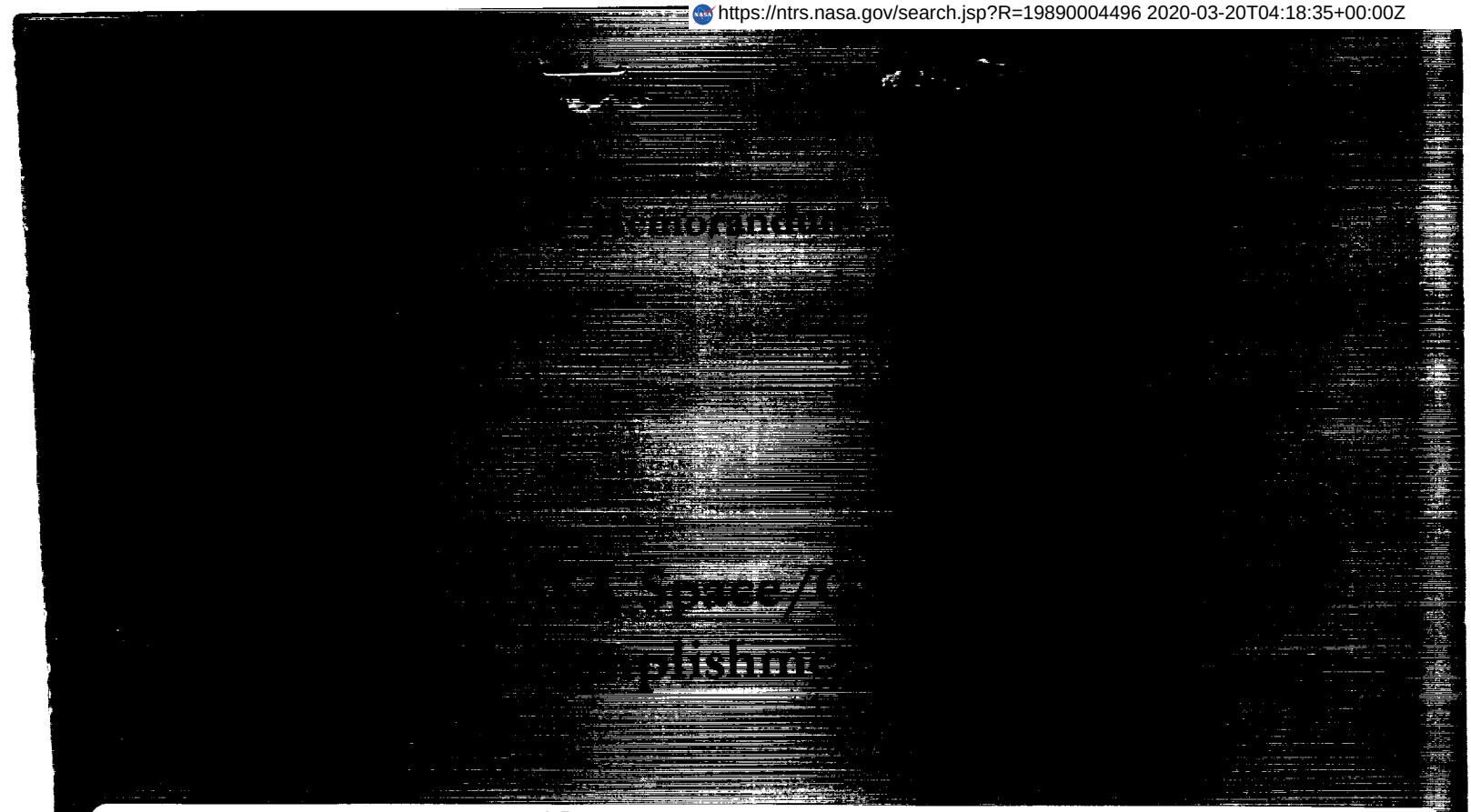

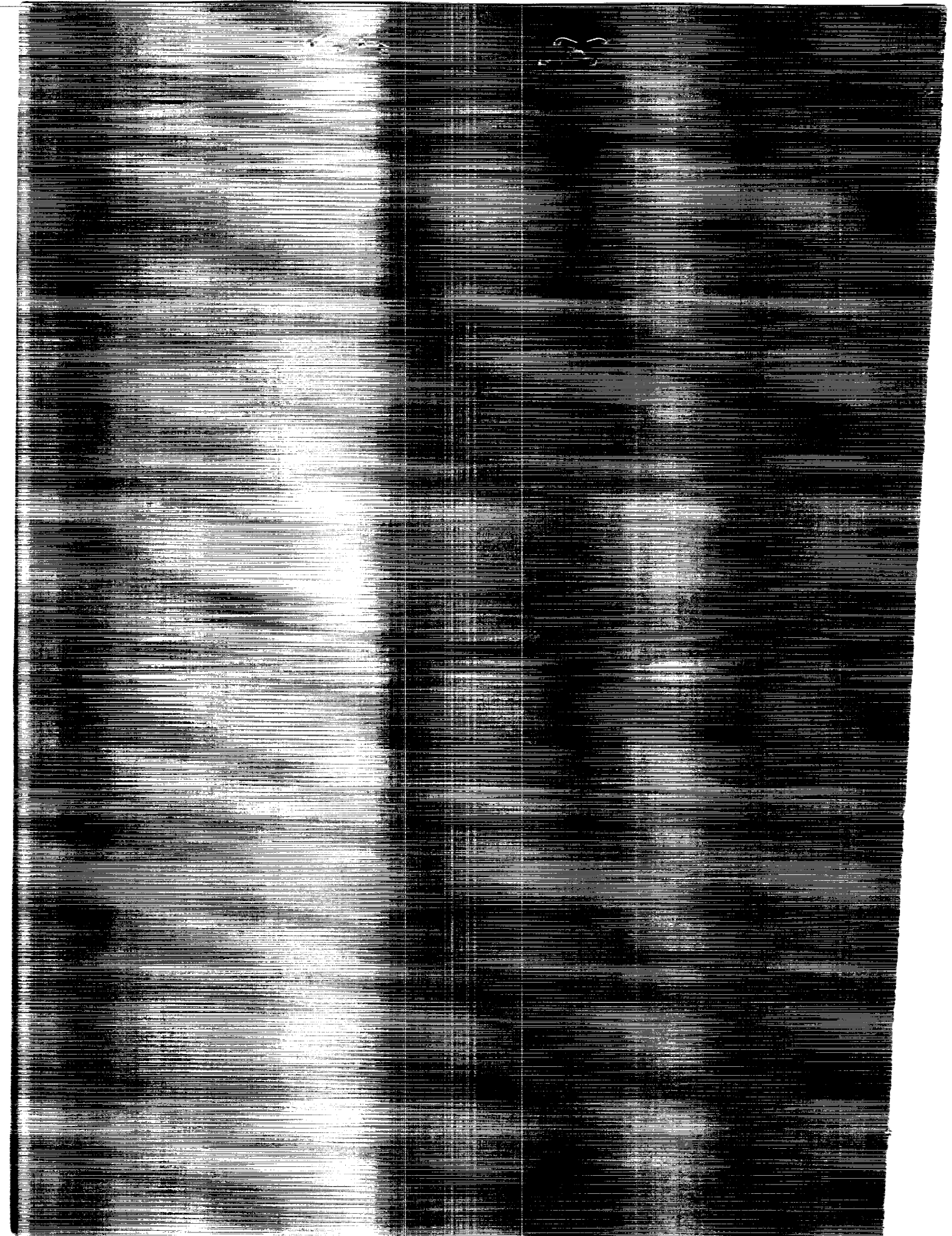

NASA Technical Memorandum 4079

1987-88 NASA Space/Gravitational Biology Accomplishments

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and Space Administration

**Scientific and Technical
Information Division**

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PREFACE

An individual technical summary of each research task within the Space/Gravitational Biology Program is presented in this publication. Each summary, prepared by the principal investigator, consists of a description of the research, a listing of the project's accomplishments, an explanation of the significance of the accomplishments, and a list of the publications resulting from the past year's research. Since spaceflight experiments, submitted in response to the Space Biology Dear Colleague letter, have become an integral part of the Program, reports on the activites of this related research are integrated in the report. Accomplishments of the scientists in the Space Biology Research Associates Program are also included. The participants in the program, which provides opportunities for postdoctoral scientists to conduct research in the fields of gravitational and space biology, have been outstanding and merit independent recognition.

This publication has two objectives: first, to provide the scientific community and NASA with an annual summary of the accomplishments of the research pursued under the auspices of the Space/Gravitational Biology Program, and second, to stimulate an exchange of information and ideas among scientists working in the Program.

Thanks are due to the Program participants whose research and cooperative response to our requests for information made this report possible. Editorial support provided by Janet V. Powers, F. Ronald Dutcher, and Katherine J. Dickson is gratefully acknowledged and appreciated, as well as the technical assistance provided by April Commodore Roy.

Additional information about this report of the Space/Gravitational Biology Program can be obtained by writing to me at the following address:

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INTRODUCTION

THE NASA SPACE/GRAVITATIONAL BIOLOGY PROGRAM

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Introduction

One of the major features of the physical environment of the surface of Earth is the constant presence of the force of gravity. The phenomenon of weightlessness encountered on spacecraft provides a unique biological research opportunity to study the importance of gravity to life on Earth. Access to space provides an opportunity to manipulate gravity from its norm of one down to almost zero, effectively providing the full spectrum of gravitational research capability for the first time. This capability, combined with the stability and pervasiveness of gravity on Earth, its obvious impact on biological evolution, and its continuing effect on the morphology, physiology, and behavior of living organisms, has led the Space/Gravitational Biology Program to concentrate its efforts and resources on investigating the biological significance of gravity and advancing knowledge in the biological sciences through the use of the microgravity environment of spaceflight.

Program Goals

The goals of the Space/Gravitational Biology Program are to: use the unique characteristics of the space environment, particularly microgravity, as a tool to advance knowledge in the biological sciences; understand the role of gravity in the biological processes of both plants and animals; and understand how plants and animals are affected by and adapt to the spaceflight environment, thereby enhancing our capability to use and explore space.

Program Scope

Research in the Program is divided into four broad areas:

1. **Gravity Perception/Sensing.** Plants and animals have developed gravity-sensing systems that facilitate orientation and locomotion within Earth's environment. The weightless environment of space provides a unique research opportunity to understand how gravity-sensing systems of different organisms have developed, and how they process and transmit information. Specific objectives are:
    a. To identify gravity-sensing organs and mechanisms, and to define how they function on Earth and adapt to weightlessness.
    b. To understand how gravitational information is transduced, processed, transmitted, and integrated into a response.
    c. To understand the role of gravity in the development and evolution of plant and animal gravity sensing systems.

