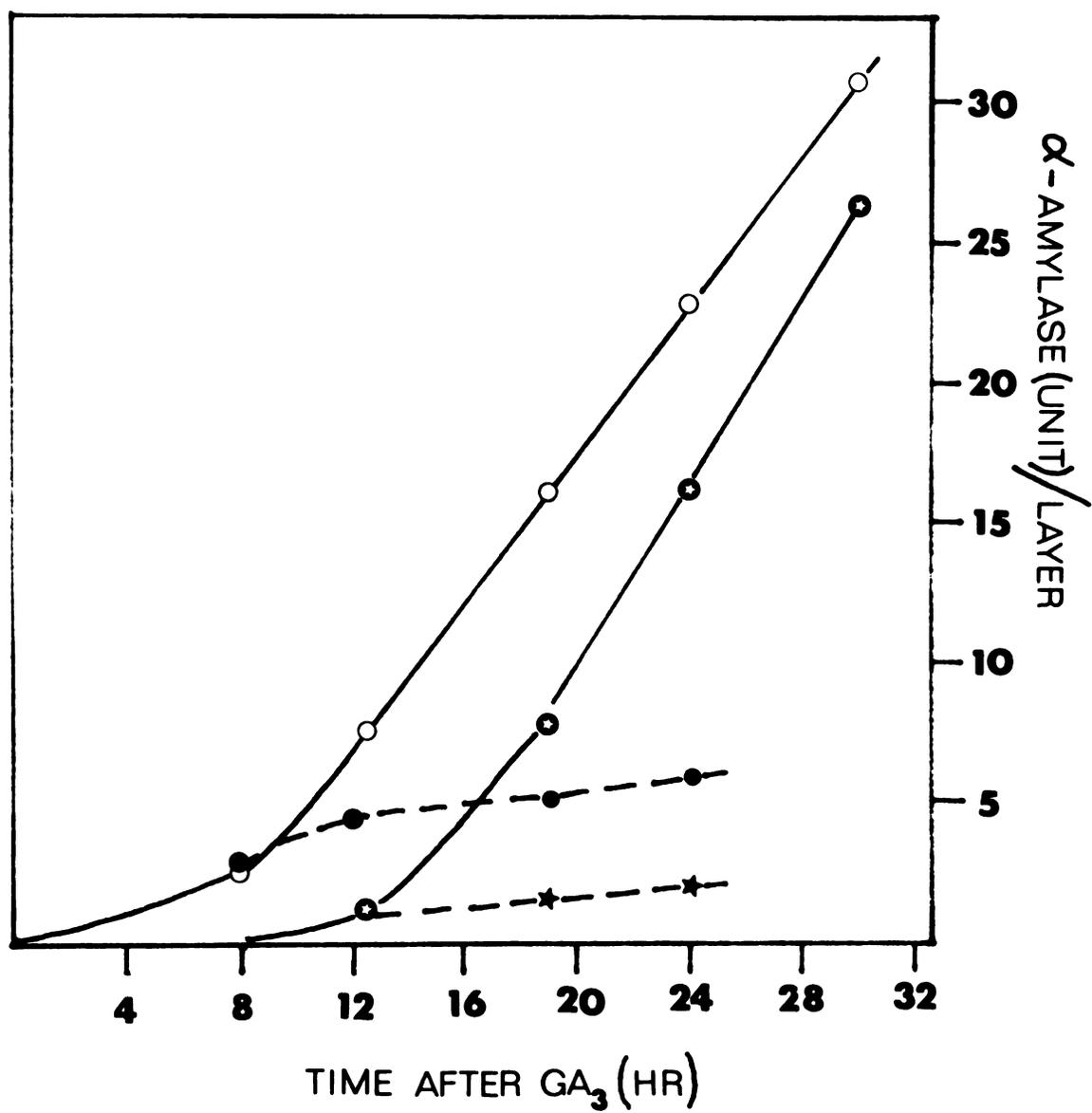
3. The third step in the process is to develop a solution. This involves identifying the most effective and efficient way to address the problem. This is often done by brainstorming ideas and evaluating them against various criteria, such as cost, time, and effectiveness. Once a solution has been identified, the next step is to implement it. This involves putting the solution into action and monitoring its progress.

4. The fourth step in the process is to evaluate the results. This involves comparing the actual results of the solution with the desired state or goal. This information is then used to determine whether the solution was effective and to identify any areas for improvement. If the solution was not effective, the process may need to be repeated.

5. The fifth and final step in the process is to document the solution. This involves creating a record of the problem, the analysis, the solution, and the results. This documentation is important for several reasons. First, it provides a clear record of what was done and why. Second, it can be used as a reference for future problems. Third, it can be used to share the solution with others who may be facing a similar problem.

Figure 4.--Time Course of the GA₃ Enhanced α -Amylase
Formation in Barley Aleurone Layers.

○ ——— ○ + GA₃ total
● ——— ● + GA₃ secreted
●-----● No GA₃ total
★-----★ No GA₃ secreted



activity of the α -amylase present at zero time was subtracted from the data presented.

Occurrence and Characterization
of Poly A-RNA

It has been reported that poly A-RNA can bind to nitrocellulose or plain cellulose, or be hybridized with immobilized oligo dT or poly U, the following methods have been tried in this study and all of them successfully bind a certain fractions of total RNA extracted from barley aleurone layers: oligo dT cellulose column chromatography, poly U cellulose column chromatography, cellulose (plain) column chromatography, poly U filtration, nitrocellulose filtration. However, only oligo dT cellulose column chromatography (for preparative purpose) and poly U filtration (for analytical purpose) were used in later studies.

From an oligo dT cellulose column (Figure 5) two major RNA peaks are eluted, one by high salt buffer (binding buffer), the other by Tris buffer without salt. A third peak eluted by buffer containing 0.1 M KCl was occasionally found may be non-specifically bound RNA. The recovery of oligo dT cellulose column chromatography varies in different batches of oligo dT cellulose. However, the specific batch used most often in this study had a recovery greater than 98%. The bound RNA is about 5 to 8% of the total RNA. The eluted peak by high salt

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3. The third part of the document focuses on the role of technology in data management and analysis. It discusses how modern software solutions can streamline data collection, storage, and reporting, thereby improving efficiency and accuracy.

4. The fourth part of the document addresses the challenges associated with data management, such as data quality, security, and privacy. It provides strategies to mitigate these risks and ensure that data is handled responsibly and in compliance with relevant regulations.

5. The fifth part of the document discusses the importance of data governance and the establishment of clear policies and procedures. It emphasizes that a strong data governance framework is essential for maximizing the value of data while minimizing associated risks.

6. The sixth part of the document explores the role of data in strategic planning and performance management. It illustrates how data-driven insights can help organizations identify trends, set goals, and track progress towards their strategic objectives.

7. The seventh part of the document discusses the importance of data literacy and training for all employees. It emphasizes that having a data-literate workforce is critical for organizations to fully leverage their data assets and drive innovation.

8. The eighth part of the document concludes by summarizing the key findings and recommendations. It reiterates the importance of a holistic approach to data management that encompasses all aspects of the organization's operations.

9. The ninth part of the document provides a detailed overview of the data collection process, including the identification of data sources, the design of data collection instruments, and the implementation of data collection protocols.

10. The tenth part of the document discusses the various methods used for data analysis, such as descriptive statistics, inferential statistics, and regression analysis. It explains how these methods can be used to interpret data and draw meaningful conclusions.

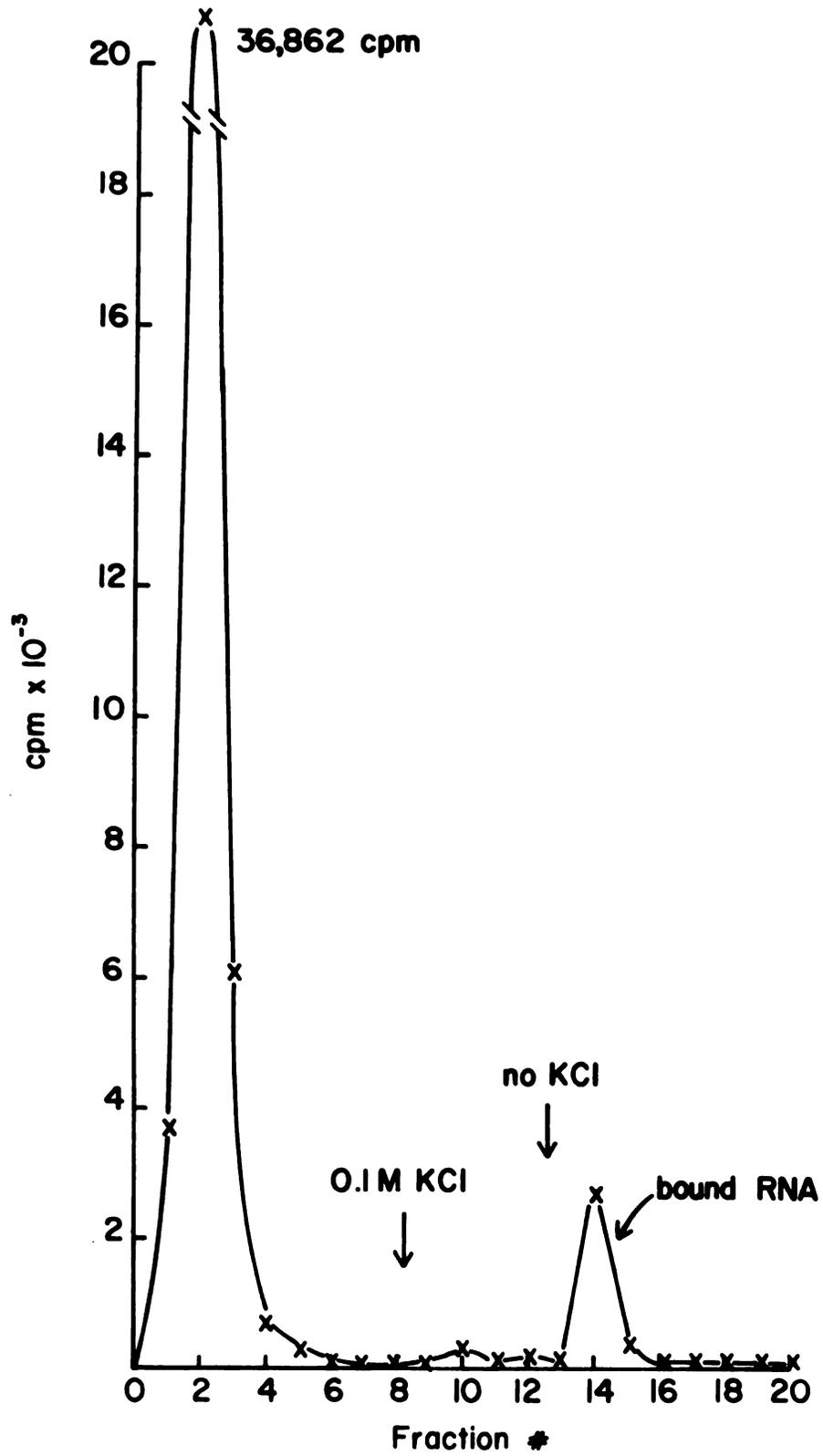
11. The eleventh part of the document focuses on the importance of data visualization in communicating complex information. It discusses various visualization techniques, such as charts, graphs, and dashboards, and how they can be used to present data in a clear and concise manner.

12. The twelfth part of the document discusses the role of data in ethical decision-making and the importance of ensuring that data is used responsibly and in compliance with ethical standards.

• *Chlorophyll a* (Chl a) is the primary photosynthetic pigment in most plants and algae. It is a green pigment that absorbs light energy in the blue-violet and red-orange regions of the visible spectrum. Chl a is essential for the light-dependent reactions of photosynthesis, where it converts light energy into chemical energy.

Figure 5.--Chromatography of total Barley RNA
on Oligo (dT)-Cellulose.

Fractions of 4.5 ml were collected.



contains rRNAs and tRNA (Figure 6A) as checked by sedimentation analysis with a sucrose density gradient. The bound RNA is heterogeneous with an average size of 14 to 17 S (Figure 6B). Since no detectable rRNA and tRNA were observed, this fraction presumably represents mRNA and probably some heterogeneous nuclear RNA. The estimation of RNA size distribution probably was not hampered by the aggregation of RNA, because the 65 °C heat treatment of RNA sample prior to sedimentation analysis described under "Material and Methods" has been used successfully to dis aggregate ovalbumin mRNA without reducing its ability to direct ovalbumin synthesis in a cell free system (23). The AMP content of bound RNA is higher than that of total RNA (TABLE 2). After RNase digestion with bound RNA, a RNase resistant segment was recovered which contain more than 90% AMP (TABLE 2 and Figure 7). Analyzing the size of this RNase resistant segment by polyacrylamide gel electrophoresis, the size of this segment was found to be heterogeneous, ranging from 80 to 200 nucleotides (Figure 8). However, based on the AMP to adenosine ratio the poly A segment was estimated to have an average size of 156 nucleotides (TABLE 3). Using the AMP content of poly A-RNA and the poly A segment as well as the size of poly A segment, one can calculate the average chain length of poly A-RNA as shown in the following diagram:

The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that every entry should be supported by a valid receipt or invoice. This not only helps in tracking expenses but also ensures compliance with tax regulations. The second part of the document provides a detailed breakdown of the company's revenue streams. It identifies the primary sources of income and analyzes their contribution to the overall financial performance. The third part of the document outlines the company's financial goals for the upcoming year. It includes a comprehensive budget and a strategy for achieving these goals. The fourth part of the document discusses the company's financial risks and the measures being taken to mitigate them. It highlights the importance of maintaining a healthy cash flow and managing debt effectively. The fifth part of the document provides a summary of the company's financial position and a forecast for the future. It concludes with a statement of confidence in the company's ability to meet its financial obligations and achieve its long-term goals.

**Figure 6.--Size Distribution of Barley Aleurone
RNA in a Sucrose Density Gradient.**

- A. RNA species not retained by oligo (dT)-
cellulose**

- B. RNA species retained by oligo (dT)-
cellulose**

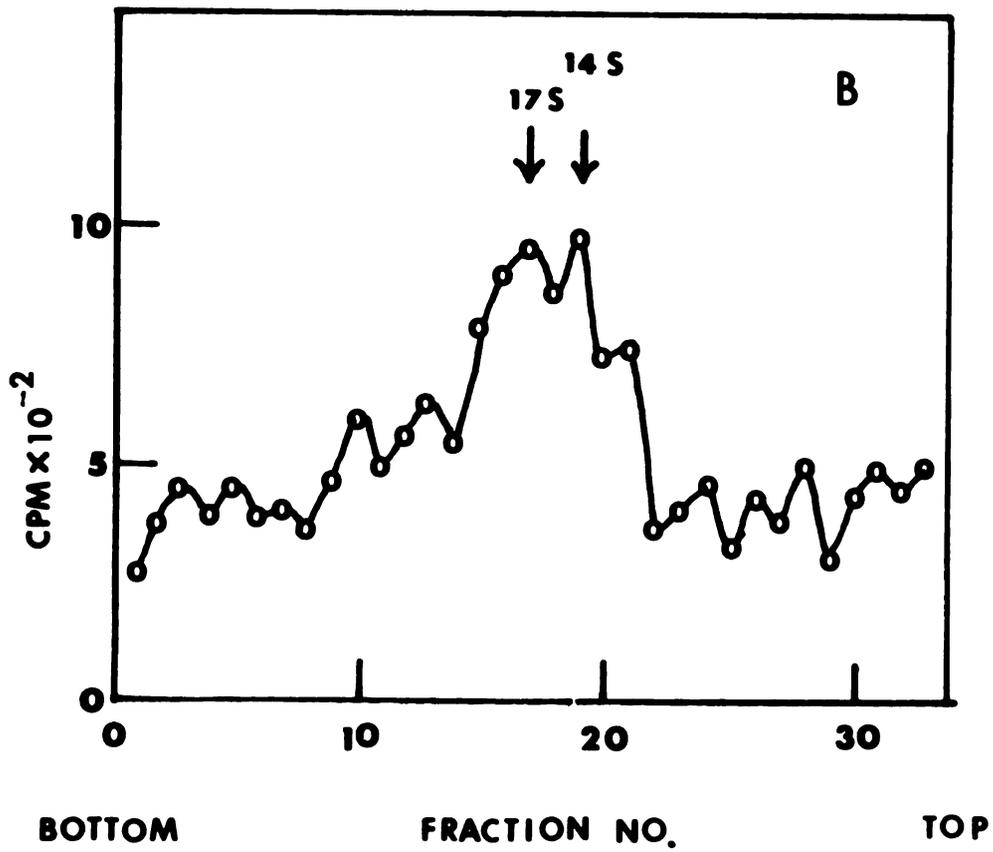
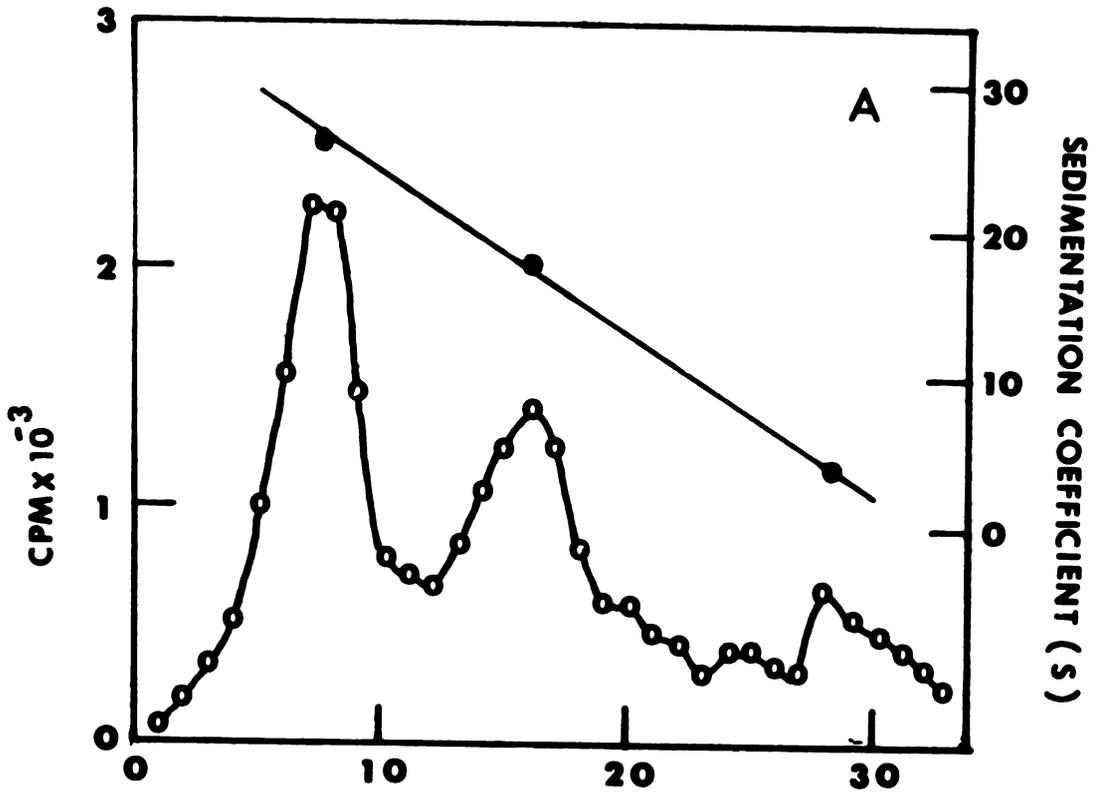


TABLE 2.--Base Composition of RNA Species Isolated from Barley Aleurone Layers

Sample	CMP	AMP	GMP	UMP
Total RNA	25.1	22.9	33.5	18.5
Bound RNA (Poly(A)-RNA)	23.7	30.2	25.1	21.0
RNase resistant (Poly(A) segment)	0.8	94.1	3.6	1.5

Figure 7.--Radioelectrophoretogram of the Hydrolyzate
of the RNase Resistant RNA Segment
Isolated from Barley Aleurone Layers.

