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METABOLISM AND ENERGETICS IN HYPOGRAVITY:

AMINO ACID METABOLISM IN HIGHER PLANTS

FINAL REPORT

by

Mendel Mazelis

Prepared under Contract No. NAS2-8685

AMES RESEARCH CENTER

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

UNCLASSIFIED

Higher Plant Metabolism and Energetics in Hypogravity: Amino Acid Metabolism

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Final Report

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The University of California

Davis, California 95616

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General Summary

This final report summarizes our laboratory's investigation into the amino acid metabolism of dwarf marigolds (Tagetes patula var. Petite Gold) exposed to an environment of simulated hypogravity. Using both in vivo, and/or in vitro studies, the following effects of hypogravitational stress have been shown:

1) Increased proline incorporation into cell wall protein.
2) Inhibition of amino acid decarboxylation.
3) Decrease in glutamic acid decarboxylase activity.
4) Decrease in the relative amount of a number of soluble amino acids present in deproteinized extracts of marigold leaves.

It is concluded from these data that there are several rapid, major alterations in amino acid metabolism associated with hypogravitational stress in marigolds. The mechanism(s) and generality of these effects with regard to other species is still unknown.

Introduction

Over the years considerable progress has been made in understanding the processes of growth and development in plants. This increased knowledge has stimulated research in several areas, one of which, plant ecology, attempts to explain a plant's response to its environment in terms of the physiological processes affected. Gravity is one environmental parameter whose morphological and physiological effect on plants has been intensely studied (1-3).

Although much data has been accumulated on geotropic responses (i.e. positive and negative geotropism and epinasty) and its hormonal regulation, the actual mechanism of gravi-perception and subsequent changes in growth habit remain unclear, especially in regard to the events occurring at the subcellular level. Indeed, the paucity of reports dealing with effect of gravitational stress at this level is surprising, although recent reports by Shen-Miller and Miller (4,5) and Waber et al. (6) plus some earlier results from the Biosatellite II mission (7) give some insight in this direction. It was the intention of this research effort to attempt a start on a fundamental understanding of the cellular consequences of gravitational stress by analyzing the effects of hypo-gravitational stress on amino acid metabolism. The subsequent sections of this report will detail our research efforts in this area.

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Section I

Effect of Simulated Hypogravity on the Incorporation of Proline into Cell Wall Protein

[1]Supported by National Aeronautics and Space Administration Contract NAS 2-6633.

Abstract

Proline-U-^{14}C was fed to shoots of intact Tagetes patula L. grown normally, on horizontal clinostats, or on vertical clinostats rotating at 15 rph. After various periods of incubation the incorporation of radioactivity into salt-extractable material from the cell walls of stems, petioles, leaves and flowers was determined. The cell walls of the gravity-compensated plants (grown on horizontal clinostats) had the highest amount of salt-extractable radioactivity. A two to nine-fold increase was observed in comparison to either the normal or vertical clinostat plant controls. Some physico-chemical properties of the salt-extractable fraction suggest that it consists of highly charged, small molecular weight entities, possibly short chain peptides. On acid hydrolysis this material yields radioactive aspartic acid, glutamic acid and proline. The presence of labelled hydroxyproline is suggested. After either base or acid hydrolysis of the cell walls of leaves, it was found that about four times the amount of ^{14}C was incorporated in the hypogravity-grown plant compared to the controls. It appears likely that extensibility changes in tissues under simulated hypogravity require additional cell wall protein.

The biosynthesis of the protein component of the primary cell wall in higher plants has been under intensive investigation for a number of years (14). The presence of a cell wall protein precursor which can be extracted from the cell wall by the use of salt solutions has been well-documented (3,5,19). The kinetics of formation of this precursor has the typical characteristics of a component in transit (19).

When plants are rotated horizontally on clinostats a typical response is the appearance of epinastic curvatures of the leaves. A similar response was observed under hypogravity conditions on orbital flights (13). It appears reasonable that during epinasty, changes in extensibility would occur in the leaf or petiole cell wall, and therefore a biochemical change correlated with this phenomenon would be an increase in the amount of cell wall protein precursors. The present report gives the results of a study comparing the synthesis of salt-extractable cell wall protein precursors in normal plants with those on a horizontal clinostat simulating hypogravity.

Materials and Methods

Growth conditions

Seeds of Tagetes patula L. var. Helen Chapman A were sown in plastic pots filled with peat moss:sand:sawdust (1:1:1) in a greenhouse at 80°/70° day/night temperatures and 16 hour day length. The pots were irrigated daily to field capacity with a modified half-strength Hoagland's solution I. The culture solution modification consisted of supplying no additional micronutrients and iron was furnished in the chelated form "Fe-138" at a concentration of 2 ppm. The ages of plants used for experiments ranged from 28 to 68 days.

Radioactive material

L-Proline-U-^{14}C with a specific activity of 200 mc/mM was supplied by International Chemical and Nuclear Corporation.

Incubation procedure

A slight modification of the cotton wick method described by Misusaki et al. (18) was followed. In our method, the wick was threaded through capillary tubing which is directly inserted into the stem of the plants. This device reduces evaporative loss.

Aliquots of the proline ^{14}C solution containing 5, 10 or 15 μc were used. After the radioactive solution had been absorbed, the vial was rinsed twice with distilled water. Plants were then mounted on horizontal or vertical clinostats rotating at 15 rph. Control plants were simply left on the bench. The incubation period was 24, 36, 68 or 72 hours. The plants were under fluorescent light with an intensity of 20 to 40 microeinsteins $m^{-2}sec^{-1}$ in the spectrum between 400 and 700 nm.

Isolation of cell walls and extraction of ionically bound wall material

Tissues were weighed and frozen in solid carbon dioxide and ground in water by means of mortar and pestle. The homogenate was centrifuged at 120 X g for 30 seconds and the pellet was washed ten times with distilled water.

The cell walls were packed into columns and eluted with 0.125 M NaCl. Preliminary experiments showed that a 0.125 M solution was a more effective extractant than a concentration of 0.25 M or 0.50 M. Aliquots of the eluates were assayed for radioactivity in a liquid scintillation counter using Bray's solution (2).

