REGULATION OF UREASE LEVELS IN JACKBEAN COTYLEDONS

A Thesis

Presented to

the Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts in Biology

by

James Arthur Cook

July 1969

REGULATION OF UREASE LEVELS IN JACKBEAN COTYLEDONS

by

James Arthur Cook

APPROVED BY:

SUPERVISOR OF THESIS Prem P Chopra

CHAIRMAN OF THE DEPARTMENT OF BIOLOGY

Graham Jr. Davis

DEAN OF THE GRADUATE SCHOOL

John M. Howell

ABSTRACT

Factors controlling urease levels in sterile cultures of jackbean cotyledons were investigated. Urease levels were determined over a period of time by successive measurements of urease activity from extracts of the cotyledons. Urease activity was measured in micro-Conway dishes by reacting aliquot portions of the enzyme solutions with an excess of standard urea solution. Units of urease activity refer to the ml of HCl equivalent to the ammonia that would be evolved in the volume of extract per gram fresh weight of the cotyledon.

Cotyledons in cultures behaved the same as cotyledons harvested from seedlings at different ages. In 4 to 5 weeks, the levels of urease fell to 1% of the initial units in soaked seeds. High temperatures, low pH, and the presence of ethylenediaminetetraacetic acid, hydroxyurea, or actinomycin D in Miller's medium led to a decrease in urease units.

Urea, thiourea, and increased growth favored higher urease levels in cultures of jackbean cotyledons. It is known that ammonium ions inhibit urease activity and possibly repress it. A continuous flow of sterile medium with and without ureas was maintained to avoid excessive accumulation of ammonia. In the control, compared to the urea-treated set, urease levels fell. A 60-70% increase in the urease levels in the urea-treated set was observed within 12 days. Thus, an increase in urease in relation to the control was attributed to substrate protection. Urea has also been shown to induce urease formation in higher plants. In experiments with thiourea added to the

culture medium, an equivalent amount of nitrogen as nitrate was substituted for ammonium salts. An increase in urease levels ranging from 155 - 199% was noted in thiourea-treated cotyledons. Thiourea inhibits nitrate reductase. Lower urease levels in the set with no thiourea may have been due to inhibition by metabolically produced ammonia. Induction of urease by thiourea has been shown in bacteria. Extracts of cotyledons in cultures with low thiourea concentrations contained 30% more ammonia than ones in high concentrations of thiourea within 5 days. In experiments with cell-free extracts, there was no indication of protective action of thiourea on urease against proteolytic activity. When cotyledons are grown on agar with thiourea, the metabolic utilization of ammonia in protein synthesis, lower protease activity and possible induction of urease may sustain higher urease levels.

A central role for ammonia as a regulator for changes in urease levels is hypothesized. Such a hypothesis explains earlier work on the phylogenetic and ontogenetic occurrence of urease as well as data presented here. Amides or compounds of the ornithine-urea cycle are abundantly present in urease rich seeds such as soybeans, jackbeans, and watermelon. Maximum synthesis of urease in cotyledons of ripening seeds may be stimulated through the incorporation of free ammonia into these compounds. Conversely, in cotyledons of germinating seeds reactions favoring breakdown and/or utilization of amides and other nitrogen reserves are predominant. Proteolytic activity rather than synthesis dominates the metabolism of cotyledons in germinating seedlings. Under these conditions an excess of ammonium ions may

inhibit urease or repress the enzyme synthesis.

Lovingly Dedicated To

My Grandmother

Mrs. Juanita Wood Swann

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to Dr. Prem P. Sehgal for encouraging my interest in the thesis topic, and for the inspiration provided by his incentive for research. I also wish to acknowledge the many opportunities that he made available to me during the course of this study.

TABLE OF CONTENTS

Paper Presented at the ASB Meeting in Memphis, Tennessee. April 18, 1969

Page

TABLES

FIGURES

INTRODUCTION

Only in a few cases are factors controlling enzyme levels in tissue cultures of cotyledons completely understood. There has been no systematic study of the mechanisms involved in regulating urease levels at the time of maturation and germination. Urease has been considered as a growth-bound enzyme, but there is no firm evidence for this assumption. Attempts to correlate it with growth and development of the seedling have been unsuccessful. Urease has been regarded by some as a storage protein utilized at germination. More recently it has been suggested that in jackbeans the urease content and the total protein may be an indirect reflection of prevailing proteolytic activity.

As conditions for urease synthesis become known, it will contribute to a better understanding of the mechanisms involved in regulating the levels of enzyme in cotyledons at the time of germination and maturation. Knowledge of the effect of differentiation and growth on enzyme levels will be valuable in understanding the role of urease. Effect of proteolytic enzymes on urease levels during germination remains unclear. If calli from germinating seeds can be made to synthesize urease like ripening seeds, it will be evidence for the totipotency of cells at the physiological level. When the optimum conditions regarding regulatory mechanisms and biosynthetic potential of the cotyledon are understood, it may be feasible to undertake large scale, specific synthesis of the enzyme.

Urease is known universally as an enzyme which catalyzes the breakdown of urea into ammonia and carbon dioxide.

$$O = C(NH_2)_2 + 2H^+ + 2H_2O \longrightarrow 2NH_4^+ + CO_2$$

$$\Delta F^\circ = -7.8K \text{ cal}$$

It is also known that urease activity can be altered by ammonia inhibition, and possibly by ammonia repression of urease synthesis. Similar inhibitory effects of ammonia have been recorded with glutamine synthetase. It appears that ammonia may be important in both plant nutrition and metabolic regulation. There have been no studies of the ontogenetic and phylogenetic distribution of urease with regard to ammonia as its regulator.

In the present study, jackbean cotyledons were cultured in sterile media under different conditions. Urease levels were determined over a period of time by successive determinations of urease activity. Factors controlling urease levels were investigated. A hypothesis is proposed concerning the distribution of urease in plants and reasons for change in urease levels in the cotyledon.

THE REVIEW OF LITERATURE

Advances in Plant Tissue Culture: The first significant advances in tissue culture of higher plants were studies of nutrient requirements of isolated organs of the plant. White (1933) maintained tomato root tips in sterile culture through numerous transfers. Loo (1945) and Ball (1946) were first to successfully culture apical portions of the stem. Van Overbeek et al. (1942) discovered that coconut milk used in culture medium permitted growth of embryos younger than had previously been grown. Dawson (1942) was among the first to employ culture technique as means for investigating metabolic pathways. He observed that tobacco root callus synthesizes nicotine and anabasine. Increasing role of plant tissue cultures in studies of enzymes and cellular metabolism was reviewed by Gautheret (1955). Staba (1963) discussed the biosynthetic potential of plant tissue cultures. His review stressed the importance of plant culture systems for answering many basic problems in plant metabolism and growth. Mention was made of future applications of plant tissue cultures in the study of plant herbicides and growth regulators, as a food source, for testing drugs, and as a source of economically or biochemically useful compounds.

Becuase of the importance of enzymes in biosynthetic processes and in the production of compounds of commercial and medicinal importance, more attention is being given to their synthesis in plant tissue cultures. Higher plants may soon contribute to such product biosynthesis in a manner already accomplished by molds and bacteria on a commercial scale.

Enzymatic Studies During Seed Germination: Considerable attention has been directed towards enzyme activation in seed germination. Young and Varner (1959) demonstrated an increase in amylase and ATP phosphatase in germinating pea cotyledons after several days. When the germinating seeds were supplied with potent inhibitors of protein synthesis such as chloramphenicol, the activities of the enzymes were curtailed. This observation led to one of two explanations: either de novo synthesis of protein from amino acids yields additional enzymes, or enzyme production in cotyledons is due to an activation of latent protein by partial hydrolysis. More evidence is accumulating in favor of activation of enzymes from preformed macromolecules. Presley and Fowden (1965) found that treatment of germinating pea seeds with azetidine-2-carboxylic acid, an inhibitor of protein synthesis, did not decrease the levels of isocitratase and acid phosphatase.

Shain and Mayer (1965) demonstrated seed germination in the presence of inhibitors of protein synthesis. Presence of an endogenous trypsin inhibitor and three distinct types of proteolytic activity was shown in germinating lettuce seeds. Neumann (1964) demonstrated germination of lettuce seeds in the presence of inhibitors of nucleic acid synthesis. Swain and Dekker (1966a and 1966b) studied the properties of some of the enzymes involved in germination of the pea. They proved that amylase in the cotyledon was similar to other known plant amylases, and that amylase was responsible for the starch hydrolysing activity in the axis of pea seedlings. Shain and Mayer (1968) observed the activation of amylopectin-1, 6-glucosidase under conditions in which protein synthesis was inhibited and amylase failed to increase in activity. They regarded this as

further proof of enzyme activation in germination by liberation and activation of percursor forms.

Physiological and Ultrastructural Studies in Cotyledons: Cotyledons are major organs of seed, and play a vital role in supplying the growing embryo with nutrients. Mayer and Poljakoff-Mayber (1963) studied changes in protease activity in soybeans during germination. The protease activity was low initially, and then increased for about 6 days. They suggested that proteins are broken down to amino acids and that part of these are deaminated. Ammonia formed from deamination is detoxicated by amide formation, predominantly asparagine and glutamine. They demonstrated that asparagine concentration decreases in the cotyledon and increases in the hypocotyl and plumule. Wiley and Ashton (1967) studied the hydrolysis of storage protein in germinating Cucurbita seeds. The storage protein was hydrolyzed at a uniform rate from 1 to 5 days and decreased from 5 to 7 days. Most hydrolytic products were transferred to axial tissue. In initial stages, seed storage protein decreased while free amino acids and amides increased. They proposed that these compounds were used as substrates for synthesis of new seedling protein or were oxidized. Penner and Ashton (1966) showed by use of protein and messenger RNA inhibitors that there is a possible de novo synthesis of proteolytic enzymes occurring in squash cotyledons during seed germination.

Changes in fats, carbohydrates and minerals have also been recorded in cotyledons. Hacker and Mohr (1966) reported that reserve fat is homogenously distributed in dormant seeds of Sinapis alba. During germination, fat was broken down in a complicated pattern. They concluded

that phytochrome did not change the pattern of fat degradation, but that it exerts a strong regulatory influence on the rate of degradation. Mayer and Poljakoff-Mayber (1963) found that in soybeans and castor beans the disapperance of fats is accompanied by the appearance of carbohydrates. Acetyl coenzyme A formed from β-oxidation of fatty acids is involved in malate production via the glyoxylate cycle. Malate is then converted to carbohydrates by a number of reactions. The presence of magnesium oxalate, calcium phosphate, and calcium carbonate inside or outside the protoplast and in the cell walls of cotyledons was confirmed by Sutcliffe (1962). During the early stages of development of seed these salts are redistributed to radicle and plumule.

Only recently has attention been devoted to ultrastructural changes in cells of cotyledons during germination. Opik (1964 and 1966) investigated the form of nuclei in storage cells and fine structural changes in germinating cotyledons of Phaseolus vulgaris. Israel and Steward (1966) studied aseptically-cultured carrot cells under conditions in which growth and morphogenesis could occur. They were the first to correlate structural changes in cells of callus with the physiological and biochemical state. Engelbrecht and Weier (1967) studied the plastids of safflower cotyledon mesophyll cells. Membranes of the plastid envelope moved to quasi crystalline prolamellar bodies by the 2nd day of germination. These new membranes separate from the plastid envelope and become connected to the prolamellar body. Butler (1967) followed changes in the fine structure of mesophyll cells in attached and detached cucumber cotyledons. The pattern of change in excised cotyledon is similar to that of intact except that chloroplast breakdown is more rapid

and ribosome loss is less rapid and less complete. Breakdown of tonoplast was delayed for 40 days.