RNase resistant RNA segment isolated
from ^{32}P RNA was described under "Mate-
rials and Methods". A 2 mm slit and
linear scale were used during the scanning.

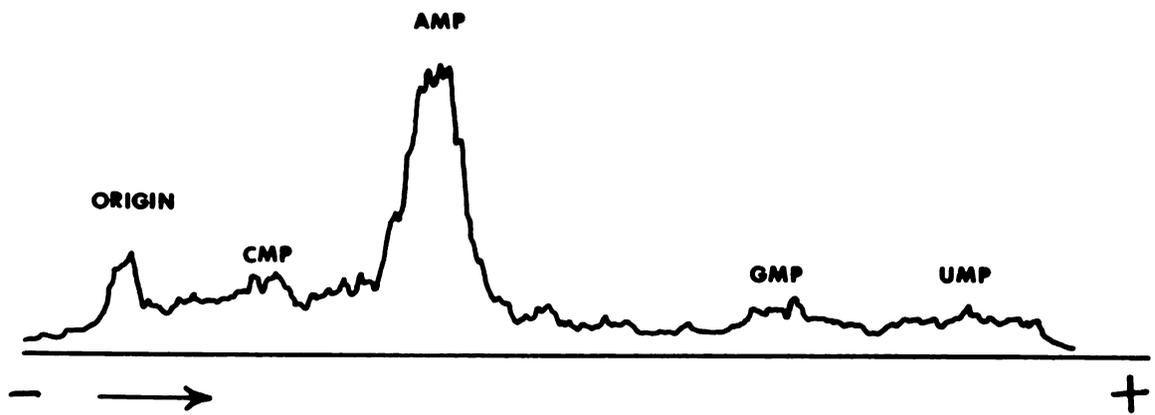


Figure 8.--Polyacrylamide Gel Electrophoresis of
Poly (A) from Barley Aleurone Layers.

Poly (A) segment labeled with ^3H -adenosine was isolated as described in "Materials and Methods" and then analyzed on 10% polyacrylamide gel. Tritiated tRNA, analyzed on a separate gel, was used as a marker.

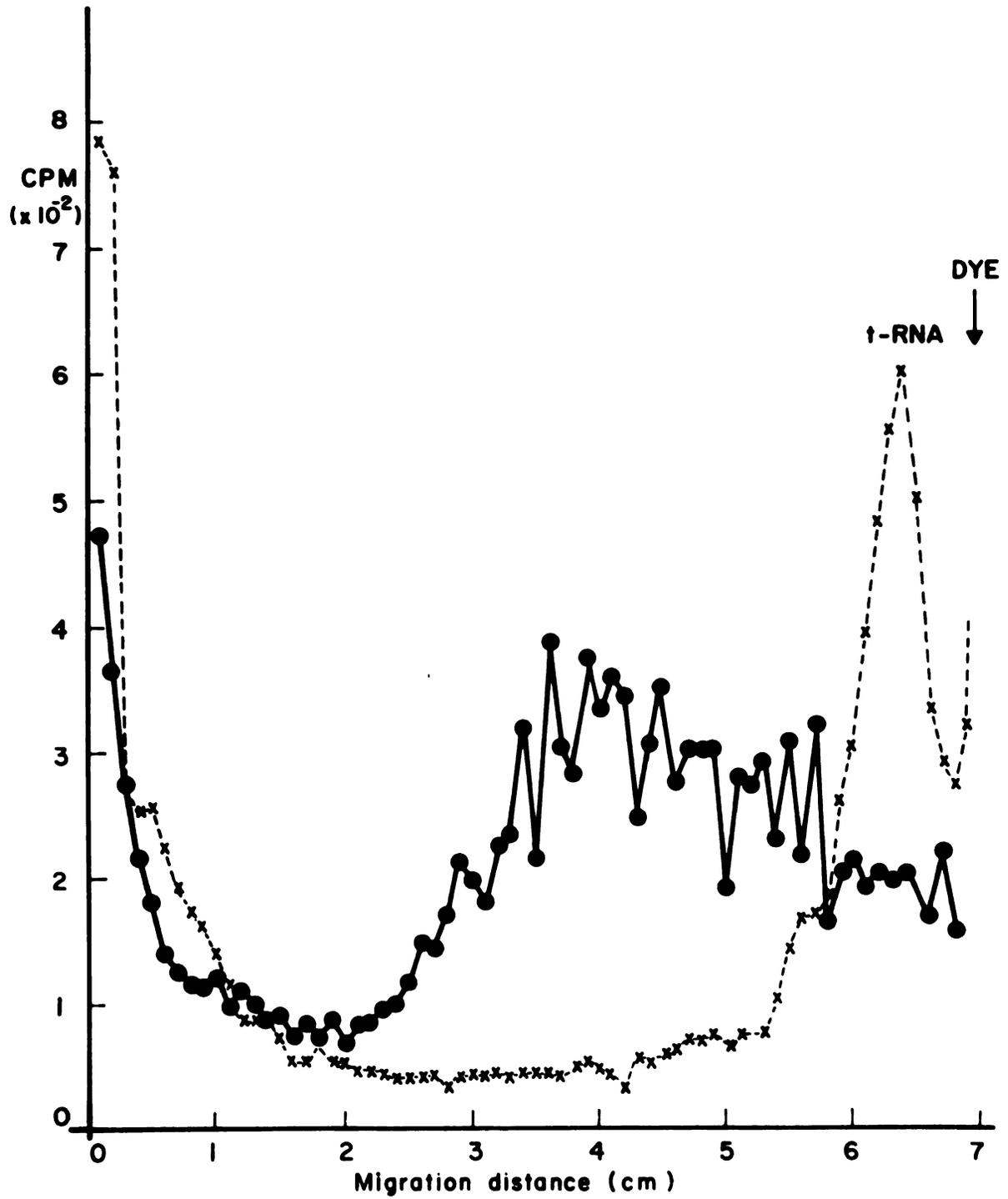
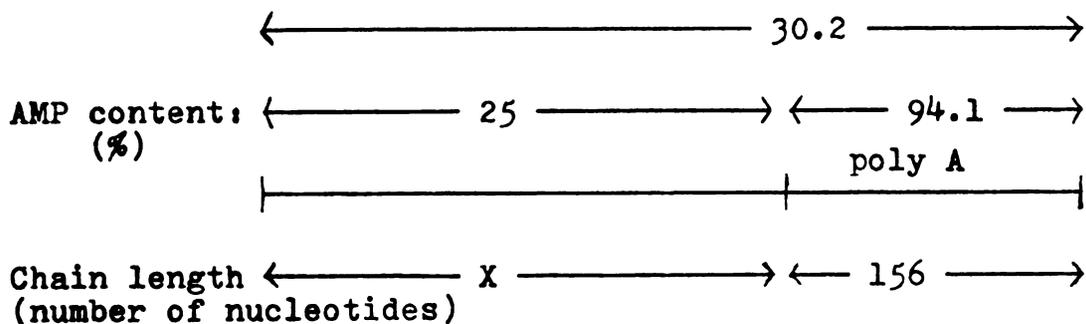


TABLE 3.--Estimation of the Size of Poly (A) Segment by the Ratio of
AMP to Adenosine

	AMP	Adenosine	Average Size of Poly A Segment (AMP/Adenosine)
CPM	37,440	239	156

Note: Poly (A) segment isolated from aleurone layers incubated with
³H-adenosine was used.



$$(X + 156) \times 30.2 = 25X + 156 \times 94.1$$

$$X = 1920$$

Therefore:

Chain length of poly A-RNA: $1920 + 156 = 2076$

M.W. of poly A-RNA: 5.6×10^5

Predicted S value of poly A-RNA: about 15 S

The calculated size of poly A-RNA is in agreement with that obtained by direct sedimentation analysis (Figure 6).

In order to study the localization of poly A segment on the RNA molecule, the 3'-OH end of RNA was selectively labeled by NaIO_4 oxidation followed by NaB^3H_4 reduction as described under "Materials and Methods". Barley aleurone poly A-RNA as well as the chemically synthesized poly A from Sigma Chemical Co. retained their label after RNase digestion (TABLE 4) indicating that the 3'-OH end of all the RNA is occupied by a poly A segment. However, the results of this experiment do not show that all poly A segment are located adjacent to the 3'-OH

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that proper record-keeping is essential for transparency and accountability, particularly in the context of public administration and financial management. The text notes that without reliable records, it becomes difficult to track expenditures, identify inefficiencies, and ensure that funds are being used for their intended purposes.

2. The second part of the document addresses the challenges associated with data collection and analysis. It highlights that gathering comprehensive data from various sources can be a complex and time-consuming process. However, the benefits of having a robust data set are significant, as it allows for more informed decision-making and the identification of trends and patterns. The document suggests that investing in data management systems and training staff can help overcome these challenges.

3. The third part of the document focuses on the role of technology in improving operational efficiency. It discusses how digital tools and software can streamline processes, reduce errors, and enhance communication. For example, the use of cloud-based systems can facilitate data sharing and collaboration across different departments. The text also mentions that automation of routine tasks can free up resources for more strategic and value-added activities.

4. The fourth part of the document discusses the importance of regular communication and reporting. It states that keeping stakeholders informed about progress and challenges is crucial for building trust and ensuring that everyone is working towards the same goals. The document recommends establishing clear communication channels and schedules for regular updates. Additionally, it suggests that reports should be concise, clear, and focused on key findings and recommendations.

5. The fifth part of the document concludes by emphasizing the need for a proactive and continuous approach to improvement. It notes that the process of refining operations and systems is ongoing and should be supported by a culture of innovation and learning. The document encourages organizations to regularly evaluate their performance, seek feedback, and implement changes as needed to stay competitive and effective in a rapidly changing environment.

TABLE 4.--Localization of Poly(A) Segment on RNA Molecule.

Sample	Counts per minute		% Remained
	Before RNase treatment	After RNase treatment	
Poly (A) from Sigma	4996.5	4493.5	89.9
<u>E. Coli</u> t-RNA	65025.0	4911.5	7.6
Barley poly (A)-RNA	73656.0	70340.5	95.5

Note: Barley samples were chemically labeled at the 3'-OH end as described in "Materials and Methods" before ribonuclease treatment.

end- the possible occurrence of internal poly A-segments is not ruled out.

Effect of Hormones
on Poly A-RNA Synthesis

Fiberglass filters with immobilized poly U were used to analyze a sample with little RNA because the filtration takes only about ten minutes and several filtrations can be handled simultaneously. As shown in TABLE 5, the poly U filter retains labeled poly A, but not poly U, and it has a maximal capacity to retain about 8 ug of poly A which is more than sufficient to analyze the small quantity of RNA used in the later experiments. The poly U filter retains a certain fraction of the short-termed labeled barley aleurone RNA and the binding can be competed out by cold poly A but not tRNA (TABLE 5). The double labeling technique described in "Materials and Methods" maximizes the sensitivity of the experiment; this design also eliminates possible artifacts generated by differential degradation during the isolation of poly A-RNA. The latter consideration is important because GA_3 is known to enhance ribonuclease activity in barley aleurone layers (11). Although a combination of several potent protein denaturants were used during RNA isolation, i.e. diethyl pyrocarbonate, SDS, and phenol-chloroform, a trace of RNase activity could be still detected in the RNA preparations. From the $^3H/^{14}C$ ratio of RNA retained

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TABLE 5.--Retention of RNA by Poly U Filtration

RNA Sample	Input cpm	Retained cpm	%
1 ug poly A(³ H)	3859.1	3859.1	99.8
2.5 ug poly A(³ H)	9647.8	9678.8	100.3
20 ug poly A(³ H)	77182.7	33130.5	42.9
100 ug poly U(³ H)	184766.1	278.6	0.1
0.5 ml barley RNA	10099.7	1305.5	12.9
1 ml barley RNA	20199.3	2667.7	13.2
1 ml barley RNA + 200 ug cold poly A	20199.3	330.2	1.6
1 ml barley RNA + 50 ug cold poly A	20199.3	452.4	2.2
1 ml barley RNA + 200 ug tRNA (<u>E.coli</u>)	20199.3	2636.6	13.1

Note: Barley RNA was extracted from 100 aleurone layers labeled with ³H-uridine for 1 hr in the absence of GA₃. The extracted RNA was dissolved in 10 ml filtration buffer (0.12 M NaCl, 10 mM Tris-HCl, pH 7.5).

• The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that this is crucial for ensuring transparency and accountability in the organization's operations.

• The second part of the document outlines the various methods and tools used to collect and analyze data. It highlights the need for consistent data collection procedures and the use of advanced analytical techniques to derive meaningful insights from the data.

• The third part of the document focuses on the interpretation of the data and the identification of key trends and patterns. It discusses how these insights can be used to inform strategic decision-making and to identify areas for improvement within the organization.

• The fourth part of the document provides a detailed overview of the findings and conclusions drawn from the data analysis. It includes specific recommendations for future actions and a discussion of the potential risks and challenges associated with the current state of affairs.

• The final part of the document serves as a summary and a call to action, urging all stakeholders to work together to implement the recommended changes and to ensure the long-term success and sustainability of the organization.

on poly U filter, one can calculate the hormonal effect as the percent enhancement of poly A-RNA synthesis over the control. For example, at 12 hr of GA₃, the ³H/ ¹⁴C ratio of the mixture of the sample containing GA₃ and the sample without GA₃ is 38.05 and that of the mixture of samples both without GA₃ is 24.22; therefore, the GA₃ enhancement is 7%. It is observed that GA₃ enhances the synthesis of poly A-RNA with a lag period 3 to 4 hours. This enhancement reaches a maximum, which is about 50 to 60% over control, at 10 to 12 hours, then decreases afterwards (Figure 9). This GA₃ effect can still be observed if uridine is used as the labeled precursor, indicating that the synthesis of the portion of the poly A-RNA molecules not containing poly A is also enhanced by GA₃. Abscisic acid, a naturally occurring plant hormone that antagonizes GA₃ mediated hydrolase synthesis in barley aleurone layers (68), prevents the GA₃ effect on poly A-RNA synthesis (TABLE 6).

The aleurone cell has a thick and rigid cell wall, and the wall is softened in the presence of GA₃ probably due to the hormone enhanced pentosanase activity (6). This fact leads to possible argument that the observed enhancement of poly A-RNA synthesis is an artifact resulting from the preferential extraction of RNA from hormone treated tissue. However, because drastic grinding in a porcelain mortar with pestle and sharp sand was used in

1. The first step in the process of identifying a problem is to recognize that a problem exists. This is often done by comparing current performance with a desired state or goal. For example, a manager might notice that sales are declining or that customer satisfaction is low. Once a problem is identified, the next step is to define it more precisely. This involves determining the scope of the problem, its causes, and its effects. For instance, a manager might define a problem as "a 10% decrease in sales over the last quarter, primarily due to a loss of market share in the competitive market." The third step is to analyze the problem. This involves gathering data, identifying key factors, and determining the underlying causes. For example, a manager might analyze sales data to identify trends, compare performance with competitors, and identify areas where the company is losing market share. The fourth step is to generate potential solutions. This involves brainstorming ideas and evaluating them based on their feasibility, effectiveness, and cost. For instance, a manager might generate several potential solutions, such as increasing marketing efforts, improving product quality, or offering discounts to customers. The fifth step is to select a solution. This involves choosing the most promising solution based on the analysis and the evaluation of potential solutions. For example, a manager might select a solution that involves increasing marketing efforts and improving product quality. The sixth step is to implement the solution. This involves putting the chosen solution into action and monitoring its progress. For instance, a manager might implement a marketing campaign and track sales and customer satisfaction over time. The seventh step is to evaluate the results. This involves comparing the actual results with the desired state and determining whether the problem has been solved. For example, a manager might evaluate sales and customer satisfaction data to determine if the marketing campaign and product improvements have led to an increase in sales and customer satisfaction. If the problem has not been solved, the manager may need to re-evaluate the solution and try a different approach.

Figure 9.--Effect of GA₃ on poly (A)-RNA
Synthesis in Barley Aleurone
Layers.

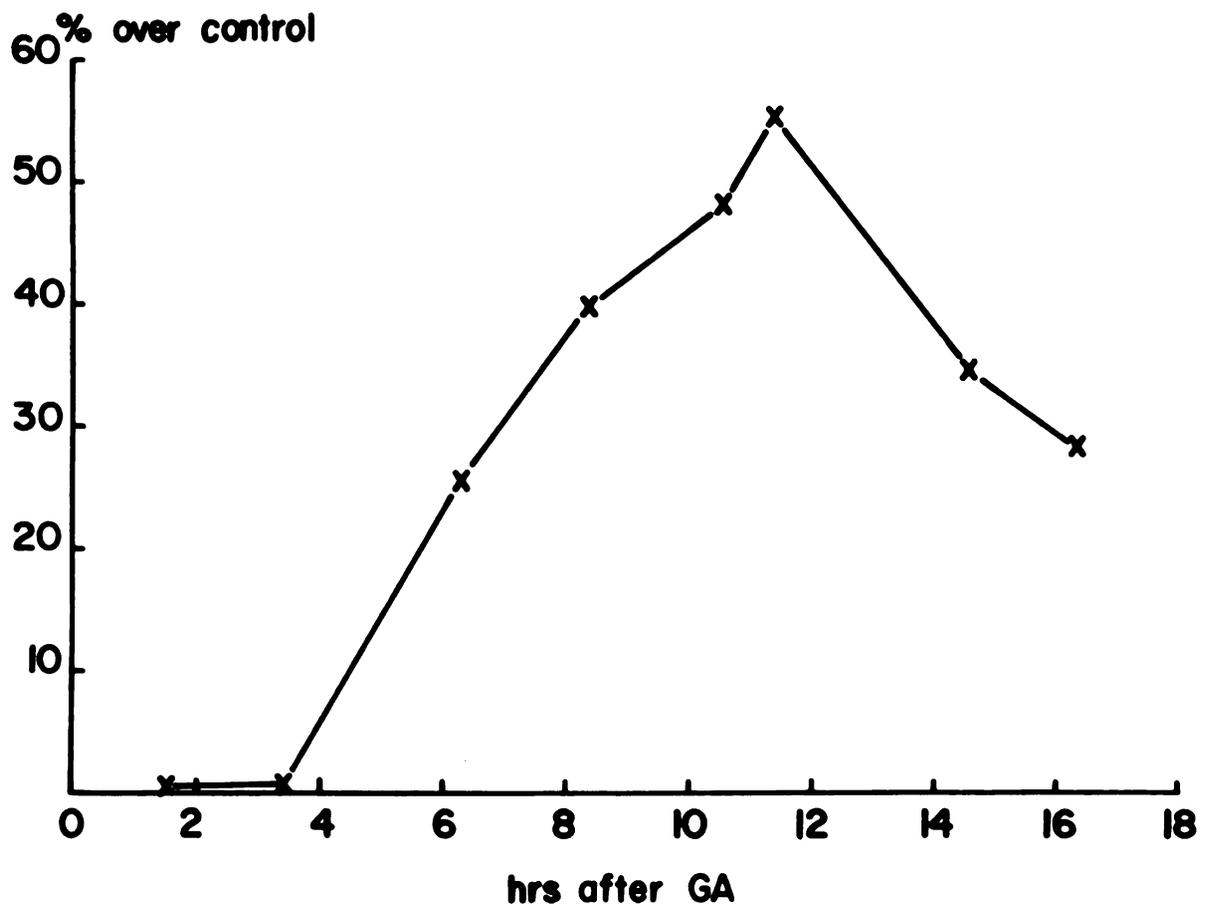


TABLE 6.--Effect of ABA on the GA₃ Enhanced Poly (A)-
RNA Synthesis

Treatment	# ³ H/ ¹⁴ C	% over Control
Control (no GA ₃)	25.70	0
+ GA ₃	38.89	51
+ GA ₃ + ABA (5 uM)	28.72	12

Note: #The experimental design was the same as described under "Materials and Methods". ABA was added at the same time as GA₃.

this study, the possibility of preferential extraction is unlikely.