Hydrolysis of cell wall material and SEM[1]

The cell walls were hydrolyzed with 6 N HCl in sealed tubes at 100° overnight. The hydrolysate was centrifuged and the supernatant solution assayed for radioactivity.

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[1]Abbreviation: SEM: Salt-extractable cell wall material.

Incorporation of label into cell wall glycoproteins was determined by hydrolysing the cell walls with 0.4 N $Ba(OH)_2$ in sealed tubes at 90° for 6 hours. The hydrolysate was neutralized with H_2SO_4 and the insoluble Ba salt removed by centrifugation. The clear supernatant solution was evaporated to dryness, dissolved in 1.0 ml 0.1 N acetic acid, and an aliquot assayed for radioactivity.

The SEM was hydrolysed with 6 N HCl by the same procedure described for the cell walls.

Chromatographic procedures

a) Ion-exchange methods

The SEM was dialysed overnight against a large volume of deionised distilled water in the cold. The internal dialysate and the external dialysis medium were each assayed for radioactivity. A portion of the former was placed on a column of DOWEX 50 (H^+) 20-50 mesh (1 X 10 cm) and eluted with 80% ethanol followed by an ethanol:ammonia solution (3:1 v/v). Aliquots of the eluate from each solvent were assayed for radioactivity.

b) Paper chromatography

The remaining portion of the dialysed hydrolysate of the SEM was evaporated on a hot water bath with a stream of air directed over the sample. The dried residue was dissolved in water and dried again as before so as to remove the HCl completely. The dried sample was then resuspended in water spotted on filter paper and irrigated with phenol:water (10:2 v/v) solution. The chromatograms were treated with either 0.2% isatin in acetone or 0.2% ninhydrin in 95% ethanol. After being heated at 100° for 10 minutes the spots obtained were compared with proline and hydroxyproline standards.

The radioactive components of the chromatographed hydrolysate of SEM were located by use of a strip counter.

Ultrafiltration

The SEM was passed through an ultrafilter under a nitrogen pressure of approximately 15 psi. All compounds having a molecular weight less than 1000 passed through the filter membrane.

Electrophoresis

Disc gel electrophoresis of the dialysed SEM was performed according to the procedure described by Davis (8) and the protein bands visualized by use of Coomassie blue.

Results

The stems and leaves of T. patula exhibited typical epinastic curvatures when plants were subjected to simulated hypogravity by rotation on horizontal clinostats. After infiltration with ^{14}C-proline the radioactivity in the SEM of cell walls from shoots of plants subjected to hypogravity was about three-fold higher than the amount found in shoots of normal control plants (Fig. 1). The amount of radioactivity in the SEM of control plants and plants rotated on certical clinostats is essentially the same.

The incorporation of radioactivity into the SEM of horizontal plants was higher in every tissue than the corresponding tissue of control plants (Fig. 2). Differences between the two treatments were found of two to nine fold depending on the particular tissue. Differences between treatments tend to be more marked in tissues from older plants compared to younger plants. This is evidenced by a comparison of Figs. 1 and 2 with the data in Table I.

The minimum time necessary for hypogravity to exert its effect on the SEM appears to be 36 hours (Table I). In shorter times, e.g. 24 hours, the SEM in horizontal plants is only slightly higher if at all than in the control. After a presentation time of 36 hours, however, the effect of hypogravity is evident and all tissues of the plants on horizontal clinostats show considerably higher

amounts of SEM than those of the controls.

After acid hydrolysis of cell walls of the leaves, the amount of label recovered was about four times greater from plants under hypogravity than the corresponding tissue of control plants. This is also true after basic hydrolysis (Fig. 3).

The ratio of incorporation in the acid hydrolysate to the base hydrolysate does not appear to be altered by the hypogravity treatment. This ratio was 2.7 and 2.6 for the control and horizontal clinostat-treated plants respectively (Fig. 3).

Physicochemical properties of SEM

The salt extractable cell wall material binds firmly to a Dowex 50 W (H^+) column and is easily eluted by ethanolic ammonia. It migrates with the buffer front in disc gel electrophoresis. It is non-dialysable for the most part since only a trace of label appears in the medium. The labelled material, however, passes through an ultrafilter membrane with a molecular weight cut off of 1000. It is ninhydrin positive. Scanning of paper strip chromatograms of the acid hydrolysate revealed the presence of labelled proline, aspartic acid, glutamic acid, and some indication of hydroxyproline.

Discussion

The salt-extractable fraction has been considered to consist of protein precursors that are ionically bound to the cell walls (3,14). It presumably represents components in transit from the cytoplasm to the cell walls (19) where they are eventually incorporated into the structural protein matrix. The physico-chemical properties of these protein precursors (SEM) were incompletely characterized in this study. However, based on their rapid electrophoretic mobility, their behavior on ultrafilters, ion exchange chromatography and paper chromatography, one may conclude that they are highly charged small molecular

weight entities, possibly short chain peptides. The low incorporation of ^{14}C into the hydroxyproline residues of the "peptides" suggests that only a few proline molecules in these peptides were hydroxylated. In intact systems, hydroxyproline synthesis is not as rapid as in loose proliferating cells in suspension cultures (21).

Old tissues were more responsive than young tissues possibly because polar transport of auxin in old tissues is more sluggish (16,17) and hence the chances for lateral displacement of auxin by hypogravity are increased. Our data suggest that hypogravity induces the synthesis of protein. This induction may be ethylene-mediated (4) since hypogravity enhances ethylene production (15). Ethylene action may also involve increased permeability of the plasmalemma (9). Thus the high amount of SEM in hypogravity tissues may be due not only to ethylene-mediated induction of protein synthesis but also to the higher rate of diffusion of the SEM across the plasmalemma. Exactly how the newly synthesized proteins participate in the characteristic epinastic curvatures of gravity-compensated plants is a matter for conjecture. It is conceivable that the new protein becomes bonded with polysaccharide chains, and such interaction might alter the microfibrillar arrangements of these polysaccharides so as to allow extensibility changes.