Studies on subcellular changes in cotyledons as a function of the nitrogen, changes in ribosomes and other organelles in relation to specific protein synthesis, the relative distribution of the organelles of the cell in relation to rates of growth, and structural differences in the cells of callus and the original explant (cotyledon) remain to be undertaken. Such data would contribute to a better understanding of the function of cotyledons and morphogenesis and organization in plants.

Hormones in Relation to Enzyme Activity: The effects of hormones on enzymes in tissue and organ cultures have been widely investigated. Bonner and Bandurski (1952) reviewed early work on the effect of auxins on enzyme levels. The first reported work in this area was by Burger and Avery (1941) on the elevation of alcohol and malic dehydrogenases in Avena coleoptile tissue. Gall (1948) cultured bean stem sections on starch agar medium. He found that 2,4-dichlorophenoxyacetic acid-treated tissue exhibited a greater capacity to degrade the starch, presumably due to increased soluble amylase and phosphorylase activity. Newcomb (1951) demonstrated an increase in ascorbic acid oxidase in cultured tobacco pith cells treated with indoleacetic acid. He mentioned that ascorbic acid oxidase may causally relate to growth because the increase precedes respiratory changes induced by IAA. Later Bryan and Newcomb (1954) demonstrated that pectin methylesterase activity is affected in a similar manner by IAA in the same tissue. Black and Humphrays (1962) investigated the effect of 2,4-D on the enzymes of the pentose phosphate cycle. Their conclusion was that treatment increased glucose catabolism

through the cycle. The increased ability of pea stems to synthesize benzoylaspartate by pretreatment with IAA was demonstrated by Venis (1964). The inducing effect was found to be suppressed by actinomycin D and by puromycin. Venis (1966) further elaborated his work by studying the effect of testosterone, hydrocortisone, and thyroxine on the synthesis of benzoylaspartate. It is noteworthy that puromycin did not reverse the inducing effect of thyroxine and that pretreatment with puromycin actually increased the induction.

Miller (1963) utilized callus of soybeans for the bioassay of kinetin and related compounds. Mann et al. (1963) demonstrated that kinetin caused a delayed and prolonged increase of tyramine methylpherase in barley embryos. They concluded that kinetin causes a marked stimulation of enzyme synthesis rather than a retardation of its inactivation. Varner and Chandra (1964) demonstrated the inductive effects of gibberellic acid on the de novo synthesis of amylase in the barley endosperm. Scott et al. (1964) investigated the effects of IAA and kinetin on the pentose phosphate cycle in tissue cultures of tobacco. They found that activity of the enzymes decreased with increasing growth rate of the plant. Galston (1951) had done earlier studies on the catalase levels of normal and crown gall tissue in several plants. He similarly concluded that catalase activity depends upon the rate of proliferation rather than on the nature of the tissue. Kinetin has been shown to decrease oxygen uptake and increase protein synthesis in tobacco tissue (Bergmann, 1965). Digby and Skoog (1966) studied the effect of kinetin on thiamine synthesis in tobacco callus culture. High concentrations (500-1000 µg/l) of kinetin activates its biosynthesis

whereas low concentrations (30-100 µg/l) causes a reduction in thiamine.

Secretion of Enzymes in Culture Media: There are several reports of enzymes in culture media in higher plants. Brakke and Nickell (1951) demonstrated the secretion of α-amylase from Rumex tumors in vitro. Lipetz and Galston (1959) studied the release of IAA oxidase and peroxidase in normal and crown gall tissue of Parthenocisscus tricuspidata. Karstens and De Messter-Manger Cats (1960) grew tobacco crown gall callus on a starch medium. The presence of starch degrading activity in the medium was mentioned. Jaspars and Veldstra (1965a) measured the release of amylase by the tissue grown on a starch medium. They isolated two isoenzymes from the tissue using gel electrophoresis (1965b). The activities of the two enzymes were dependent upon the organ of the plant and the nature of the tissue. Straus (1962) proved the presence of invertase in the cell walls of Libocedrus, Cupressus, Ephedra, Rosa, and Pyrus tissue cultures. Straus and Campbell (1963) presented data on the release of enzymes by 10 higher plants. The presence of phosphatase, peroxidase and IAA oxidase was investigated. Indoleacetic acid oxidase was released by all 10 tissues when treated with 0.05 M $CaCl_2$. They suggested that enzymes released by the tissue may serve to modify the medium.

Enzymatic Differences in Tissue: Other metabolic studies involving enzymes in tissue cultures have been investigated. Enzymatic differences between carrot taproot secondary phloem and tissue cultures derived from secondary phloem were studies by Wiesner and Guttman (1966). Peroxidase, acid phosphatase, and malic dehydrogenase were present in both preparations but the cultured material contained more peroxidase

and less malic dehydrogenase. Gainor and Crisley (1961) studied the proteolytic activity of crude stem extracts in normal and tumorous tissues in plants. They discovered that the proteolytic activity in tobacco stem segments, tomato and Phaseolus is greater in tumorous tissue than in normal tissue. They demonstrated an increase in proteolytic activity in tumor stem tissue from Nicotiana treated with such sulfhydryl activators as cysteine hydrochloride (1962). Becker et al. (1964) studied the synthesis of extracellular polysaccharides in Acer pseudoplatanus. Such polysaccharides were of slightly different composition than that obtained in cambial tissue of the intact sycamore tree.

Regulation of Enzymes in Plant Tissues: Although induction of enzyme synthesis has been thoroughly investigated in microbial systems recently, extensive investigations with higher plants have not been undertaken. Hageman and Flesher (1960) studied the effect of an anaerobic environment on the development of alcohol dehydrogenase in maize seedlings. Tolbert and Cohan (1953) studied the activation of glycolic acid oxidase in plants. The adaptive formation and physiological significance of indoleacetic acid oxidase was investigated by Galston and Dalberg (1954). Product repression by glucose and its derivatives on acid invertase levels in sugar cane was demonstrated by Galsziou and Waldron (1964). Evans and Nason (1953) were the first to study nitrate reductase in higher plants. Their attempts to demonstrate induction of the enzyme activity were inconclusive. Beevers et al. (1965) showed that both nitrate and light play a role in the induction of nitrate reductase in radish cotyledons and maize seedlings. Inhibition

of the enzyme by actinomycin D, chloramphenicol, and puromycin provided evidence supporting de novo synthesis. Ferrari and Varner (1969) studied the substrate induction of nitrate reductase in barley aleurone layer. Recently Filner et al. (1969) reviewed enzyme induction in higher plants. They suggested that specific control of enzyme degradation will be significant in the control of metabolism in higher organisms.

In microorganisms, Eckert et al. (1964) studied the effect of different nitrogen sources, including urea, on enzymes of the ornithine-urea cycle in Endomycopsis and Torulopsis. Bollard et al. (1968) have observed that urea increases urease activity in Spirodela oligorrhiza. Matsumato et al. (1968) showed that urea (1.5×10^{-1}M) induces the de novo synthesis of urease in leaves of jackbean. They suggested that ammonia represses urease activity in vivo by product inhibition and possibly repression of new urease synthesis. Hoare and Laidler (1953), König et al. (1966), and Mehta et al. (1967) also have shown that ammonia can inhibit urease activity. Heilmeyer et al. (1967) found that glutamine synthetase can be repressed by ammonium ions. Adenylation of the enzyme glutamine synthetase can be influenced by the substrate and product from glutamine metabolism (Kingdon et al., 1967). Enzymes leading to ammonia formation and utilization are widely prevalent in higher plants (Cohen and Brown, 1960). Egami et al. (1957) investigated the nitrate reducing systems in cotyledons and seedling embryos of soybeans during germination.

Compounds of the Ornithine-Urea Cycle and Their Analogues: The biosynthesis of urea represents a unique biological system which involves the primary fixation of both carbon dioxide and ammonia. In higher

plants, incorporation of $C^{14}O_2$ into citrulline via carbamyl phosphate in Phaseolus was among the earlier data supporting presence of the ornithine-urea cycle (Bone, 1959). Several reactions of the ornithine-urea cycle have since been demonstrated in plants. The presence of urea in higher plants is still controversial, due to insufficient assay techniques. It appears that urea may not necessarily be synthesized. Arginine can be used directly in plants as a nitrogen source (Bollard, 1959). The arginine analogue, canavanine, acts as a substrate for arginase and occurs in high concentrations in jackbeans and other leguminous plants. The metabolic role of canavanine is unclear, although it is known to cause an array of effects in higher and lower plants (Table I). The urea analogue, thiourea and other substituted Thiourea compounds are commonly used as herbicides. Thiourea exhibits a variety of physiological and biochemical effects, including the inhibition of nitrate reductase and induction of urease synthesis (Table II). Hydroxyurea is an effective inhibitor of urease and is employed as an antitumor agent.

There have been numerous investigations of hydroxyurea as an antitumor agent. Gale (1964) reported that hydroxyurea inhibits the growth of Pseudomonas aeruginosa, while the growth of fungi, yeast, and other bacteria were unaffected. It was suggested that the drug interferes with nucleic acid metabolism by reducing the DNA/RNA ratio. Hydroxyurea has been shown to reduce the rate of incorporation of thymidine in Ehrlich ascites tumor cells (Gale et al., 1964). Hydroxyurea has been effective in treating mouse leukemia. It reduces white blood cell count and spleen size upon oral and intravenous administration, with little or no

Table I

Effects of Canavanine

Category		Reference
A. Biochemical Effects	1. It is incorporated into soluble RNA fraction of liver	Allende et al., 1965.
	2. Increases rate of DNA synthesis of normal Hela cells; blocks incorporation of arginine C^{14} into histones.	Ackermann et al., 1965.
	3. Represses ornithine transcarbamylase in E coli.	Faanes and Rogers, 1968.
	4. Inhibits yeast alcohol dehydrogenase activity.	Tschiersch, 1966.
	5. Decomposes into canaline in jackbean leaves during germination.	Nakatsu et al., 1964.
	6. At 0.8 mM will inhibit proliferation of human cells cultured in vitro.	Miedema, 1966.
	7. Represses synthesis of alkaline phosphatase in E coli.	Gallant and Stapleton, 1964.
B. Physiological Relations: Lower Plants	1. Inhibits gametogenesis in Chlamydomonas (0.1mM).	Hipkiss, 1967.

Table I cont.

Category		Reference
	2. Canavanine killed E coli unable to support reproduction of T-even bacteriophage.	Schachtele and Rogers, 1968.
	3. Lethal effect may be due to incorporation into membrane bound proteins; interrupts normal organization.	Schachtele et al., 1968.
	4. Interfers with biosynthesis of arginine and its uptake in Neurospora and Saccharomyces.	Konobu and Suzuki, 1963. Grenson et al., 1966.
	5. Amino acid content of mutant resistant yeast reduced to 1/2; secrete 3 times as much into medium.	Morfaux and Dupuy, 1966.
	6. Inhibits Peronospora sporulation; can be reversed by arginine.	Shepherd and Mandryk, 1964.
C. Physiological Relations: Higher Plants	1. Occurs in wide variety of plants especially in Papilionaceae.	Tschiersch, 1963.
	2. Is synthesized in pods and translocated to the seed.	Tschiersch, 1961. Williams and Hunt, 1967.
	3. Tissues suspended in it may develop a negative water balance.	Mueller, 1963.

Table I cont.

Category		
4.	Causes necrotic changes in the petioles and veins of leaves when fed to bean plants.	Tschiersch, 1963.
5.	Amount in cotyledon, plumule, hypocotyl, root and seed coat decreases during germination.	Nakatsu et al., 1964. Ho and Shen, 1966.
6.	Inhibits germination of pollen tube growth in members of Papilionacea; stimulates some at low concentration.	Simola, 1967.
7.	Inhibits germination and seedling growth in Cattleya.	Raghavan, 1964.