Effect of Cordycepin on
the Production of α -Amylase

Cordycepin, 3'-deoxyadenosine, is believed to work as a chain terminator during RNA synthesis. In animal tissue, cordycepin preferentially inhibits poly A synthesis during short-termed incubation (12). However, it also inhibits the formation of major RNA species if the incubation is longer than a few minutes. In order to check the effectiveness of the cordycepin effect on enzyme induction in plant tissue, I tested the effect of cordycepin on the induction of nitrate reductase in barley aleurone layers, because it is known that nitrate reductase induction depends on the continuous synthesis of RNA (15). As shown in Figure 10, cordycepin, no matter whether added at the same time or 3.5 hours after KNO_3 quickly abolishes the induction. Therefore, together with the data of cordycepin effect on RNA synthesis (to be presented in the next section), this result indicates that cordycepin is quickly taken up by the tissue and is quickly effective. As for the GA_3 enhanced α -amylase formation, cordycepin inhibits most of the hormone increased α -amylase activity if it is added at the same time as the hormone. On the other hand, 2'-deoxyadenosine, even at much higher concentration, is not inhibitory (TABLE 7).

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1. The first part of the text discusses the importance of maintaining accurate records of all transactions and activities related to the business. This includes keeping track of income, expenses, and assets. Proper record-keeping is essential for determining the business's financial health and for reporting to tax authorities.

2. The second part of the text focuses on the importance of understanding the tax implications of various business decisions. This involves consulting with a tax professional to ensure that the business is taking full advantage of available tax deductions and credits, and that it is complying with all applicable tax laws.

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Figure 10.--Effect of Cordycepin on the Substrate
Induction of Nitrate Reductase.

0—0 50 mM KNO_3
●-----● No KNO_3
x 50 mM KNO_3 + Cordycepin (added at 0 time)
Δ-----Δ 50 mM KNO_3 + Cordycepin (added at 3.5 hours
after KNO_3)

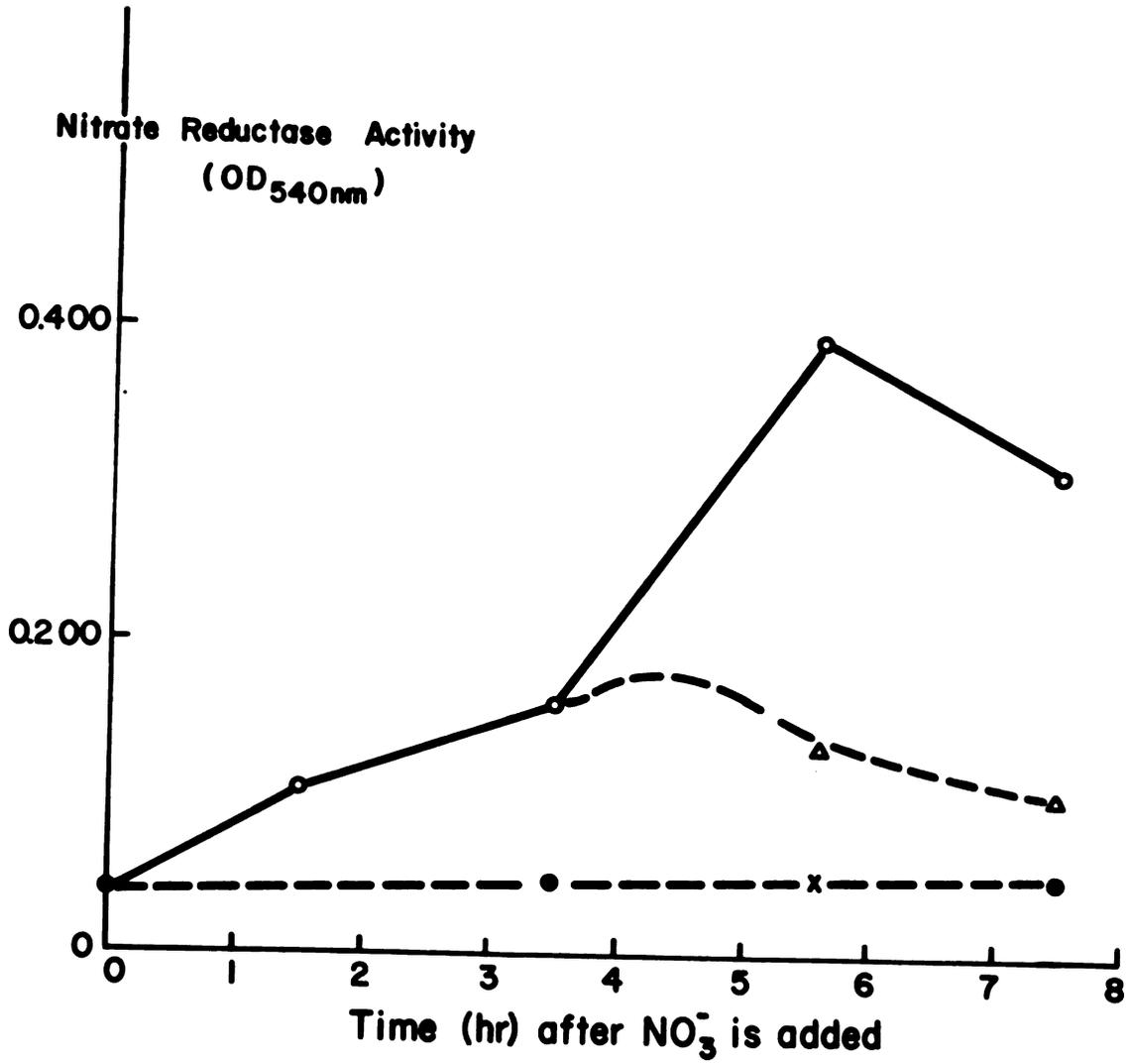


TABLE 7.--Effect of Cordycepin and 2'-Deoxyadenosine on the GA₃ Enhanced Formation of α-Amylase in Barley Aleurone Layers

Treatment	α-Amylase Activity (units/layer)	%
+ GA ₃ only	39.6	100
0.1 mM Cordycepin	6.3	15.9
0.1 mM 2'-Deoxyadenosine	36.1	91.1
01.0 mM 2'-Deoxyadenosine	33.0	83.3

Note: Cordycepin or 2'-deoxyadenosine was added at the same time as GA₃ and enzyme was assayed 24 hours after addition of the hormone.

Cordycepin at a concentration of 0.1 mM (about 25 ug/ml) can give maximal effect (Figure 11), therefore, 0.1 mM was used in most of the experiments. Cordycepin treated tissue usually retains more of the α-amylase in the layer (as much as 50%). The inhibitory effect of cordycepin on α-amylase production is not fully reversible. However, results to be shown in Part II of this thesis strongly suggest that the inhibitory effect of cordycepin is not via general toxicity to the cell. If cordycepin is added after GA₃, the inhibitory effect becomes progressively less and less as the time of addition is delayed (Figure 12). There is essentially no inhibitory effect on α-amylase production if the cordycepin is added 12 hours or

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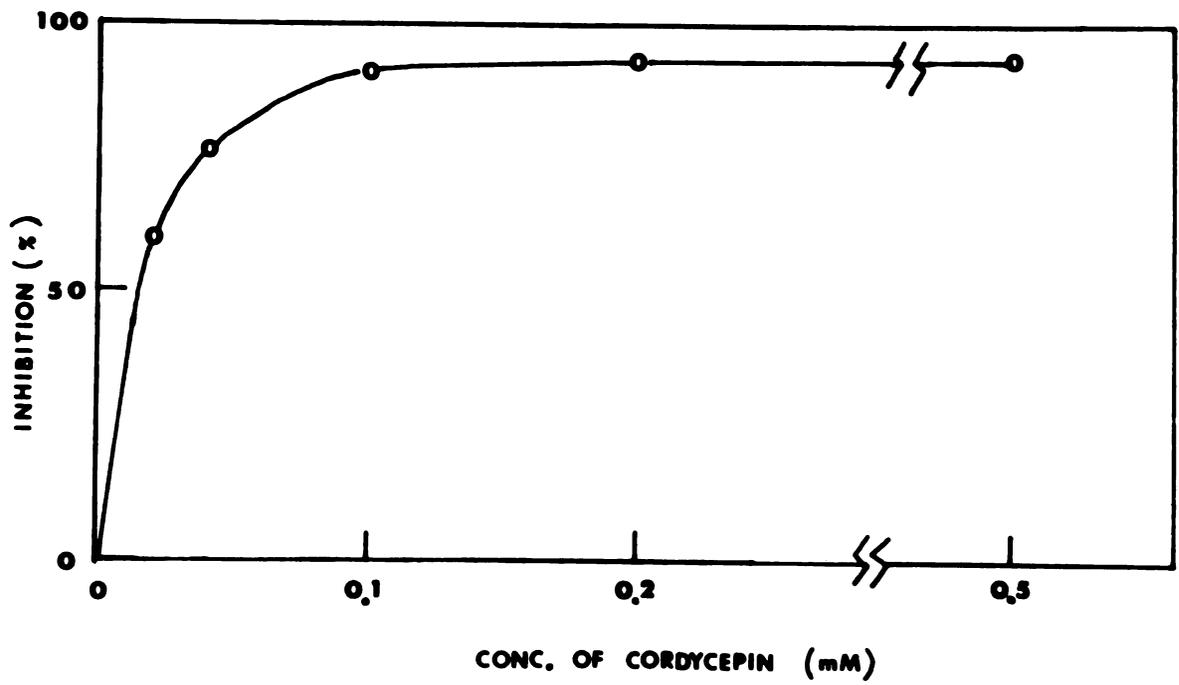
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• The first step in the process of identifying a problem is to define the problem clearly. This involves identifying the symptoms and the underlying causes of the problem.

• The second step is to gather information about the problem. This involves collecting data and consulting with experts in the field.

Figure 11.--Inhibition of α -Amylase Formation by
Various Concentrations of Cordycepin.

Cordycepin was added at the same time
as GA_3 and α -amylase was assayed at
24 hours after the addition of GA_3 .



more after GA₃ (Figure 12). Similar inhibitory effects of cordycepin were observed on the GA₃-enhanced protease production (TABLE 8). Since the length of incubation with cordycepin becomes shorter and shorter when the inhibitor is added later and later after GA₃ addition, one might argue that the decreasing inhibitory effect results from the shortening incubation time with the inhibitor. However, this has been shown not the case, because no inhibitory effect was observed if cordycepin was present 12 to 30 hours after GA₃ (18 hr incubation) while cordycepin added at 6 hr after GA₃ (6 to 24 hr; also 18 hr of incubation) inhibited α -amylase production by 40% (TABLE 9). Increasing the concentration of cordycepin up to 0.7 mM (about 175 ug/ml) caused no inhibition when the cordycepin was added 12 hours after the addition of GA₃ (TABLE 10). Occasionally α -amylase production is even higher when cordycepin is added after 12 hours of GA₃ treatment (TABLE 10). However, the mechanism of this "superinduction" is not known.

All the data presented in this section suggest that the α -amylase after 12 hours of GA₃ treatment is translated from a stable mRNA. In the next three sections evidence will be presented to answer the following three questions which are necessary to prove the above suggestion

- 1). Does cordycepin effectively inhibit the synthesis of RNA, especially mRNA (poly A-RNA)

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that this is crucial for ensuring transparency and accountability in the organization's operations.

2. The second part of the document outlines the various methods and tools used to collect and analyze data. It highlights the need for consistent and reliable data collection processes to ensure the validity of the findings.

3. The third part of the document describes the results of the data analysis, including the identification of key trends and patterns. It notes that these findings provide valuable insights into the organization's performance and areas for improvement.

4. The fourth part of the document discusses the implications of the findings and the recommendations for future actions. It suggests that the organization should focus on strengthening its internal controls and improving its reporting mechanisms to enhance its overall efficiency and effectiveness.

5. The fifth part of the document provides a summary of the key findings and conclusions. It reiterates the importance of ongoing monitoring and evaluation to ensure that the organization remains compliant with all relevant regulations and standards.

6. The sixth part of the document includes a list of references and sources used in the research. It acknowledges the contributions of various authors and organizations that provided valuable information and support throughout the project.

7. The seventh part of the document contains a list of appendices and supporting documents. These include detailed data tables, charts, and other relevant information that provide further context and detail to the main findings.

8. The eighth part of the document provides a list of contact information for the authors and the organization. It includes email addresses and phone numbers for those who may have questions or need further information.

9. The ninth part of the document includes a list of acknowledgments and thanks. It expresses appreciation to the individuals and organizations that provided assistance and support during the course of the project.

10. The tenth part of the document contains a list of footnotes and references. It provides additional information and citations for the sources used in the document.

11. The eleventh part of the document includes a list of tables and figures. These provide a visual representation of the data and findings, making it easier to understand the results of the analysis.

12. The twelfth part of the document contains a list of glossary terms and definitions. It provides clear and concise explanations of the key terms and concepts used throughout the document.

13. The thirteenth part of the document includes a list of appendices and supporting documents. These include detailed data tables, charts, and other relevant information that provide further context and detail to the main findings.

14. The fourteenth part of the document provides a list of contact information for the authors and the organization. It includes email addresses and phone numbers for those who may have questions or need further information.

Figure 12.--Effect of Cordycepin on α -Amylase
Activity.

Cordycepin was added at different times after GA_3 as indicated, and aleurone layers were further incubated until 24 hours after the addition of GA_3 , when the activity of α -amylase was assayed.

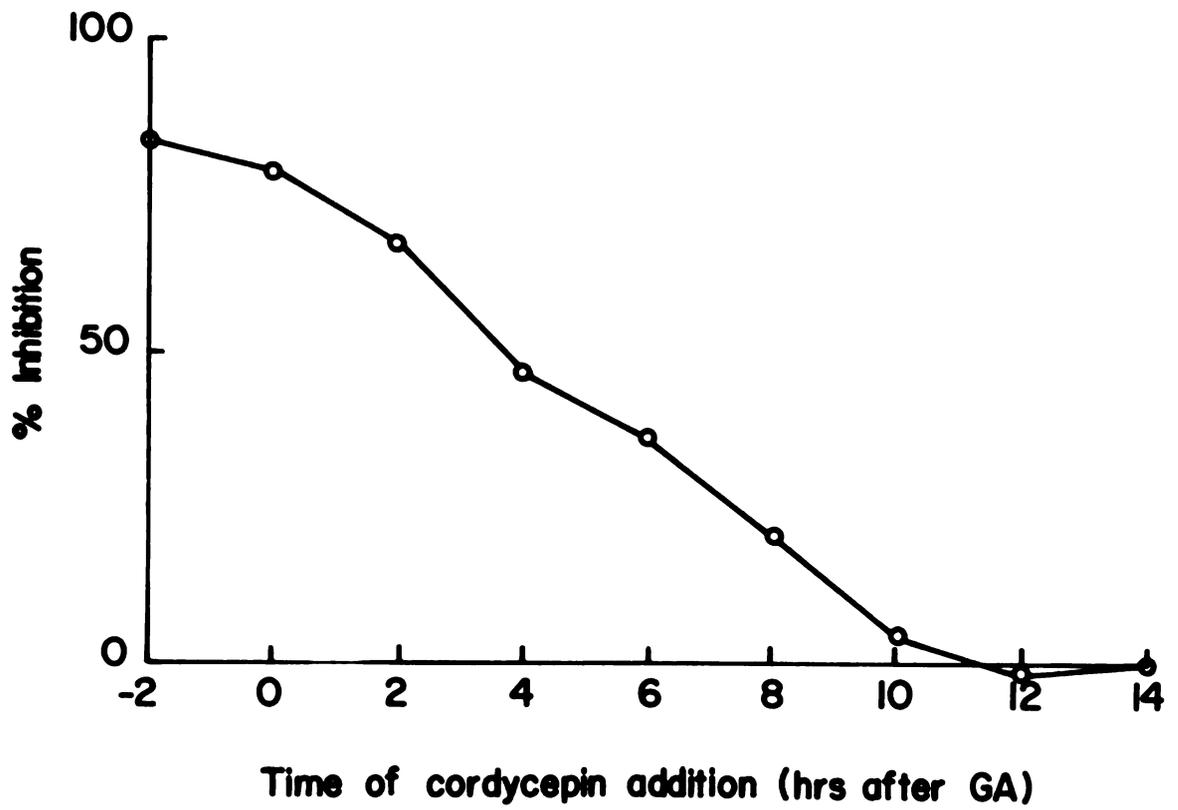


TABLE 8.--Effect of Cordycepin on the GA₃-Enhanced Protease Formation

Time of Cordycepin Addition (hours after GA ₃)	Protease Activity (units/10 layers)	% Inhibition
+ GA ₃ only	7.47	0
0 time	2.66	64.4
2	2.54	66.0
4	2.97	60.0
6	4.31	42.3
8	4.43	40.7
10	4.88	34.7
12	6.52	12.7
14	6.90	7.6

Note: Cordycepin was added at different times after GA₃ as indicated, and aleurone layers were further incubated until 24 hours after the addition of GA₃, when the activity of protease was assayed.

TABLE 9.--Comparison of the Effect of Cordycepin Added
at Different Times after GA₃ on the Production
of α-Amylase

Treatment	α-Amylase Activity (units/layer)	%
+ GA ₃ only (24 hr)	24.9	100
+ GA ₃ + Cordycepin (6 to 24 hr)	15.1	60
+ GA ₃ only (30 Hr)	34.2	100
+ GA ₃ + Cordycepin (12 to 30 hr)	34.8	102

TABLE 10.--Effect of Various Concentrations of Cordycepin on the
Production of α -Amylase

Concentration of Cordycepin (mM)	α -Amylase Activity (units/layer)	%
0	16.1	100
0.1	22.0	136
0.2	19.4	120
0.4	20.8	129
0.7	16.8	104
1.0	12.7	79

Note: Cordycepin was added 12 hours after GA_3 addition, and the aleurone layers were further incubated until 24 hours after GA_3 , when the enzyme was assayed. α -Amylase produced before 12 hours of GA_3 was subtracted.

when added 12 hours after GA₃ addition ?

- 2). Is the rapid increase of α -amylase activity after 12 hours of GA₃ treatment due to the de novo synthesis of the enzyme molecule ?
- 3). Does cordycepin inhibit both the synthesis and degradation of α -amylase which tend to compensate with each other ?

Effect of Cordycepin on RNA Synthesis

Cordycepin, at a concentration of 0.1 mM, has a profound effect on the synthesis of total RNA in barley aleurone layers as measured by uridine incorporation (Figure 13). Cordycepin has no significant effect on the uptake of the radioactive precursor. When it is added at the same time as GA₃, the inhibitory effect of cordycepin on RNA synthesis is about 60%. However, the inhibitor causes nearly complete inhibition of RNA synthesis in one hour if it is added 12 hours after GA₃ (Figure 13). The more effective inhibition of cordycepin after 12 hours of GA₃ may be due to the faster uptake of the inhibitor. Poly A-RNA and on-poly A-RNA are inhibited by cordycepin to 84% and 93% respectively when the inhibitor is added 12 hours after GA₃ (Figure 14). Thus, unless the analog has some unexpected selectivity one must conclude that the formation of all the RNAs is inhibited and that at 12 hours α -amylase message is present in non-limiting amounts and is stable for an additional 18 hours.

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that proper record-keeping is essential for transparency and accountability, particularly in the context of public administration and financial management. The text notes that without reliable records, it becomes difficult to track expenditures, identify inefficiencies, and ensure that funds are being used for their intended purposes.