Apart from effects on ethylene production, hypogravity may effect epinastic curvatures through other auxin dependent processes such as:

(a) activation of cell wall loosening enzymes: For example, Bara and Gordon (1), have reported an increase in cellulase activity when seedlings of Helianthus annuus were subjected to hypogravity.

(b) stimulation of a H^+ pump on the plasmalemma (6,20): Secretion of H^+ from the cytoplasm to the cell wall leads to a lowered pH in the latter. The more acid pH may exert an effect directly on certain chemical bonds or

indirectly by providing a more optimum pH (12) for the activity of certain enzymes.

(c) osmotic effects: Hypogravity enhances the synthesis of starch and sugars (10) which results in an increased osmotic potential. The subsequent absorption of water would result in cell elongation.

Although the exact sequence of events resulting in epinastic curvature is yet to be clarified, it appears likely that the primary motive force is provided by either auxin transport, synthesis or a combination of both.

Acknowledgments

The authors wish to thank Dr. W. P. Hackett and the staff of the Department of Environmental Horticulture for providing the greenhouse facilities.

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Table I. Incorporation of ^{14}C from L-proline-^{14}C into SEM as a function of incubation time.

Twenty eight-day-old T. patula plants were infiltrated with L-proline-U-^{14}C as described in "Materials and Methods". SEM was isolated from the specific tissue as described in the text.

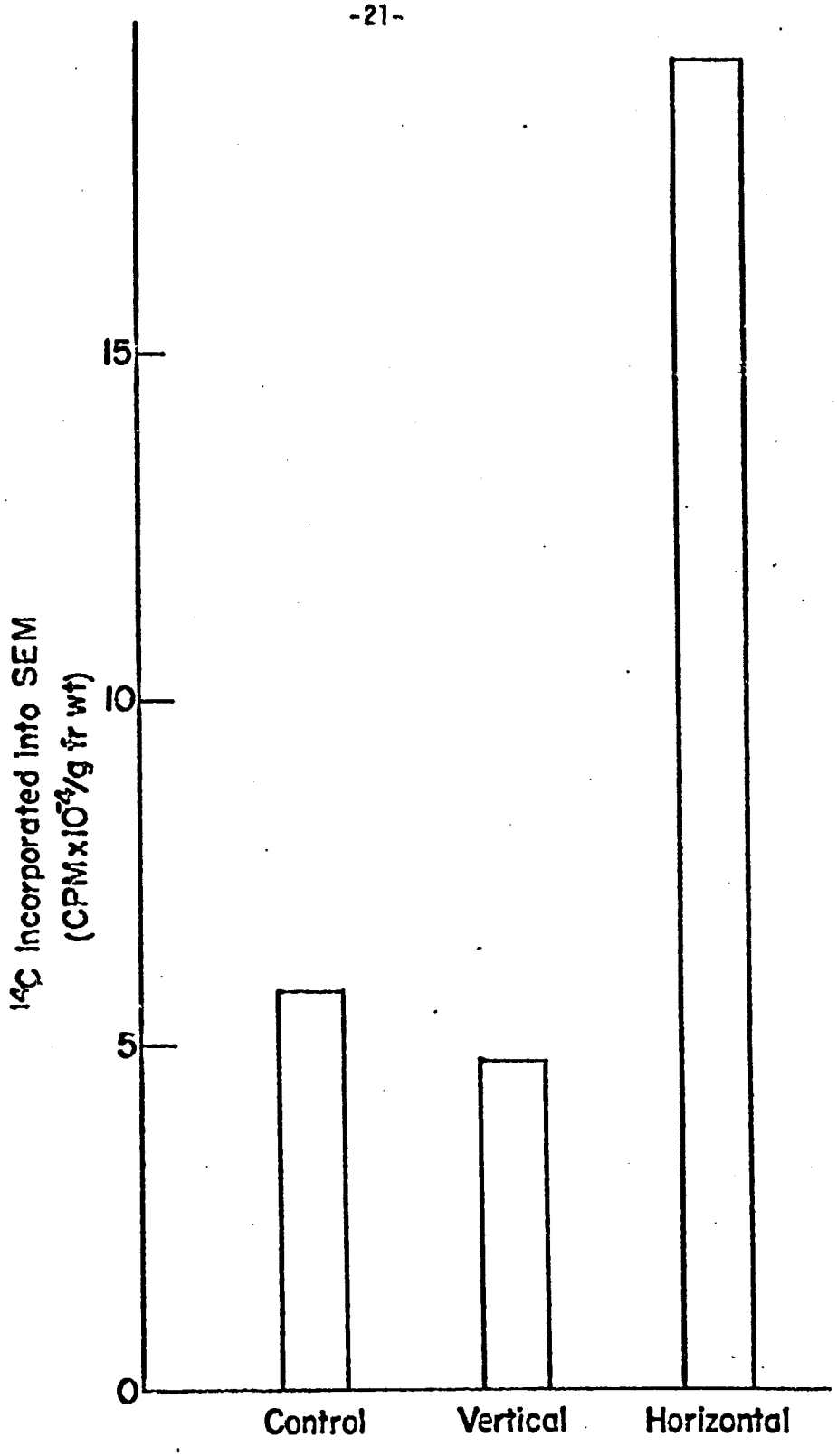
Incubation time (Hours)	^{14}C Incorporated (cpm X 10^{-3} X g. fr. wt.$^{-1}$)					
	Stem		Petiole		Leaf	
	Control	Horizontal clinostat	Control	Horizontal clinostat	Control	Horizontal clinostat
24	10.3	9.5	14.2	18.7	10.3	18.7
36	15.0	53.3	14.5	34.7	16.6	51.1

Figure Legends

Fig. 1. Amount of ^{14}C incorporated into SEM of 68 day-old *T. patula* shoots after 36 hours feeding with L-proline-U-^{14}C. Normal plants are compared with plants fed while mounted on vertical and horizontal clinostats.

Fig. 2. Effect of hypogravity treatment on the amount of ^{14}C in the SEM of different tissues of 50 day-old *T. patula* after 68 hours infiltration with L-proline-U-^{14}C.