Table II

Effects of Thiourea

Category		Reference
A. Biochemical Effects	1. Inhibits the activities of polyphenol oxides, ascorbic acid, oxidase and nitrate reductase.	Nason and Evans, 1955.
	2. It activates uricase.	Leone, 1955.
	3. a. Does not inhibit urease even at 10^{-3}M.	
	b. Causes reversible inhibition depending upon pH and concentration.	Kisiakowski and Shaw, 1953.
	4. Induced urease synthesis in Azotobacter.	Mehta et al., 1967.
	5. Increased protein synthesis in bacteria.	Ruban and Lobyreva, 1966.
	6. Oxidation of ammonia to hydroxylamine is sensitive to thiourea.	Hoffman and Lees, 1953.
B. Physiological Relations: Lower Plants	1. Antibacterial action on Staphylococcus.	De Ritis and Scalfi, 1946.
	2. Inhibits nitrosomanas in pure culture.	Jensen and Sorenson, 1952.
	3. Inhibits soil nitrification at low concentrations.	Quastel and Scholefield, 1949.

Table II.cont.

Category		Reference
	4. Supports growth of fungi.	Jensen, 1957.
	5. *Penicillium* breaks thiourea.	Lashen and Starkey, 1964.
	6. *Botrytis*, *Phythium* and *Verticillium* form thiourea in culture.	Ovcharov, 1937. Zelenin, 1939.
	7. *Chlorella* do not grow on either urea or nitrate in the presence of thiourea (0.002M).	Hodson, 1966.
	8. Greater inhibition of growth in *Aspergillus* in media with nitrate. Reduced toxicity in the presence of ammonium salts.	Fluey, 1948.
	9. Fixation of iodine in thallus of *Laminaria* is inhibited.	Roche and Yagi, 1952.
	10. Used as a fungicide.	Anderson, 1962.
C. *Physiological relations: Higher Plants*	1. Thiourea isolated from seeds of *Laburnum*.	Klein and Farkas, 1930.
	2. Large amount of thiourea occurs in rust-infected leaves of *Alchemilla*, *Rhamnus*, and *Rubus*. Only traces occur in healthy plants.	Ovcharov, 1937.
	3. Increase chlorophyll breakdown.	Ovcharov, 1937.

Table II cont.

Category		Reference
4.	Arrests growth or toxic to higher plants at a concentration of 0.2-5 parts/1000.	Nicolas and Nicolas, 1925.
5.	Protects against radiation-induced chromosome aberation in Tradescantia.	Mikaelson, 1955.
6.	Removes apical dominance in potato shoots; stimulates formation of thick shoots in potato tubers at 2% level.	Satarova, 1953. Pallidina and Pervosa, 1965.
7.	It is active in lettuce seed germination bioassay for cytokinin activity.	Kefford et al., 1965.
8.	It breaks secondary dormancy induced by high temperatures.	Johnson, 1946.
9.	It may substitute for low temperature treatment in the promotion of after-ripening in many seeds.	Johnson, 1946.
10.	It depresses catalase activity but stimulates peroxidase activity in vivo in germinating lettus seeds.	Poljakoff-Mayber, 1953.

gastrointestinal toxcity (Fishbein and Carbone, 1965). Frenkel et al. (1964) showed that hydroxyurea inhibits conversion of cytidine monophosphate to deoxycytidine monophosphate in bone marrow.

Davidson and Winter (1963) hydrolyzed urea and hydroxyurea in serum with urease in tris-phosphate buffer at pH 7.4. They reported that hydroxyurea acted as a substrate, but only at a rate 10^{-3} that of urea. Hydroxyurea has been shown to be a potent inhibitor of urease, causing 50% inhibition of urease at 5×10^{-5}M. A tentative mechanism of inhibition involving hydrolysis of hydroxyurea by urease to form the actual inhibitor has been proposed (Gale, 1965). Fishbein and Carbone (1965) postulated that hydroxyurea probably acts simultaneously as substrate and as an irreversible inhibitor of urease.

Some Studies on Urease: The occurrence of urease in plants, and its molecular and physical properties, has been reviewed by Bollard (1959) and Varner (1960). The enzyme does not occur universally in higher plants (Takeuchi, 1909; Kiesel, 1911). Urease is present in high concentrations in many legumes of the sub-family papilionatae. Its presence, however, in Cicer arietinum, Vigna catiang, Phaseolus mungo Lens esulenta could not be detected by Nandi (1958). Damodaran and Sivaramakrishnan (1937) found jackbean, watermelon, and squash to be better sources of urease than soybeans. The presence of urease in wheat was demonstrated by Gupta and Das (1959). Annet (1914) tested Oryza sativa and Setaria italica for urease activity without success. Tai (1953) was also unsuccessful in tests for urease with Zea mays, Triticum vulgare, Hordeum vulgare, Brassica napus and Cucumis melo.

There has been no systematic study of regulatory mechanisms for

urease at the time of seed germination and maturation. Granick (1938) studied the distribution of urease in different parts of jackbeans and soybeans. He considered urease as a growth-bound enzyme and that changes in urease levels were related to changes in protoplasmic activity. No firm evidence was presented for this assumption. Williams (1950) proposed the possibility that urease may be functioning as reserve protein. Williams and Sharma (1954), however, could not correlate the breakdown of total protein with the degradation of urease. They observed that watermelon seedlings pass through a 'urease stage' before an abrupt fall after the sixth or seventh day. Detached cotyledons smeared with vaseline maintained urease activity over a longer period, indicating that urease levels may be connected with respiration and water content. Bollard (1959) postulated that urease may be functioning as only part of the reserve protein during germination, but a part which is used immediately after germination. It has been shown that urease and arginase variations are almost parallel in soybeans during germination, and an involvement in arginine metabolism has been suggested (Suzuki, 1952). Sehgal and Naylor (1966) clarified earlier work by Granick on the changes in urease levels in different parts of the jackbean plant throughout its life cycle.

METHODS AND MATERIALS

The experiments conducted involved (1) the extraction of urease from the jackbean (soaked and cultured cotyledons) (2) determination of enzymatic activity in the extract, and (3) protein determination of the extract.

Jackbeans used for the present investigations were obtained from Mr. Ernest Nelson of Route #1, Waldron, Arkansas. Seeds selected for experiments were of uniform size and age (1968 harvest).

Culture and Extraction Techniques: All transfers and treatments were conducted under a sterile hood pre-exposed to 15 minutes of germicidal ultraviolet light. The jackbean seeds were initially sterilized by soaking for 30 minutes in 0.05% (vol./vol.) sodium hypochlorite. Sodium hypochlorite is a strong oxidant and does not inactivate urease. The seeds were rinsed thoroughly by three washings of sterile distilled water, and allowed to soak 6 to 8 hours. The seed coat was removed by hand from fully swollen seeds, and naked seeds were retreated for 5 minutes in 0.025% (vol./vol.) sodium hypochlorite. They were then rinsed again by three washings of sterile distilled water. One-fourth of the embryo end of each seed was chopped using s sterile scalpel and forceps. The cotyledons were transferred to the appropriate culture media. All cultures except the urea set were incubated in a growth chamber in the dark at 27°C; one set was incubated at 33°C. A continuous flow system was devised for the urea experiment. It consisted of a sterile enclosed and inclined plate, and a 1 liter reservoir of culture medium. The cotyledons were placed on the plate and the culture

medium was regulated to drain over them at a constant rate.

Nutrient conditions for the culture medium were basically those described by Miller (1963)*, unless stated otherwise. Modications of Miller's medium involved changes in the proportion of NH_4NO_3 to KNO_3, deletion of ammonium salts, changes in pH, and the addition of Cleland's reagent (10^{-5}M), urea (1.66×10^{-2}M), thiourea (1.66×10^{-2}M), hydroxyurea (1.66×10^{-2}M), ethylenediaminetetraacetic acid (1.34×10^{-3}M), and actinomycin D (10^{-3}M). In experiments involving ammonia deletion, an equivalent amount of nitrogen was substituted as nitrate. In the urea experiments, sterile distilled water containing Cleland's reagent was used as the control medium to avoid possible infection in the continuous flow system. All culture media were sterilized 30 minutes in an autoclave. Both urea and actinomycin D were sterilized with ultraviolet light and then added to the cooled culture medium. These compounds decompose at high temperatures.

In some experiments large samples of cotyledons (3 to 4 gm, in

*Stock cultures of the jackbean tissue have been maintained on a medium containing (mg/liter): KH_2PO_4,300; KNO_3,1000; NH_4NO_3,1000; $Ca(NO_3)_2 \cdot 4H_2O$,500; $MgSO_4 \cdot 7H_2O$,71.5; KCl,65; $MnSO_4 \cdot 4H_2O$,14; Fe citrate, 18.0; $ZnSO_4 \cdot 7H_2O$,3.8; H_3BO_3,1.6; $Cu(NO_3)_2 \cdot 3H_2O$,0.35; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$,0.1; KI, 0.8; i-inositol,100; nicotinic acid, 0.5; pyridoxine.HCl, 0.1; tiamin·HCl, 0.1; α-naphtaleneacetic acid, 2; kinetin,0.5; sucrose, 30,000; and Bacto-agar, 10,000. The pH was adjusted to 5.8(NaOH).

Tables III and IV, and 10 gm in Table V) were used for each analysis. In other experiments (Tables VI, VII, and VIII), matched cotyledons from each seed were used, one as an experimental and the other as a control. In such cases, individual variations among seeds were reduced to a minimum. Each sample of cotyledons was weighed and homogenized in a pestle and mortar with acid-washed sand. Fifteen ml of 0.05 M K_2HPO_4 and KH_2PO_4, pH 7 buffer containing 10^{-5}M Cleland's reagent were used per gram fresh weight during extraction. The homogenate was centrifuged at 27,000 x g for 15 minutes. The residue was extracted a second time with the buffer and centrifuged again. Both extracts were combined and enzyme activity and protein were determined.

Determination of Urease Activity: Enzyme activity was determined in micro-Conway dishes (Conway, 1962). The procedure was described by Sehgal and Naylor (1966). The dishes were routinely cleaned with a dilute solution of alconox, followed by a thorough washing with running water. They were next treated with dilute HCl for 2 to 3 hours and rinsed thoroughly with running tap water. They were autoclaved for 30 minutes, rinsed with distilled water, and dried at 105°C. Treatment with 1% siliclad solution (Clay - Adams, New York) provided a water repellent layer that prevented the substrate and enzyme drops from spreading and mixing. The dishes were retreated with siliclad every 5 to 6 cycles of cleaning.

A 0.5% boric acid in 20% alcohol solution containing an indicator was used to absorb the liberated ammonia. The indicator consisted of bromocresol green (0.033%) and methyl red (0.066%) in 95% alcohol. Indicator was used at 10.0 ml per 990 ml of the alcohol-boric acid solution. A saturated solution of K_2CO_3 was used to stop the enzyme-

substrate reaction after 5 minutes, and to promote the liberation of ammonia from the reaction mixture. Diffusion was allowed to occur at room temperature for 2 to 3 hours. 100λ of 0.05 M urea in phosphate buffer, pH 7.0 (2.8% anhydrous KH_2PO_4 and 6.8% anhydrous Na_2HPO_4) and 50λ of urease solution (diluted in 0.05 M phosphate buffer, pH 7.0 containing Cleland's reagent) were placed side by side. The central well contained 2 ml of boric acid and indicator solution, and the lower end of the outer well contined 1 ml of saturated K_2CO_3. Micro-Conway dishes were sealed air tight by glass covers that had been smeared on the edges with green surgical soap. Each dish was lifted and tapped lightly to mix the enzyme solution and substrate. Great care was taken not to mix the saturated K_2CO_3 with the reaction mixture in the outer well. The reaction was stopped after 5 minutes by tilting each dish to mix the K_2CO_3 with the reaction mixture. The mixture was swirled around the central well to assure uniform diffusion. Jackbean urease has a value of .003 M for K_m in buffer at pH 7.0 (Varner, 1960). The concentration of the substrate used in the experiments was 17 times the K_m for urease. There is a linear increase in the reaction up to 20% hydrolysis of the substrate within 5 minutes. All experiments involved hydrolysis of less than 2% of the substrate.