2. **Developmental Biology.** Research in this area examines the influence of gravity and weightlessness on genetic integrity, reproduction, growth, development, life span, senescence, and subsequent generations of plants and animals. Specific objectives are:
    a. To determine if organisms and multiple generations of organisms can develop normally in microgravity.

b. To identify gravity-sensitive developmental stages, systems, and mechanisms in both plants and animals.
c. To understand the effects of gravity and weightlessness on gravity-sensitive developmental stages, systems, and mechanisms.

3. **Biological Adaptation.** All biological species on Earth have evolved under the influence of a gravity level of 1 g. In response to this force, organisms have developed structures to withstand gravity loads, as well as regulatory systems to function optimally. The objective is to understand how gravity affects and controls the physiology, morphology, and behavior of organisms; how gravity and other environmental stimuli and stresses interact in this control; and the biological mechanisms by which living systems can respond and adapt to altered gravity, particularly that of the space environment. It includes the use or removal of gravity's physiological effects to explore biological problems. Specific objectives are:
   a. To understand the influence of gravity on the evolution, regulation, and function of biological support structures.
   b. To determine the role of gravity in regulating metabolism, metabolic rate and products, fluid dynamics, and biorhythms.
   c. To understand basic mechanisms of mineral and hormonal homeostasis and the role of calcium as a mediator of gravitational effects.
   d. To identify the effects on organisms of the interaction of environmental factors (e.g., temperature and light) with gravity, and determine the mechanisms involved.

4. **Cell Biology.** Cells that are building blocks of systems (e.g., plant root caps), individually functioning units of certain tissues (e.g., blood cells), and unicellular organisms (e.g., paramecia) have been shown to be sensitive to gravity. Research focuses on how gravitational loading influences cell functions and the molecular mechanisms regulating them. The objective is to determine at what level gravity affects cells and where and how they are affected. Specific objectives are:
   a. To investigate the role of gravity in maintaining normal cellular and molecular function.
   b. To determine how and where gravity affects the cell.
   c. To distinguish direct from indirect, extracellular, or systemic, gravitational effects on cells.
   d. To discriminate between the influences of cosmic rays, microgravity, and other environmental factors.
   e. To assess the permanence of effects on cells exposed to microgravity.

Focus of Program

The program focuses on research that promises to answer basic scientific questions that can contribute to the resolution of biological problems of fundamental importance on Earth and/or to space exploration.

Understanding how plants develop, metabolize and grow in space is essential for a space based Controlled Ecological Life Support System (CELSS). Biomineralization and the mechanisms controlling the structural integrity of bone and bone turnover are important to osteoporosis on Earth and the calcium loss and bone changes of spaceflight. The reconstruction and modeling of the functional organization of mammalian gravity sensors are expected to lead to increased understanding of how information is processed by

biological systems. In a sense this is all basic research with an eye on application — especially for Earth.

Research Opportunities

While the research supported and encompassed by the Space/Gravitational Biology Program is primarily ground-based, spaceflight experiments are an essential component of the program. Spaceflight provides the validation for experimental hypotheses developed in ground-based research, while gravitational experiments on Earth hone the questions, provide the necessary baseline data, and develop spaceflight experimental protocol.

The experimental approach of the ground-based studies is to manipulate gravity on Earth and develop weightless simulation models to: (a) develop and test gravitational hypotheses, (b) identify gravity-sensitive biological systems and interacting environmental response mechanisms, (c) analyze biological systems and mechanisms known to be gravity-sensitive, (d) analyze flight experiment data and iteratively expand ground research capability, and (e) plan and design future space experiments. Research is also conducted to understand how the uncontrollable biodynamic factors of the spacecraft and behavior of components of the environment in weightlessness affect the results of flight experiments.

The Space Shuttle is currently the only U.S. developed spacecraft capable of carrying biological experiments. Two avenues are available to propose Shuttle flight experiments through NASA. An Announcement of Opportunity, "Life Science Investigations in Space 1986-91," formally solicits proposals. The next proposal due date is February 1, 1989 with selection to be announced in March 1990.

Unsolicited proposals for flight experiments received in response to a 1983 Dear Colleague Letter on "Emerging Opportunities in the Space Biology Program" have been accepted on an ad hoc basis since the announcement. This opportunity, created for biological experiments to be flown in the Shuttle Orbiter mid-deck on a space available basis, is temporarily closed but should become available again in 1989.

The limited opportunities to conduct biological experiments on spacecraft have stimulated the examination of alternative means to conduct space research. Biosatellites offer such an alternative. In fact, they offer a valuable supplemental capability. While requiring an increased level of automation beyond the "mid-deck" experiment approach, they have the significant advantage of extended stay in space. Currently a retrievable reusable biosatellite called LifeSat is under study with the support of international space agencies.

The research of the Space/Gravitational Biology Program is dependent upon several dynamic factors: the requirements of NASA, the characteristics of flight experiment opportunities, the sensitivity of specific biological systems to gravity, the scientific value of the research, the state of knowledge and technology in the specific scientific areas, the interest of scientists in studying the biological questions, and the availability of funds to support the research.

ACCOMPLISHMENT HIGHLIGHTS

SPACE/GRAVITATIONAL BIOLOGY
ACCOMPLISHMENT HIGHLIGHTS

PLANT

Gravitropism: Sensing

- Nature of the receptor
    - Roots of a starchless *Arabidopsis* mutant that nevertheless respond to gravity do not respond as well as the wild type when exposed to repeated short periods of gravistimulation. (Sack)
    - Plastids of this starchless *Arabidopsis* mutant are the heaviest and most moveable components of its root gravisensing cells. (Sack)
    - Plastids in this mutant and in wild type plants as well are in direct contact with the endoplasmic reticulum. (Sack)
    - Starch in chloroplasts, the likely gravisensor organelle in cereal grain shoots, gradually disappears during the course of upward bending of gravistimulated shoots. (Kaufman)
    - Tissue which loses the ability to respond to gravity due to loss of starch in darkness can still respond to hormones. This suggests that treatment eliminating starch affects a stimulus perception element rather than a growth response component. (Brock)
    - An inhibitor specific for the sensory steps of gravitropism in maize roots, the gadolinium ion, has been established. (Pickard)
- Electrical potential
    - The effect of an applied electrical potential upon both growth and endogenous auxin concentrations in the shoot is dependent on the polarity of the applied potential. (Desrosiers, Bandurski)
    - Applied electrical potentials affect the transport of IAA, glucose, and calcium. This effect also is dependent on the polarity of the applied potential. (Desrosiers, Bandurski)

Gravitropism: Transduction Mechanism

- Disruption of cell-to-cell cytoplasmic connections by means of plasmolysis/deplasmolysis of root cap cells prevents gravi-induced polar calcium movement but does not prevent gravitropism or gravi-induced auxin redistribution. These results may cause some reassessment of the calcium transduction model. (Evans)
- Evidence has been found that links the role of calcium to operation of a second messenger system: the phosphatidyl inositol messenger system. (Leopold)

- Various components of the phosphoinositide pathway have been detected in corn root tips and linked to light and gravitropic signal transduction. (Poovaiah)

- Results from experiments using calcium and aluminum ions and calcium-binding chemicals suggest that auxin transport is calcium-dependent. (Evans)

- Small pieces of plasma membrane have been isolated that contain all of the necessary components of the polar auxin transport system and a native calcium-dependent protein kinase. (Lomax)

- A close correlation between the kinetics of gravity-induced auxin movement across the root cap and the kinetics of gravitropic curvature has been demonstrated . (Evans)

- Gravistimulation results in differential gibberellic acid metabolism between upper and lower halves of stems. This effect does not occur in "lazy" gravity mutants (plants that initially respond but then lose the ability to respond to gravity). (Kaufman)

- The responsiveness of cereal stems to applied hormones was altered by gravistimulation. It inhibited the normal response produced by auxin at moderate to high concentrations; at the same time, gravity induced a responsiveness to gibberellic acid that otherwise would not have been present. (Brock, Kaufman)

- Sensitivity to applied auxin is different for upper and for lower tissues in gravistimulated stems. Upper tissues exhibit a reduced sensitivity compared to lower tissues. (Salisbury)

- A protein is being isolated that may participate in the regulation of auxin transport across the cell membrane. (Pickard)

- Studies with a gravitropically sluggish and auxin-insensitive mutant of tomato indicate a possible auxin/ethylene feedback mechanism for the control of auxin transport. (Rayle)

- Epidermal and cortex tissue do not appear to play a direct role in gravitropism. The endodermis, however, is essential and appears to play a role in the transport of or response to the signal. (Björkman)

- A gradient of gravitropic effectors (agents capable of inducing a gravitropic response) occurs in the mucilage surrounding gravistimulated roots. A gradient of calcium occurs in the mucilage and in epidermal cells as well. (Moore)

- Light Initiation of Gravitropic Capability

  - In dark-grown roots that require light for normal gravitropism, there is no polar calcium or auxin transport and auxin sensitivity is low. Light stimulates growth, induces calcium and auxin transport, and increases auxin sensitivity. (Evans)

  - Red light shifts growth of certain types of corn roots from a state of horizontal growth to one of downward growth. The horizontal growth appears to be

independent of calcium regulation, while the downward growth direction is calcium-dependent and has been linked to operation of the phosphatidylinositol messenger system. (Leopold)

- Light induces rapid, specific, and apparently calcium-mediated, changes in protein phosphorylation. These changes are observed only in root tips (which are considered to be the site of light and gravity perception). (Poovaiah)

Gravitropism: Growth Response

- Growing stems appear to inhibit growth on the upper stem surface by simply ceasing wall loosening, not by actively rigidifying the walls of the upper surface. (Cosgrove)

- Wall loosening during stem growth is a dynamic process. A simple viscoelastic or enzymatic mechanism does not appear to be sufficient to account for the observed kinetics of relaxation and the dynamics of recovery from relaxation. (Cosgrove)

- Application of calcium and calcium binding agents to isolated cell walls has little effect on wall extensibility, which is evidence against the idea that extracellular calcium rigidifies the wall during gravitropism. (Cosgrove)

- Cell wall stiffening and loosening do not appear to be calcium-dependent; rather, they appear to be regulated by wall pH, which likely modulates the activity of wall loosening and wall stiffening enzymes. Proteins (probably enzymes) are necessary for both of these processes. (Rayle)

- Gravistimulation results in greater invertase activity, higher proportions of 1-glucan and arabinan, and less cellulose and starch in the lower halves of upward bending stems. (Kaufman)

- Role of peroxidase

  - A monoclonal antibody has been produced that is specific for one particular cell wall peroxidase (a cell wall enzyme involved in wall cross-linking). Staining reveals it to be present in the wall. (Roux)

  - Light causes a rapid decrease in this peroxidase; changes are red/far red-light reversible and therefore are mediated by phytochrome. (Roux)

  - The preceding result is the first report of a phytochrome-induced change in the content of a wall enzyme. It is also the first report of a change in peroxidase content in walls following a stimulus that affects gravitropic growth. (Roux)

  - This particular peroxidase is present in shoots and leaves but not in roots, which indicates there may be different enzymatic wall mechanisms regulating growth in shoots and roots. (Roux)