Fig. 3. Amount of ^{14}C present in cell wall protein of control and horizontal clinostat plants as determined by acid and base hydrolysis. The cell walls were obtained from 28 day-old *T. patula* leaves after 24 hours of incubation with L-proline-^{14}C.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10
5
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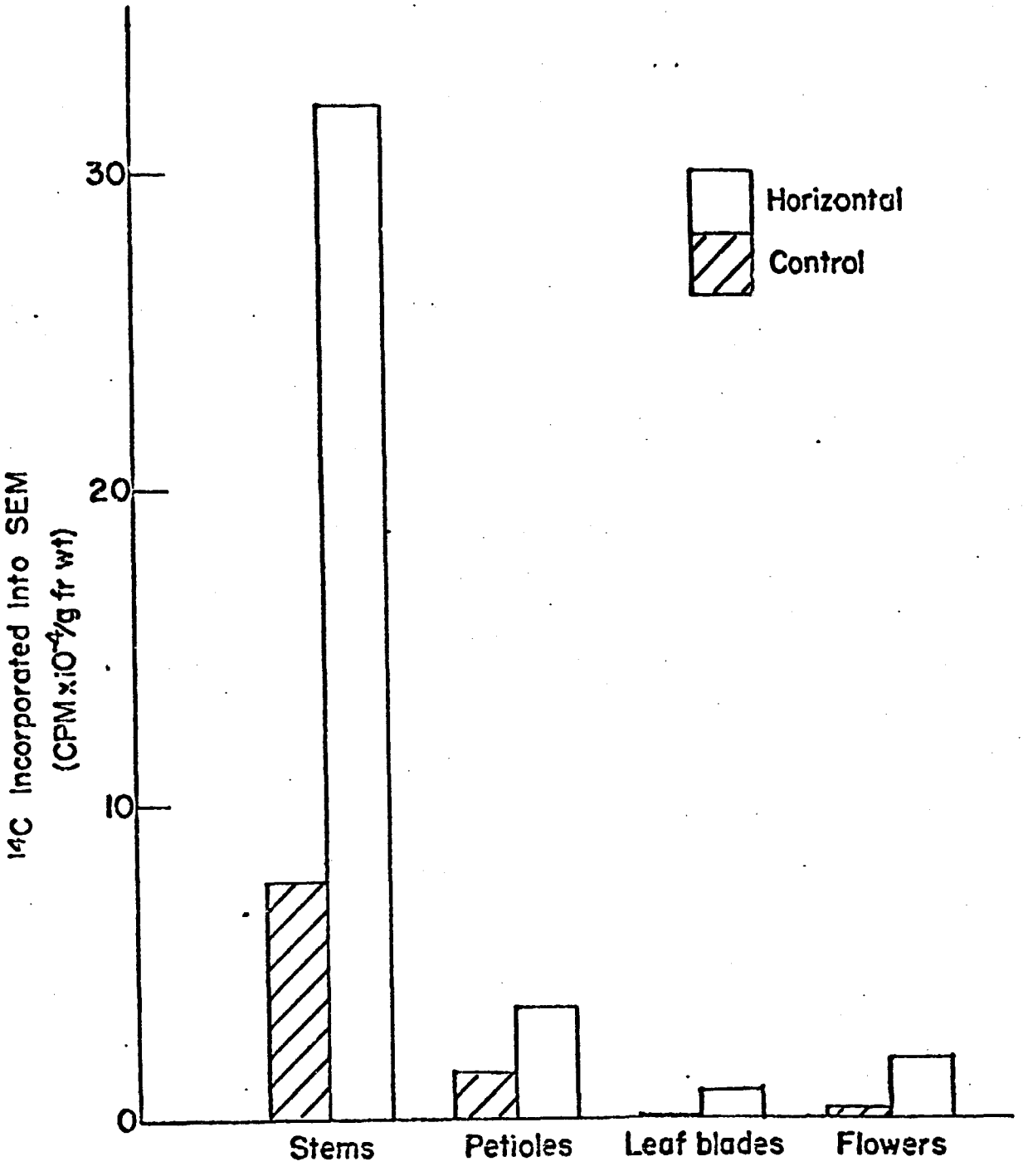
^{14}C incorporated into SEM
(CPM x 10^{-4}/g fr wt)
Control
Vertical
Horizontal

Figure 1


30
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^{14}C Incorporated into SEM
(CPM x 10^{-4}/g fr wt)
Horizontal
Control
Stems
Petioles
Leaf blades
Flowers