The amount of ammonia liberated was measured by titrating the solution in the central well with standard 0.002 N HCl. The end point was sharp and easily reproducible. Each determination was replicated 3 to 4 times.

Definition of the Urease Unit: One urease unit was defined as the amount of ammonia required to neutralize 1 ml0.002 HCl. The standard

HCl used in titrations is directly proportional to the quantity of ammonia produced from 100λ of 0.05 M urea solution in pH 7.0 phosphate buffer in the micro-Conway dish at room temperature. The reaction time was 5 minutes and the amount of enzyme solution used was 50λ. Total enzyme units in the extract were based upon the amount of HCl (in ml) required to titrate the total extract as calculated from 50λ diluted aliquots of the extract. Units per gram fresh weight were equal to the total units divided by the weight of the sample:

$$\frac{\text{ml HCl} \times \frac{1000\lambda}{50\lambda} \times \text{Vol. Extract} \times \text{Dilution Factor}}{\text{Fresh Weight Sample}} = \text{Units/gm F. W.}$$

Protein Determination: Protein determinations were made with Biuret reagent. Procedures and composition of reagent were according to Gornall et al. (1949).* Protein was precipitated from solution by adding 5 ml of 20% trichloracetic acid to 5 ml of each test sample. The mixture was allowed to stand at room temperature for 10 minutes, after which it was centrifuged at 27,000 x g for 15 minutes. The precipitate was dissolved in 5 ml 1.0 N NaOH.

One ml of the dissolved precipitate was added to 4.0 ml of Biuret reagent and mixed by swirling. The solution was allowed to stand 30 minutes at room temperature. Optical densities were read on a Spectronic 20 spectrophotometer at 560 mμ. One ml of 1.0 N NaOH was added to 4 ml of Biuret reagent as a blank. The amount of protein in unknown solutions was calculated from a standard curve for bovine serum albumin (1-10 mg) measured at 560 mμ.

*Biuret reagent: 1.50 g $CUSO_4 \cdot 5H_2O$ (cupric sulfate and 6.0 g $NaKC_4H_4O_6 \cdot 4H_2O$ (sodium potassium tartrate) were dissolved in 500 ml of H_2O. 300 ml of 10% sodium hydroxide was added and the solution was diluted to 1 liter. The reagent was stored in a dark colored bottle.

RESULTS AND DISCUSSION

Sehgal and Naylor (1966) studied the urease content of cotyledons harvested from seedlings at different ages. They found that the urease content of both light and dark grown cotyledons goes through three phases over a period of 4 to 5 weeks. During the first few days of germination, the urease remained uniform. The second phase was characterized by a gradual declining urease content, and the last phase by a marked decline in urease. They suggested that in jackbean cotyledons the total protein, including urease, may be an indirect reflection of prevailing proteolytic activity.

In the present investigation, jackbean cotyledons were cultured in a complete medium over a period of 35 days (Table III). The urease content of the cotyledons also exhibited decrease in units. Units decreased from 100% in the control, 1 day soaked seeds, to 1% after 35 days. Decrease in units was not linear. Individual differences among seeds may cause minor variations in urease content. It is feasible, however, that the relatively constant levels of urease from day 4 to 11 represent a phase in cultured cotyledons in which proteases are breaking down other proteins faster than urease. In both harvested and cultured cotyledons, there seems to be some mechanism contributing to decline in urease units. Urease content of fresh callus removed from cultured cotyledons over 30 days old was very low (less than 1% of the initial units present in the explant). Modifications of Miller's medium involving a change in the proportion KNO_3 to NH_4NO_3 caused no significant change in the rate of decline of urease over a period of 14 days, as compared to the control (Table IV).

TABLE III

Changes in Urease Content of Cotyledons* at 27°C

Time in days on Miller's medium	Urease units per g Fresh Weight	Percentage units
Control (soaked 1 day) zero time in Miller's medium	2400	100
2	1490	62
4	1150	48
6	935	39
8	1170	49
11	915	38
14	566	24
15	479	20
19	334	14
24	100	4
35	27	1

*For each analysis about 3 to 4 g of cotyledons were used.

Low pH (4.5) and high temperatures led to a faster decrease in urease levels. The addition of urea or Cleland's reagent (dithiothreitol) to the medium, or greater aeration of the medium by shaking seems to preserve urease activity in relation ot the control (Table V).

Tanis (1968) demonstrated that the polymeric state of jackbean urease is dependent upon pH. Urease was separated into different polymers by ultracentrifugation and sedimentation rates were determined. He found that the 18s urease fraction could be converted to the 12s form by lowering the pH from 5.3 to 4.8 and vice versa. In the present experiments, pH of the medium was lowered from control, pH 5.8, to 4.5. Urease activity of cotyledons cultured at pH 4.5 was 20% lower than in those cultured at 5.8. It appears doubtful that any shift in the polymeric state of urease accounted for the change in activity of the extract, because both the control and lower pH set are assayed at pH 7. It may be that shifts to a smaller polymeric state of urease *in vivo*, caused by lowering the pH, renders the molecule more susceptible to action of proteases. Higher temperatures (33°C) than control (27°C) caused a 32% reduction in urease activity. One would expect less favorable conditions for growth at higher temperatures, increased protein denaturation and probably increased protease activity.

The urease molecule is rich in sulfhydryl groups--40 to 75 SH groups per molecule (Varner, 1960). Oxidation of these groups by compounds such as iodosobenzoate, ρ-mercuribenzoate and heavy metals causes complete enzymic inactivation. Urease activity can be protected by various activators (protein, amino acids and gum arabic), which function by binding heavy metals and thus protecting the SH groups. When Cleland's

TABLE IV

Changes in Urease Acitvity* on Modified Miller's Medium at 27°C

Urease Units per g Fresh Weight

Time in days on medium	Complete Miller's medium*	Modified Miller's medium*	Percent change
Soaked seeds (zero time)	2400	2400	
2	1490	1390	-7.0
4	1150	1160	+1.0
6	935	950	+1.5
8	1170	1115	-4.6
11	915	985	+7.0
14	566	570	+0.5

*For each analysis 3 to 4 g of cotyledons were used.

Complete Miller's medium contained 1.0 g NH_4NO_3/1 and 1.0 g KNO_3/1.

Modified Miller's medium contained 1.5 g NH_4NO_3/1 and 0.5 g KNO_3/1.

TABLE V

Factors Affecting Urease Content in Jackbean Cotyledons*

Experimental Conditions	Urease units per g Fresh Weight after 2 days in solution	Percentage Units
Control in Miller's medium (no shaking, 27°C, pH 5.8, no agar)	1645	100
Aerated on shaker	1770	107
Miller's medium plus Cleland reagent (10^{-5}M)	2097	127
Miller's medium plus urea (1.66×10^{-2}M)	2222	134
Miller's medium at pH 4.5	1309	80
Miller's medium at 33°C	1110	68

*Before putting cotyledons in flasks, sterilized seeds were soaked in water for 1 day. For each analysis about 10 g of cotyledons were used.

reagent (Table V) was added to the medium, a 27% increase in activity was observed over the control. This compound is rich in SH groups, and probably prevents oxidation of urease - SH groups, similarly as other known activators. Aeration of the medium by continuous shaking gave a slight increase in urease activity (7%) over the control. The greater abundance of oxygen in the culture medium may promote an increase in respiration, which in turn may facilitate increased protein synthesis, including urease. Addition of urea to the medium caused the greatest increase in urease activity within 2 days. However, determinations of enzyme activity on later days revealed a decrease in units in the urea-treated-set compared to the control, and an increase in ammonia in the culture medium.

Ammonium ions inhibit urease activity (Hoare and Laidler, 1953; Mehta et al., 1967) and repress new enzyme synthesis (König et al., 1966; Matsumato et al., 1968). Ammonia is produced in sterile cultures of jackbean cotyledons when urea is added to the medium. To avoid excessive accumulation of ammonium ions, a sterile continuous flow system was designed. Culture medium (water and Cleland's reagent) with and without urea flowed at a constant rate over an inclined, enclosed sterile plate. The results in Table VI show a 60 to 70% increase in urease units over the control within a period of 12 days. Urea, as a substrate, enhances urease activity. The ammonia which accumulates in cotyledons inhibits urease and thus prevents further breakdown of urea. Excess amounts of urea supplied freshly in a continuously flowing system leads to urea - urease binding. Such a complex may be less likely broken by proteases than the urease in cotyledons untreated with urea. Possible induction of urease synthesis cannot be ruled out. There is

increasing evidence that urea may induce urease formation in higher plants (Matsumato et al., 1968; Bollard et al., 1968). In microorganisms, urea also accelerates formation of enzymes of the ornithine-urea cycle (Eckert and Kating, 1968).

Thiourea is structurally very similar to urea and differs only in that the carbon-sulfur bond replaces the carbon-oxygen bond. There are several effects of thiourea on plants that make it suitable for investigations of urease levels in cultured jackbean cotyledons. It inhibits the activities of several metal-requiring enzymes such as polyphenol oxidase, ascorbic acid oxidase and nitrate reductase (Nason and Evans, 1955). Kistiakowski and Shaw (1953) demonstrated that thiourea causes reversible inhibition of urease depending upon pH and concentration of thiourea. Increased protein synthesis was observed in bacteria treated with thiourea (Ruban and Lobyreva, 1966). Mehta et al. (1967) induced urease synthesis in Azotobacter with thiourea. Asparagine and ammonia inhibited urease synthesis in their system. Thiourea has been isolated from seeds of higher plants (Klein and Farkas, 1930). It is known to affect seed germination. Mayer and Evanari (1951) demonstrated that thiourea stimulates seed germination in conjunction with 2-4D. In germinating lettuce seeds, thiourea depresses catalase but stimulates peroxidase activity (Poljakoff-Mayber, 1953). Kefford et al. (1965) showed that thiourea enhances lettuce seed germination. In potato, thiourea removes apical dominance and stimulates formation of thick shoots from the tubers (Satarova, 1953; Pallidina and Pervosa, 1965). The effect of thiourea as a growth inhibitor in Aspergillus was shown by Fluey (1948). There is greater inhibition of growth in media

TABLE VI

Changes in Urease Activity Under Continuous Flow of Solutions*

	Units per g Fresh Weight		
Time in Days	Control (water)	Urea Solution (1.66×10^{-2}M)	Percent Change
1	1500	1530	+2.0
2	1985	2072	+4.4
5	1270	1390	+9.4
7	1100	1380	+25.5
9	1010	1720	+70.3
12	1025	1670	+63.0

*Matched cotyledons from the same seed were used for analysis. One ml B-mercaptoethanol/l was added in both solutions. The experiment was performed at room temperature.

containing nitrate. Toxicity is reduced in the presence of ammonium salts.