  - These findings are also of interest because there is evidence that calcium is involved in regulation of wall peroxidases. (Roux)

- Role of amine oxidase, lignification

  - Amine oxidase, the enzyme that produces the substrate used by peroxidase-catalyzed reactions and that might therefore be a forerunner to the peroxidase-mediated cell wall cross-linking that may play a role in gravitropism (through short-term inhibition of wall extensibility and growth) has been shown to be localized in the plant cell wall. (Slocum)

  - The distribution of amine oxidase in plants correlates with known sites of lignification and is thought to be part of the lignification process. (Lignification is irreversible cross-linking mediated through wall-bound peroxidases.) (Slocum)

  - There is no lateral asymmetry in the activity of amine oxidase across gravistimulated organs, though there are asymmetries in wall peroxidase and polyamine substrates across such organs. (Slocum)

  - Two enzymes involved in cellular differentiation and wall lignification in isolated leaf mesophyll cells are being characterized. (Galston)

Shoot Inversion

- Shoot inversion restricts stem elongation by decreasing cell length and increasing stem diameter, and causes a decrease in the concentration of auxin in the growth region of the inverted shoot. (Cline)

- Shoot inversion causes the genes for hydroxyproline-rich glycoprotein (a cell wall-strengthening protein) to be activated. (Cline)

Mechanical Stress

- Inhibition of growth induced by mechanical stress appears to be associated with an accumulation of calcium in the zone of maximum stem elongation. Growth inhibition occurs more rapidly than calcium accumulation, however. (Mitchell)

- Mechanical stress affects plant reproduction at certain discrete periods of development (e.g., full seed pod stage). (Mitchell)

Atmospheric Environment

- Mung bean seedlings grow better at low atmospheric pressure than at ambient pressure, which suggests that growth of these seedlings may be somewhat limited by diffusion of contaminants. (Musgrave)

- Respiratory metabolism in mung bean responds normally to ambient partial pressure of oxygen, regardless of the total atmospheric pressure. (Musgrave)

Cell Biology

- Hypogravity does not have a significant effect on the fern spore cell cycle, although the application of hypergravity does have an effect. (Raghavan)

- The gene involved in the process of chromosomal segregation during cell division in yeast was successfully cloned, sequenced, and characterized. This lays the groundwork for a flight experiment examining, at the molecular level, the effect of spaceflight on chromosome partitioning during cell division. (Bruschi)

ANIMAL

Gravity receptors

- Studies of the surface morphology of the gravity receptor of jellyfish showed that cilia length can be used to determine the location and time of formation of the mechanosensory hair cells. (Spangenberg)

- A reliable method of cloning jellyfish for a specific morphological feature was developed. This approach will be useful for the scheduled jellyfish flight experiment as well as other studies. (Spangenberg)

- The number of statoconia (stone-like cell components that fall in response to gravity) in molluscs appear to increase with the weight of an animal, but not with its age, which suggests that statoconia production is related to whole-body metabolism. (Wiederhold)

- Glycoproteins and glycosaminoglycans, chemicals involved in a range of cellular activities including mineralization of an organic template, and known to be part of the adult rodent's statoconial membrane, are also integral parts of the chick's statoconial membrane. (Fermin)

- The presence of muscarinic acetylcholine receptors in mammalian vestibular sensory epithelia was demonstrated, the first direct demonstration of a presumptive neurotransmitter or neuromodulator receptor site in mammalian vestibular sensory organs. (Kerr)

- Macular receptors, which act as bioaccelerometers, contain weighted neural networks that process information in parallel. (Ross)

- No two macular receptive fields are identical. (Ross)

- As maculas mature, certain circuits begin to emerge so that there are identifiable, largely repeatable responses to specific kinds of input. Weighting between non-functional and functional stages can change. (Ross)

- Perturbations such as microgravity cause initial disarray in macular fields but then the neural elements readjust their weighting, i.e., they adapt to the new situation. (Ross)

Neurovestibular

- Amphibian embryos subjected to the stress of higher gravity have the ability, under some situations, to regulate or compensate (in terms of neural development) for environmentally induced stress and to regulate their own developmental progress. (Phillips)

- The phasic response of the bullfrog utricle (part of the vestibular system) to very small movements is extraordinarily acute. The sinusoidal steady-state response is directly proportional to the rate of change of acceleration (i.e., to "jerk"). (Lewis)

- The gravity vector apparently acts as a lever to amplify "jerk" on the bullfrog utricle during vertical head rotation. (Lewis)

- Oculomotor neurons respond vigorously to static tilt in the larval frog, compared with the response of the adult frog. The decreased response in adults is probably due to rearrangement, during metamorphosis, of neuronal pathways central to the VIIIth nerve input but prior to the nerve-muscle synapse. (Cochran)

- Vestibular responses were recorded in bird embryos as young as embryonic day 15. This is the first time observations have been made of embryonic electrophysiological activity in the vestibular portion of the VIIIth nerve. (Jones)

- The recordings mentioned above were made using a new approach that should provide a means of quantifying vestibular response dynamics (the timing of neural activation and transmission) throughout development. (Jones)

- An *in vivo* method was developed to mechanically stimulate the semicircular canals of pigeons. The use of this technique *in vivo* allows vestibular system dynamics to be studied in the peripheral as well as the central nervous system pathways. (Dickman)

- The mammalian vestibulo-ocular reflex receives inputs mainly, if not exclusively, from regular afferent neurons (as opposed to inputs from irregular afferents). It receives a selective afferent input, and not an indiscriminate mixture of inputs from afferents with varying physiologic properties. These findings are consistent with our theoretical understanding of this system. (Minor)

- Most otolith units in the rat show "tonic" responses in which the neural discharge rate is proportional to head position and is independent of the velocity of transmission. The remaining otolith units have "phasic-tonic" responses in which an overshoot or undershoot during transition is followed by a rapid return to a new steady-state. (Blanks)

Development

- A specific mRNA has been located in the amphibian egg vegetal hemisphere. It is being tracked to more accurately characterize the cytoplasmic rearrangements that occur during gravity orientation of the egg. (Malacinski)

- A brief cold shock diminishes cytoplasmic viscosity in amphibian eggs, which suggests that the cytoskeleton (e.g., microtubules) plays a role in stabilizing the egg density compartments. (Malacinski)

- Following exposure to hyper g, female mice (but not males) weighed less than controls, and were more likely than males to show elongation and compression of skulls, indicating that females adapt more readily to gravitational changes. (Duke)

- Effects of centrifugation on fetus size and bone size and shape were diminished in fetuses resulting from pairings of centrifuged females with 1 g males, compared with those produced from pairings of two centrifuged animals. This suggests that some event regulating fetal skeletal growth occurs during early reproductive stages, and that this regulatory event can be altered by gravity. (Duke)

- A range of observed abnormalities in embryonic amphibian nerve and muscle cells grown in culture in simulated microgravity suggest that this condition exerts profound effects on the ability of embryonic cells to mature and to communicate with each other. (Gruener)

Bone and Muscle

- Levels of somatomedin C (an insulin-like growth factor) decrease in the growth plates of unweighted rat bones. This result, combined with an inability of growth hormone to reverse the inhibition of bone formation caused by unloading, suggests that the unloaded bone may have an abnormal response to growth hormone. (Bikle)

- The preceding results could be a key to understanding why bone formation is inhibited by unloading, since somatomedin C stimulates bone formation and its production by bone is thought to be under growth hormone control. (Bikle)

- Bone formation is accelerated when rats are allowed to recover after hindlimb unloading; the deficit in bone mass is nearly completely reversed in 2 weeks. (Bikle)

- Results from several spaceflight experiments indicate that blood vessels in bone are affected by microgravity. In ground experiments, blood flow in bone is reduced during the first 2 days of non-weight-bearing, and returns to normal by 5-7 days. (Doty)

- Ground experiments also show that osteoblasts (bone-forming cells) adjacent to blood vessels in bone are affected by non-weight-bearing. (Doty)

- The two preceding sets of results suggest that blood flow and mechanical stress are both necessary for normal maintenance of bone. (Doty)

- When compared with a spaceflight experiment, the ground-based, unloaded rat model duplicated many, but not all of the effects of spaceflight. Muscle and bone changes were similiar but testicular atrophy was greater with the model. (Morey-Holton)

- Changes in muscle and bone caused by ground-based unloading may be transient, since neither system showed major changes in a long-duration (40-day) study. (Morey-Holton)

- A strong correlation was found between the energetics of induced electrical fields (stress-generated potentials) caused by functional loading in birds and the remodeling response of bone to those same functional loading patterns. (McLeod)

- An ability to predict the bone remodeling response from stress-generated-potential energetics may lead to the design of a mechanical loading regimen that could maximize the stimulus to the bone while at the same time minimizing the time and physical energy required to maintain bone mass. (McLeod)

- Production of osteoblast (bone-forming cell) precursor cells was found to be depressed uniformly both day and night in simulated weightlessness tests, which

suggests that disturbance of the circadian rhythm is not the mechanism by which simulated weightlessness decreases the number of preosteoblasts. (Roberts)

- Differentiation to a preosteoblast is enhanced by a decrease in cell density. An observed decrease in cell density in a ground-based experiment suggests that preosteoblast production may be suppressed as a result of a relative increase in cell density due to loss of extracellular fluid during spaceflight. (Roberts)

- Dexamethasone, a synthetic glucocorticoid that slows the rate of growth of bone cells in culture, apparently affects entry of the cells into S-phase (the DNA synthesis stage). (Hughes-Fulford)

- Dexamethasone causes a decrease in prostaglandin synthesis in bone cells with the greatest effect occurring in late G_1 at the entry of cells into S-phase. This suggests a possible causal relationship between prostaglandin E_2 and cell growth. (Hughes-Fulford)

- Epidermal growth factor and a tumor promotor called PMA cause an increase in collagenase inhibitor in osteoblast cells, which is paralleled by increases in the messenger RNA for this protein. (Partridge)

- Osteoblasts cultured in roller bottles produced more collagenase inhibitor messenger RNA and procollagen messenger RNA than those cultured in stationary flasks indicating responsiveness to motion and/or gravity. (Partridge)

- Unloaded muscle (and possibly denervated muscle as well) may act to slow or regulate its rate of atrophy by reducing the rate of protein degradation. (Tischler)

- Stretch alone can prevent only some of the effects of unloading in muscle. (Tischler)

Regulatory Biology

- Hyperdynamic environments result in extended depressed rat body temperatures, multiple-day recovery periods for the temperature rhythms, and greater depression of circadian regulation of body temperature. These results indicate that the effects of gravity on temperature regulation are multiple and distinct. (Fuller)

- Body temperature rhythms may not synchronize to the 24-hr light/dark cycle upon exposure to simulated microgravity. (Fuller)

- A computer model was developed to predict and simulate the electrical activity of a rat hippocampal pyramidal cell, including the effects of temperature. The model is based on a simulation of currents passing through ion channels located in the pyramidal cell membrane. (Horowitz)