Figure 2

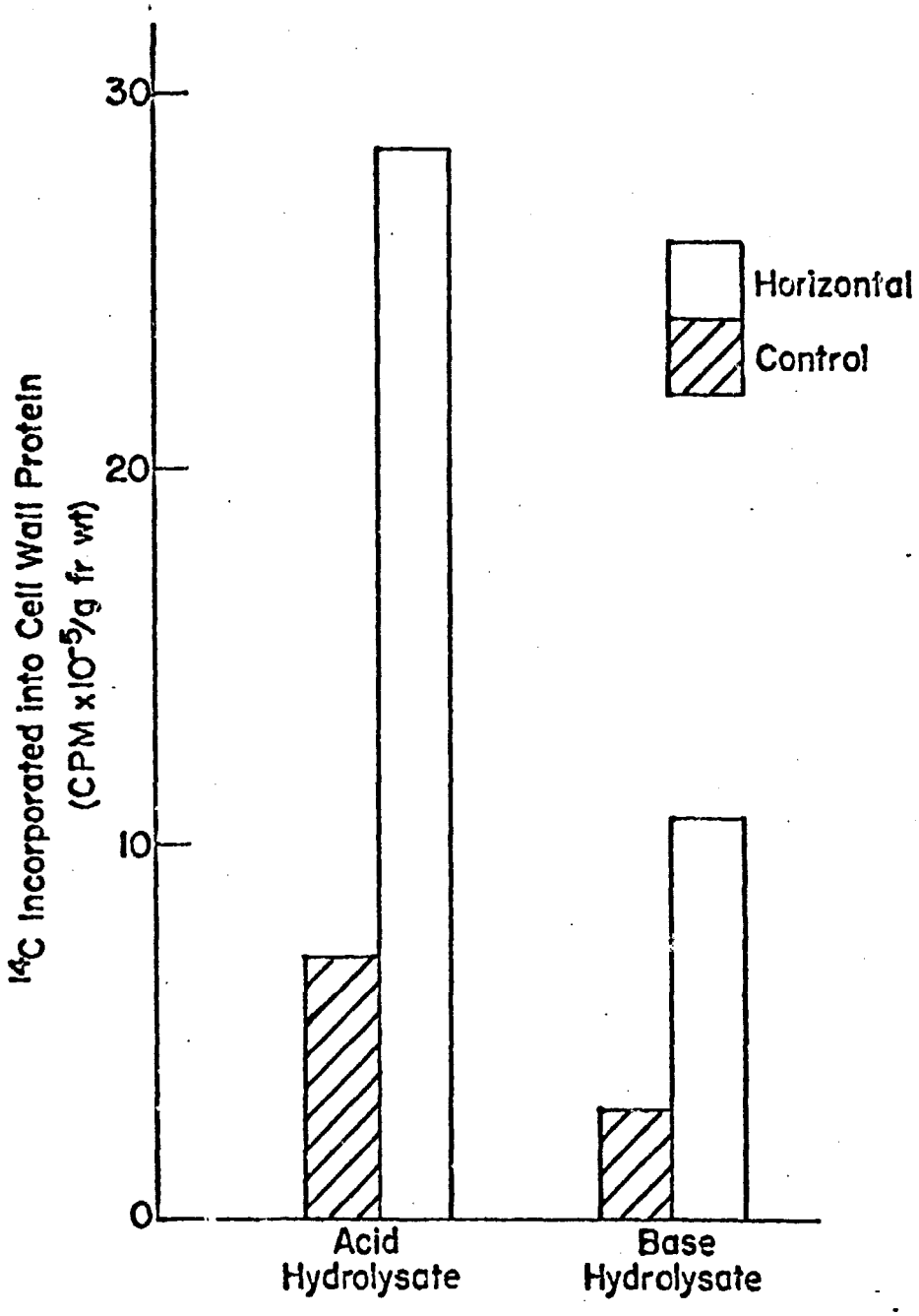
30
20
10
0
^{14}C Incorporated into Cell Wall Protein
(CPM x 10^{-5}/g fr wt)
Horizontal
Control
Acid
Hydrolysate
Base
Hydrolysate

Figure 3

Phytochemistry, 1977, Vol. 16, pp. 673–675. Pergamon Press. Printed in England.

SIMULATED HYPOGRAVITY AND PROLINE INCORPORATION INTO SALT-EXTRACTABLE MACROMOLECULES FROM CELL WALLS

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(*Revised received* 5 *November* 1976)

Key Word Index—*Tagetes patula*; Compositae; marigold; hypogravity; proline; cell walls.

Abstract—Proline [U-^{14}C] was fed to shoots of intact *Tagetes patula* grown normally, on horizontal clinostats, or on vertical clinostats rotating at 15 rev/hr. After various periods the incorporation of ^{14}C into salt-extractable material from the cell walls of stems, petioles, leaves and flowers was determined. The cell walls of the gravity-compensated plants (grown on horizontal clinostats) has the highest amount of salt-extractable radioactivity. A 2- to 9-fold increase was observed in comparison to either the normal or vertical clinostat plant controls. Some physicochemical properties of the salt-extractable fraction suggest that it consists of highly charged, low MW entities, possibly short chain peptides. On acid hydrolysis this material yields radioactive aspartic acid, glutamic acid and proline. The presence of labelled hydroxyproline is suggested. After acid hydrolysis of the cell walls of leaves, it was found that *ca* 4 times the amount of ^{14}C was incorporated in the hypogravity-grown plant compared to the controls. It appears likely that extensibility changes in tissues under simulated hypogravity required additional cell wall protein.

INTRODUCTION

The biosynthesis of the protein component of the primary cell wall in higher plants has been under intensive investigation for a number of years [1]. The presence of a cell wall protein precursor which can be extracted from the cell wall by the use of salt solutions has been well-documented [2–4]. The kinetics of formation of this precursor has the typical characteristics of a component in transit [4].

When plants are rotated horizontally on clinostats a typical response is the appearance of epinastic curvatures of the leaves. A similar response was observed under hypogravity conditions on orbital flights [5]. It appears reasonable that during epinasty, changes in extensibility would occur in the leaf or petiole cell wall, and therefore a biochemical change correlated with this phenomenon would be an increase in the amount of cell wall protein. The present report gives the results of a study comparing the synthesis of salt-extractable cell wall macromolecules in normal plants of *Tagetes patula* with those on a horizontal clinostat under simulated hypogravity. These macromolecules could be precursors of new cell wall protein.

RESULTS

The stems and leaves of *T. patula* exhibited typical epinastic curvatures when plants were subjected to simulated hypogravity by rotation on horizontal clinostats. After infiltration with proline-[^{14}C] the radioactivity in the salt-extractable macromolecules (SEM) in the cell walls from shoots of 68-day-old plants subjected to hypogravity amounted to 192 000 cpm per g fr. wt as compared to 57 500 in shoots of normal control plants, about a 3-fold increase. The amount of radioactivity in the SEM of control plants and plants rotated on vertical clinostats (47 000 cpm/g fr. wt) was essentially the same.

The incorporation of radioactivity into the SEM of horizontal plants was higher in every tissue than the corresponding tissue of control plants (Table 1). Differences of 2- to 9-fold between the two treatments were found depending on the particular tissue. Differences between treatments tend to be more marked in tissues from older plants compared to younger plants. This is evidenced by a comparison of the data in Tables 1 and 2.

The minimum time necessary for hypogravity to exert its effect on the SEM appears to be 36 hr (Table 2). In shorter times, e.g. 24 hr, the SEM in horizontal plants is only slightly higher if at all than in the control. After a presentation time of 36 hr, however, the effect of hypogravity is evident and all tissues of the plants on horizontal clinostats show considerably higher amounts of SEM than those of the controls.