2. The second part of the document addresses the challenges associated with data collection and analysis. It highlights that gathering comprehensive data from various sources can be a complex and time-consuming process. However, the benefits of having a robust data infrastructure are significant, as it enables decision-makers to base their actions on evidence and insights derived from the data. The text suggests that investing in modern data management systems and training personnel in data analysis techniques can greatly enhance the organization's ability to handle large volumes of information effectively.

3. The third part of the document focuses on the role of technology in improving operational efficiency. It discusses how digital tools and automation can streamline processes, reduce manual errors, and accelerate the flow of information. For example, the implementation of cloud-based systems can facilitate collaboration and data sharing across different departments and locations. The text also mentions that technology can be used to monitor and optimize resource allocation, ensuring that the organization is operating at its maximum capacity.

4. The fourth part of the document explores the importance of continuous improvement and innovation. It states that organizations should not be satisfied with the status quo and should actively seek ways to enhance their performance. This can be achieved through regular audits, benchmarking against industry best practices, and encouraging a culture of innovation where employees are empowered to propose and implement new ideas. The text emphasizes that staying current with the latest trends and technologies is crucial for long-term success in a competitive environment.

5. The fifth and final part of the document discusses the need for strong leadership and governance. It argues that clear vision, strategic direction, and effective communication are essential for the success of any organization. Leaders should ensure that the organization's goals and values are well-defined and consistently communicated to all stakeholders. Additionally, the text stresses the importance of maintaining high standards of integrity and ethical conduct, as these are fundamental to building trust and credibility with the public and other stakeholders.

- H_2O is a polar molecule. It has a partial positive charge on the hydrogen atoms and a partial negative charge on the oxygen atom.
- The partial positive charge on the hydrogen atoms is attracted to the partial negative charge on the oxygen atom of another water molecule.
- This attraction between the partial positive charge on one water molecule and the partial negative charge on another water molecule is called a hydrogen bond.
- Hydrogen bonds are relatively weak compared to covalent bonds, but they are strong enough to hold water molecules together in a network.
- This network of hydrogen bonds is what gives water its unique properties, such as its high boiling point and its ability to form a solid structure (ice) that is less dense than its liquid form.

Figure 13.--Effect of Cordycepin on the Incorporation of Uridine into RNA.

The amount of ^3H -uridine used was 2.5 uCi/ml. RNA was extracted at specific times as described under "Materials and Methods".

- A. No Preincubation with GA_3 -- Cordycepin was added at the same time as GA_3 .
- B. Preincubated with GA_3 for 12 hours -- Cordycepin was added 12 hours after GA_3 .

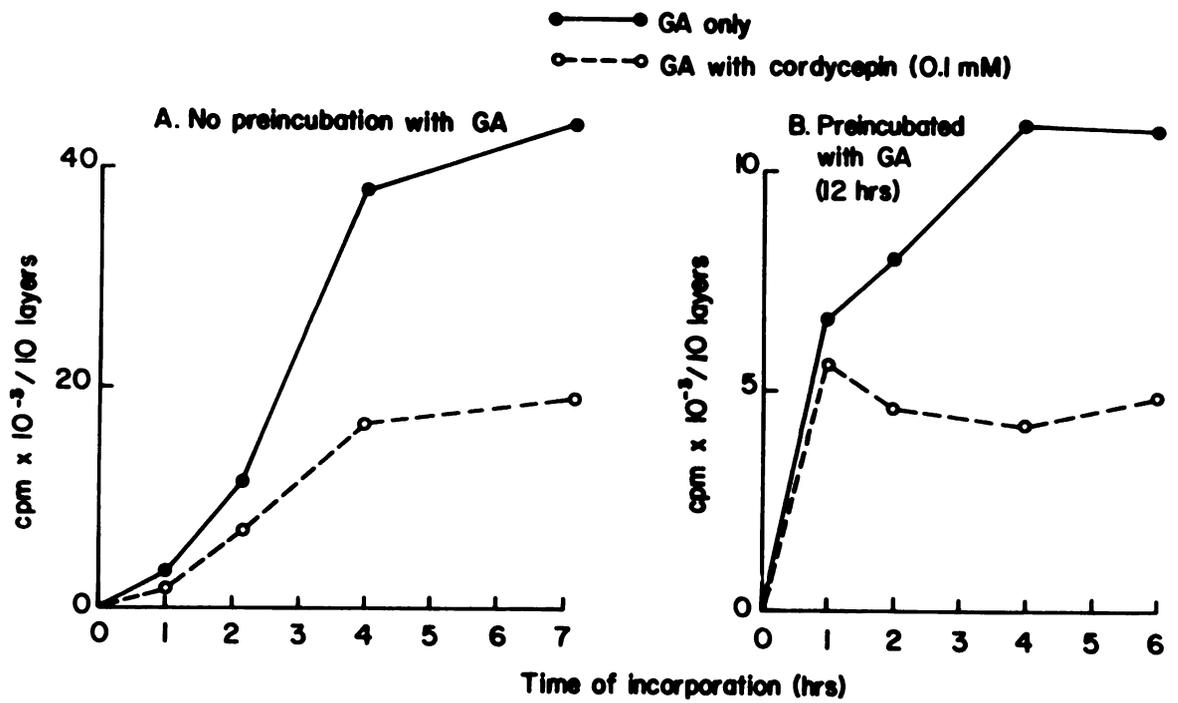
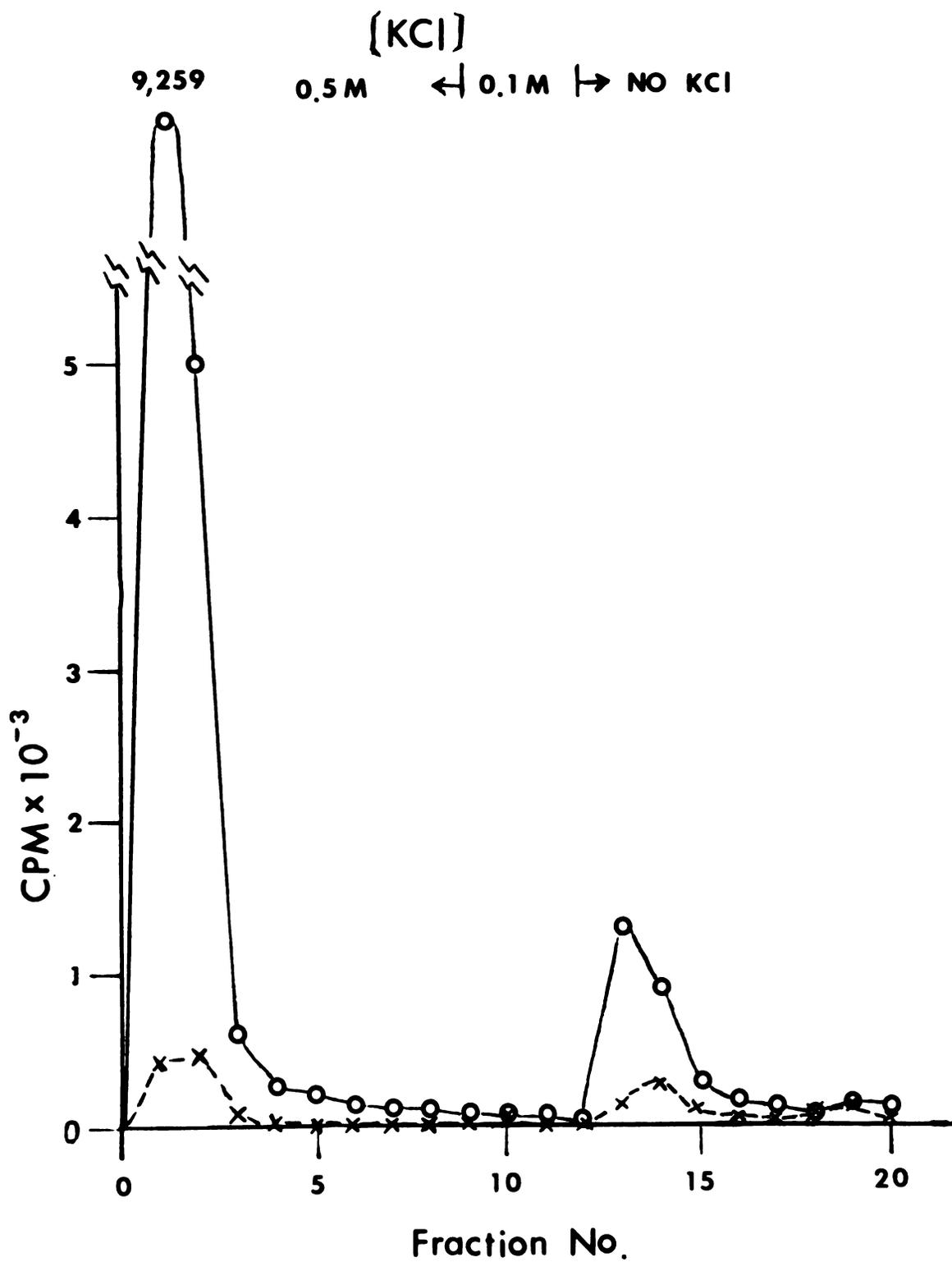


Figure 14.--Effect of Cordycepin on RNA Synthesis.

The two samples, 80 aleurone layers each, were pretreated with GA_3 for 12 hours and cordycepin was then added to one of the samples. Label (3H -uridine, 5 uCi/ml) was introduced from 13.5 to 18 hours after GA_3 . RNA was extracted and fractionated by oligo dT cellulose chromatography as described under "Materials and Methods". The first peak (fractions 1 to 4) eluted by 0.5 M KCl consists of RNA species not containing poly A segment. The peak (fractions 13 to 15) eluted in the absence of KCl contains poly A-RNA.

0—0 Control (+ GA_3 only)
x-----x Cordycepin treated



De Novo Synthesis of α -Amylase

The GA_3 enhanced α -amylase activity has been shown to be due to the de novo synthesis of the enzyme molecule after addition of hormone (17,63,64). However, since α -amylase activity does not increase until 8 to 10 hours after GA_3 , one can raise the possibility that an inactive precursor of α -amylase is formed in the lag period and is converted into active form when the rapid increase of α -amylase is observed. In order to check this possibility, a density labeling experiment was performed in which ^{13}C -amino acids were introduced to the aleurone layers either before or during the rapid increase of α -amylase activity, and the buoyant density of α -amylase molecules was then measured by isopycnic centrifugation in a CsCl density gradient. The amino acids necessary to support protein synthesis in barley aleurone layers are derived from the degradation of reserve proteins in this tissue (17). Therefore, the degradation of reserve proteins had to be slowed down in order to have an efficient use of the exogenously supplied ^{13}C -amino acids. This is accomplished by including 5 mM $KBrO_3$ in the incubation medium, because $KBrO_3$ inhibits barley aleurone protease effectively (42). The production of α -amylase (TABLE 11) and the inhibitory effect of cordycepin (TABLE 12) were unchanged in the presence of 5 mM $KBrO_3$ if exogenous amino acids were supplied. As shown in Figures 15 to 19 and summarized

QUESTION 1: THE BIRTH OF THE NATION

1. The American Revolution was a struggle for independence from British rule, but it was also a struggle for a new political system. The framers of the Constitution sought to create a government that would be both strong and just, one that would protect the rights of the people and ensure the stability of the nation. This was a difficult task, as the framers had to balance the interests of different groups and regions, and they had to create a system that would be able to adapt to the changing needs of the country over time.

2. The framers of the Constitution were faced with a number of challenges. One of the most significant was the need to create a government that would be able to enforce the law and maintain order. The framers had to create a system of checks and balances, which would allow the different branches of government to keep each other in check and prevent any one branch from becoming too powerful. This was a novel idea, and it was one that had never been tried before.

3. Another challenge was the need to create a government that would be able to protect the rights of the people. The framers had to create a system of government that would be able to protect the rights of the people from the government itself. This was a difficult task, as the framers had to create a system that would be able to protect the rights of the people without being so weak that it would be unable to do anything.

4. The framers of the Constitution also had to create a system of government that would be able to adapt to the changing needs of the country over time. The framers had to create a system of government that would be able to deal with the challenges of a growing nation, and they had to create a system that would be able to deal with the challenges of a changing world. This was a difficult task, and it was one that the framers had to face with courage and wisdom.

5. The framers of the Constitution were able to create a government that has lasted for over two centuries. This is a testament to the wisdom and foresight of the framers, and it is a testament to the strength and resilience of the American people. The framers of the Constitution created a government that has been able to adapt to the changing needs of the country over time, and they have created a government that has been able to protect the rights of the people and ensure the stability of the nation.

TABLE 11.--Effect of Bromate and Amino Acids on the
Production of α -Amylase in Barley Aleurone
Layers

Treatment	α -Amylase (units/layer)			
	Medium	Layer	Total	%
GA ₃ only	32.1	4.7	36.8	100.0
GA ₃ + Bromate	7.0	2.2	9.2	25.0
GA ₃ + Bromate + Amino Acids	29.4	10.3	39.7	108.0

Note: The concentration of bromate was 5 mM and the concentration of amino acids was 10 mg casein hydrolyzate plus 0.5 mg tryptophan per ml.

TABLE 12.--Effect of Cordycepin on α -Amylase Production
in the Presence of Bromate and Amino Acids

Treatment	α -Amylase (units/layer)	%
GA ₃ + Amino Acids	35.4	100
GA ₃ + Amino Acids + Cor- dycepin	43.0	121
GA ₃ + Amino Acids + Bromate + Cordycepin	44.9	127

Note: The concentration of bromate and amino acids are the same as described in TABLE 11.

in TABLE 13, because density of α -amylase molecule was much higher if ¹³C-amino acids were present after 12

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Figure 15.--Density Labeling of Barley Aleurone
Proteins with ^{13}C -Amino Acids (I).

Control: Aleurone layers were incubated
with ^{12}C -amino acids for 24 hours. Fifty
uCi of ^3H -leucine were added after 12
hours of GA_3 .

• • • • • ^3H - Radioactivity
0—0 α -Amylase activity peak
x—x Density gradient

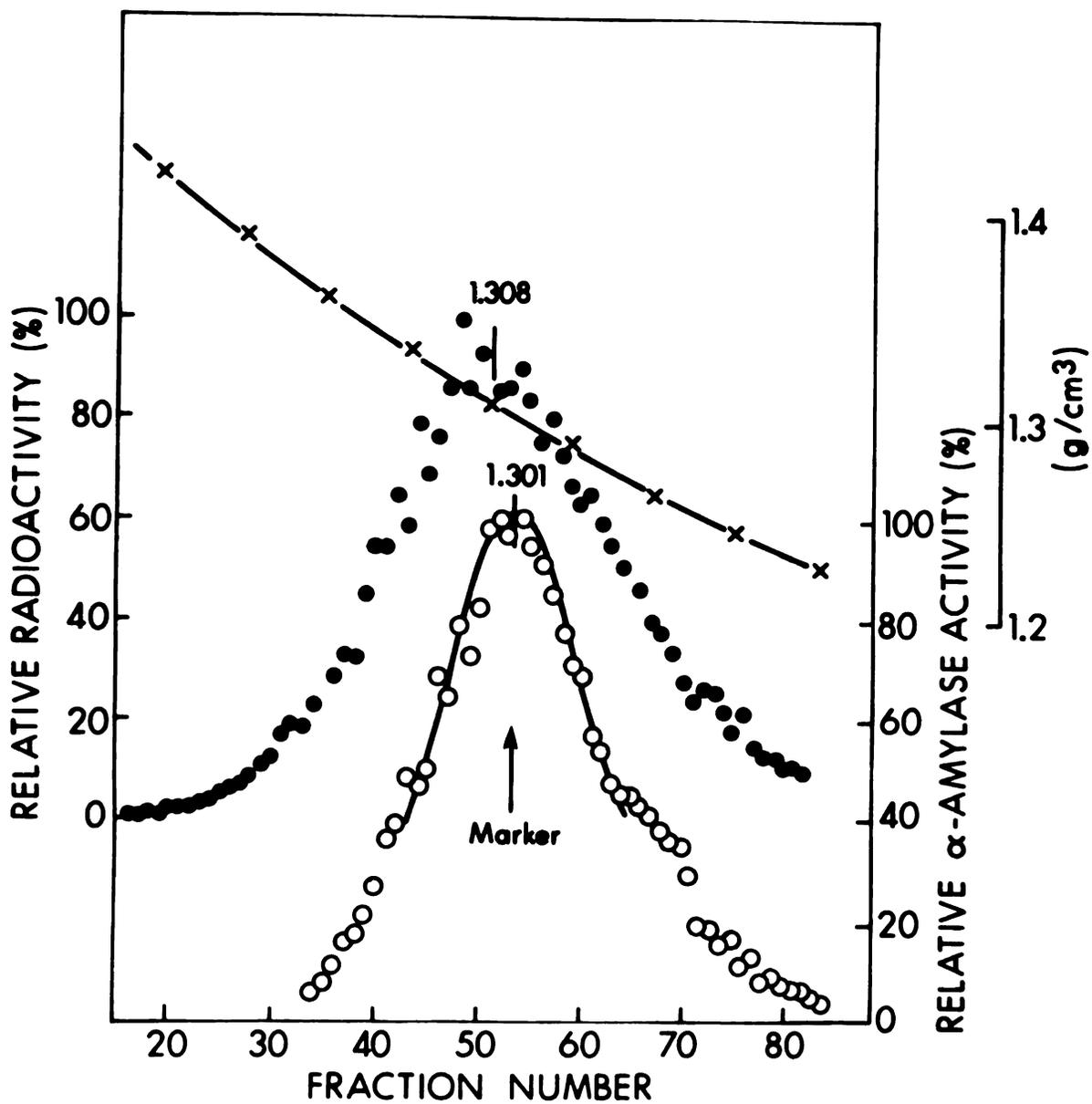


Figure 16.--Density Labeling of Barley Aleurone
Proteins with ^{13}C -Amino Acids (II).

Aleurone layers were incubated with ^{13}C -amino acids for 24 hours. Fifty μCi of ^3H -leucine were added to the incubation medium at 12 hours after the addition of GA_3 .