- Hippocampal pyramidal cell model simulations correspond to experimental data over a range of temperature from 40°C to 35°C. (Horowitz)

- Studies of the renin angiotensinogen system suggest that there may be direct neural as well as neuroendocrine control of angiotensinogen secretion. (Ganong)

- It was shown that the ventromedial nuclei affect renin secretion but not angiotensinogen concentrations, which indicates that an additional hypothalamic mechanism may play a role in regulating salt and water homeostasis and maintaining blood pressure. (Ganong)

Cell Biology

- A controllable direct-current electromagnet has been developed for biological investigations. It will be used in conjunction with magnetic microparticles to apply mechanical tension to cell surfaces, to study the effects of physical forces on cell metabolism. (Ingber)

- A closed vial, cell culture system holding numerous vials has been developed and is suitable for maintaining functional (growth-hormone releasing) rat pituitary cells for 9 days. This system will be used in an upcoming flight experiment. (Hymer)

PLANT PROJECTS

EFFECT OF MICROGRAVITY ON GROWTH HORMONE CONCENTRATION AND DISTRIBUTION IN PLANTS

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Description of Research

Moving a plant from a vertical to a horizontal orientation in a 1 g environment causes a change in the distribution of growth hormone such that the plant grows back into a vertical orientation. It is also known that a change in the gravitational vector causes a membrane depolarization and changes in the distribution of calcium. There are uncertainties as to the mechanism by which the plant senses the gravitational vector; how the gravitational vector change causes membrane depolarization; and how the depolarization is transduced into chemical changes such as the asymmetric distribution of growth hormone, indole-3-acetic acid (IAA) and calcium. Despite the gaps in our knowledge, plants provide an attractive system for studying the effects of gravity on living organisms since they provide the most reductionist system available. If we can understand this relatively simple system, we may better understand the effects of gravity on the relatively more complex animal systems.

It is not known what will happen to the distribution of calcium or IAA in a microgravity environment. In preparation for a Shuttle flight experiment, we have used the time awaiting a flight opportunity to improve our knowledge of the homeostatic mechanisms used by a plant to control its endogenous levels of IAA. This increased knowledge will enable us to better understand effects of weightlessness on IAA content and turnover as may be observed during the flight experiment. We have used two major approaches: (1) We have attempted to determine whether a seedling plant synthesizes any of its IAA *de novo*, or whether it obtains all of its IAA by hydrolysis of IAA conjugate reserves; and (2) We have attempted to determine the relationship between seedling growth rate and IAA content. In addition, we have also begun to optimize our analytical procedures so as to make optimum use of the scarce space-grown material.

Accomplishments

(1) The mechanism of biosynthesis of indole-3-acetic acid is not known. It has been repeatedly postulated that the plant first synthesizes tryptophan, and then the tryptophan is decarboxylated and deaminated to form indole-3-acetic acid. However, the postulated route through tryptophan is uncertain, and we have shown that seedling plants of *Zea mays* do not convert 5-[^{3}H]-tryptophan to labeled IAA. Owing to uncertainties about the biosynthetic route, we used the most general precursor possible, that is, growing the plants on 30% deuterium oxide (D_2O). If *de novo* synthesis of IAA occurs, there will be incorporation of deuterium into non-exchangeable positions in the indole ring — that is, positions 2,4,5,6, and 7. If no incorporation occurs, then we will know that our seedling plant system is a closed system with no *de novo* synthesis. That knowledge will considerably simplify interpretation of the results of our flight experiment.

For the study of deuterium incorporation into IAA by corn seedlings, the kernels were imbibed with 30% D_2O for 24 hr, then grown with 30% D_2O for 96 hr. The IAA was then isolated, treated with hot alkali to remove exchangeable deuterium, then methylated and examined by gas chromatography-mass spectrometry. The results showed no deuterium incorporation into stable

positions of the indole ring. Using a different variety of corn and different growth conditions, we had earlier observed deuterium incorporation into IAA. Thus, we feel confident that ***under our current growth conditions, plants do present a simple and closed system with no de novo IAA synthesis.*** Measuring the rate of depletion of endosperm IAA conjugate reserves will provide an estimate of the rate of use of IAA by the seedling. This is important for the flight experiment.

(2) A second important consideration is the relationship between growth rate and the amount of endogenous IAA in the plant. Owing to the low concentrations of free IAA and the huge concentrations of phenyl propene acids in plants, it has not previously been possible to obtain accurate measurements of the amount of IAA in the growing tissue. We introduced a gas chromatographic-mass spectrometric assay using 4,5,6,7 tetradeutero IAA as an internal standard. This method was sensitive and gave certainty as to what was being measured. More recently, the method has been further improved using IAA labeled with 6 atoms of ^{13}C in the benzene ring as an internal standard. A second change, in addition to the change in assay procedure, was the use of the mesocotyl of the seedling as experimental material. With this tissue, it is possible to remove the vascular tissue, and do an assay for IAA on cortical tissues.

The results of our assays are shown in Figure 1. The reciprocal of the growth rate is plotted against the reciprocal of the amount of free IAA in the cortical tissues. We varied the endogenous free IAA in two ways, first by decapitation of the shoot, since this procedure is known to deprive the shoot of endogenous IAA or, second, by exposing the seedlings to a flash of red light (since this is known to reduce the growth rate of the mesocotyl tissue). Many interesting conclusions may be drawn from this plot. First, the relationship between growth rate and IAA amount is arithmetic and not logarithmic. This means ***that all of the "free" IAA is bound to a receptor.*** Secondly, it can be seen that the plant is operating at about the K_m for IAA, since the amount of free IAA in the cortex is 5.6×10^{-8} M whereas the K_m for IAA is 6.7×10^{-8} M.

Significance of the Accomplishments

It is important to know what effects, if any, microgravity will have on endogenous plant growth hormones. Such knowledge will be of value in the Controlled Ecological Life Support System (CELSS) program and also of importance in understanding the effects of a gravitational stimulus at a molecular level. Of particular importance is the relative simplicity of a plant seedling system. We have essentially defined all the pathways for the metabolism and transport of IAA in the corn seedling system. Now, hopefully from this reasonably well-defined system will come a reductionist system for studying the pervasive effects of gravity on living material.

Publications

Bandurski, R.S., Schulze, A., Desrosiers, M., and Epel, B. Transduction of the Gravitational Stimulus (Abstract). *ASGSB Bulletin* 1: 28, 1988.

Bandurski, R.S., Schulze, A., Domagalski, W., Komoszynski, M., Lewer, P., and Nonhebel, H.M. Synthesis and Metabolism of Conjugates of Indole-3-Acetic Acid. In: *Proceedings of the International Symposium on Conjugated Plant Hormones, Structure, Metabolism, and Function* (ed. by K. Schreiber, H.R. Schütte, and G. Sembdner), pp. 11-20, 1988.

Desrosiers, M.F. and Bandurski, R.S. Effect of an Applied Voltage Upon IAA Transport (Abstract). *ASGSB Bulletin* 1: 21, 1988.

Domagalski, W., Schulze, A., and Bandurski, R.S. Isolation and Characterization of Esters of Indole-3-Acetic Acid From the Liquid Endosperm of the Horse Chestnut (*Aesculus* sp.). *Plant Physiology* 84: 1107-1113, 1987.

Lewer, P. Preparation of 7-Hydroxy-2-Oxoindolin-3-ylacetic Acid and its [$^{13}C_2$], [5-n+-^{3}H], and [5-n-^{3}H]-7-0-Glucosyl Analogues for Use in the Study of Indol-3-ylacetic Acid Catabolism. *Journal of Chemical Society Perkin Transactions* I: 753-757, 1987.

Lewer, P. and Bandurski, R.S. Occurrence and Metabolism of 7-Hydroxy-2-Indolinone-3-Acetic Acid in *Zea mays*. *Phytochemistry* 26: 1247-1250, 1987.

Leznicki, A. and Bandurski, R.S. Partial Purification and Characterization of Uridine Diphosphoglucose-Indole-3-Acetyl-Glucose Transferase (Abstract). *Plant Physiology* 83(Suppl.): 94, 1987.

Schulze, A. and Jensen, P.J. An Evaluation of the Function of the Coleoptile Tip (Abstract). *Plant Physiology* 83(Suppl.): 102, 1987.

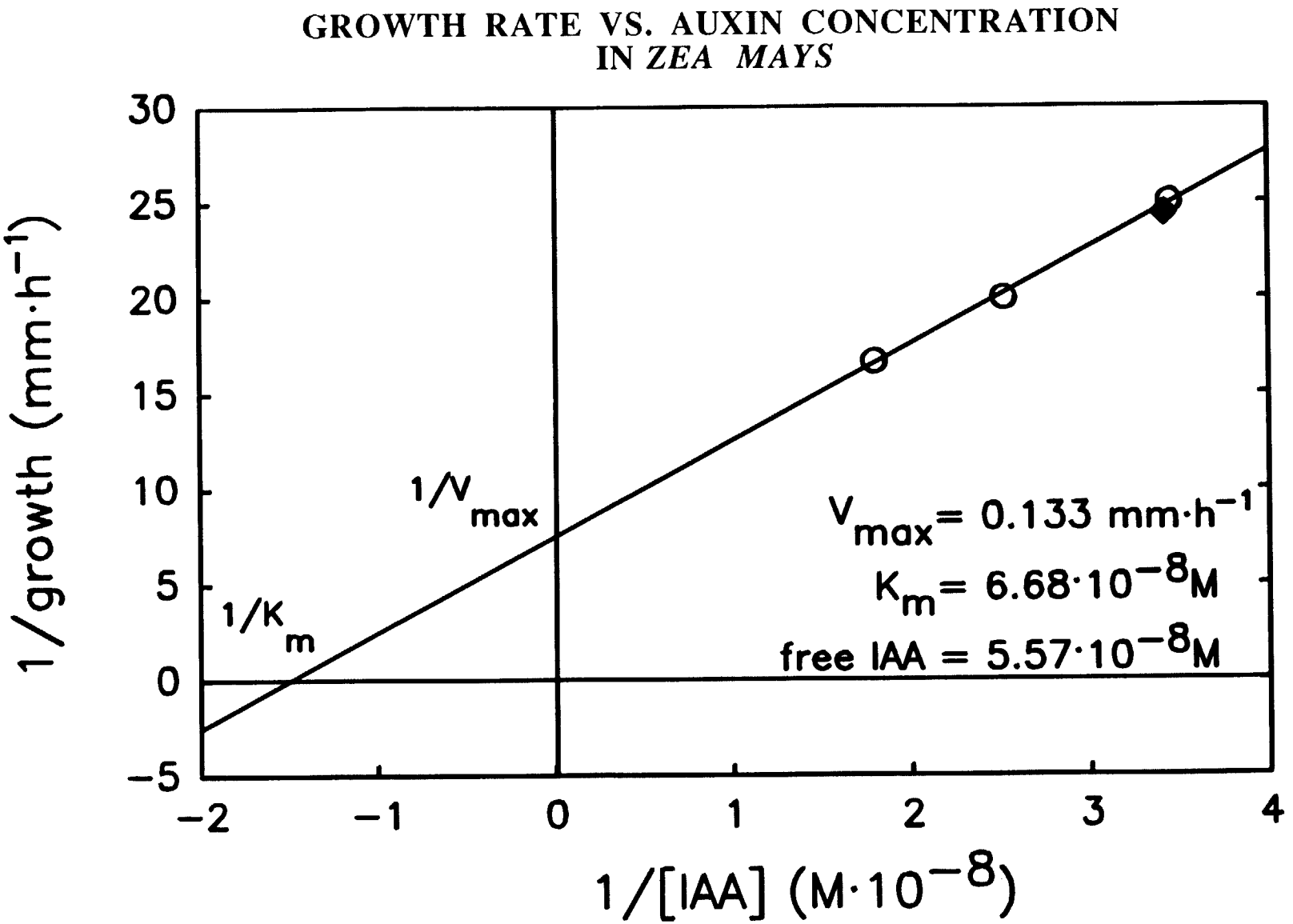

Figure 1. A plot of the reciprocal of the growth rate of a 1-cm portion of the mesocotyl of a seedling of *Zea mays* against the reciprocal of the concentration of free IAA in that same section of the mesocotyl cortex. An apparent maximum velocity of growth may be determined from the slope. The apparent affinity of the IAA binding site for IAA may be determined from the ordinate intercept. It is of interest that the concentration of free IAA in the mesocotyl cortex is