After acid hydrolysis of the cell walls of the leaves, the amount of label recovered was *ca* 4 times greater

Table 1. ^{14}C incorporation into SEM in different plant tissues. 50-day-old *T. patula* plants were infiltrated with L-proline [U-^{14}C] for 68 hr as described in 'experimental' and SEM was isolated from each tissue.

Treatment	^{14}C Incorporation into SEM (cpm × 10^{-4}/g fr. wt)			
	Stem	Petiole	Leaf	Flower
Normal control	7.4	1.4	Trace	0.3
Horizontal clinostat	31.9	3.5	0.8	1.9

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ection II

Decarboxylation of Amino Acids in Compensated Plants

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Hypogravity-Induced Inhibition of CO_2 Production from Amino Acids in Higher Plants

The effect of hypogravity on the growth and physiology of higher plants has been simulated by means of the horizontal rotation of the plant on a clinostat[1]. The appearance of leaf epinasty has been the only criterion utilized for determining when hypogravity has affected the normal physiology of the plant. We have been investigating the effect of simulated hypogravity on the metabolism of certain amino acids in higher plants. L-proline-U-^{14}C was infiltrated by use of a wick[2] into 50-day-old marigold plants (*Tagetes patula*) mounted on vertical and horizontal clinostats rotating at 4 rph (revolutions per h). Normal plants were similarly infiltrated. After 24 h of incubation the various tissues of the plants were extracted with aqueous ethanol and the free amino acid fraction examined by two-dimensional paper chromatography and radioautography. A major radioactive constituent in every case was γ-aminobutyric acid. Since the conversion of proline to glutamate is a well-known route of proline metabolism[3], the presence of an active glutamate decarboxylase was indicated.

The in vivo conversion of L-proline-U-^{14}C to $^{14}CO_2$ in normal and hypogravity plants under continuous illumination was then measured and compared with plants similarly treated with L-glutamic-U-^{14}C and others with L-valine-U-^{14}C. Marigold plants (var. Petite Gold), between 30 to 50 days old, were mounted on horizontal clinostats rotating at a speed of 15 rph and left for at least 4 days. Control plants were rotated on vertical clinostats. The labelled amino acid was injected into the stem of the plant at the desired time by means of a syringe. Immediately after the injection the plants were placed in clinostat gas exchange chambers mounted on the clinostats. The chambers were swept with compressed air from a cylinder. The air passing over the plant was bubbled through 5 ml of a 1 *M* solution of hyamine hydroxide so as to trap the released $^{14}CO_2$. The trapping solution was replaced every 10 min with fresh hyamine. Aliquots of the hyamine solutions were mixed in 10 ml Bray's solution[4] and then assayed for radioactivity in a liquid scintillation counter. At the conclusion of the experiment the plants were cut just above the point of label application and weighed.

The results of Figures 1 and 2 demonstrate very clearly that $^{14}CO_2$ production is much higher in the control plants than in those subjected to hypogravity. Since

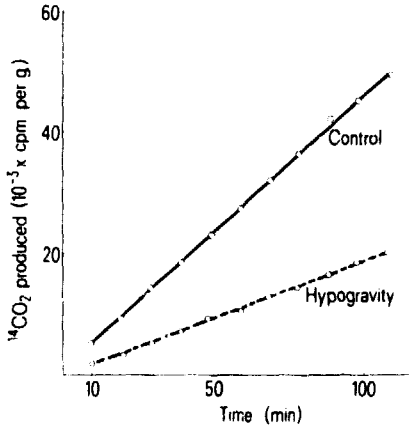

Fig. 1. The production of $^{14}C_2O$ from control plants compared to those rotated on horizontal clinostats after infiltration of 5 μCi of L-glutamic acid-U-^{14}C (specific activity 175 mCi per mmole). 42-day-old plants were rotated on the clinostats for 4 days before infiltration. The results are given on a fresh weight basis.

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Section III

The Effect of Hypogravity on the Activity of Glutamic Acid Decarboxylase in Crude Extracts of Marigold Leaves

It has previously been shown (Section II), that the rate at which $^{14}CO_2$ is released from plants injected with uniformly labeled ^{14}C-glutamic acid is rapidly and substantially lowered by horizontal clinostat rotation (7). This finding suggested that the activity of the enzyme glutamic acid decarboxylase (GAD) was abnormally low in gravi-compensated plants. The data presented in Figure 1 confirms this suggestion, and illustrates that the activity of this enzyme is indeed lower in extracts prepared from leaves of horizontally rotated plants. The evidence that the released $^{14}CO_2$ does come from the C-1 carbon of glutamic acid is shown in Table I.

The observed changes in GAD activity are also reflected in the amounts of certain soluble amino acids detected in deproteinized extracts of marigold leaves. Table II contains the results of some preliminary amino acid analyses of extracts prepared from plants rotated for two hours on either vertical (control) or horizontal (treated) clinostats. From these data (expressed as a ratio: $\frac{\text{treated}}{\text{control}}$) it can be seen that there is a large change in the amount of glutamine and therefore in the size of the glutamine-glutamic acid pool. The other decreases noted cannot be considered significant due to the small number of analyses performed to date. We conclude from these data, that the drop in GAD activity is correlated with a change in the size of the glutamate-glutamine pool.

It is also evident from Figure I, that although the GAD activity is lowered by gravi-compensation, this activity is slowly rising in both treated and control plants. This continuous elevation is due, at least in part, to the vibrational stress associated with clinostat rotation and/or vibration caused by the cycling of the compressor attached to our experimental chamber.

Preliminary experiments designed to study the effect of vibrational stress on the GAD level in leaves (Figure II) seem to bear out the suggestion, that in

addition to hypogravitational stress, the activity of GAD in leaves is also sensitive to vibrational stress.

Although we have demonstrated that indeed the GAD activity extracted from leaves of gravi-compensated plants is lower, this effect can only be consistently observed in plants that have three pairs of true leaves and a small bud (Table III). The inability of plants with two pair of true leaves and fairly well developed flower bud to respond is puzzling. It is suggested that the physiological changes associated with the transition from vegetative to flowering growth can mask and/or preclude the hypogravity response.