. ^3H -Radioactivity
0—0 α -Amylase activity peak
x—x Density gradient

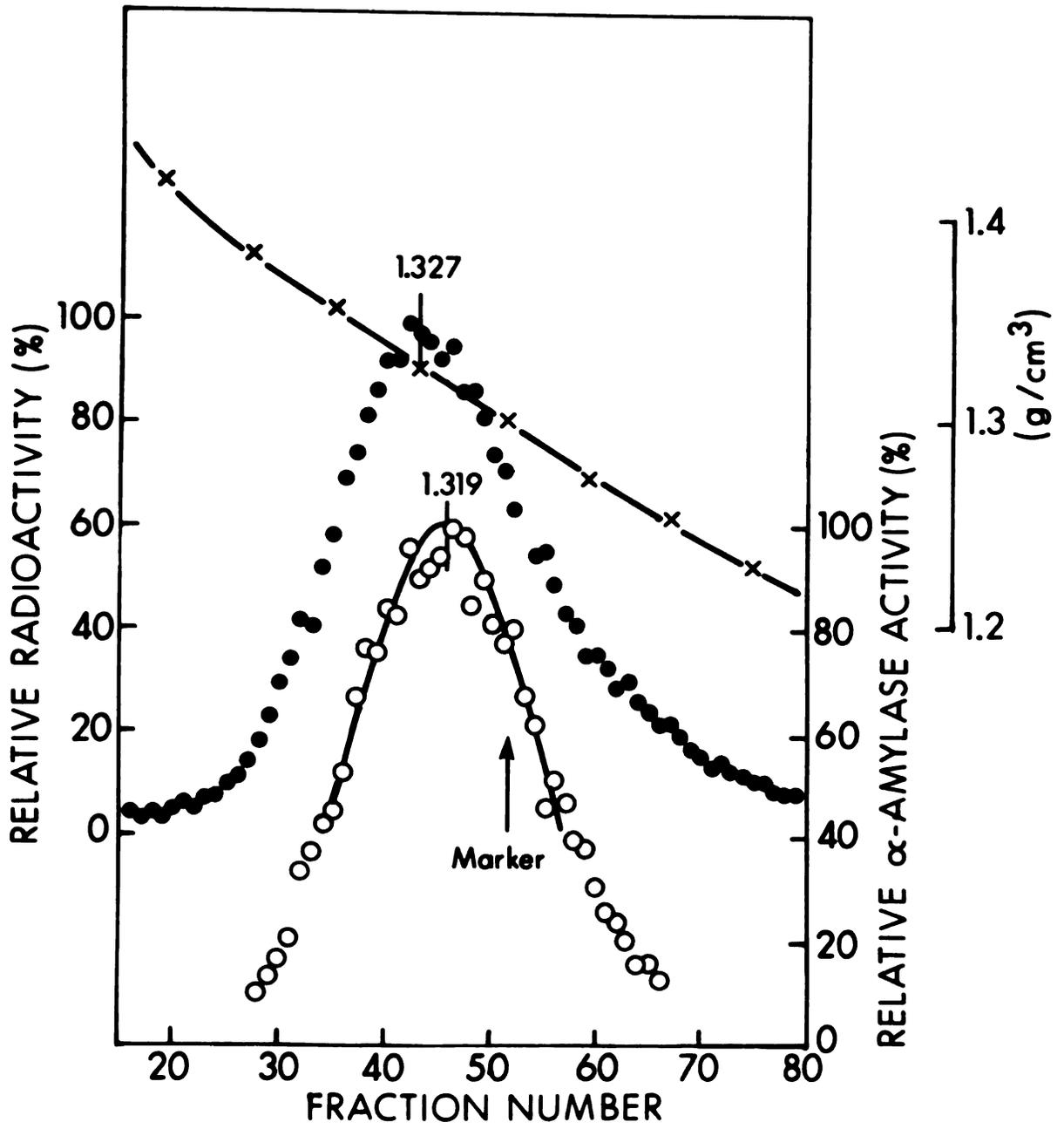


Figure 17.--Density Labeling of Barley Aleurone
Proteins with ^{13}C -Amino Acids (III).

Aleurone layers were incubated with ^{12}C -
amino acids for 12 hours and then further
incubated with ^{13}C -amino acids for
another 12 hours. Fifty μCi of ^3H -
leucine was added to the incubation
medium at 12 hours after the addition
of GA_3 .

• • • • • ^3H -Radioactivity

0—0 α -Amylase activity peak

x—x Density gradient

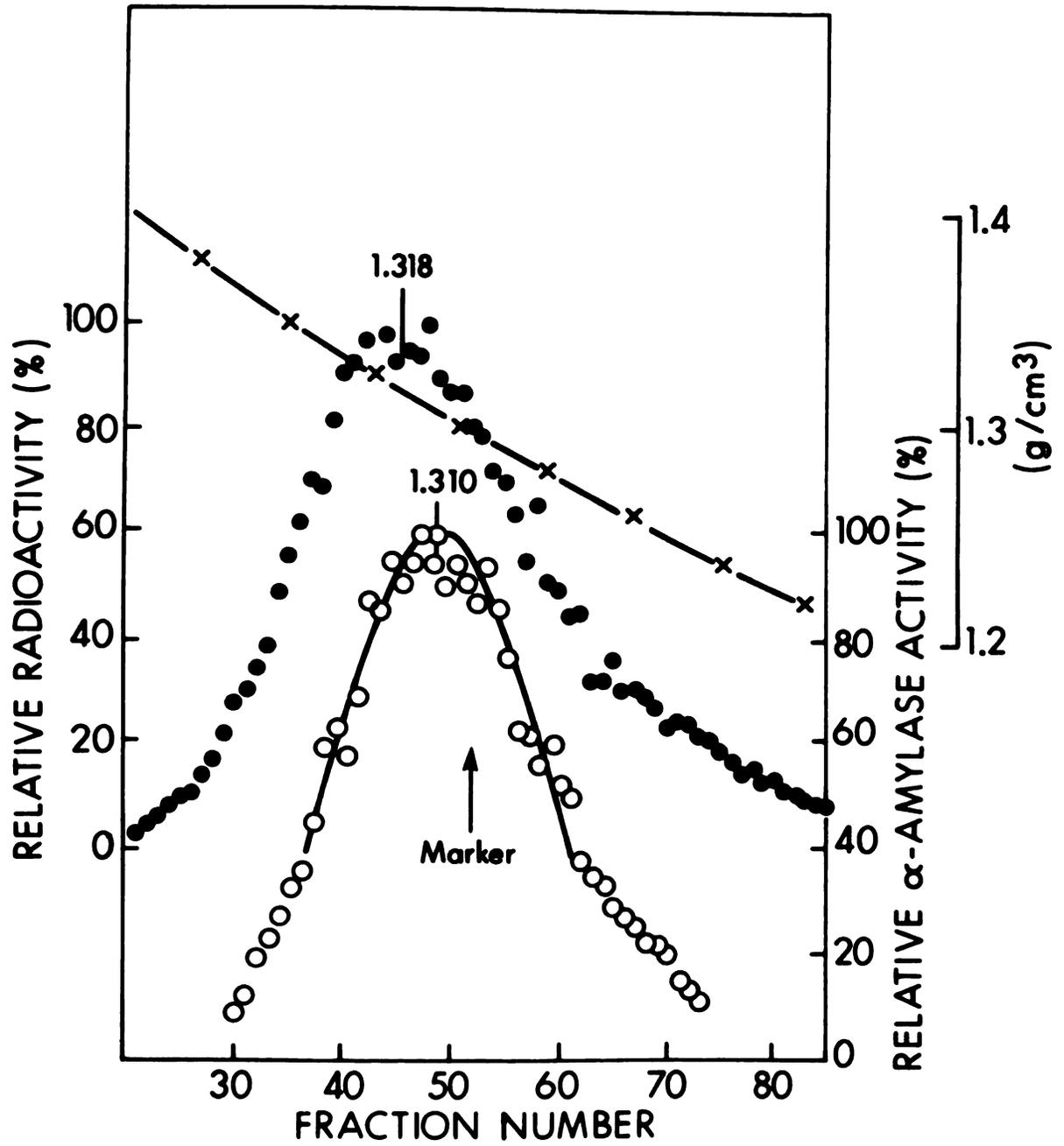


Figure 18.--Density Labeling of Barley Aleurone Proteins with ^{13}C -Amino Acids (IV).

Aleurone layers were incubated with ^{13}C -amino acids for 12 hours and then further incubated with ^{12}C -amino acids for another 12 hours. Fifty μCi of ^3H -leucine was added to the incubation medium at 12 hours after the addition of GA_3 .

• • • • • ^3H -Radioactivity
0—0 α -Amylase activity peak
x—x Density gradient

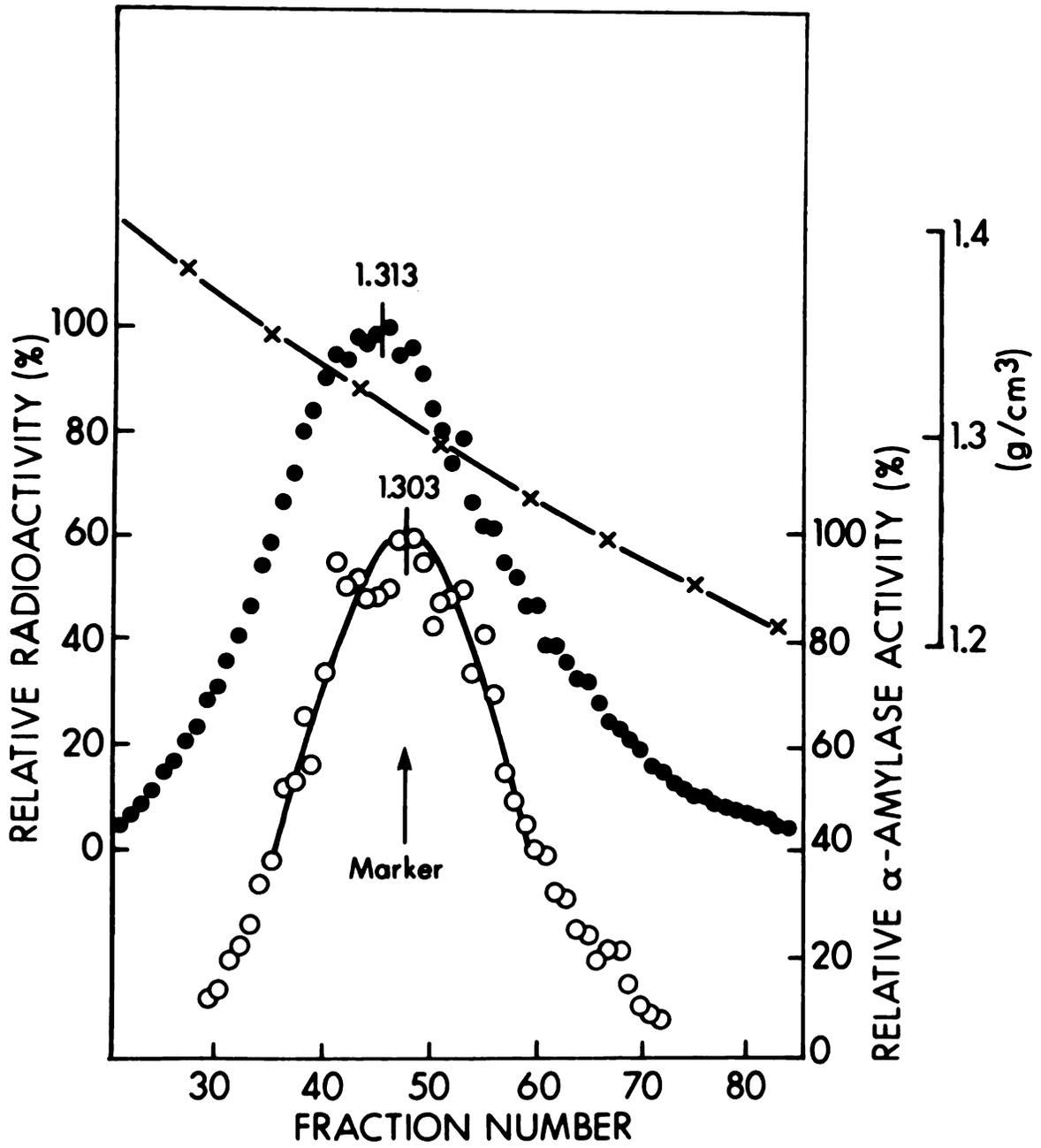
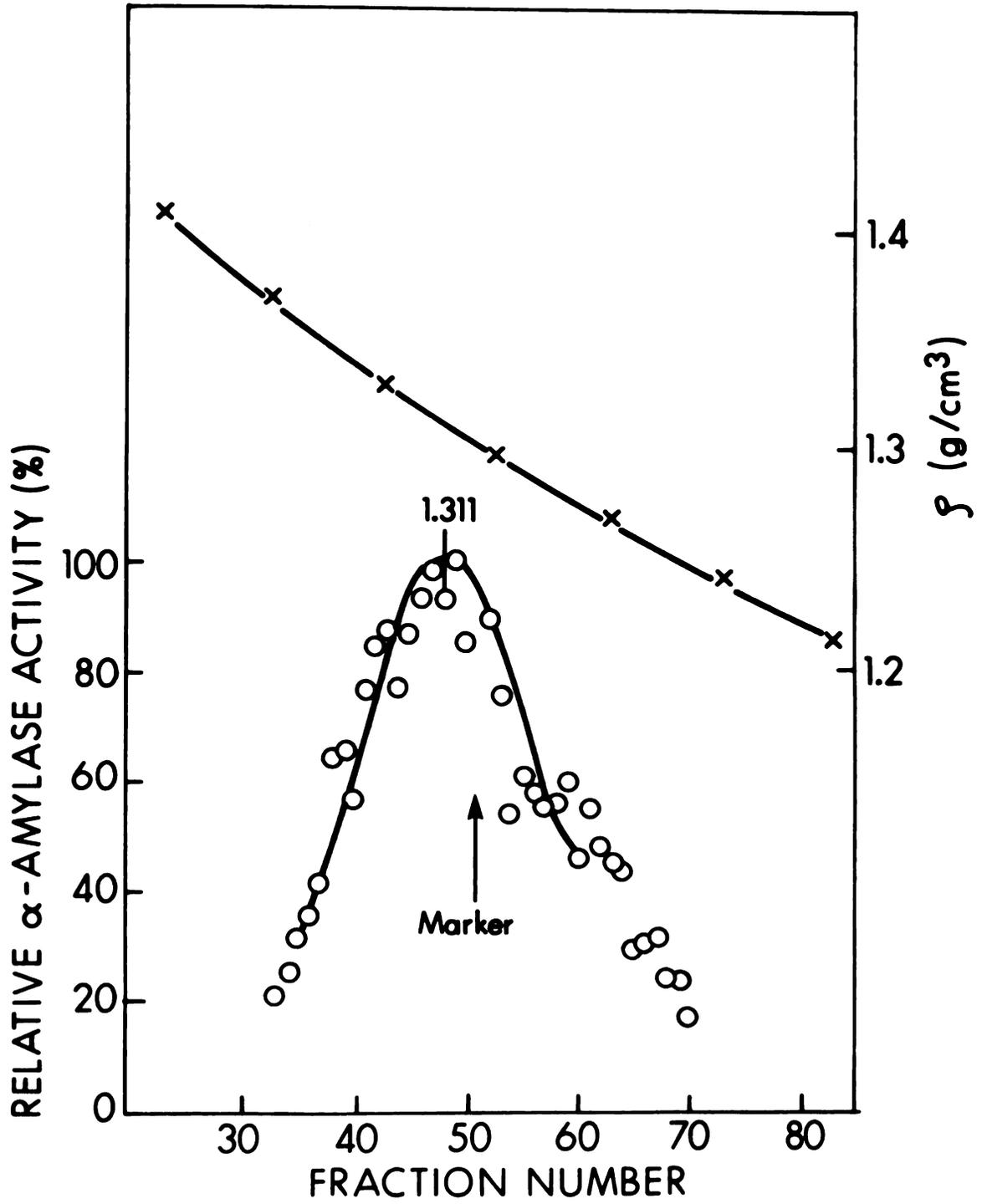


Figure 19.--Density Labeling of Barley Aleurone
Proteins with ^{13}C -Amino Acids (V).

The experimental procedures were the same as described in Figure 18, except the aleurone layers were incubated with ^{12}C -amino acids for 12 hours and then further incubated with ^{13}C -amino acids and cordycepin for another 12 hours.

0———0 α -Amylase activity peak

x———x Density gradient



hours of GA₃ treatment, the rapid increase of α-amylase activity after 12 hours of GA₃ is due to the de novo synthesis of the enzyme molecule. Furthermore, by also adding a radioactive amino acid during the experiment but after 12 hours of GA₃ treatment, it was possible to measure the density shift of the newly synthesized proteins which would represent the maximal density shift (100% de novo synthesis) for that period. By comparing the density shift of α-amylase and that of the total newly synthesized proteins (radioactive peak), one should be able to estimate the extent of de novo synthesis of α-amylase molecule. As shown in TABLE 13, essentially all α-amylase activity that appears after 12 hours is due to the de novo synthesis.

Lack of Cordycepin Effect on the Degradation of α-Amylase

The degradation of α-amylase, measured at the time when its synthesis was shut down by cycloheximide is moderate with a half life about 13 hours (Figure 20). The disappearance of α-amylase in the medium is much slower than that of α-amylase in the layer; however, disappearance from the layer might result from the continuous secretion of α-amylase even though protein synthesis was inhibited (65). Cordycepin does not slow down the degradation of α-amylase. Therefore, the insensitiveness of α-amylase production to cordycepin after 12 hours of GA₃

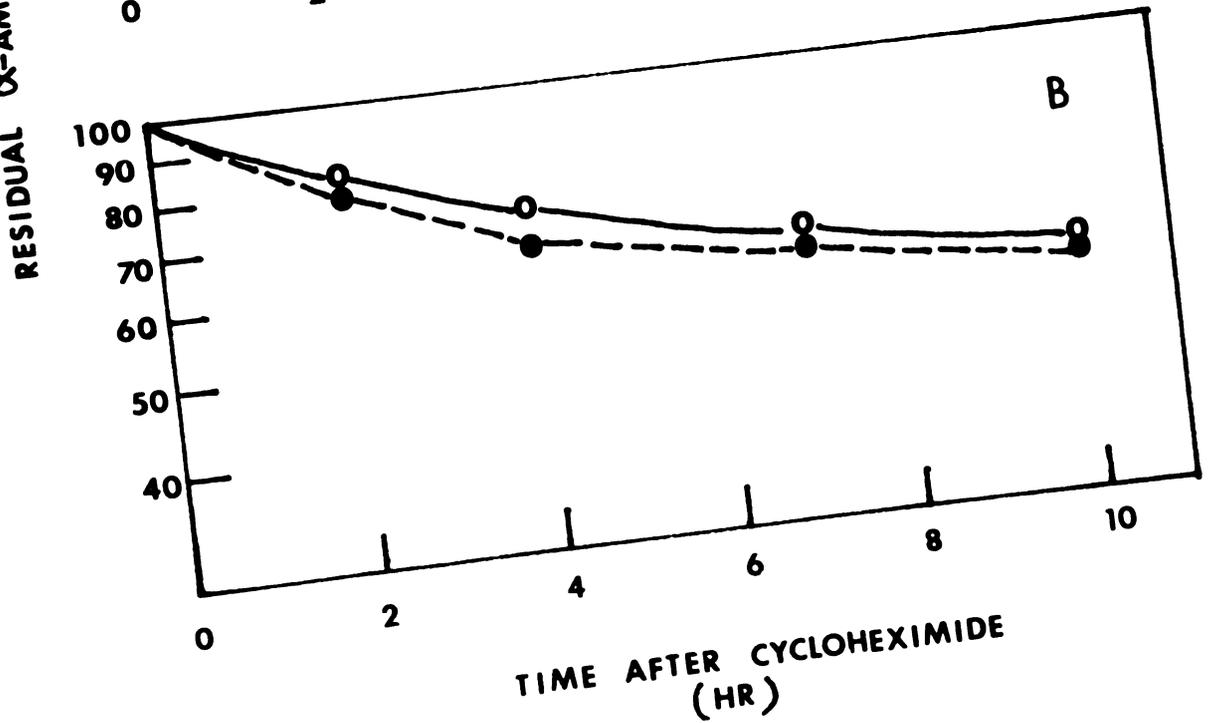
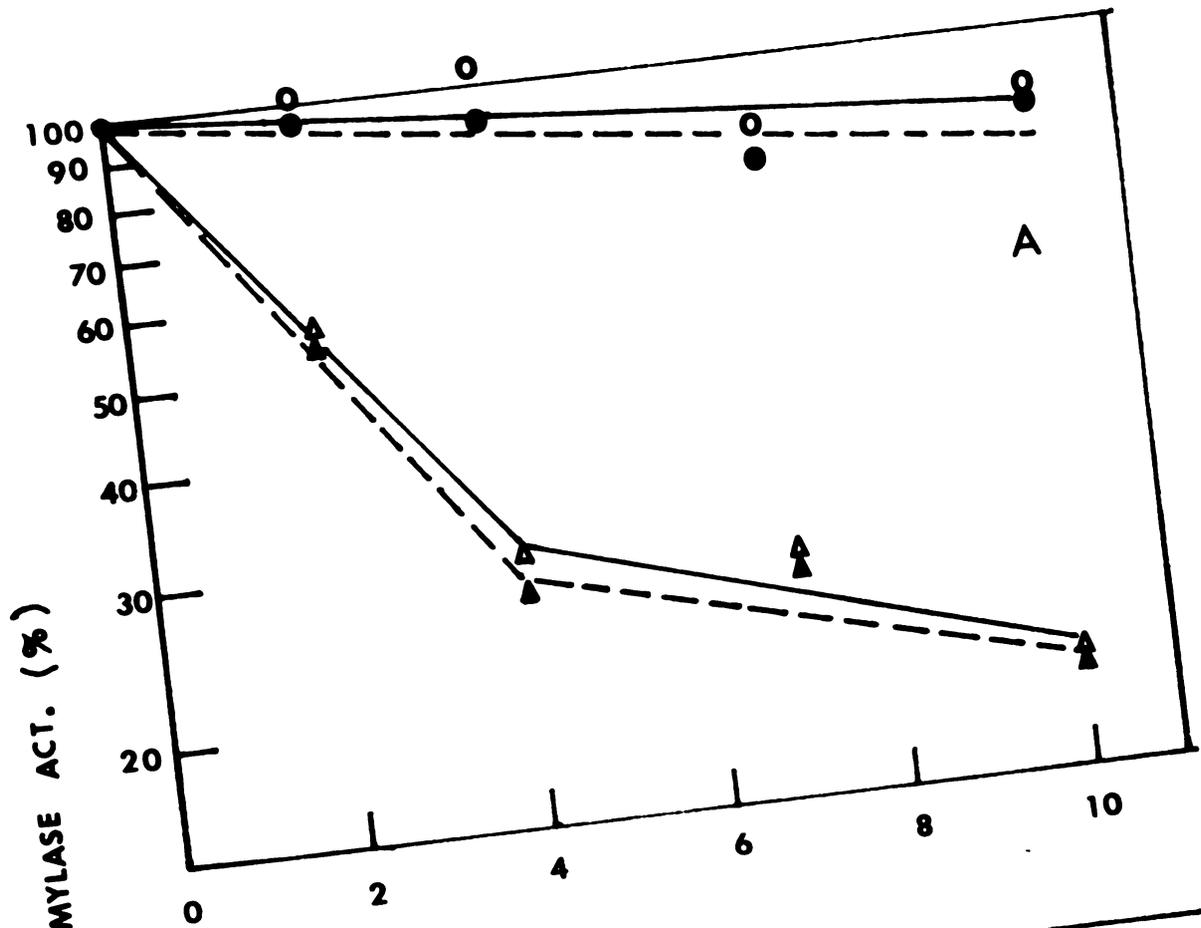
TABLE 13.--Summary of the ^{13}C -Amino Acids Density Labeling Experiments