LOCALIZATION AND IDENTIFICATION OF THE GRAVITY SENSING MECHANISM OF PLANTS

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Description of Research

A gravitational stimulus induces the sequence of events illustrated in Figure 1. (1) A membrane depolarization occurs within seconds after the stimulus (Behrens, H.M., et al., *Planta* 163: 463-472, 1985); (2) Within minutes, an asymmetric distribution of the plant growth hormone indole-3-acetic acid (IAA) develops (Bandurski, et al., In: *Second Messengers and Phosphoinositids in Plants*, A. Liss, in press); (3) About simultaneously, an asymmetric distribution of calcium develops (Slocum and Roux, *Planta* 157: 481-492, 1983); (4) IAA and calcium asymmetries are followed within an hour by an asymmetric distribution of various nutrients for growth (Goswami and Audus, *Annals of Botany* 40: 49-64, 1976); and, finally, (5) Asymmetric growth occurs and this restores the plant to its normal orientation relative to the gravitational vector. The intent of this research is to explain this sequence of events at the molecular level.

Accomplishments

(1) The working theory we have developed for the above sequence of events has been published and is gaining acceptance (Bandurski, R.S., et al. In: *Second Messengers and Phosphoinositids in Plants,* A. Liss, in press; Bandurski, R.S., et al., *Advances in Space Research* 6: 47-54, 1986; Bandurski, R.S., et al., *Physiologist* 27 (6, Suppl.): 123-126, 1984). The major advantage of the theory is that it provides a mechanism for utilizing the membrane depolarization that occurs rapidly following either a gravity or light stimulus to cause the development of asymmetric chemical distributions such as that of IAA and calcium. Briefly stated, the postulates of the theory are that the gravity or light stimulus causes a membrane depolarization which, in turn, opens and/or closes potential-gated IAA and calcium transport channels between cells and between vascular and cortical tissues. A diagrammatic sketch of the postulated reaction sequence is shown in Figure 2.

(2) An initial test of the potential gating theory was to determine if application of a small electrical potential to growing plant shoot tissue would cause growth effects. Our data show that a ***5- or 10-volt DC potential, applied along the length of the shoot, is sufficient to almost totally inhibit growth when the tip of the plant is made positive relative to the roots, whereas that same small potential gives almost no growth inhibition if the tip is made negative relative to the roots*** (Desrosiers and Bandurski, *Plant Physiology*, in press). Current flow is the same with either polarity and the potential applied is only on the order of 0.6 mV per 10 micrometer cell. These data confirm and extend earlier studies (Cholodny and Sankewitsch, *Plant Physiology* 12: 385-408, 1937) in a statistically valid manner and using procedures that produce sufficient tissues for chemical analysis.

(3) A test of the potential-gating theory was to determine whether a gravity-stimulated stem could selectively unload IAA from the stele into the surrounding cortical cells. For this experiment, 5-[^{3}H]-IAA was injected into the kernels of vertically held seedlings of *Zea mays*. A gravity stimulus was then given by moving the seedlings to a horizontal position. The results show that the radioactivity moves selectively into the lower half of the horizontal seedling. ***This***

experiment demonstrates that the plant can control the flow of IAA from stele to cortex.

(4) As shown in the data of Table I (Desrosiers and Schulze, unpublished), ***a 5-volt potential will alter the movement of ^{45}Ca from seed to shoot.*** As demonstrated by the data of Table II (Schulze, Desrosiers, and Jensen, unpublished) ***a 5-volt potential will cause an increase in the amount of esterified IAA in the stele of a tip-positive seedling.*** Whether these effects of potential application are sufficient to explain the observed growth inhibition remains to be demonstrated.

Table I. Effect of a 5-volt potential on the transport of ^{45}Ca from endosperm to shoot.

	(dpm·plant^{-1} + S.D.)
Control	107 ± 24
Top positive	74 ± 16, **, t = 2.80
Top negative	148 ± 65, t = 1.45

Table II. Effect of a 2-hr application of a 5-volt potential on the endogenous ester IAA in the stele of the mesocotyl of *Zea mays* seedlings.

	pmol·plant^{-1}
Control	2.30 ± 0.45
Top positive	4.32 ± 1.97
Top negative	2.30 ± 0.39

Significance of the Accomplishments

If the potential-gating theory is correct, it will mean that a target for the gravity stimulus has been found — to wit — the plasmodesmatal channels that connect the vascular stele of the plant with the surrounding cortical tissue (Figure 3). It would then be possible to state that the gravitational stimulus is transduced by increasing or decreasing the resistance to solute movement between stele and cortex. We plan in the future to make direct tests of the control of solute movement between stele and cortex.

Publications

Desrosiers, M. and Bandurski, R.S. Effect of an Applied Voltage on Growth Rate of *Zea mays* Seedlings (Abstract). *Plant Physiology* 83(Suppl.): 19, 1987.

Epel, B.L. and Bandurski, R.S. Selective Tissue-Tissue Communication in *Zea mays* Seedlings (Abstract). *Plant Physiology* 83(Suppl.): 66, 1987.

Schulze, A. and Bandurski, R.S. A Gravity Induced, Asymmetric Unloading of Indole-3-Acetic Acid from the Stele of *Zea mays* into the Mesocotyl Cortex (Abstract). *Plant Physiology* 83(Suppl.): 102, 1987.

TIME COURSE FOR GRAVITY INDUCED ASYMMETRIES

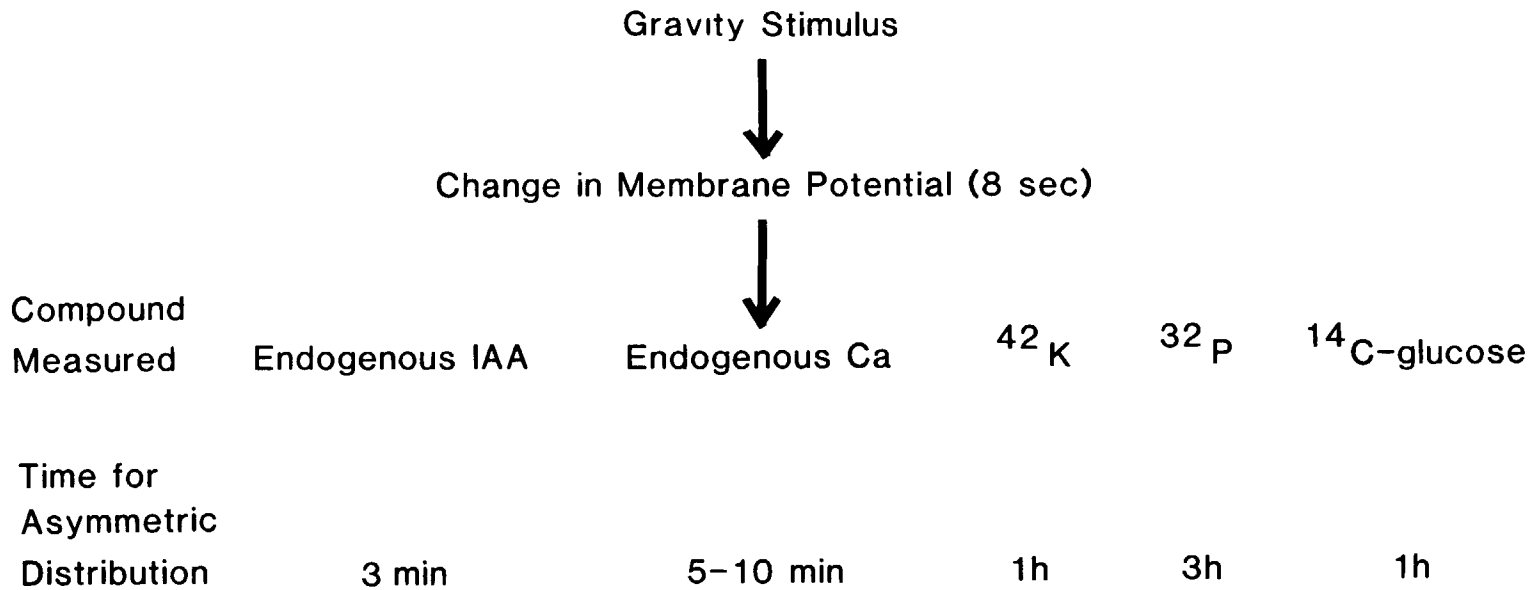

Figure 1. Time course for physicochemical changes following movement of a plant from a vertical to a horizontal orientation.