Ammonium salts are typically present as a nitrogen source in plant culture media. Experiments were conducted in which an equivalent amount of nitrogen as nitrate was substituted for ammonium salt in the culture medium, and urea was replaced with thiourea (Table VII). Cotyledons in thiourea show enhanced urease activity. An increase ranging from 155 to 199% is noted in thiourea-treated cotyledons over controls. The increase of urease in thiourea-treated cotyledons cannot be accurately compared to the 60 - 70% increase in urea-treated cotyledons; both nutrients supplied and conditions of culture were different. There are several explanations of the results in Table VII. Ammonia can be produced metabolically from amides in seeds (Cohen and Brown, 1960). Ammonia can also be produced from nitrate in the medium by action of nitrate reductase, from glutamic acid by glutamic acid dehydrogenase and other enzymes in a variety of higher plants. It is likely that nitrate reductase is present in jackbeans because Egami et al., (1957) isolated the enzyme from soybeans. Inhibition of nitrate reductase by thiourea should cause a reduction of urease inhibition and repression by decreasing the amount of metabolically produced ammonia. This idea is supported by the fact that when nitrate is the only source of nitrogen, ammonia is found (5.5 μM/gm fresh weight) in extracts from cotyledons cultured for 7 days in a medium containing 1.66×10^{-2} M thiourea. At lower concentrations of thiourea (1.66×10^{-3} M), 7.1 μM ammonia/gm fresh weight is detectable in comparable cotyledons. Induction of urease and other proteins by thiourea as shown in microorganisms

TABLE VII

Effect of Thiourea on Urease Activity in Cotyledons*

Time in Days	Urease Units per g Fresh Weight		Percentage Change in Total Units on per g Fresh Weight Basis	Percentage Change in Specific Activity (Total units/Total proteins)
	Control Modified Miller's Medium (no thiourea)	Thiourea (1.66×10^{-2} M) In Modified Miller's Medium		
1	1675	1955	+21.8	+23.3
2	1520	1840	+21.5	+17.1
4	1570	2020	+28.8	+11.2
7	668	1340	+101.0	+105.0
8	512	1525	+198.0	+176.0

may also be possible in cotyledons.

Assuming that changes in urease levels are an indication of an imbalance between synthesis and degradation of the enzyme in cells, effect of thiourea on cell-free extracts was tried (Table VIII). In such clarified extracts, synthesis of urease is most probably lacking, and protease activity is quite high. Clarified extracts were incubated for 8 to 72 hours at 27°C in the presence of thiourea (1.66×10^{-2}M). The proteolytic acitvity of the extracts at pH 7.0 was increased by adding chymotrypsin (10 mg/ml) and bacterial protease (5 mg/ml). Thiourea-treated extracts showed a decrease of 8 to 12% in urease units in 48 hours when compared to controls. There was no indication of protective action of thiourea on urease against proteolytic activity. Thiourea added to the substrate in the reaction mixture (urea at 0.05 M, thiourea at 0.025 M and 0.25 M, pH 6.0 to 7.0) did not inhibit urease activity.

Compared to the cell-free extracts, dividing and growing cells of cotyledons have increased protein synthesis and relatively low protease activity. In growing cells one would also expect that free pools of ammonia are smaller because of increased protein synthesis accompanying cell division and growth. Growth was increased in cotyledons cultured on agar nutrient medium containing thiourea as compared to cotyledons in a liquid nutrient medium also containing thiourea. The changes in fresh weight were taken as an indication of growth, and this was compared to total urease units following 12 days of culture (Figure I). Increased growth in cotyledons cultured on agar accompanies higher levels of urease in relation to cotyledons in the same nutrient medium lacking agar. The

TABLE VIII

Effect of Thiourea and Protease on Urease Activity in Cell Free Extracts

Units per g Fresh Weight

Time in hours	Control*	Thiourea*	Percent change
24	1625	1495	-8.0
48	1610	1420	-11.8
72	1665	1270	-23.2

*The control contained 10 ml extract in 0.05 M phosphate buffer, pH 7. The extract with thiourea contained 10 ml 0.25 M thiourea in 0.05 M phosphate buffer, pH 7.0. 0.1 ml of B-mercaptoethanol was added to 10 ml of each extract. 10 mg/ml of chymotrypsin and 5 mg/ml of bacterial protease were added to the extracts.

The results were explained by assuming the inhibition of nitrate reductase by thiourea, the utilization of metabolically produced ammonia because of increased protein synthesis, a lower protease activity prevailing in growing cells and possibly, induction of urease.

Thiourea effects were not obtained by the addition of EDTA (1.35×10^{-3} M) or hydroxyurea (1.66×10^{-2} M) in Miller's medium, modified by substituting an equivalent amount of nitrogen as KNO_3 (Table IX). Both compounds caused a definite decrease in urease units per gram fresh weight, compared to the control. It was assumed that EDTA may partially mimic the effect of thiourea by inhibiting the metal-containing enzyme nitrate reductase. This proved not to be the case, and a 50.5% reduction in urease activity was observed after 5 days. EDTA, because of its potent metal-binding capacity, probably non-selectively inhibits all metal-containing enzymes and cofactors. Essential metabolic pathways involved in urease synthesis may therefore be blocked. Hydroxyurea may bind with urease at the active site (Fishbein and Carbone, 1965). It was assumed that such a complex would be less susceptible to action by proteases. However, the effects of hydroxyurea on urease of cotyledons in culture support earlier *in vitro* studies (Gale, 1965) that it acts as an inhibitor. A 79% decrease in urease activity was observed in the hydroxyurea treated after 4 days. Actinomycin D (10^{-2} M) in modified Miller's medium caused a 10.6% decrease in urease levels after 5 days. Actinomycin D is an effective inhibitor of RNA synthesis. The effects of this inhibitor indicate that some *de novo* synthesis of urease may occur in the germinating cotyledon.

FIGURE 1

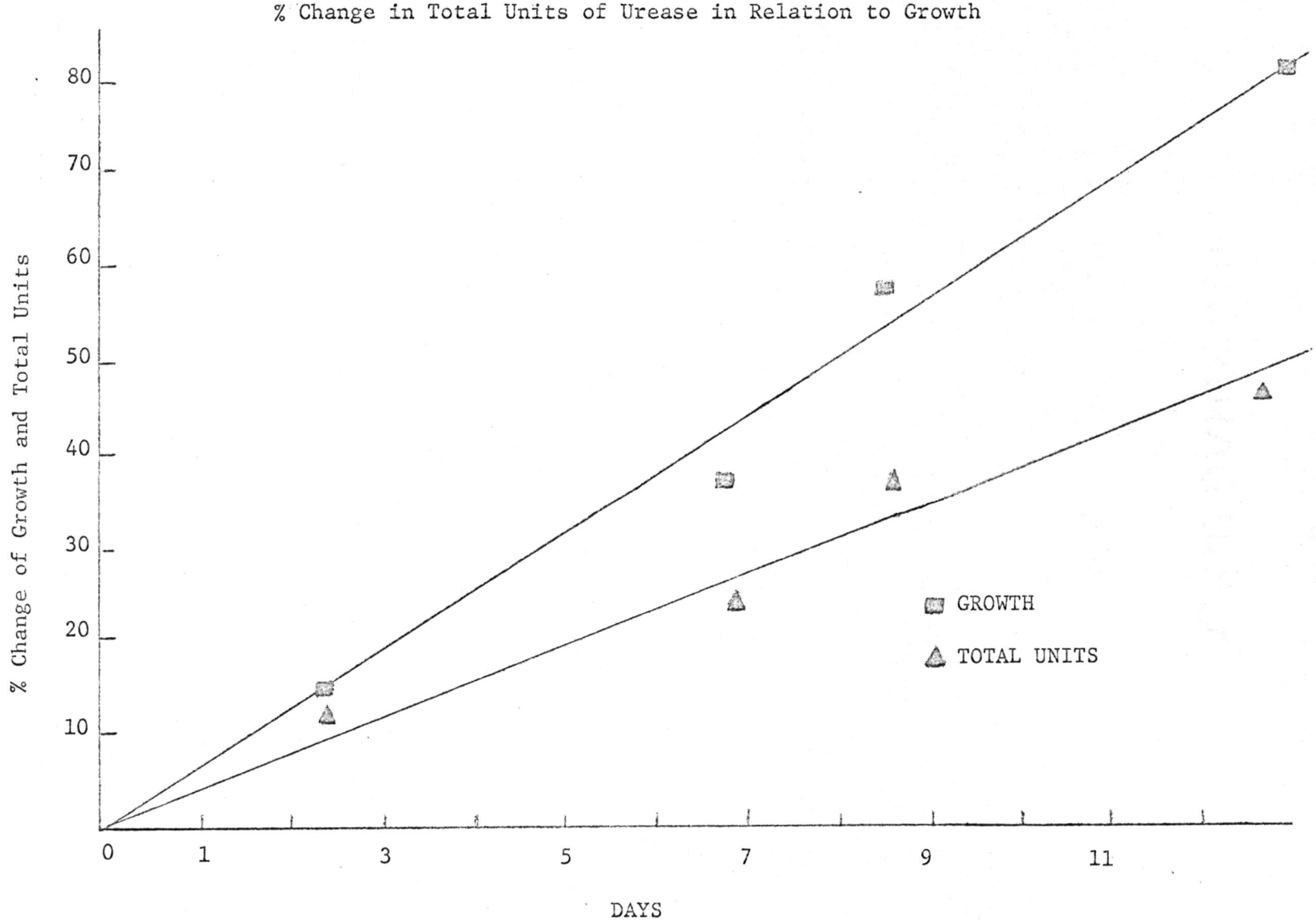

% Change in Total Units of Urease in Relation to Growth
% Change of Growth and Total Units
80
70
60
50
40
30
20
10
0
1
3
5
7
9
11
DAYS
GROWTH
TOTAL UNITS

TABLE IX

Effect of EDTA, Actinomycin D, and Hydroxyurea on Urease Activity in Cotyledons*

Time in days	Treatment	Urease Units per g Fresh Weight: Control*	Treated*	Percent change
3	EDTA (1.34×10^{-3} M)	2720	2260	-20.3
4		1791	1272	-40.8
5		1810	860	-52.5
2	Actinomycin D (1×10^{-3} M)	2960	2850	-3.7
5		2720	2430	-10.6
4	Hydroxyurea (1.66×10^{-2} M)	2958	230	-79.0

*Matched cotyledons from the same seed were used for analysis. Miller's medium was modified in both control and treated. Ammonium nitrate was deleted, and an equal amount of N as KNO_3 was added. Cleland's reagent was present in all media at 10^{-5} M. Incubation was done at 24°C.