Treatment	α -Amylase Peak (g/cm ³)	Half-height width x 10	Radioactivity peak (ρ)	% of α -Amylase synthesized
^{12}C (0 to 24 hr)	1.300	0.603	1.308	
^{13}C (0 to 24 hr)	1.317	0.640	1.326	95%
^{12}C (0 to 12 hr) then				
^{13}C (12 to 24 hr)	1.310	0.652	1.318	100%
^{13}C (0 to 12 hr) then				
^{12}C (12 to 24 hr)	1.302	0.624	1.311	
^{12}C (0 to 12 hr) then				
^{13}C (12 to 24 hr) with cordycepin	1.311	0.706		

Figure 20.--Effect of Cordycepin on the Degradation of α -Amylase.

Cordycepin was added at 12 hours after GA_3 and cycloheximide (10 ug/ml) was added at 15 hours after GA_3 . α -Amylase activity was assayed at specific times after the addition of cycloheximide and the activity of α -amylase at 15 hours after GA_3 was assigned as 100%.

- A. \bigcirc — \bigcirc + GA_3 only (α -amylase in the medium)
 Δ — Δ + GA_3 only (α -amylase in the layer)
 \bullet ----- \bullet + GA_3 and cordycepin (α -amylase in the medium)
 \blacktriangle ----- \blacktriangle + GA_3 and cordycepin (α -amylase in the layer)
- B. \bigcirc — \bigcirc + GA_3 only (total α -amylase)
 \bullet ----- \bullet + GA_3 and cordycepin (total α -amylase)



cannot be explained by a slowing of α -amylase degradation by this inhibitor, which in turn would compensate for a slower rate of synthesis.

Lack of Effect of Osmoticum on
the Stability of α -Amylase mRNA

It has been shown in mammalian tissue that the average half life of mRNA associated with membrane bound polysomes appears to be relatively short compared to mRNA associated with free polysomes (47). Since α -amylase is a secretory protein which is synthesized by membrane bound polysome, it would be interesting to know whether stripping the polysomes from the membrane has any effect on the stability of α -amylase mRNA as measured by the sensitivity of α -amylase synthesis to an RNA synthesis inhibitor such as cordycepin. It has been known that high concentrations of osmotica, such as mannitol or polyethylene glycol, can prevent protein synthesis in barley aleurone layers (35) and other plant tissues (26) by exerting water stress on the cells. Electronmicroscopic and biochemical evidences indicate that water stress induced by these osmotica is to reduce the binding of ribosomes to the endoplasmic reticulum and this reduction in membrane bound polysome formation does not result from reduced ribosome activity as measured by peptidylpuromycin formation (1). When aleurone layers were treated with 0.8 M mannitol together with cordycepin between 12 to 24 hours after GA₃ addition

and mannitol was removed afterwards, the production of α -amylase between 24 to 36 hours had the same rate as that of layers treated with 0.8 M mannitol but without cordycepin (TABLE 14). Since the mannitol effect on the binding of ribosomes to endoplasmic reticulum is reversible and the presence of cordycepin does not influence the resumed α -amylase production after mannitol is removed it appears that stripping ribosomes from ER by osmotic stress does not affect the stability of α -amylase mRNA.

It was recently reported that mRNA of membrane-bound polysomes in mammalian cells remain associated with the membrane even after ribosomes are removed (39) and the poly A segment appears to be attached to the membrane directly (39,44). If a similar situation occurs in plant cells, then one would expect that stripping off membrane bound ribosomes would not influence the association of α -amylase mRNA with microsome membrane.

TABLE 14.--Effect of Water Stress on the Sensitivity
of α -Amylase Synthesis to Cordycepin Treatment

Treatment	α -Amylase (units/layer)	%
+ GA ₃ only (26 hr)	44.4	100
- GA ₃ (26 hr)	11.4	26
+ GA ₃ and Cordycepin (0 to 26 hr)	12.6	28
+ GA ₃ and Cordycepin (12 to 26 hr)	45.4	102
+ GA ₃ \longrightarrow + GA ₃ and Mannitol (12 to 26 hr) \longrightarrow + GA ₃ only (26 to 36 hr)	16.0	100
+ GA ₃ \longrightarrow + GA ₃ and Mannitol and Cordycepin (12 to 26 hr) \longrightarrow + GA ₃ and Cordycepin (26 to 36 hr)	17.0	106

Note: The concentration of mannitol was 0.8 M. For the mannitol treated layers only the activity of α -amylase produced between 26 to 36 hr after GA₃ is shown.

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DISCUSSION

This study shows that poly A-RNA is present in barley aleurone layers. The poly A-RNA is polydisperse in size and its synthesis is enhanced in the presence of GA₃. This enhancement begins after a lag period of 3-4 hours and reaches a maximum at 10 to 12 hours after the addition of GA₃. ABA can prevent the GA₃ enhancement on poly A-RNA synthesis. The barley aleurone layer is a non-dividing tissue, and neither its respiration nor its energy charge is changed significantly by the application of hormone (9). Therefore, the GA₃ enhanced poly A-RNA synthesis can not be a nonspecific result of hormone-enhanced growth or hormone-enhanced energy metabolism.

In barley aleurone layers which have been treated with GA₃, about 40% of the newly synthesized protein is estimated to be α-amylase (66). The percentage of the sum of all kinds of hydrolases in terms of total protein is even higher. Thus, if GA₃ causes the synthesis of mRNAs for hydrolases, there should be a detectable enhancement of the synthesis of total mRNA. Gibberelic acid also causes an increase in the activity of two enzymes, phosphorylcholine cytidyl transferase and phosphorylcholine-glyceride transferase, which are required for the

biosynthesis of lecithin, a major component of membrane phospholipids (38). However, GA_3 appears to enhance the activity of these two enzymes by some activation process not involving protein synthesis or RNA synthesis (4). Therefore, it may be that enhanced synthesis of poly A-RNA is not necessary for the observed membrane proliferation.

In principle, the control of hormone-enhanced specific proteins could be either on the transcriptional or the post-transcriptional level. It has been shown that hormone-controlled synthesis of egg white proteins in chick oviduct is due to the accumulation of sepecific mRNAs (7,54). Palmiter (48) has found that ovalbumin has a fairly stable mRNA whose synthesis is enhanced by estrogen. It was recently concluded that withdrawal of estrogen will decrease the half life of ovalbumin mRNA, indicating that this mRNA is somehow stabilized in the hormone-treated tissue (50).

In barley aleurone layers the GA_3 enhanced synthesis of α -amylase is no longer sensitive to a transcriptional inhibitor such as cordycepin after 12 hours of exposure to the hormone. Therefore, a post-transcriptional control is proposed because the synthesis of α -amylase specific mRNA cannot be rate-limiting at this stage. This is not analogous to the situation in terminally differentiated cells such as erythrocyte or galea cells in silk moth, in which the transcriptional control mechanism

ceases to exist when the cells engage in the rapid and massive synthesis of cell specific proteins. However, transcriptional control mechanisms can still exist in the barley aleurone after the first 12 hours of GA₃ addition. The evidence for this is that the synthesis of α-amylase in barley aleurone cells after GA₃ treatment can be prevented by another hormone, ABA, whose effect appears to be dependent on the continuous synthesis of a short-lived RNA (Part II). Thus, although α-amylase synthesis at this stage is under post-transcriptional control, yet the aleurone cells apparently maintain their potential for control the synthesis of specific RNAs.

Two concurrent events take place in barley aleurone cells during the first 12 hours of GA₃ treatment, namely the proliferation of membrane and the enhanced mRNA formation. The membrane proliferation, which is a prerequisite for later secretory protein synthesis, is initiated by the hormone mediated activation of phosphorylcholine transferases. The hormone enhanced formation of mRNAs presumably those specific for α-amylase and other hydrolases, must reflect the existence of a transcriptional control mechanism at this stage.

Another fact to be considered in the study of the control mechanism in barley aleurone cells is that GA₃ does not increase the total protein synthesis in these cells. This is not necessary contradictory to the

discovery of hormone enhanced mRNA formation, because hydrolases have long lived mRNA. Therefore, a selective destruction of other enzymes' mRNAs would maintain the total mRNA population at a relatively constant level while the population of hydrolase specific mRNAs would be greatly increased. This is a situation analogous to the recent concept of Palmiter and Schimke (49) in which they suggest that more efficient translation of a long-lived mRNA would lead to an increased rate of production of its corresponding protein. This increase in efficiency would be due to more favorable competition for some rate limiting factor once the more labile messages started to disappear. Since mRNA associated with membrane bound polysomes are more stable than those associated with free polysomes, a selective stabilization of hydrolase (secretory protein) specific mRNAs by their association with the ER which is abundant in the hormone treated tissue, could occur in barley aleurone cells leading to the enhancement of relative population of hydrolase mRNAs.

In mammalian hepatoma cell cultures, an RNA synthesis inhibitor, such as Actinomycin D, not only slows down the synthesis of tyrosine aminotransferase but also to a greater extent causes a decrease of the degradation of this enzyme (37). However, this does not occur in the cordycepin treated aleurone layer because cordycepin does not influence the degradation of α -amylase. Thus the

1. The first step in the process of identifying a problem is to recognize that a problem exists. This is often done by comparing current performance with a desired state or goal. For example, a manager might notice that sales are declining or that customer satisfaction is low. Once a problem is identified, the next step is to define it more precisely. This involves determining the scope of the problem, its causes, and its effects. For instance, a manager might define a problem as "a 10% decrease in sales over the last quarter, primarily due to a loss of market share in the competitive market." This definition helps to narrow down the focus of the problem and provides a clear starting point for further investigation.

2. The second step in the process is to gather information about the problem. This involves collecting data and facts that are relevant to the problem. For example, a manager might gather data on sales trends, market conditions, and customer feedback. This information is then used to identify the underlying causes of the problem. For instance, a manager might discover that the loss of market share is due to a combination of factors, including increased competition, changes in consumer preferences, and a lack of innovation in the product line. This information is crucial for developing an effective solution.

3. The third step in the process is to analyze the information and identify the root cause of the problem. This involves using logical reasoning and problem-solving techniques to determine the underlying factors that are contributing to the problem. For example, a manager might use a fishbone diagram to identify the root cause of the sales decline. This diagram helps to visualize the relationship between different factors and their impact on the problem. In this case, the root cause might be identified as a lack of innovation in the product line, which is leading to a loss of market share.

4. The fourth step in the process is to develop a solution. This involves brainstorming ideas and evaluating them to determine the most effective and feasible solution. For example, a manager might brainstorm ideas such as developing new products, improving customer service, and increasing marketing efforts. Each idea is then evaluated based on its potential to address the root cause of the problem and its feasibility in terms of cost and resources. In this case, the most effective and feasible solution might be to develop new products that meet the needs of the competitive market.

5. The fifth and final step in the process is to implement the solution and monitor its progress. This involves putting the solution into action and tracking its performance over time. For example, a manager might implement the solution by developing and launching new products, improving customer service, and increasing marketing efforts. The manager then monitors the performance of the solution, comparing it to the desired state or goal. If the solution is not working, the manager may need to make adjustments or develop a new solution. This step is crucial for ensuring that the problem is effectively resolved and that the organization is able to maintain its performance over time.

evidence presented in this study indicates that α -amylase and probably protease are translated from stable mRNAs. Since the synthesis of α -amylase is not influenced by the presence of cordycepin for at least 12 hours one would expect the half life of α -amylase mRNA exceeds 50 hours. This would not be an astonishing because the biosynthesis of cell specific proteins is usually associated with long lived messages. For example, the mRNA for cocoonase of silk moth has a half life of 100 hours (36), that for ovalbumin of chick oviduct is 18 hours (48) and reticulocytes can synthesize hemoglobin for at least 48 hours after extrusion of the nucleus (55).

• The first step in the process of identifying a problem is to recognize that a problem exists. This is often done by comparing current performance to a desired state or goal. For example, a manager might notice that sales are down or that customer satisfaction is low. Once a problem is identified, the next step is to define it more precisely. This involves determining the scope of the problem, the resources available, and the time frame for addressing it. A clear definition of the problem is essential for developing an effective solution. The third step is to analyze the problem and identify its causes. This is often done by using tools such as the fishbone diagram (Ishikawa diagram) or the 5 Whys technique. These tools help to trace the problem back to its root cause, rather than just addressing the symptoms. Once the root cause is identified, the next step is to develop a solution. This involves brainstorming ideas, evaluating them, and selecting the most feasible and effective one. The final step is to implement the solution and monitor its progress. This involves setting up a system to track the results of the solution and making adjustments as needed. The process of problem-solving is an iterative one, and it may be necessary to revisit some of the steps as more information is gathered.

PART II
THE RESPONSE OF BARLEY ALEURONE
LAYERS TO ABSCISIC ACID

INTRODUCTION

Abscisic acid is known to reverse or prevent many responses of barley aleurone layers to GA_3 . These include the GA_3 enhanced syntheses of α -amylase (9,28) and protease (29), membrane bound polysome formation (14), incorporation of ^{32}P into membrane phospholipids (38), poly A-RNA synthesis (25), and the activation of phosphorylcholine transferases (4,31). However, abscisic acid has no effect on general cellular metabolism as measured by oxygen consumption (9).

Although ABA can prevent the response to GA_3 , no direct effects of ABA in aleurone cells have been observed. However, the failure of the aleurone cells to respond to GA_3 in the presence of ABA does not result from simple competition between these two hormones because a high concentration of GA_3 cannot completely overcome the ABA effect (9,28).

Because most of the effects of GA_3 in aleurone cells depend on cellular metabolism, elg1 transcription and translation, it has been difficult to study the mode of action of ABA because application of metabolic inhibitors would inhibit GA_3 effects directly. It has been demonstrated in the first part of this study that α -amylase is

translated from stable mRNA that is synthesized before the rapid increase of α -amylase activity. Because α -amylase synthesis after 12 hours of GA_3 treatment is no longer sensitive to transcription inhibitors such as cordycepin (3'-deoxyadenosine), while ABA at this stage still effectively inhibits α -amylase production, it provides us a good opportunity to study whether or not there is a requirement for RNA synthesis for the effect of ABA when it prevents the tissue's response to GA_3 .

MATERIALS AND METHODS

Source of Chemicals and Seed

Same as described in Part I.

Preparation and Treatment of Aleurone Layers

The methods described in Part I were modified. Embryo-less half-seeds are surface sterilized by sodium hypochlorite (5-fold dilution of commercial bleach) for 20 min and rinsed several times with sterile deionized water, and further stirred in 0.01 N HCl for 10 min to destroy any trace of sodium hypochlorite remaining on their surface. After thorough rinsing with water and with 20 mM sodium succinate buffer, pH 5.0, containing 10 mM CaCl₂, the half-seeds were imbibed on sterilized sand moistened with the same sodium succinate buffer. Aleurone layers were peeled from 3-day imbibed half-seeds and incubated with sodium succinate buffer, with different combinations of hormones and inhibitors, in a reciprocal metabolic shaker (120 oscillations/min) at 25 °C. The concentration of GA₃ used in this work was 2.5 μM. Using sodium succinate buffer slightly increases the production of α-amylase in the presence of GA₃. Further, it also decreases the base level of α-amylase production (in the absence of GA₃) by more than

50% (TABLE 15). Thus the GA₃ enhancement of α-amylase production in succinate buffer can reach about 25 to 40 fold instead of the 3 to 10 fold usually obtained in acetate buffer. Less α-amylase is released into the medium in the presence of succinate buffer (TABLE 15). The reason for these effects is unclear.

Extraction and Assays of Enzyme

α-Amylase was extracted and assayed as described in part I.

Sodium Dodecylsulfate (SDS) Gel Electrophoresis

The procedures were modified from those of Laemmli (40). Gels with 2.5 cm long stacking gel (4%) and 10.5 cm long separation gel (12%) were used. Samples for gel electrophoresis were prepared by grinding 30 to 40 aleurone layers with 0.5 ml 0.2 M NaCl containing 10 mM KBrO₃ with a mortar and pestle. The mortar was rinsed with another 1 ml of NaCl solution and the solutions were combined. After centrifuging for 30 min at 12,000g, the supernatant was decanted and 1.0 ml SDS reagent containing 0.125 M Tris-HCl, pH 6.8, 4% SDS and 10% β-mercaptoethanol was added to the supernatant. This supernatant is referred to as salt-soluble protein preparation. To the washed pellet 1.0 ml SDS reagent was added. After storage at room temperature for several days, the pellet with SDS reagent was centrifuged. The resulting supernatant was diluted with an equal volume of distilled water and referred to as the salt

TABLE 15.--GA₃ Enhancement of the Production of α -Amylase in Different Buffers

Treatment	α -Amylase (units/layer)			GA ₃ Enhancement (fold)	
	Medium	Layer	Total		
2 mM Na Acetate	+GA ₃	20.6	2.0	22.6	9.8
	No GA ₃	1.6	0.7	2.3	
20 mM Na Succinate	+GA ₃	17.2	8.6	25.8	25.8
	No GA ₃	0.4	0.6	1.0	

Note: The pH of the buffers was 5.0 and α -amylase produced in the first 24 hours was assayed.

insoluble protein preparation. A 100 ul sample was applied to each gel and electrophoresis was carried out with constant voltage of 80 V and an initial current of 40 mA per 12 gels. The molecular weights of the radioactive bands in the gels were determined by comparing their mobility with those of marker proteins of known molecular weight (Figure 21).