SEQUENCE OF EVENTS IN POTENTIAL-GATING

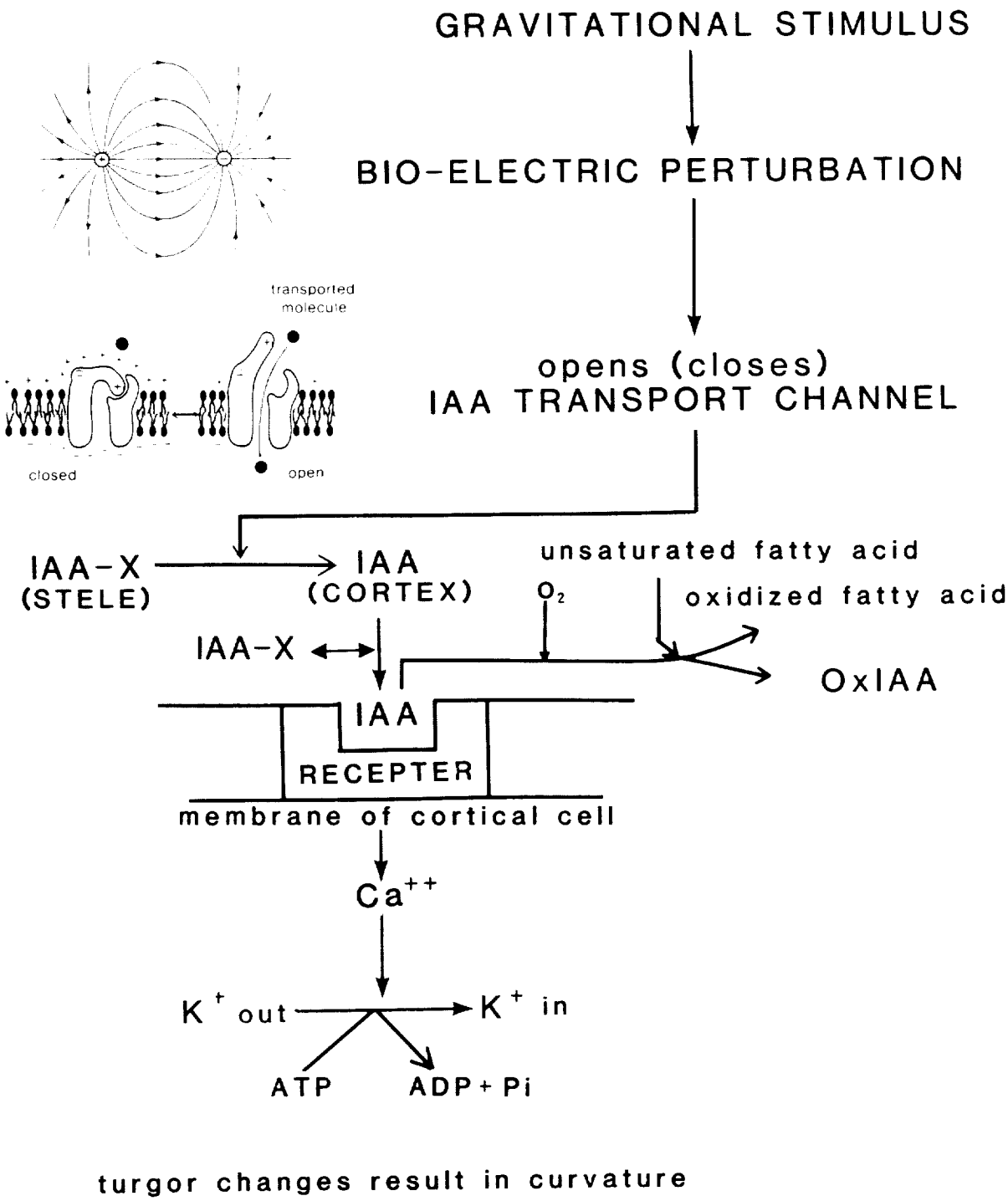

Figure 2. Diagrammatic representation of the potential-gating theory. The environmental stimulus causes membrane depolarization, which opens and/or closes potential-gated channels and gap junctions between cells and, in particular, between the stele and cortex. IAA is "selectively leaked" from one side of the stele into one side of the cortex. The IAA-receptor complex can then induce a second messenger cascade, resulting in turgor changes and tropic growth.

POTENTIAL-GATING IN PLASMODESMATAL CHANNELS

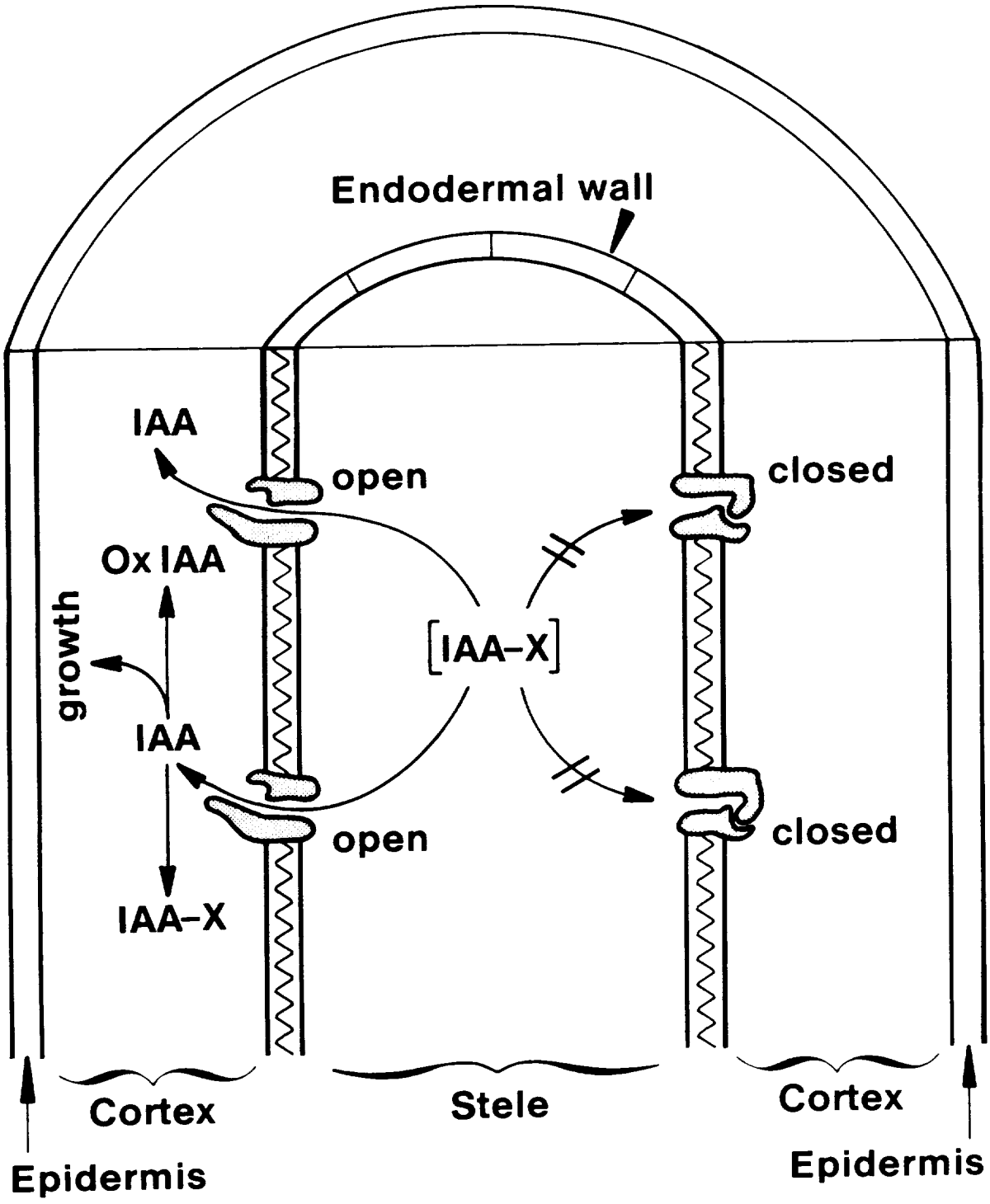

Figure 3. Diagrammatic representation of how the plasmodesmatal channels between the vascular tissues of the plant and the surrounding cortical tissues might be potential-gated in a manner analogous to animal gap junctions.

PLANT MORPHOGENESIS AND PHYSIOLOGICAL BEHAVIOR IN RELATION TO GRAVITY

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Description of Research

The long-range goal is to improve our understanding of how gravity is important for plant development and physiological behavior. The unique advantage offered by experimental access to protracted microgravity is the ability to observe plant growth and function and to perform experiments in protracted hypogravity. To accomplish these efforts it is necessary to use not only an orbiting laboratory but also a centrifuge of suitable design so that tests can be conducted throughout the g-force region from essentially zero to unit g and above. Both exploratory research investigations and critical tests of theory have been the subjects of our past and, hopefully, future studies in true hypogravity. Our prediction that research on plants in hypogravity would be scientifically fruitful and would produce some surprises has already proven to be correct, which disposes us to be optimistic about future scientific experimentation with plants in space laboratories.

Methodology: Experimental manipulation of the g-force magnitude is unambiguously achievable in space. Flight opportunities are infrequent, however. Therefore, utilization of hypergravity (centrifugation) and of simulated hypogravity (clinostat rotation), both conveniently attainable in Earth-bound laboratories, have been exploited, often as preliminary to a definitive flight experiment. Since our experimental materials (seedlings and small plants) usually are too large for appropriate use of fast rotating clinostats (ca. 0.8 Hz), we use slowly rotating clinostats (ca. 1.7 to 17 mHz). Much if not most of our work with clinostats requires rotation simultaneously on two orthogonal axes, one of which must be as nearly horizontal as possible.

In some cases comparison of results from clinostat simulation experiments and from space experiments (preferably with the same apparatus) can be used to test the validity of the simulation method. Whenever possible we try to plan experiments in such a way that this kind of validation test can be achieved, if only as a secondary but nonetheless very important scientific objective.

Accomplishments

(1) ***Developed a preliminary version of an analysis system (VDAS) for processing FOTRAN video images in near real time during flight.***

(2) ***Developed a system for processing GTHRES video images in near real time during flight.*** FOTRAN and GTHRES VDAS will be needed, especially in the early stage of the International Microgravity Laboratory-1 mission, to confirm the suitability of inertial (centripetal) and photic test stimuli in time to make allowances for unexpectedly larger (or smaller) plant responses than had been anticipated.

(3) Conducted a series of Investigators' Ground Studies (IGS-1) for both FOTRAN and GTHRES experiments using flight hardware Gravitational Plant Physiology Facility (GPPF) at NASA Ames Research Center.

(4) Developed a detailed test plan for IGS-2, scheduled for June 1988.

(5) Collected (processed) additional data from FOTRAN stimulus lamps' performance during KC-135 parabolic flight tests.

(6) Developed a modified test plan for a second series of KC-135 flight tests scheduled for mid-June 1988.

(7) Hosted a visiting investigator, Dr. M.A. Benjaminson, who conducted a series of centrifuge test runs at different levels of hypergravity to obtain data on aspects of development of *Dictyostelium.*

Publications

Chapman, D.K. and Brown, A.H. Validation of Clinostat Simulations - A Long Standing Problem (Abstract). In: *Space Life Sciences Symposium: Three Decades of Life Science Research in Space,* Washington, D.C., June 21-26, p. 44, 1987.

Chapman, D.K. and Brown, A.H. Variable Speed Centrifuges Create Any Test Condition in Space From μG to 1.3G (Abstract). *ASGSB Bulletin* 1: 38, 1988.

Chapman, D.K., Heathcote, D.G., and Brown, A.H. Light Output from Tungsten Filament Lamps During Low Gravity Exposure on KC-135 Parabolic Flights (Abstract). *ASGSB Bulletin* 1: 37, 1988.