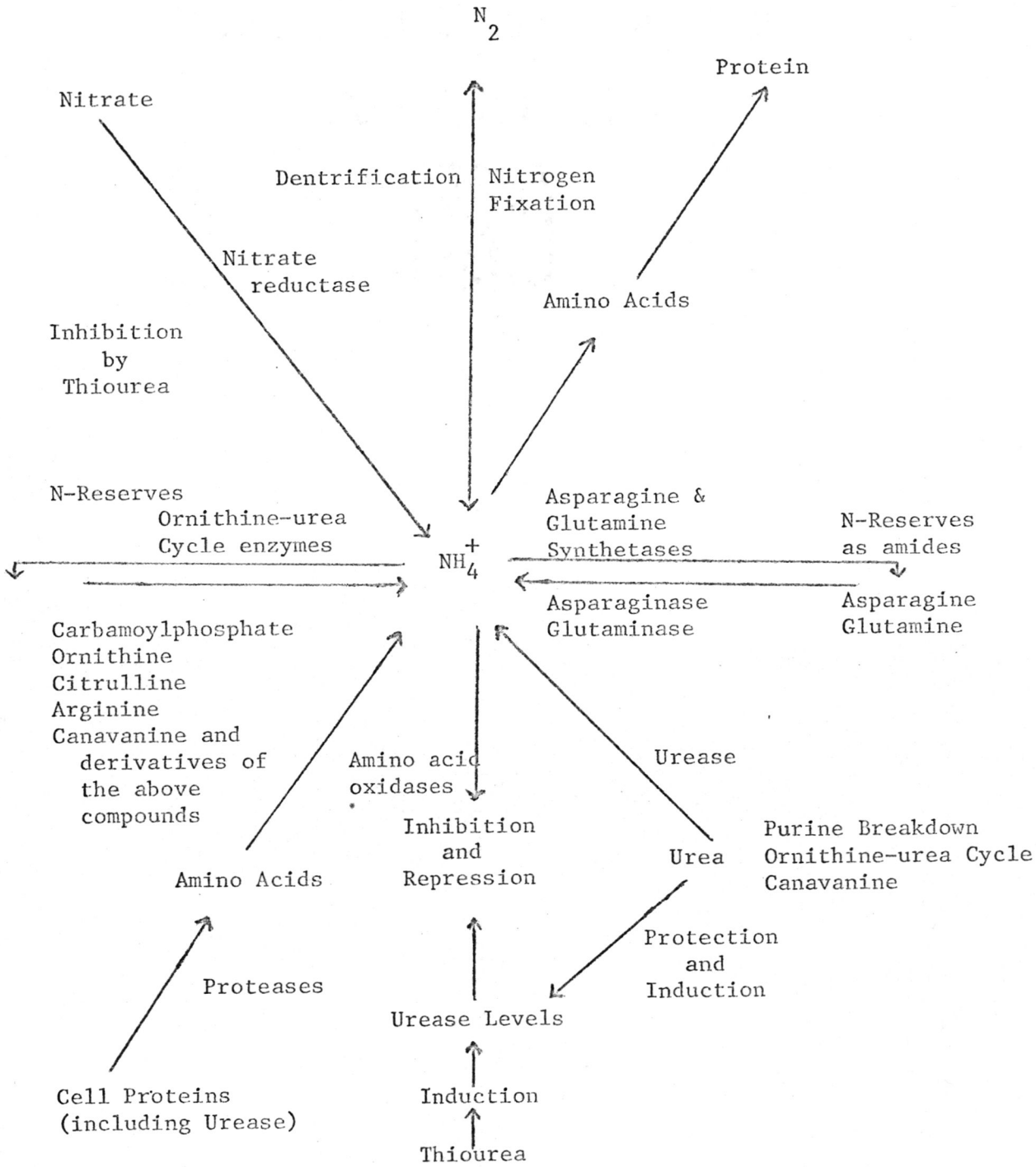
A central role for ammonia as a regulator is hypothesized for changes in urease levels. Earlier data of Sehgal and Naylor (1966), as well as that presented here, can be explained by the above hypothesis. When seeds are being formed in pods, urease synthesis and its storage is at its maximum (Sehgal and Naylor, 1966). In cotyledons of ripening seeds the reactions resulting in utilization of ammonia, for example, the synthesis of amides and/or the compounds of the ornithine-urea cycle, including their derivatives, must be very active. This hypothesis is strengthened by the fact that amides and compounds of the ornithine cycle are abundantly present in soybeans, jackbeans and watermelon (Meister, 1965). These plants are also known to be the richest sources of urease. Conversely, in cotyledons of germinating seeds, reactions favoring breakdown and/or utilization of amides and other nitrogen reserves like canavanine, ornithine, citrulline and arginine are predominant. Under these conditions free ammonium ions inhibit and repress urease synthesis. In growing seedlings of jackbeans and soybeans, the cotyledons are non-growing tissues. Proteolytic activity rather than synthetic reactions dominates the metabolism of cotyledons. The other parts of the germinating seedling may still, however, show increased amide content and protein synthesis.

Canavanine, an analogue of arginine, occurs in a wide variety of plants, especially those belonging to the Papilionaceae (Turner and Harbone, 1967). It is synthesized in pods and translocated to the seeds. In ripening seeds, the amount of canavanine as a percentage of dry weight remains constant (Tschiersch, 1961; Williams and Hunt, 1967). During germination, on the other hand, the levels of canavanine in cotyledon,

plumule, hypocotyl, root and seed coat decrease (Nakatsu et al., 1964; Ho and Shen, 1966). These observations on levels of canavanine during seed germination and ripening further support the hypothesis outlined in (Figure 2).

Figure 2

PROPOSED ROLE OF AMMONIA IN REGULATING UREASE LEVELS



SUMMARY

Factors controlling urease levels in sterile cultures of jackbean cotyledons were investigated. Urea, thiourea, and increased growth favor higher urease activity. A continuous flow of sterile medium with and without urea was maintained to avoid excessive accumulation of ammonia. Higher urease levels in the urea-treated set were attributed to substrate protection and/or induction of urease. In the experiment with thiourea, nitrate was substituted for ammonium salts in the medium. Lower urease activity in the absence of thiourea may be due to inhibition of urease by metabolically produced ammonia. Utilization of ammonia in protein synthesis, lower protease activity, and possible induction of urease by thiourea in growing tissues of cotyledons may lead to higher urease activity. High temperatures, low pH, and the presence of EDTA, hydroxyurea or actinomycin D cause a faster decrease in urease units. A central role for ammonia as a regulator for changes in urease levels is hypothesized. Such an hypothesis explains earlier work on the phylogenetic and ontogenetic occurrence of urease, as well as data presented here. Maximum synthesis of urease in cotyledons of ripening seeds may be facilitated through the storage of free ammonia as amides or compounds of the ornithine-urea cycle. In cotyledons of germinating seeds, proteolytic activity and reactions favoring breakdown and/or utilization of amides are predominant. Under these conditions, ammonium ions inhibit urease or repress new enzyme synthesis.

LITERATURE CITED

Ackermann, W. W., D. C. Cox, and S. Dinka. 1965. Control of histone and DNA synthesis and canavanine, puromycin, and poliovirus. Biochem. Biophys. Res. Commun. 19(6):745-750.

Allende, G. E., C. C. Allende, A. Caronado, E. Mardones, M. Gatica, J. Celis, and M. Matamala. 1965. The activating enzymes of amino acids. Int. Amer. Symp. Peacful Appl. Nucl. Engery 5:47-53. (Chem. Abstr. 63:11929a)

Anderson, A. M. 1962. Effect of gibberellic acid, kinetin-like substance, ceresan, and phencridane chloride on the germination of Panicum ramosum seeds. Proc. Int. Seed Test Ass. 27:730-741. (Chem. Abst. 58:6134b)

Annet, H. E. 1914. The urease content of certain Indian seeds. Biochem. J. 8:449-452.

Ball, E. 1946. Development in sterile culture of stem tips and subjacent regions of Tropaeolum majus L. and Lupinus albus L. Amer. J. Bot. 33:301-318.

Becker, G. E., P. A. Hui, and P. Albertshein. 1964. Synthesis of extracellular polysaccharide by suspensions of Acer pseudoplatanus cells. Plant Physiol. 39:913-920.

Beevers, L., L. E. Schrader, D. Flesher, and R. H. Hageman. 1965. The role of light and nitrate in the induction of nitrate reductase in radish cotyledons and maize seedlings. Plant Physiol. 40:691-698.

Berger, J., and G. S. Avery. 1941. Dehydrogenases of the Avena coleoptile. Amer. J. Bot. 30:290-297. (Chem. Abstr. 37:4768)

Bergmann, L. 1965. The effect of kinetin on the metabolism of plant tissue cultures, p. 171 to 178. In P. R. White and A. R. Grave (ed.) Proceedings of an international conference on plant tissue culture. McCutchan Publ. Corp., Berkeley, Calif.

Black, C. C., Jr., and T. E. Humphrays. 1962. Effects of 2, 4-dichlorophenoxyacetic acid on enzymes of glycolysis and the pentose phosphate cycle. Plant Physiol. 37:67-73 (Chem. Abstr. 52:7452d)

Bollard, E. G. 1959. Urease, urea and ureides in plants. Symp. Soc. Exp. Biol. 13:304-329.

Bollard, E. G., A. R. Cook, and N. A. Turner. 1968. Urea as sole source of nitrogen for plant growth. I. The development of urease activity in Spirodella oligorrhiza.

Bone, D. H. 1959. Metabolism of citrulline and ornithine in mung bean mitochondria. Plant Physiol. 34:171-175.

Bonner, J., and R. S. Bandurski. 1952. Studies on the physiology, pharmacology and biochemistry of the auxins. Annu. Rev. Plant Physiol. 3:59-86.

Brakke, M. K., and L. G. Nickell. 1951. Secretion of α-amylase by Rumex virus tumors in vitro properties and assay. Arch. Biochem. Biophys. 32:28-41.

Bryan, W. H., and E. H. Newcomb. 1954. Stimulation of pectin methylesterase activity of cultured tobacco pith by indoleacetic acid. Physiol. Plant 7:290-297.

Butler, R. D. 1967. The fine structure of senescing cotyledons of cucumber. J. Exp. Bot. 18:535-543. (Biol. Abstr. 48:109567)

Cohen, P. P., and G. W. Brown. 1960. Ammonia metabolism and urea biosynthesis. p. 161 to 231. In M. Florkin and H. S. Mason (ed.) Comparative Biochemistry. Vol. II. Academic Press, New York.

Conway, E. J. 1962. Microdiffusion analysis and volumetric error. 5th ed. Crosby Lockwood and Son Ltd., London. 467p.

Damodaran, M., and P. M. Sivaramakrishnan. 1937. New sources of urease for the determination of urea. Biochem. J. 31:1041-1046.

Davidson, J. D., and T. S. Winter. 1963. Analyzing for hydroxyurea in biological fluids. Cancer Chemother. Rep. 27:97.

Dawson, R. F., 1942. Nicotine synthesis in excised tobacco roots. Amer. J. Bot. 29:813-815.

De Ritis, F., and L. Scalfi. 1946. Antibacterial action of thiourea. III. Demonstration of its bacteriostatic nature. Boll. Soc. Ital. Biol. Sper. 22:699-701. (Chem. Abstr. 4:3167)

Digby, J., and F. Skoog. 1966. Cytokinin activation of thiamine biosynthesis in tobacco callus cultures. Plant Physiol. 41:647-652.

Eckert, L. K. Kleczkowski, and H. Kating. 1964. Intersuchungen zum Stoffwechsel des Harnstoffs in Mikroorganismen. V. Die spezifishen Aktivitäten der Enzyme des Ornithin-Cyclus bei Endomycopsis vernalis and Torulopsis utilis in Abangigkeit von der N-Quelle. Arch. Mikrobiol. 55:54-65. (Chem. Abstr. 65:20550)

Eckert, L., and H. Kating. 1968. Investigations on the metabolism of the urea in microorganism. VII. Dependence of the specific activity of the enzymes of the ornithine cycle and the urease on the culture period and N. source. Arch. Mikrobiol. 64:173-202.

Egami, F. K., Ohmachi, K. Lida, and S. Taniguchi. 1957. The nitrate-reducing systems in the cotyledons and seedling embryo of Vigna sesquipedalis during the stages of germination. Biokhimiya 22:122-134. (Chem. Abstr. 51:11484c)

Engelbrecht, A. H. P., and T. E. Weier. 1967. Chloroplast development in the germinating safflower (Carthamus tinctorius) cotyledon. Amer. J. Bot. 54:844-855.

Evans, H. J., and A. Nason. 1953. Pyridine nucleotide nitrate reductase from extracts of higher plants. Plant Physiol. 28:233-254.