In conclusion, we have demonstrated that the level of GAD in marigold leaves can be altered by environmental stress. The magnitude and direction of this alteration can be effected or modified by any one of the following:

1) Type of stress
2) Duration of stress
3) Plant species
4) Developmental stage of test organism.

It appears clear, therefore, that we are dealing with an extremely complicated response which is regulated by an, as yet, unknown mechanism.

Table I. Specificity of glutamic acid decarboxylase.

	dpm/min/mgm*
DL-glutamic acid [1-^{14}C]	833
DL-glutamic acid [5-^{14}C]	0

*The numbers represent disintegrations of $^{14}CO_2$ per minute/minute incubation time/mgm protein in reaction mixture.

Table II. Change in soluble amino acid content induced by two hours of simulated hypogravity.

Amino acid	Ratio*
Glutamic acid	0.96
Glutamine	0.65
Glutamic acid + glutamine	0.80
γ-Amino butyric acid	0.80
Alanine	0.82
Aspartic acid	0.92

*Computed by dividing the relative amount of each amino acid found in extract from horizontally rotated plants by the relative amount found in similar extracts from vertically rotated plants.

Table III. Effect of hypogravity on the activity of glutamic acid decarboxylase of marigolds possessing the indicated number of true leaf pairs and a flower bud*.

	2 or 2-1/2 leaf pair	3 leaf pair
Horizontal clinostat	1018 ± 80	688 ± 28
Vertical clinostat	995 ± 45	946 ± 150

*The numbers represent the disintegrations per minute/minute of incubation time/mgm of protein in the reaction mixture plus or minus the standard error of the mean.

Figure Legends

Fig. I. The effect of clinostat rotation on the activity of glutamic acid decarboxylase extracted from leaves of twenty or twenty-one day old marigolds. The plants were maintained in the designated condition for the times shown. All values are averages ± standard error of mean.

Fig. II. The effect of vibrational stress on the activity of glutamic acid decarboxylase is presented. Except where noted, the enzyme was extracted from marigold leaves.

Figure I

EFFECT OF HYPOGRAVITY ON THE ACTIVITY
OF GLUTAMIC ACID DECARBOXYLASE

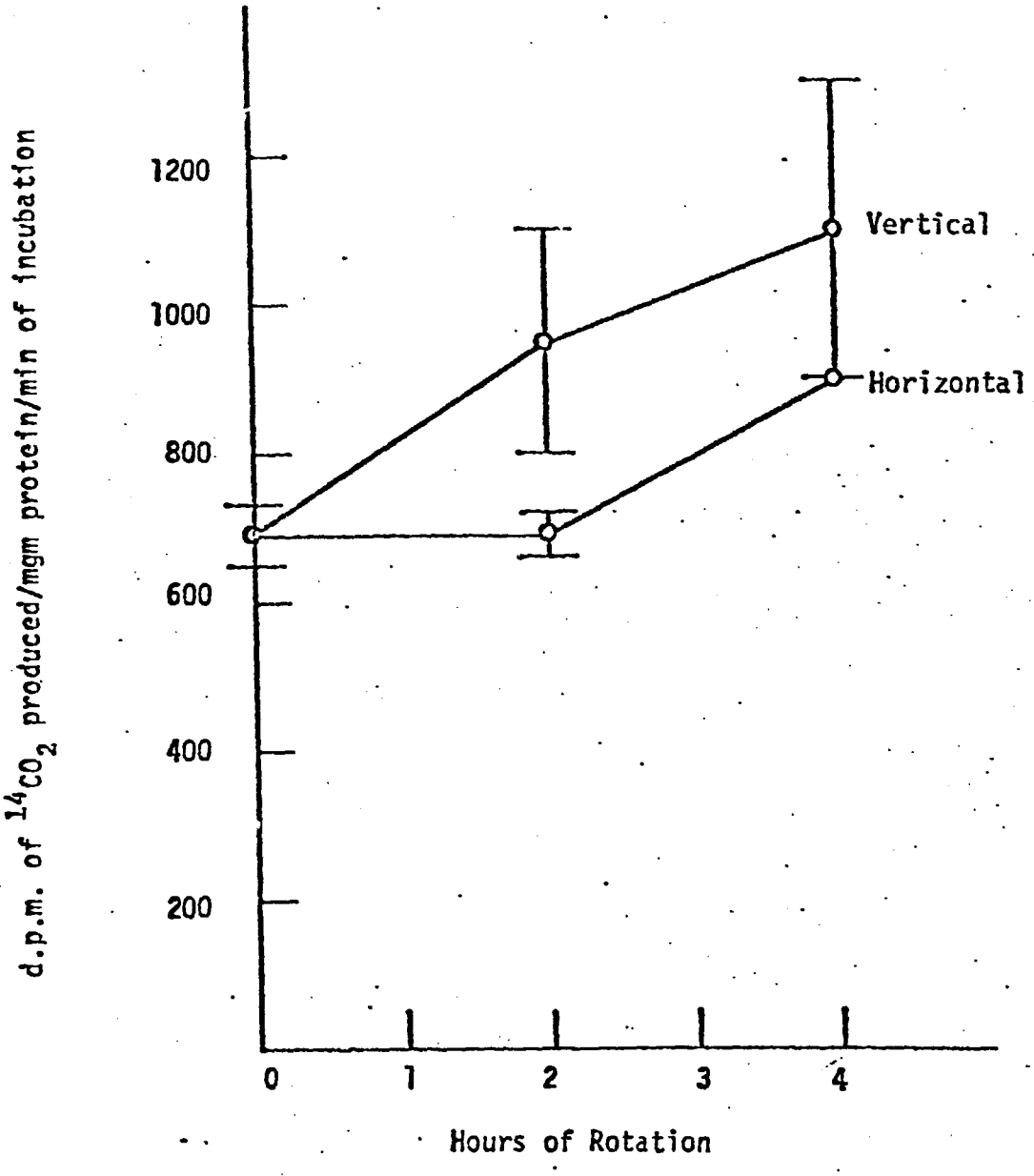
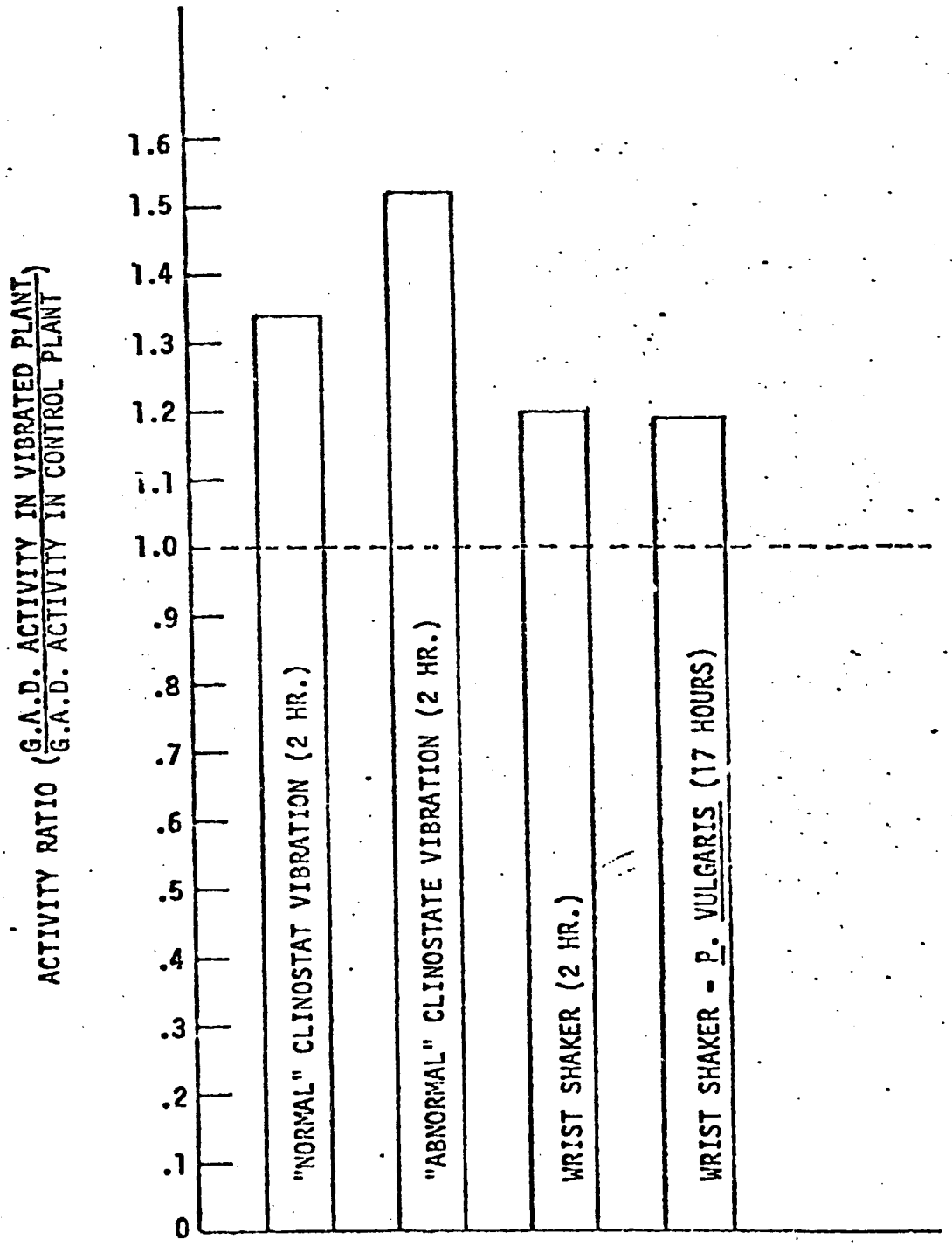