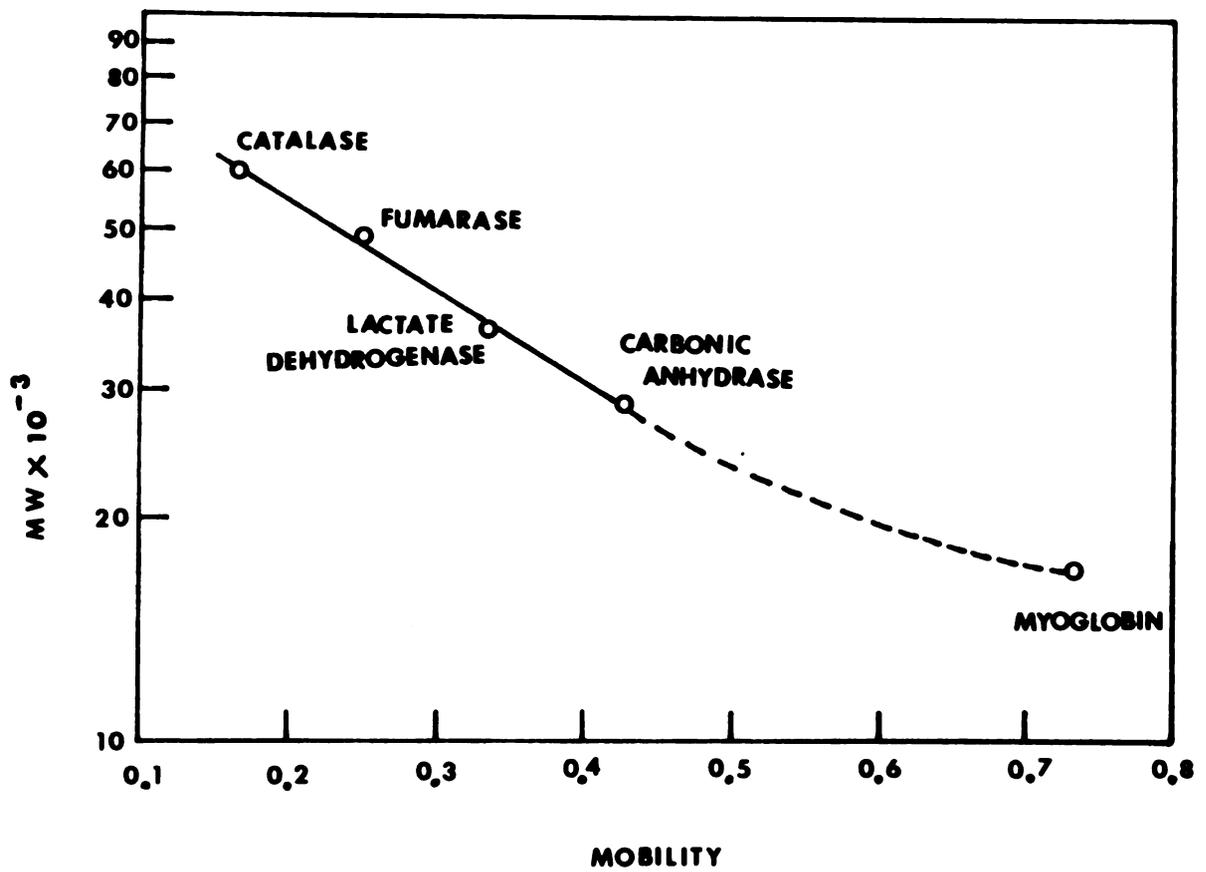
Figure 21.--Calibration Curve for Molecular Weight Determination on SDS Gel Electrophoresis.

Marker proteins with known molecular weight of their subunits were treated with SDS reagent and then subjected to electrophoresis as described under "Materials and Methods". The mobility is described as:

$$\text{Mobility} = \frac{\text{Distance of protein migration}}{\text{Distance of dye migration}}$$

The swelling of gel during staining and destaining was insignificant, therefore, no correction was made.

The source and molecular weights of the marker proteins (subunit) are: Catalase (Beef liver, M.W. 60,000); Fumarase (Pig heart, M.W. 49,000); Lactate Dehydrogenase (Chicken heart, M.W. 36,000); Carbonic Anhydrase (Bovine erythrocyte, M.W. 29,000); Myoglobin (Equine skeletal muscle, M.W. 17,200).



RESULTS

Effect of Cordycepin on GA₃ Enhanced α -Amylase Formation

Cordycepin acts as a chain terminator during RNA synthesis and inhibits in barley aleurone layers the formation of both poly A-RNA and RNA species not containing the poly A segment (Part I). Cordycepin is equally effective as a transcription inhibitor whether it is added at the same time or 12 hours after GA₃ (Part I). As in acetate buffer, the inhibitory effect of cordycepin on GA₃ enhanced α -amylase synthesis in succinate buffer is less and less as cordycepin is added later and later after the hormone and no inhibitory effect is observed if cordycepin is added 12 hours or later after the addition of GA₃ (TABLE 16). In fact, an enhancement of α -amylase formation by cordycepin added 12 hours after GA₃ was occasionally observed (TABLE 16). Because the α -amylase molecules are synthesized at the time that this increase in enzyme activity is observed (Part I), and cordycepin has no effect on the degradation of the enzyme, it is concluded that α -amylase is translated from stable mRNA.

TABLE 16.--Effect of Cordycepin on the GA₃ Enhanced Synthesis of α-Amylase

Treatment	α-Amylase Activity (units/layer)		%
	Total	Increase over Control	
GA ₃ only	27.7	26.6	100
Control (No GA ₃)	1.1		
GA ₃ + Cordy. (added at 0 time)	6.0	4.9	18
GA ₃ + Cordy. (added at 4 hours after GA ₃)	10.9	9.8	37
GA ₃ + Cordy. (added at 8 hours after GA ₃)	25.0	23.9	85
GA ₃ + Cordy. (added at 10 hours after GA ₃)	27.6	26.5	100
GA ₃ + Cordy. (added at 12 hours after GA ₃)	31.2	30.1	113

Note: Cordycepin (Cordy., 0.1 mM) was added at different time as indicated and the aleurone layers were further incubated for a total of 24 hours of GA₃ treatment when α-amylase was extracted and assayed.

Effect of ABA on α -Amylase Formation

Abscisic acid, at a concentration of 5 μM , effectively inhibits α -amylase formation if it is added at the same time as GA_3 . However, higher concentrations of ABA (10 to 25 μM) are needed to prevent α -amylase formation when ABA is added 12 hours after GA_3 . It has been reported that ABA, at a concentration of 38 μM forms a complex with fungal α -amylase, resulting in inhibition of the enzyme activity (43,46). However, as shown in TABLE 17, ABA, at concentrations up to 50 μM , has no significant effect on barley aleurone α -amylase activity in a cell free enzyme preparation after a 23 hour incubation at 25 $^{\circ}\text{C}$. Since the concentration of ABA we used for this study was 25 μM and the time span of the experiment was about 12 hours (from 12 hours to about 24 hours after GA_3), we conclude that the ABA effect on α -amylase production must be via a physiological process.

Effect of Cordycepin on the Action of ABA

Abscisic acid added 12 hours after GA_3 gradually inhibits further accumulation of α -amylase (Figure 22) (9). Because the accumulation of α -amylase in response to GA_3 , both before and after 12 hours is due to de novo synthesis (Part I) and because α -amylase accumulation after 12 hours of GA_3 is not inhibited by cordycepin, it is clear that ABA inhibits translation of α -amylase and not transcription of α -amylase mRNA. However, when ABA is added 12 hours after

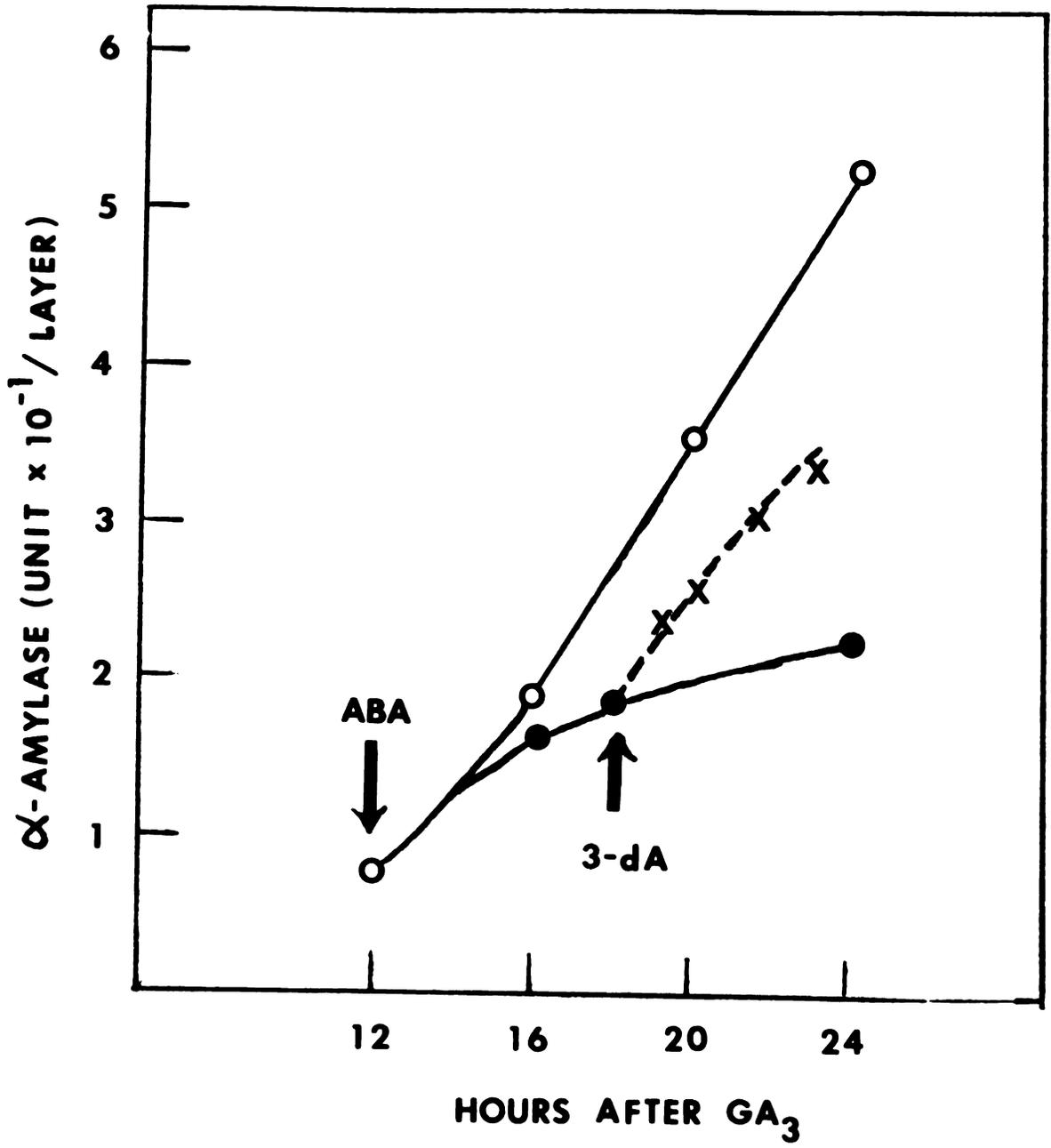
TABLE 17.--Lack of Direct Effect of Abscisic Acid on α -Amylase Activity

Incubation Time (at 25 °C)	No ABA		10 μ M ABA		50 μ M ABA	
	α -amylase %	ABA	α -amylase %	ABA	α -amylase %	ABA
0 hr	41.0	100	41.0	100	41.0	100
3 hr	41.6	101	40.9	99.8	40.8	99.6
8 hr	40.2	98	39.5	96	37.2	91
23 hr	38.6	94	37.2	91	37.5	91

Note: α -Amylase extracted from 24-hour GA_3 treated aleurone layers was used. The unit of α -amylase is $A_{620}/\text{min, ml}$.

Figure 22.--Effect of Midcourse Addition of ABA
and Cordycepin on the Synthesis of
 α -Amylase.

o—o GA_3 only
●—● GA_3 and ABA
x—x GA_3 , ABA, and Cordycepin (3-dA)



GA₃ and cordycepin is added at the same time or later, the accumulation of α -amylase either does not stop, or is quickly resumed (Figures 22 and 23). The effect of cordycepin cannot be due to a generally toxic effect because α -amylase production remains normal in the presence of cordycepin without ABA. Thus the effect of ABA depends on the continuous synthesis of a short-lived RNA which is inhibited by cordycepin. Because the rate of α -mylase accumulation after cordycepin addition is close to that of tissue treated with GA₃ alone (Figures 22 and 23), it appears that the amount of α -amylase mRNA is not limiting in the presence of ABA and cordycepin, i.e. ABA has no significant effect on the stability of α -amylase mRNA.

In order to determine whether ABA specifically prevents the synthesis of α -amylase (and perhaps other GA₃ enhanced hydrolases as well) or whether ABA slows down protein synthesis in general, the profile of newly synthesized proteins was checked by SDS gel electrophoresis. α -Amylase is detected as the predominant radioactive band on SDS gel with a molecular weight of about 50,000 daltons. As shown in Figure 24 for salt-soluble proteins and Figure 25 for salt-insoluble proteins, the synthesis of α -amylase is substantially decreased in the presence of ABA, while the amount of radioactivity in most of the minor bands remains essentially the same as in control tissue (+GA₃-ABA).

1. $\frac{1}{x^2} = x^{-2}$

$\frac{d}{dx} x^{-2} = -2x^{-3} = -\frac{2}{x^3}$

2. $\frac{d}{dx} \frac{1}{x^3} = \frac{d}{dx} x^{-3}$

$= -3x^{-4} = -\frac{3}{x^4}$

3. $\frac{d}{dx} \frac{1}{x^4} = \frac{d}{dx} x^{-4}$

$= -4x^{-5} = -\frac{4}{x^5}$

Figure 23.--Effect of Cordycepin Added at Different Times on the Synthesis of α -Amylase in the Presence of both GA_3 and ABA.

ABA was added at 12 hours after GA_3 . The arrows indicate the time of cordycepin addition.

0—0 GA_3 only

●—● GA_3 and ABA

x-----x GA_3 , ABA, and cordycepin

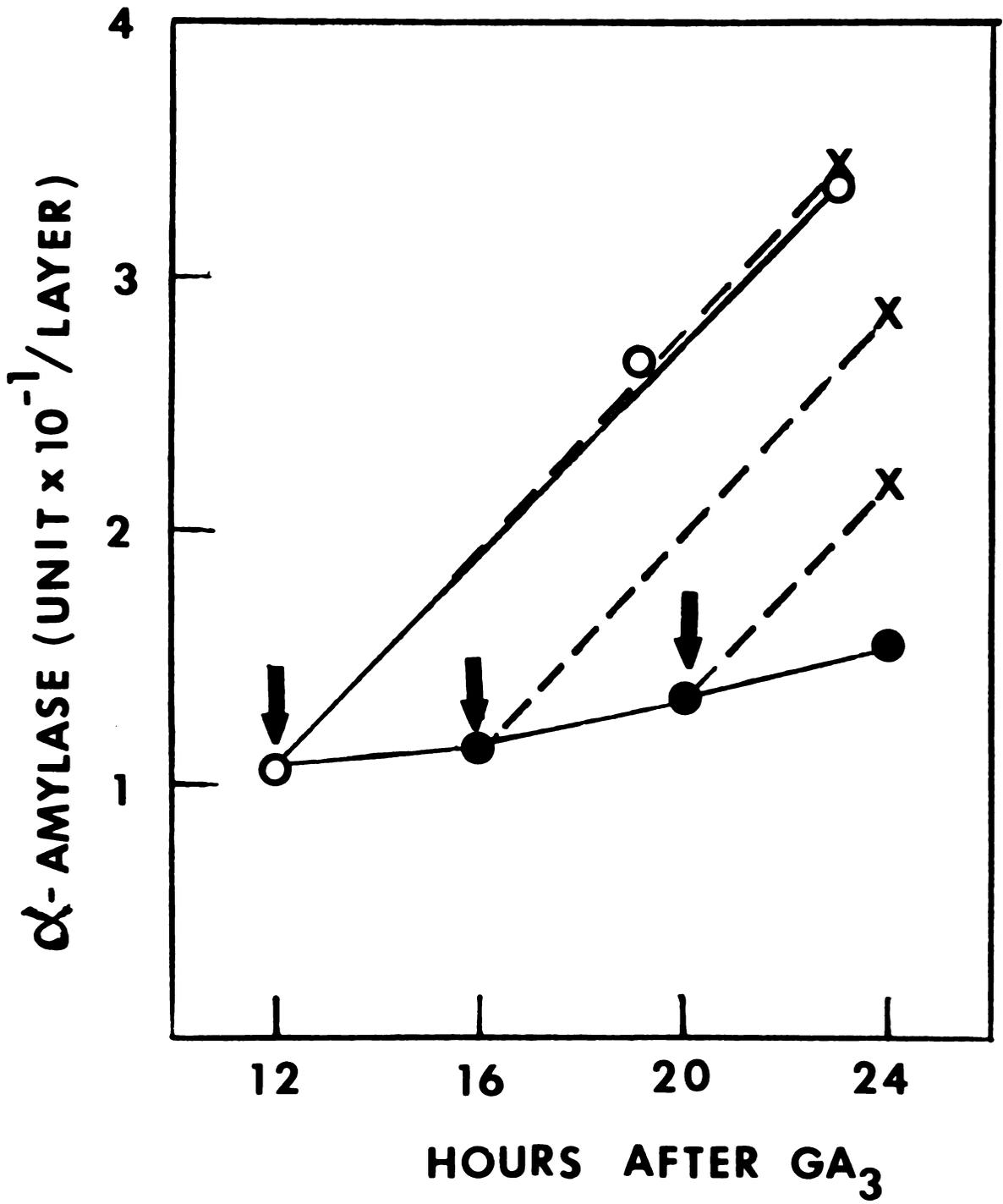


Figure 24.--Profile of Newly Synthesized Salt-Soluble Proteins on SDS Gel.

Aleurone layers (30 to 40) were labeled with ^3H -leucine (15 uCi/ml) for 2 hours (18 to 20 hours after GA_3). Salt-insoluble proteins were extracted as described under Materials and Methods. One mm thick gel pieces were sliced and digested in 0.5 ml NCS solubilizer (9 parts of full strength NCS solubilizer and 1 part of distilled water) at 50 °C for two hours. Ten milliliter of toluene based scintillation fluid (6 g PPO and 75 mg POPOP/liter) were used in each sample.