Faanes, R., and P. Rogers. 1968. Roles of arginine and canavanine in the synthesis and repression of ornithine transcarbamylase by Escherichia coli. J. Bacteriol. 96:409-420. (Biol. Abstr. 49:41945e)

Ferrari, T. E., and J. E. Varner. 1969. Substrate induction of nitrate reductase in barley aleurone layer. Plant Physiol. 44: 85-88.

Filner, P., L. Wrary, and J. E. Varner. 1969. Enzyme induction in higher plants. Science 165:358-366.

Fishbein, W. N., and P. P. Carbone. 1965. Urease catalysis. II. Inhibition of the enzyme by hydroxyurea, hydroxylamine, and acetohydroxamic acid. J. Biol. Chem. 240:2407-2414.

Floyd, A., and H. Beevers. 1963. Proteolytic enzymes from germination seeds of Cucurbita maxima. Plant Physiol. 38:xxi.

Fluey, C. 1948. Action of thiourea on Aspergillus niger. The special role of nitrogen. Biol. Aoc. Suisse Botan. No. 58:462-477. (Chem. Abstr. 43:3880d)

Frenkel, E. P., W. N. Skinner, and J. D. Smiley. 1964. A metabolic effect induced by hydroxyurea. Cancer Chemother. Rep. 40:19.

Gainor, C., and F. D. Crisley. 1961. Proteolytic activity of crude stem extracts from normal and tumor tissues of plants. Nature 190-1031-1032.

Gainor, C., and F. D. Crisley. 1962. Factors affecting proteolytic activity of extracts from normal and tumor tissues of tobacco. Nature 193:1076-1077.

Gale, G. R., 1964. Effect of hydroxyurea on the incorporation of thymidine in Ehrlich ascites tumor cells. Biochem. Pharm. 13: 1377-1382. (Chem. Abstr. 61:15197g)

Gale, G. R., S. M. Kendall, H. H. McLain, and S. Dubois. 1964. Effect of hydroxyurea on Pseudomonas aeruginosa. Cancer Res. 24:1012-1019. (Chem. Abstr. 61:4741g)

Gale, G. R. 1965. Inhibition of urease by hydroxyurea. Biochem. Pharm. 14:693-698.

Gall, H. J. F. 1948. Effects of 2, 4-dichlorophenoxyacetic acid on starch digestion and reducing activity in bean tissue cultures. Bot. Gaz. 110:319-323. (Chem. Abstr. 43:2286d)

Gallant, J., and R. Stapleton. 1964. Depression of alkaline phosphate synthesis by chloramphenicol and canavanine inhibition. J. Mol. Biol. 8(4):442-451. (Biol. Abstr. 45:69624)

Galston, A. W. 1951. Sur la relation entre le croissance des cultures des tissue végétaux et leur teneur en catalase. Compt. Rend. 232:1505-1507.

Galston, A. W., and L. V. Dalber. 1954. The adaptive formation and physiological significance of indolacetic acid oxidase. Amer. J. Bot. 41:373-380.

Gautheret, R. J. 1955. The nutrition of plant tissue cultures. Annu. Rev. Plant Physiol. 6:433-484.

Glasziou, K. T., and J. C. Waldron. 1964. The regulation of acid invertase levels in sugar-cane: Effects of sugars, sugar derivatives and polyhydric alcohols. Aust. J. Biol. Sci. 17:609-618.

Goksöyer, J., E. Boeri, and R. K. Bonnichsen. 1953. The variation of ADH and catalase activity during the germination of green pea (Pisum sativum). Acta Chem. Scand. 7:657-662.

Gornall, A. G., C. J. Bardawill, and M. M. David. 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177:751.

Granick, S. 1938. Urease distribution in Soja max. Plant Physiol. 13: 29-54.

Grenson, M., M. Mousset, J. M. Wiame, and J. Bechet. 1966. Multiplicity of amino acid permeases in Saccharomyces cerevisiae. I. Evidence for a specific arginine transporting system. Biochim. Biophys. Acta 127:325-338. (Chem. Abstr. 66:630r)

Gupta, Y. P., and N. B. Das. 1959. Urease in wheat leaves. Ann. Biochem. Exp. Med. (Calcutta) 19:199-204. (Biol. Abstr. 35:24969)

Haeker, M., and H. Mohr. 1966. The degradation of reserve fat in cotyledons of Sinapis alba L. under the influence of phytochrome Planta 68:215-223.

Hageman, R. H., and D. Flesher. 1960. The effect of an anaerobic environment on the activity of alcohol dehydrogenase and other enzymes of corn seedlings. Arch. Biochem. Biophys. 87:203-209.

Heilmeyer, L., J. D. Macke, and H. Holzer. 1967. Reactivation of ammonium-inactivated glutamine synthesis in Eshcerichia coli. Europe. J. Biochem. 2:399-402.

Hipkiss, A. R. 1967. The effect of streptomycin on gametogenesis in Chlamydomonas reinhardi. Life Sci. 6(6):669-672. (Chem. Abstr. 66:92712)

Ho, Hon-Kai, and Chi Shen. 1966. The distribution of canavanine in Tiawan plants and its metabolic change during germination. Chung Kuo Nung Yeh Hua Hsueh Hui Chih, Spec. Issue (Dec.), p. 23-31. (Chem. Abstr. 67:97625b)

Hoare, J. P., and K. J. Laidler. 1953. The molecular kinetics of the urea-urease system. II. The inhibition by products. J. Amer. Chem. Soc. 75:866-871.

Hodson, R. C. 1966. Studies on urea metabolism in the alga Chlorella. Ph.D. dissertation, 159 p., Cornell Unv. Authorized Reprint by Microfilm--Xerography, Unv. Microfilm, Ann arbor, Michigan. (Diss. Abstr. 66-5594)

Hoffman, T., and H. Lees. 1953. The biochemistry of the nitrifying organisms. IV. The respiration and intermediary metabolism of Nitrosomonas. Biochem. J. 54:579-583.

Israel, H. W., and F. C. Steward. 1966. The fine structure of quiescent and growing carrot cells. Its relation to growth induction. Ann. Bot. 30:63-79.

Jaspars, E. M. J., and H. Veldstra. 1965a. An α-amylase from tobacco crown-gall tissue cultures. I. Purification and some properties of the enzyme. Pattern of α-amylase isoenzymes in different tobacco tissues. Physiol. Plant. 18:604-625.

_____. 1965b. An α-amylase from tobacco crown-gall tissue cultures. II. Measurements of activity in media and tissues. Physiol. Plant. 18:626-634. Ibid

Jensen, H. L., and H. Sorenson. 1952. Influence of some organic sulfur compounds and enzymes on Nitrosomas europea. Acta Agr. Scand. 2:295-308.

Jensen, H. L. 1957. Biological transformation of thiourea. State Lab. Soil Crop Res. Lyngby Denmark. Arch. Mikrobiol. 28:145-152. (Chem. Abstr. 53:14213e)

Johnson, L. P. V. 1946. Effect of chemical treatments on the germination of forest tree seeds. Forestry Chron. 22:17-24.

Karstens, W. H. K., and V. De Messter-Manger Cats. 1960. The cultivation of plant tissues in vitro with starch as a carbon source. Acta Bot. Neerl. 9:263-274.

Kefford, N. P., J. A. Zwar, and M. I. Bruce. 1965. Enhancement of lettuce seed by some urea derivatives. Planta 67:103-106.

Kiesel, A. 1911. Über den fermentation Abbau des Arginins in Pflanzen. Hoppe-Seyl. Z. Physiol. Chem. 75:169-196.

Kingdon, A. S., B. M. Shapiro, and E. R. Stadtman. 1967. Regulation of glutamine synthetase. VII. ATP: glutamine synthetase adenyltransferase, and enzyme that catalyzes alterations in regulatory properties of glutamine synthetase. Proc. Nat. Acad. Sci. (U. S.) 58:1703-1710.

Kinoshita, S., H. Samejima, C. Fujita, K. Tanaka, and M. Suzuki. 1959. L-canavanine L-glutamate. Kyowa Fermentation Industry Co. Ltd. Japan. 1933(66). Feb. 10, Appl. April 11, 1 p. (Chem. Abstr. 64:14275g)

Kistiakowski, G. B., and W. H. R. Shaw. 1953. On the mechanism of the inhibition of urease. J. Amer. Chem. Soc. 75:866-871. (Chem. Abstr. 47.5969)

Klein, G., and E. Farkas. 1930. Der mikrochemische Nachweis der alkaloide der Pflanze. XIV. Der Nachweis von cytisin. Österr. Bot. Z. 79:107. In: H. S. Mckee. 1962. Nitrogen Metabolism in Plants. Clarendon Press, Oxford, 290 p.

Kobashi, K., J. Hase, and K. Uehara. 1962. Specific inhibition of urease by hydroxamic acids. Biochim. Biophys. Acta 65:380-383.

König, C., H. Kaltwasser, and H. G. Schlegel. 1966. Die Bildung von Urease nach Verbrauch der äusseren N-Quelle bei Hydrogenomonas H 16. Arch. Mikrobiol. 53:231-241.(Biol. Abstr. 64:82979)

Konobu, K., and T. Suzuki. 1963. Action of canavanine on the growth of Neurospora crassa arginine-requiring mutants. Yakugaku Zasshi 83:25-29. (Chem. Abstr. 59:1990f)

Lashen, E., and R. L. Starkey. 1964. Dissimilation of thiourea and substituted thioureas. Bacteriol. Proc. 64:5.

Leone, E. 1955. Uricase, p. 485 to 489. In S. P. Colowick and N. O. Kaplan (ed.). Methods in enzymology. Vol. II. Academic Press, New York.

Lipetz, J., and A. W. Glaston. 1959. Indoleacetic acid oxidase and peroxidase activities in normal and crown-gall tissue cultures of Parthenocissus tricuspidata. Amer. J. Bot. 46:193.

Loo, S. W. 1945. Cultivation of excised stem-tips of asparagus in vitro. Amer. J. Bot. 32:13-17.

Mann, J., C. Steinhart, and S. Mudd. 1963. Alkaloid and plant metabolism. V. The distribution of tyramine methylpherase during germination of barley. J. Biol. Chem. 238:676-681.

Matsumato, H., Y. Hasegawa, M. Kobayashi, and E. Takahashi. 1968. Inducible formation of urease in Canavalia ensiformis. Physiol. Plant. 21:872-881.

Mayer, A. M., and M. Evanari. 1951. Influence of two germination inhibitors (coumarin and 2, 4-D) on germination in conjuction with thiourea and cysteine. Bull. Res. Council Israel 1:125-129. (Chem. Abstr. 46:6311b)

Mayer, A. M. 1954. Polyphenol oxidases of lettuce seeds and their inhibitors. Enzymologia 16:277-284.

Mayer, A. M., and A. Poljakoff-Mayber. 1963. The germination of seeds. Pergamon Press, New York. 236 p.

Mehta, S. L., M. S. Naik, and N. B. Das. 1967. Induced synthesis of urease in Azotobacter vinelandii. Indian J. Biochem. 4:194-196. (Chem. Abstr. 68:75891d)

Meister, A. 1965. Biochemistry of amino acids. Vol. II, p. 685-707. Academic Press, New York.

Miedema, E., and P. F. Kruse. 1966. Effect of canavanine on proliferation and metabolism of human cells in vitro. Proc. Soc. Exp. Biol. Med. 121:1220-1222. (Biol. Abstr. 47:97669)

Mikaelson, K. 1955. The protective property of thiourea against radiation induced chromosome aberrations. Exp. Cell. Res. 8:400-403.

Miller, C. O. 1963. Kinetin and kinetin-like compounds, p. 194-202. In: H. F. Linskens and M. V. Tracey (ed.). Methods of plant analysis. Vol. VI. Springer Verlag, Berlin.