Figure II

EFFECT OF VIBRATIONAL STRESS ON THE ACTIVITY OF GLUTAMIC ACID DECARBOXYLASE



Section IV

Tryptophan Decarboxylase Studies

Tryptophan decarboxylase is an extremely important enzyme, in that it could catalyze the first reaction in a proposed pathway leading from tryptophan to indole-3-acetic acid (IAA). Since many gravitational responses are mediated by IAA and decarboxylase systems seem to be affected by hypogravity, an attempt was made to detect this enzyme in marigolds. Although we have not been able to detect the decarboxylase with an *in vitro* assay, we have shown by use of the *in vivo* feeding technique that marigolds do possess the ability to decarboxylate tryptophan. It is presently not known if tryptophan-decarboxylase will prove to be as useful as glutamic acid decarboxylase in demonstrating a biochemical response to gravi-compensation.

Section V

Elemental Analysis of Marigolds

This laboratory is currently in the process of developing a procedure that will allow us to routinely analyze for almost every element above atomic number 11 present in our plants. The actual measurements involve ion-excited X-ray analysis of a powder prepared from dried plant material.

Table I presents the results of a typical analysis. The amount of each element being expressed as a ratio to calcium. It should be noted, that these same ratios also appeared in our twelfth quarterly report along with the absolute amount of each element. Unfortunately, we now feel that these absolute values are questionable and therefore should not be used as such although we do consider the ratio values to be correct within acceptable limits.

We are currently working to surmount the problems we have encountered in adapting this relatively new physical tool to solving biological problems. Once we have these problems in hand, we feel that applications of this technique to our research program will be extremely fruitful.

Table I. Preliminary mineral analysis data.

	46 $\mu E/m^2/sec$[a]	64 $\mu E/m^2/sec$[b]
	Ratio to Ca	Ratio to Ca
Mg	2.59×10^{-1}	1.82×10^{-1}
P	3.44×10^{-1}	2.34×10^{-1}
S	1.28×10^{-1}	1.44×10^{-1}
K	1.06	1.36
Ca	1.000	1.000
Mn	8.40×10^{-3}	1.38×10^{-2}
Fe	9.58×10^{-2}	4.02×10^{-2}
Zn	7.71×10^{-3}	1.14×10^{-2}

[a] n = 1 leaf (1st).

[b] n = 4 leaves (1st).