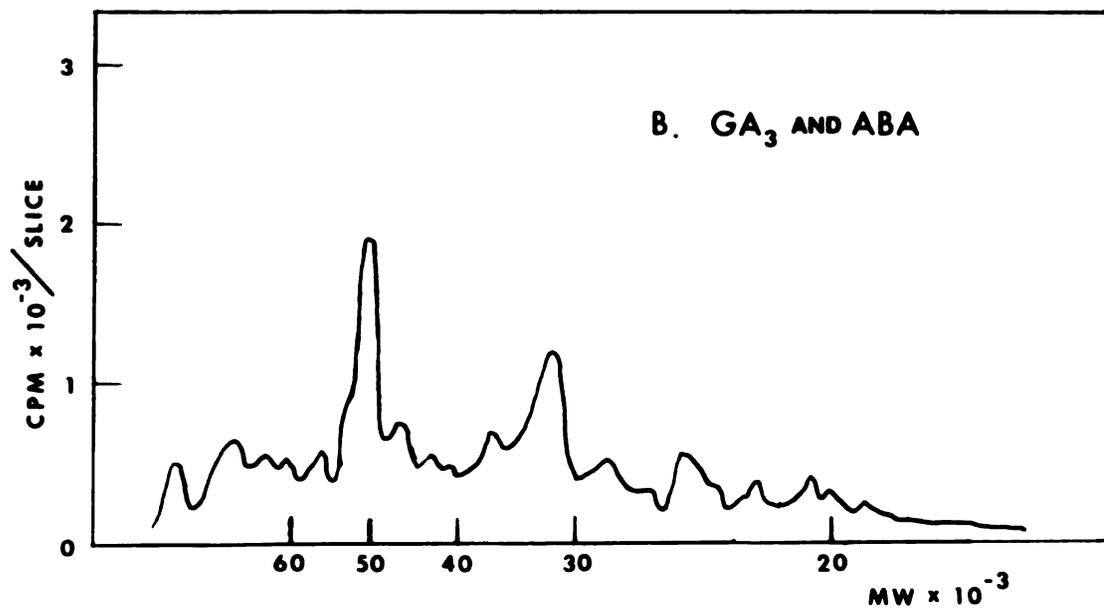
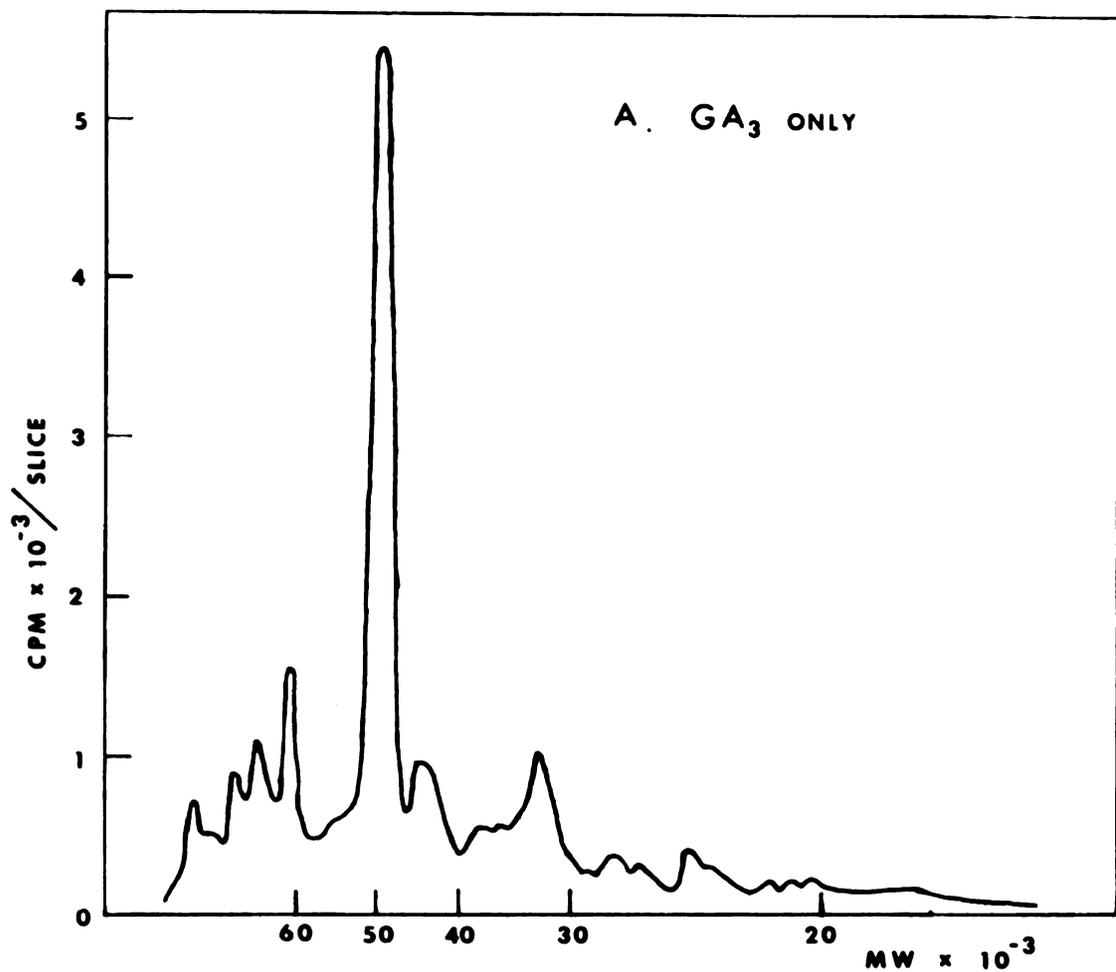
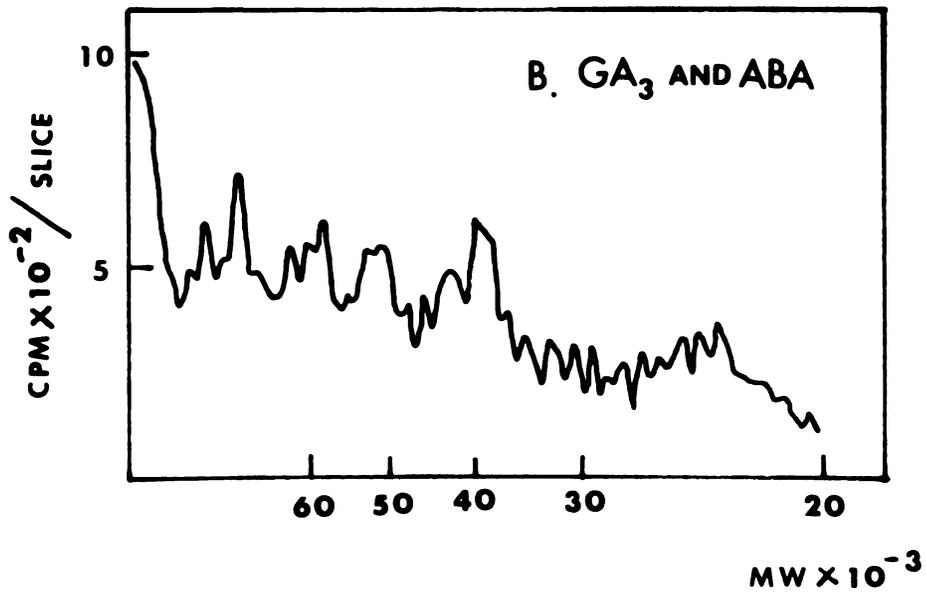
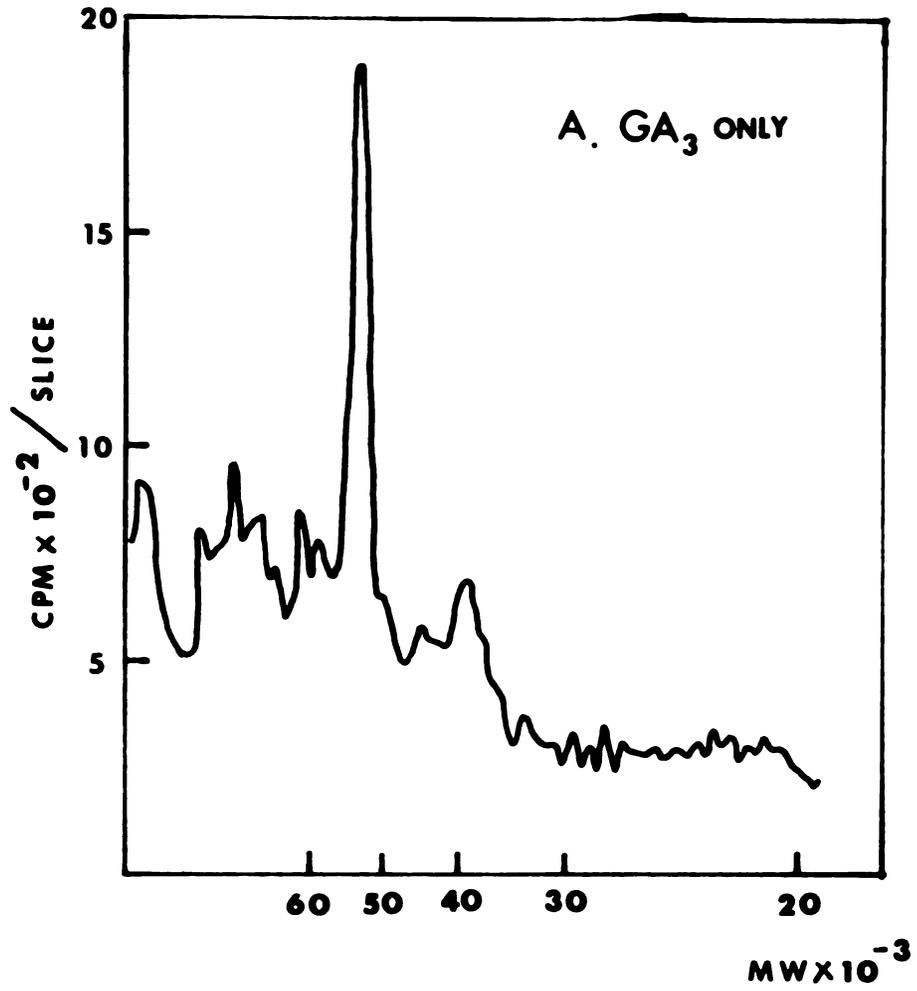


Figure 25.--Profile of Newly Synthesized Salt-
Insoluble Proteins on SDS Gel.

Experimental methods were the same as
described in Figure 24.



The major predominant salt-insoluble protein (Figure 25) has a molecular weight of about 52,000 daltons, which is slightly larger than that of α -amylase. Whether this protein is a precursor of α -amylase or an α -amylase covalently linked to cell wall or certain membrane fractions is unknown.

DISCUSSION

Although the synthesis of α -amylase after 12 hours of GA₃ treatment is no longer subject to transcriptional control, the inhibitory effect of ABA on α -amylase production at this same stage apparently depends on the continuous synthesis of short-lived RNA (regulator-RNA in Figure 4). I propose that this regulator-RNA, or its translation product, can decrease the rate of translation of α -amylase mRNA without influencing protein synthesis in general. The mRNA of α -amylase is stable for at least 12 hours after exposure of the tissue to GA₃, and its stability is maintained in the presence of ABA.

Ihle and Dure (27), working with precociously germinating cotton embryos, obtained evidence that the translation of carboxypeptidase mRNA was inhibited by ABA. Because they found that actinomycin D prevented the ABA inhibition, they proposed that a suppressor molecule had to be formed to bring about the ABA inhibition (27).

The action of ABA thus appears to be similar in the two systems, the precociously germinating cotton embryos where gibberellins probably have no regulatory role, and the mobilization of reserve nutrient in the germinating

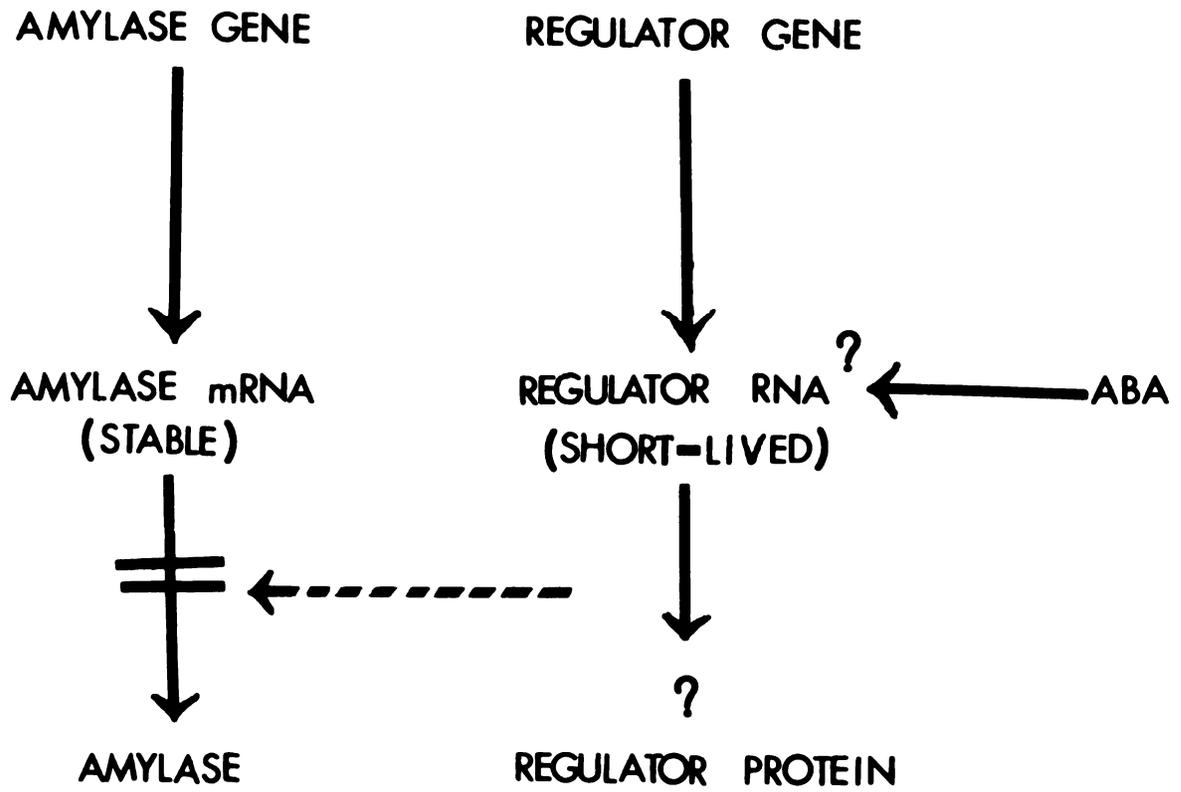
barley seed where ABA prevents the GA_3 enhanced α -amylase production in the aleurone cells. Therefore, it seems reasonable to suggest that some ABA effects depend on transcription although there is no evidence for a requirement of transcription in some fast effects of ABA such as that of preventing the activation of phosphorylcholine glyceride transferase (4) and that of stomatal closure (52).

There are two alternative sites of action for ABA (Figure 26): 1) ABA might derepress the regulator-RNA and cause the synthesis of regulatory-RNA and/or regulator-protein, or 2) the regulator-RNA is under continuous turnover, ABA activates it or works with it to prevent the translation of α -amylase mRNA. In human reticulocytes a new species of low molecular weight RNA has been reported to be able to preferentially stimulate the synthesis of one of the globin chain (19,20).

The mechanism proposed here is by no means the only mode of action of ABA one might propose in barley aleurone cells. It has been shown that ABA prevents the GA_3 enhanced poly A-RNA synthesis (Part I) indicating besides the translational control ABA can also stress its effect on the transcriptional machinery before 12 hr of GA_3 .

Because of the many convenient properties of barley aleurone tissue and because of the ability of ABA to modify the tissue's response to GA_3 , as reported in this study, it is felt that aleurone tissue is an important system

Figure 26.--Postulated Mechanism of ABA Action.



for the further study of how ABA modifies a tissue's response to GA₃.

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9. The ninth part of the document is a list of figures. These figures illustrate the results of the study in a visual format.

10. The tenth part of the document is a list of abbreviations. These abbreviations are used throughout the document to simplify the text.

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3. The third part of the document describes the process of data analysis. This involves identifying patterns and trends in the data, and then using these insights to draw conclusions about the research topic. It is important to be objective and unbiased in this process, and to clearly communicate the findings to the relevant stakeholders.

4. The fourth part of the document discusses the ethical considerations that must be taken into account when conducting research. This includes issues such as informed consent, confidentiality, and the potential for harm to participants. It is essential to ensure that the research is conducted in a responsible and ethical manner.

5. The fifth part of the document provides a summary of the key findings and conclusions of the study. This is an important part of the research process, as it allows the researcher to communicate the results of their work to a wider audience. It is important to be clear and concise in this part of the document, and to provide a clear and logical argument for the conclusions drawn.

6. The sixth part of the document discusses the implications of the research findings. This involves considering the potential impact of the research on the field of study, and on the wider community. It is important to be thoughtful and reflective in this part of the document, and to consider the broader context of the research.

7. The seventh part of the document provides a list of references and sources used in the research. This is an important part of the document, as it allows the reader to verify the accuracy of the information presented, and to explore the research further if they are interested. It is important to use a consistent and appropriate citation style in this part of the document.

8. The eighth part of the document provides a list of appendices and supplementary materials. This is an important part of the document, as it allows the reader to access additional information that may be relevant to the research. It is important to provide a clear and logical structure for these materials, and to ensure that they are easy to access and use.

9. The ninth part of the document provides a list of acknowledgments and thanks. This is an important part of the document, as it allows the researcher to express their appreciation to the people and organizations that have supported their work. It is important to be sincere and specific in this part of the document, and to clearly identify the individuals and organizations being thanked.

10. The tenth part of the document provides a list of contact information and a closing statement. This is an important part of the document, as it allows the reader to get in touch with the researcher if they have any questions or comments. It is important to provide accurate and up-to-date contact information, and to express a willingness to engage in further discussion.

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1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that this is crucial for ensuring transparency and accountability in the organization's operations.

2. The second part of the document outlines the various methods and tools used to collect and analyze data. It highlights the need for consistent data collection procedures and the use of advanced analytical techniques to derive meaningful insights from the data.

3. The third part of the document focuses on the role of technology in data management and analysis. It discusses how modern software solutions can streamline data collection, storage, and analysis processes, thereby improving efficiency and accuracy.

4. The fourth part of the document addresses the challenges associated with data management, such as data quality, security, and privacy. It provides strategies to mitigate these risks and ensure that the data remains reliable and secure throughout its lifecycle.

5. The fifth part of the document discusses the importance of data governance and the establishment of clear policies and procedures. It stresses that effective data governance is essential for maximizing the value of the organization's data assets.

6. The sixth part of the document explores the role of data in decision-making and strategic planning. It illustrates how data-driven insights can inform key business decisions and drive the organization's long-term success.

7. The seventh part of the document discusses the importance of data literacy and training for all employees. It emphasizes that having a data-literate workforce is critical for the organization to fully leverage its data capabilities.

8. The eighth part of the document discusses the importance of data ethics and the responsible use of data. It highlights the need to ensure that data is used in a fair, transparent, and ethical manner, respecting individual privacy and rights.

9. The ninth part of the document discusses the importance of data security and the implementation of robust security measures. It emphasizes that protecting sensitive data from unauthorized access and breaches is a top priority for any organization.

10. The tenth part of the document discusses the importance of data integration and the ability to connect data from different systems and sources. It highlights that integrated data provides a more comprehensive view of the organization's operations and performance.

11. The eleventh part of the document discusses the importance of data visualization and the use of dashboards and reports. It emphasizes that visualizing data makes it easier to understand complex information and identify trends and patterns.

12. The twelfth part of the document discusses the importance of data archiving and the long-term preservation of data. It highlights that maintaining a secure and accessible archive of data is essential for compliance and historical analysis.

13. The thirteenth part of the document discusses the importance of data backup and disaster recovery planning. It emphasizes that having a robust backup and recovery strategy is critical to ensuring business continuity in the event of a data loss or system outage.

14. The fourteenth part of the document discusses the importance of data migration and the smooth transition of data between systems. It highlights that careful planning and execution are essential to ensure data integrity and availability during the migration process.

15. The fifteenth part of the document discusses the importance of data collaboration and the sharing of data across different departments and teams. It emphasizes that data collaboration is essential for breaking down silos and fostering a data-driven culture throughout the organization.

16. The sixteenth part of the document discusses the importance of data innovation and the exploration of new data-driven opportunities. It highlights that staying at the forefront of data technology and innovation is key to maintaining a competitive edge in the market.

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