Zachariassen, E., Johnsson, A., Brown, A.H., Chapman, D.K., and Johnson-Glebe, C. Influence of the g-force on the Circumnutation of Sunflower Hypocotyls. *Physiologia Plantarum* 70: 447-452, 1987.

MICROGRAVITATIONAL EFFECTS ON CHROMOSOME BEHAVIOR

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Description of Research

The long-term goal of this research project is to assess and quantitate the potential genetic risk of the space environment to man. To achieve this goal, we are: (1) studying at the basic level the molecular mechanisms involved in the correct separation of chromosomes, and (2) constructing a microbiological system to be used as a model for detection of genetic alterations during a spaceflight experiment inside Spacelab, aboard the Space Shuttle.

The focus of the experiments conducted during the past year has been the molecular cloning of the yeast cell-division-cycle *cdc6* gene, whose product is involved in correct chromosome segregation during mitosis. By screening a yeast genomic DNA library, we have been able to clone a gene able to correct a defect in the cell's *cdc6* gene. This gene has been shown by recombinant DNA and genetic engineering technology to be located on the same chromosome as the original mutation. This is further proof that we have cloned the expected gene and not another gene that suppresses the abnormal phenotype. Moreover, we have determined the DNA sequence of this gene and therefore deduced the protein sequence and structure by computer analysis. Recently, we have obtained preliminary evidence that the *cdc6* gene, which appears activated at particular times during the cell cycle, is involved in the repair of ultraviolet radiation damage to the cell.

At the same time, we have analyzed the effect of a temperature-sensitive mutation of this gene on the level of DNA recombination and chromosome loss. Our results show that chromosome loss, as detected by genetic analysis, is the most important anomaly generated by the mutation.

What we have now is one individual gene that is essential for the normal division of the cell and whose alteration induces errors in the mechanism of chromosome replication and partitioning during the cell cycle. This will allow us to further investigate at the molecular level the intimate mechanism of transmission of genetic information from one cell to another during cell division.

Accomplishments

(1) ***Molecular cloning, sequencing, and characterization of the yeast cdc6 gene involved in the process of chromosomal segregation during cell division was accomplished.***

(2) Accomplished genetic quantitation of the alterations occurring at the level of chromosome loss and recombination in a strain that carries a mutation in the *cdc6* gene.

(3) Completed construction of yeast strains with a genetic background suitable for detection of space-induced genetic alterations.

Significance of the Accomplishments

In order to understand why exposure to the space environment affects chromosome structure and segregation during cell division, we must first understand how chromosomes behave in normal conditions. To this end, it is of the utmost significance to isolate and characterize those gene

products that are responsible for the correct succession of events leading to the duplication and then the division of chromosomes. With the first accomplishment, we have isolated the molecular information responsible for one of these products and therefore we can now study its expression, regulation, and mechanism of action. In addition, secondary events not immediately visible at the level of an organism may be produced by the exposure to spaceflight conditions. Among these, alteration of the recombination frequency between genes can lead to the expression of hidden mutations and, ultimately, to cancer.

The second accomplishment, which relates the alteration of chromosome transmission to an abnormal level of DNA recombination, is of significance to the study of the secondary effects of the spaceflight environment in eukaryotic organisms. If the discovery of a role of the *cdc6* gene in DNA repair is confirmed, the damage occurring in a *cdc6* mutant could very well serve as a basic model to mimic the effects of spaceflight in a microbial system.

The genomic abnormalities observed in *cdc6* mutants seem structurally and functionally analogous to those observed in other microbiological systems exposed to the spaceflight environment, for example, root tip cells of oat plants. This, together with the modelistic value of the yeast system and its experimental genetic manipulability, provides an ideal system to study large numbers of genetic events in space with reduced handling, biohazard, and cost.

Publications

Bruschi, C.V. and Chuba, P.J. Nonselective Enrichment for Yeast Adenine Mutants by Flow Cytometry. *Cytometry* 9: 60-67, 1988.

Colasanti, J.J. and Bruschi, C.V. Molecular Analysis of *CDC6* Gene Function in *Saccharomyces cerevisiae* (Abstract). In: *Yeast Cell Biology*, Cold Spring Harbor Laboratory, p. 36, 1987.

THE ROLE OF GRAVITY IN APICAL DOMINANCE

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Description of Research

That lateral bud outgrowth is sensitive to gravity can be easily demonstrated by orienting a herbaceous shoot at the three following positions: (1) upright, at which no lateral buds sprout; (2) horizontal, at which several lateral buds grow out at apparently random positions on the stem; and (3) inverted, at which several lateral buds near the base of the stem will sprout and grow upwards. Hence, the location at which the buds sprout is greatly influenced by the orientation of the shoot.

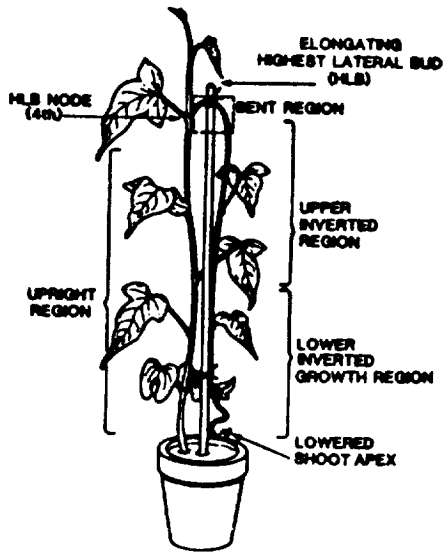

Figure 1. Depiction of growth of the highest lateral bud after inversion of Japanese Morning Glory shoots.

In Japanese Morning Glory, we have found that if the upper shoot is bent down at the fourth node and if the inverted apex is secured to a stake with a string, the highest lateral bud, adjacent to the bend in the stem, will begin to grow out within 24-36 hr (Figure 1). This shoot inversion-induced release of apical dominance is correlated with enhancement of ethylene production, the inhibition of elongation, and the accumulation of glycoprotein and lignin in the inverted shoot. It appears that the outgrowth of the highest lateral bud is due to restriction of elongation of the inverted shoot via inversion-induced ethylene production.

In addition to the gravity stress associated with the displaced orientation of shoot inversion, there appears to be a restraining stress imposed upon the inverted shoot due to the prevention of gravicurvature by the string secured to a stake. The degree to which the restraining stress contributes to the total stress is unknown.

The long-term goal of this project has been to understand how gravity affects lateral bud outgrowth. Some of the specific objectives during the past year have been: (1) to determine the generality of the shoot inversion-induced responses of ethylene production and growth inhibition in the inverted shoot; (2) to analyze the effects of shoot inversion on stem structure and on auxin content/transport; and (3) to determine if gravity controls cell wall glycoprotein production in the inverted shoot at the gene level.

Accomplishments

(1) ***Shoot inversion-induction of ethylene and the inhibition of elongation in the inverted shoot, previously observed in morning glory, were also found in corn, peas, soybean, and tomato.*** The enhanced ethylene production in all cases appeared to be due to activation of ACC synthase, the key enzyme in the ethylene biosynthetic pathway. Shoot inversion promotion of ethylene production was not observed to be a persistent response. It could be reversed and restimulated by appropriate manipulation of the shoot.

(2) In a collaborative study with Fred Sack, also of Ohio State University, we determined that after 72 hr of shoot inversion, ***there was an increase in stem diameter, accompanied by an increase both in cell number and in cross sectional area of pith and of vascular tissue. There appeared to be no effect on cell wall thickness.*** Restriction of shoot elongation was also correlated with a significant decrease in the length of pith cells.

(3) ***Preliminary results indicated that shoot inversion caused a 29% decrease in the concentration of endogenous auxin in the growth region of the inverted shoot.*** When the auxin transport inhibitor triidobenzoic acid (TIBA) was applied to the stem tissue just above the highest lateral bud of a plant with an inverted shoot, there was a substantial increase in bud outgrowth in the TIBA-treated plant over that of the control.

(4) Additional evidence demonstrated that it was not merely the terminal bud but rather the entire 13-cm growth region of the morning glory shoot that influenced outgrowth of the lateral bud in the release of apical dominance.

(5) ***Genes for hydroxyproline-rich glycoprotein (HRGP), the cell wall strengthening protein, were found in preliminary data to be activated by shoot inversion in soybean. Qualitative evidence for enhanced messenger-RNA accumulation following 48 hr of inversion was determined by RNA blot hybridization.***

Significance of the Accomplishments

Finding #1: The finding that shoot inversion-induction of ethylene production and the inhibition of elongation in the inverted shoot occurs in one monocot and in four other dicots in addition to morning glory suggests that these two responses are general.

Finding #2: Shoot inversion restricts stem elongation by decreasing cell length and increases stem diameter by increasing tangential cell division and the cross sectional area of the pith and the vascular tissue.

Finding #3: The preliminary finding of a lower auxin content in the growth region of the inverted shoot than in that of the upright shoot suggests that enhanced ethylene production in the inverted shoot is not due to the accumulation of auxin but rather to a more direct effect of gravity stress on ACC synthase. The finding that lateral bud outgrowth in the plant with the inverted shoot was enhanced by TIBA treatment of the stem region just above the bud is consistent with

the hypothesis that auxin depletion in the bud via gravity and ethylene inhibition of upward auxin transport in the inverted shoot is responsible for release of apical dominance.

Finding #4: Although the mechanism for controlling lateral bud outgrowth is present within the terminal bud (approximately 0.5 cm in length), it is clear from our present findings that the entire 13-cm growth region does influence lateral bud outgrowth.

Finding #5: Just as the stress of mechanical wounding and pathogen infection results in the transcriptionally induced production of HRGP (an important cell wall structural protein which plays a vital defensive and protective role), so apparently does the stress of shoot inversion cause (via gene activation) the accumulation of HRGP which, in some way, perhaps by inhibition of shoot elongation, assists the plant in adapting to the displaced orientation of inversion.

Publications

Prasad, T.K. and Cline, M.G. Gibberellin-enhanced Elongation of Inverted *Pharbitis nil* Shoot Prevents the Release of Apical Dominance. *Plant Science* 49: 175-179, 1987.

Prasad, T.K. and Cline, M.G. Shoot Inversion and Ethrel Enhancement of Hydroxyproline and Lignin Production in *Pharbitis nil* Shoots (Abstract). *Plant Physiology* 83(Suppl.): 43, 1987.

Prasad, T.K. and Cline, M.G. Shoot Inversion and Gene Expression for Glycoprotein Production (Abstract). *ASGSB Bulletin* 1: 21, 1988.

Prasad, T.K. and Cline, M.G. Shoot Inversion Inhibition of Stem Elongation in *Pharbitis nil*: A Possible Role for Ethylene-induced Glycoprotein and Lignin. *Plant Physiology* 85: 104-108, 1987.