_____. 1965. Evidence for the natural occurrence of zeatin and derivatives: Compounds from maize which promote cell division. Proc. Nat. Acad. Sci. 54:1052-1058.

Morfaux, J. N., and P. Dupuy. 1966. Comparison of excretion of amino acids by a strain of Saccharomyces cerevisiae and a mutant resistant to canavanine. C. R. Compt. Rend. Acad. Sci., Paris 263:1224-1226. (Chem. Abstr. 66:26790v)

Mueller, E. 1963. Physiological action of canavanine. Naturwiss. 50(13):479. (Chem. Abstr. 59:7846e)

Nakatsu, S., S. Haratake, Z. Sakurai, N. J. Z. Nishihara, and M. Hayashida. 1964. The change of quantity of canavanine in leguminous plants in the process of germination, growth, and fructification. Seikagaky 36:467-471. (Chem. Abstr. 62:829b)

Nandi, D. L. 1958. Studies on the amino acid composition and urease activity of some leguminous seeds during germination in different organic nitrogen media. Naturwiss. 45:246-247.

Nason, A., and H. J. Evans. 1955. Nitrate reductase from Neurospora, p. 411-419. In: S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology. Vol. II. Academic Press, New York.

Neumann, J. 1964. The effect of actinomycin D on lettuce seedlings and its differential uptake by roots and shoots. Physiol. Plant. 17:363:370.

Newcomb, E. H. 1951. Effect of auxin on ascorbic acid oxidase activity in tobacco pith cells. Proc. Soc. Exp. Biol. Med. 76:504-509.

Nicolas, E., and G. Nicolas. 1925. Observations sur L' influence de l'uree, de la sulfo-uree et de l'allylsulfo-uree sur les végétaux syperius. Compt. Rend. Acad. Sci. 180:1286-1289.

Opik, H. 1965. The form of nuclei in the storage cells of the cotyledons of germinating seeds of Phaseolus vulgaris L. Exp. Cell. Res. 38: 517-522.

_____. 1966. Changes in cell-fine structure in the cotyledons of Phaseolus vulgaris L. during germination. J. Exp. Bot. 17:427-439.

Ovcharov, K. E. 1937. The production of thiourea by fungi. Compt. Rend. Acad. Sci. U. R. S. S. 16:461-464. (Chem. Abst. 32:1745)

Pallidina, T. A., and Y. A. Pervosa. 1965. Effect of germination stimulators on potato shoots and tubers. Fisiol. Fast. 13:283-289. (Chem. Abstr. 64:20538)

Penner, D., and F. M. Ashton. 1966. Proteolytic enzyme control in squash cotyledons. Nature 212:935-936.

Poljakoff-Mayber, A. 1953. Peroxidase activity in germination lettuce seeds. Enzymologia 16:122-124. (Chem. Abstr. 48:9478i)

Presley, H. J., and L. Fowden. 1965. Acid phosphatase and isocitrase production during seed germination. Phytochemistry 4:169-176.

Quastel, J. H., and R. G. Scholefield. 1949. Influence of organic nitrogen compounds on nitrification in soil. Nature 164:1068.

Raghavan, V. 1964. Effect of certain organic nitrogen compounds on growth in vitro of seedlings of Cattleya. Bot. Gaz. 125(4):260-267. (Chem. Abstr. 62:8346g)

Roche, J., and Y. Yagi. 1952. Fixation of I^{131} by algae-iodinated constituents of Laminaria. Compt. Rend. Soc. Biol. 146:642-645.

Roon, R. J., and B. Leuenberg. 1968. An adenosine triphosphate-dependent, avidin-sensitive enzymatic cleavage of urea in yeast and green algae. J. Biol. Chem. 243:5213-5215.

Ruban, E. I., and L. B. Lobyreva. 1966. Effect of thiols on accumulation of free amino acids in bacterial cultures. Mibrobiologiya 35:784-786. (Chem. Abstr. 8960j)

Satarova, N. A. 1953. Effect of chemical stimulants on changes of colloido-chemical properties of protoplasm and activity of peroxidase in potato tubers. Doklady Akad. Nawk. S. S. S. R. 93:1110-1122. (Chem. Abstr. 48:5298g)

Schachtele, C. F., and P. Rogers. 1968. Mechanism of canavanine death in Escherichia coli. I. Effect of canavanine on macromolecular synthesis. J. Mol. Biol. 33:843-860. (Chem. Abstr. 69: 33785n)

Schachtele, C. F., D. L. Anderson, and P. Rogers. 1968. Mechanism of canavanine death in Escherichia coli. II. Membrane-bound canavanyl-protein and nuclear disruption. J. Mol. Biol. 33:861-872. (Chem. Abst. 69:33786)

Scott, K. J., J. Daly, and H. H. Smith. 1964. Effect of indoleactic acid and kinetin on the activities of enzymes of the hexose monophosphate shunt in tissue cultures of Nicotiana. Plant Physiol. 39:709-711.

Sehgal, P. P., and A. W. Naylor. 1966. Ontogenetic study of urease in jackbeans, Canavalia ensiformis (L.). D. C. Bot. Gaz. 127:27-34.

Shain, Y., and A. M. Mayer. 1965. Proteolytic enzymes and endogenous trypsin inhibitor in germinating lettuce seeds. Physiol. Plant. 18:853-859. (Chem. Abstr. 63:16775g)

_____. 1968. Activation of enzymes during germination: Amylopectin-1, 6-glucosidase in peas. Physiol. Plant. 21:765-776.

Shepherd, C. J., and M. Mandryk. 1964. Effects of metabolites and antimetabolites on the sporulation of Peronospora tabacina on tobacco leaf discs. Australian J. Biol. Sci. 17(4):878-891. (Chem. Abstr. 62:3338h)

Simola, L. K. 1967. The effect of some nonprotein amino acids on pollen-tube growth in 5 species of the Vicieae. Planta 77: 287-297. (Chem. Abstr. 68:19648t)

Staba, E. J. 1963. The biosynthetic potential of plant tissue cultures. In: Developments in Industrial Microbiology 4:193-198. AIBS (Amer. Inst. Biol. Sci.), Washington, D. C.

Steinhart, C. E., J. D. Mann, and S. H. Mudd. 1964. Aklaloid and plant metabolism. VII. The kinetin produced elevation in tyramine methylpherase levels. Plant Physiol. 39:1030-1038.

Straus, J. 1962. Invertase in cell walls of plant tissue cultures. Plant Physiol. 37:342-348.

Straus, J., and W. C. Campbell. 1963. Release of enzymes by plant tissue cultures. Life Sci. 1:50-62.

Sutcliffe, J. F. 1962. Mineral salts absorption in plants. Pergamon Press, New York. 194 p.

Suzuki, Y. 1952. Physiological function of urease. Kagaku (Science), Tokyo 22:264-265. (Chem. Abstr. 46:10306d)

Swain, R. R., and E. E. Dekker. 1966a. Seed germination studies. I. Purification and properties of an α-amylase from cotyledons of germination peas. Biochim. Biophys. Acta 122:75-86.

_____. 1966b. Seed germination studies. II. Pathways for starch degradation in germination pea seedlings. Ibid. 122:-87-100.

Tai, K. 1953. Distribution of urease in plant seeds. Science Repts. Shiga Agr. Coll. Ser. 1(4):57-59. (Chem. Abstr. 6598g)

Takeuchi, T. 1909. On the occurrence of urease in higher plants. Coll. Agr. Tokyo Imp. Univ. 1:1-14.

Tanis, R. J., and A. W. Naylor. 1968. Physical and chemical studies of a low-molecular-weight form of urease. Biochem. J. 108:771-777.

Tolbert, N. E., and M. S. Cohan. 1953. Activation of glycolic acid oxidase in plants. J. Biol. Chem. 204:639-648.

Tschiersch, B. 1961. The biosynthesis of canavanine. Flora 150:87. (Chem. Abstr. 59:11900g)

_____. 1963. Canavanine as the cause of the incompatibility of Canavalia grafts. Flora 153:73-86. (Chem. Abstr. 63:4658f)

_____. 1966. Non-competitive inhibition of enzymatic (alcohol dehydrogenase from yeast) reactions by canavanine. Tetrahedron Lett. 28:3237-3241. (Biol. Abstr. 48:56238)

Turner, B. L., and J. B. Harbone. 1967. Distribution of canavanine in the plant kingdom. Phytochemistry 6:863-866.

Van Overbeek, J., E. M. Conklin, and A. F. Blakeslee. 1942. Cultivation in vitro of small Datura embryos. Amer. J. Bot. 29: 472-477.

Varner, J. E. 1960. Urease, p. 247-251. In: P. D. Boyer, H. Lardy, and K. Myrback (ed.). The enzymes. 2nd ed., Vol. IV. Academic Press, Inc., New York. 631p.

Varner, J. E., and G. R. Chandra. 1964. Hormonal control of enzyme synthesis in barley endosperm. Proc. Nat. Acad. Sci. (U.S.) 52:100-106.

Venis, M. A. 1964. Induction of enzymatic activity by indolyl-3-acetic acid and its dependence on synthesis of ribonucleic acid. Nature 202-900-901.

_____. 1966. Induction of enzyme activity in pea tissue by animal hormones and by puromycin. Nature 210:534-535.

White, P. R. 1933. Liquid media as substrata for the culturing of isolated root tips. Biol. Zentralbl. 53:359-364.

Wiesner, W. R., and H. N. Guttman. 1966. Enzymatic differences between carrot taproot secondary phloem and tissue cultures derived from secondary phloem. Exp. Biol. Med. 121:523-524.

Wiley, L., and F. M. Ashton. 1967. Influence of the embryonic axis on protein hydrolysis in cotyledons of Cucurbita maxima. Physiol. Plant. 20:688-695. (Chem. Abstr. 67:90271z)

Williams, S. E., and G. E. Hunt. 1967. Canavanine distribution in jackbean fruit during fruit growth. Planta 77(2):192-202. (Biol. Abstr. 49:36691)

Williams, W. T. 1950. Function of urease in Citrullus seeds. Nature 165:79.

Williams, W. T., and P. C. Sharma. 1954. The function of urease in Citrullus seeds. J. Exp. Bot. 5:136-157.

Young, J. L., and J. E. Varner. 1959. Enzyme synthesis in the cotyledons of germinating seeds. Arch. Biochem. Biophys. 84:71-78.

Zelenin, M. M. 1939. Quantitative determination of thiourea in fungal extracts by spectral method. Bull. Acad. Sci. U. R. S. S. Sér. Biol. 832-837. (Chem. Abstr. 34:4404)

(149)

Regulation of Urease in Cotyledons

PREM P. SEHGAL AND JAMES A. COOK
East Carolina University

The factors controlling urease levels in cotyledons of jack beans were studied under sterile conditions. In culture, the urease content declined as rapidly as in cotyledons harvested from seedlings of different ages. High temperatures (35°) or low pH (4.5) caused a faster decrease in urease activity as compared to the controls (75°F, pH 5.8). The addition of dithiothreitol — a sulfhydryl compound, urea or thiourea lead to increased urease activity on fresh weight and protein basis. In experiments with urea, a continuous flow of sterile medium was maintained to avoid excessive accumulation of ammonium ions in cotyledons. Higher urease activity in urea is attributed to substrate protection. Thiourea, a competitive inhibitor of the enzyme gave best preservation of urease in contrast to urea-treated and untreated cotyledons. Thiourea and other substituted ureas are used as herbicides. Possible mechanism of action of thiourea will be discussed.

Reprinted from *The ASB Bulletin*,
Vol. 16, No. 2, April 1969, p. 67.