MECHANISM OF DIFFERENTIAL GROWTH DURING STEM GRAVITROPISM

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Description of Research

In this project we are using gravity as a tool to help elucidate some of the cellular and biophysical mechanisms controlling cell expansion and growth of plants. Gravitropism is the bending of plant stems, roots, and other organs in response to a gravity stimulus (Figure 1). One of the intriguing aspects of gravitropism is that cells very close to one another — often less than 1 or 2 mm — respond in opposite fashion. For example, in our research on cucumber stem growth, within 10 min of placing the stem horizontal, the cells on the upper surface cease elongation entirely while those on the lower surface double their elongation rate.

We have measured the intracellular pressure, or turgor pressure, of the cells on the upper and lower stem surface, and found only a negligible change in turgor. These results, in combination with other measurements, indicate (1) that the essential hydraulic properties of the growing cells do not change during the gravitropic response, and (2) that water flow into the expanding cells imposes little restriction on their growth rate. From these results we have concluded that the yielding properties of the wall entirely control the gravitropic growth response. Therefore, we have been studying the nature of these yielding properties, how they are controlled, and the possible influence of extracellular calcium on these properties. Wall calcium has been hypothesized to inhibit wall expansion, either through direct effects on the wall or by influencing enzyme action inside and outside the cell.

Accomplishments

Yielding Properties of Plant Cell Walls

(1) To gauge wall yielding properties, *in vivo* relaxation of vertical cucumber stems was measured with the pressure-block method. About 5 min after the start of wall relaxation, the rate of relaxation was observed to increase 2-5 fold. Total wall relaxation reduced turgor pressure from about 4 bars to about 0.5 bar.

(2) When relaxation was complete, the plants were placed horizontal and the pressure block was maintained. No further curvature was observed. This indicates that ***gravitropism does not induce a further lowering of the wall yield threshold on the lower (fast-growing) stem surface.***

(3) After 1 hr in a horizontal pressure block, the pressure was released. There was no immediate bending of the stem. This indicates that while in the horizontal, pressure-blocked condition, ***the walls on the upper (nongrowing) surface did not become mechanically stiffened or rigidified because of a gravitropism response.***

(4) The dynamic changes in wall properties (Accomplishment #1) were further investigated by applying pressure steps to the growing region and observing the consequent growth behavior. Two conclusions emerged from such experiments: (a) the plants were able to compensate for pressure steps as large to 2-3 bars by adjusting their wall yielding properties; and (b) much of the relaxation measured in (1) entails a rapid decrease in the yield threshold.

Thus, the yield threshold measured by the pressure-block technique is best thought of as the lowest yield threshold ultimately attainable.

(5) Further evidence for the dynamic nature of the control of cell wall yielding properties was obtained by applying a sinusoidally varying pressure to the growing region of the cucumber seedlings, again using the pressure-block instrument. The induced oscillations in growth rate were complex and their pattern varied with the frequency of the oscillation.

Influence of Extracellular Calcium on Wall Properties

(6) Using calcium-specific microelectrodes inserted into the extracellular space of cucumber stems, ***the average free calcium activity was measured to be about 0.25 mM.*** Optical techniques, using calcium sensitive probes, are currently being explored to confirm and extend these results.

(7) Stress relaxation of isolated cell walls was carried out with and without added calcium and EDTA (a calcium chelator), using the method pioneered by Y. Masuda. Remarkably, ***addition or subtraction of calcium had little effect on the relaxation spectrum.***

(8) Long-term extension of isolated cucumber wall was measured with and without added calcium. Concentrations as high as 10 mM only slightly inhibited wall extension.

(9) Cucumber seedlings were found to grow significantly faster when calcium was added to their rooting medium. Calcium concentrations had to reach levels as high as 100 mM before they became inhibitory. Likewise, calcium in the 1-10 mM range had little effect on cucumber section growth.

Significance of the Accomplishments

Finding #1 supplies basic information about the control of wall yielding and shows that a simple viscoelastic or enzymatic mechanism is not sufficient to account for the kinetics of relaxation and the dynamics of recovery from relaxation. Findings #4 and #5 confirm and extend this basic conclusion. It appears that cell expansion is acting like a feedback-controlled process. The nature of the "growth sensor" is entirely unknown, although stretch-activated channels in the plasma membrane may be speculated to play a role. Thus, the membrane channels described by Edwards and Pickard (*1986-87 NASA Space/Gravitational Biology Accomplishments*) may be involved in this growth control process.

Findings #2 and #3: We can imply two facts about the way that growth is modified during the gravitropic response. ***First, the growing stem appears to inhibit growth on the upper stem surface simply by ceasing wall loosening, not by actively rigidifying the walls of the upper surface.*** This would seem to exclude new, additional crosslink formation (e.g., by peroxidases) as a means to induce stem bending. Also, the kinetics of the growth response make such peroxidase crosslink formation within the wall unlikely. Second, the response to gravitropic stimulus does not induce a further reduction in the wall yield threshold (e.g., beyond that attainable in the vertical plant during the pressure-block procedure).

Finding #6: This is apparently the first report of an *in situ* measurement of free calcium in plant cell walls. Findings #7-9 relate to the idea that extracellular calcium may rigidify the wall and thereby inhibit cell expansion. These results show little effect of calcium on wall extensibility properties. Moreover, calcium actually stimulated growth of intact plants; this is contrary to reports of calcium effects in other plants like the oat coleoptile, and suggests the role of calcium may be quite different in these plants. These results do not offer any support for the idea that

gravitropism is mediated wall-stiffening associated with a possible movement of extracellular calcium to the upper (nongrowing) half of a horizontal stem.

Publications

Cleland, R.E., Cosgrove, D., and Tepfer, M. Long-term Acid-induced Wall Extension in an *In-vitro* System. *Planta* 170: 379-385, 1987.

Cosgrove, D.J. Altered Wall Properties During Stem Gravitropism: Relaxation Experiments and the Role of Calcium (Abstract). *ASGSB Bulletin* 1: 20, 1988.

Cosgrove, D.J. Linkage of Wall Extension With Water and Solute Uptake. In: *Physiology of Cell Expansion During Plant Growth* (ed. by D.J. Cosgrove and D.P. Knievel). Rockville: American Society of Plant Physiologists, pp. 88-100, 1987.

Cosgrove, D.J. The Mechanics of Plant Cell Growth. In: *Cytomechanics* (ed. by J. Bereiter-Hahn, R. Anderson, and W. Reif). Berlin: Springer-Verlag, pp. 215-229, 1987.

Cosgrove, D.J. Wall Relaxation and the Driving Forces for Cell Expansive Growth. *Plant Physiology* 84: 561-564, 1987.

Cosgrove, D.J. Wall Relaxation in Growing Stems: Comparison of Four Species and Assessment of Measurement Techniques. *Planta* 171: 266-278, 1987.

Cosgrove, D.J. and Knievel, D.P. (Eds.). *Physiology of Cell Expansion During Plant Growth*. Rockville: American Society of Plant Physiologists, 318 pp., 1987.

Melan, M.A. and Cosgrove, D.J. Evidence Against the Involvement of Ionically Bound Cell Wall Proteins in Pea Epicotyl Growth. *Plant Physiology* 86: 469-474, 1988.

GRAVITROPIC GROWTH RESPONSE IN CUCUMBER SEEDLING

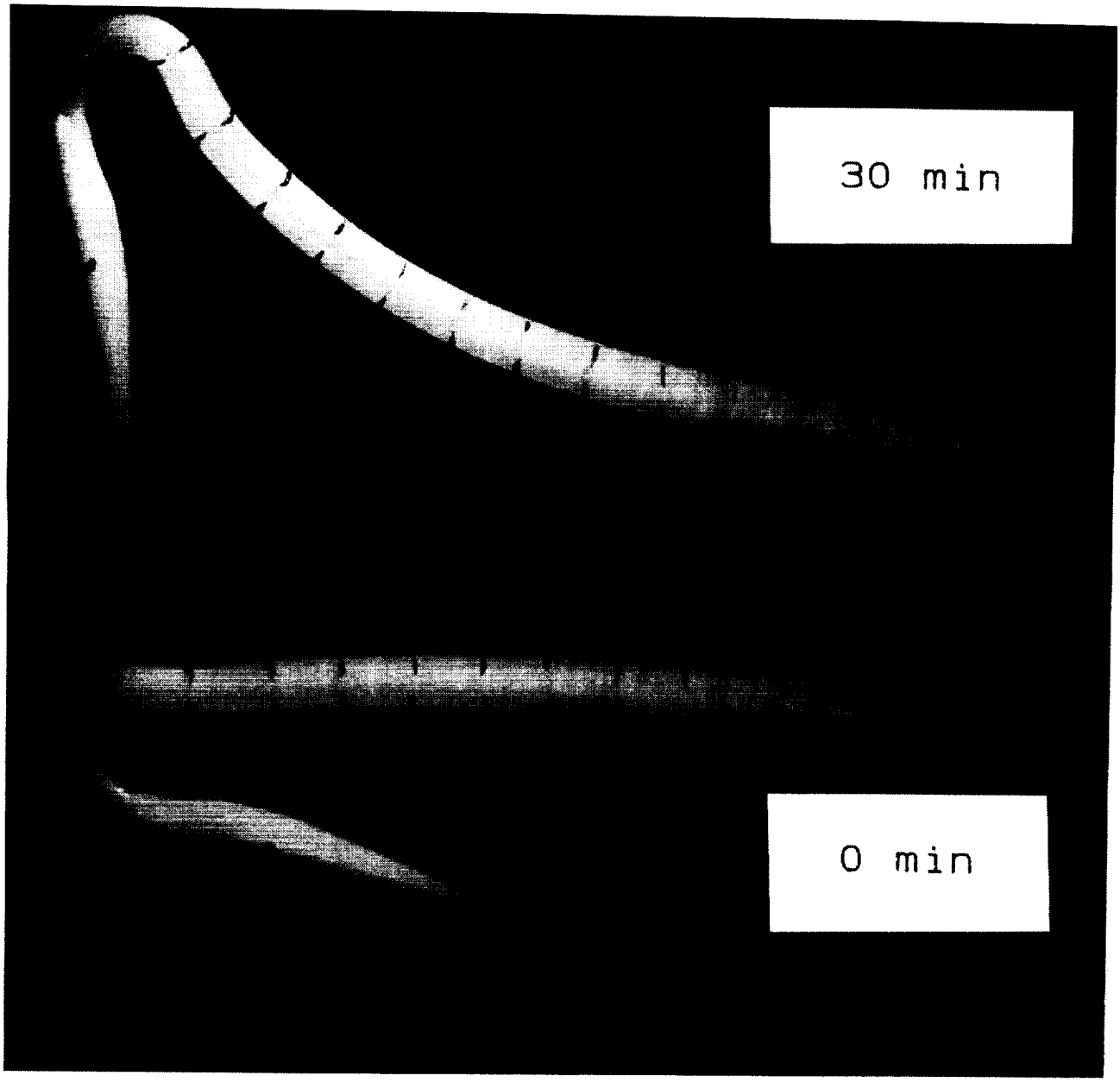
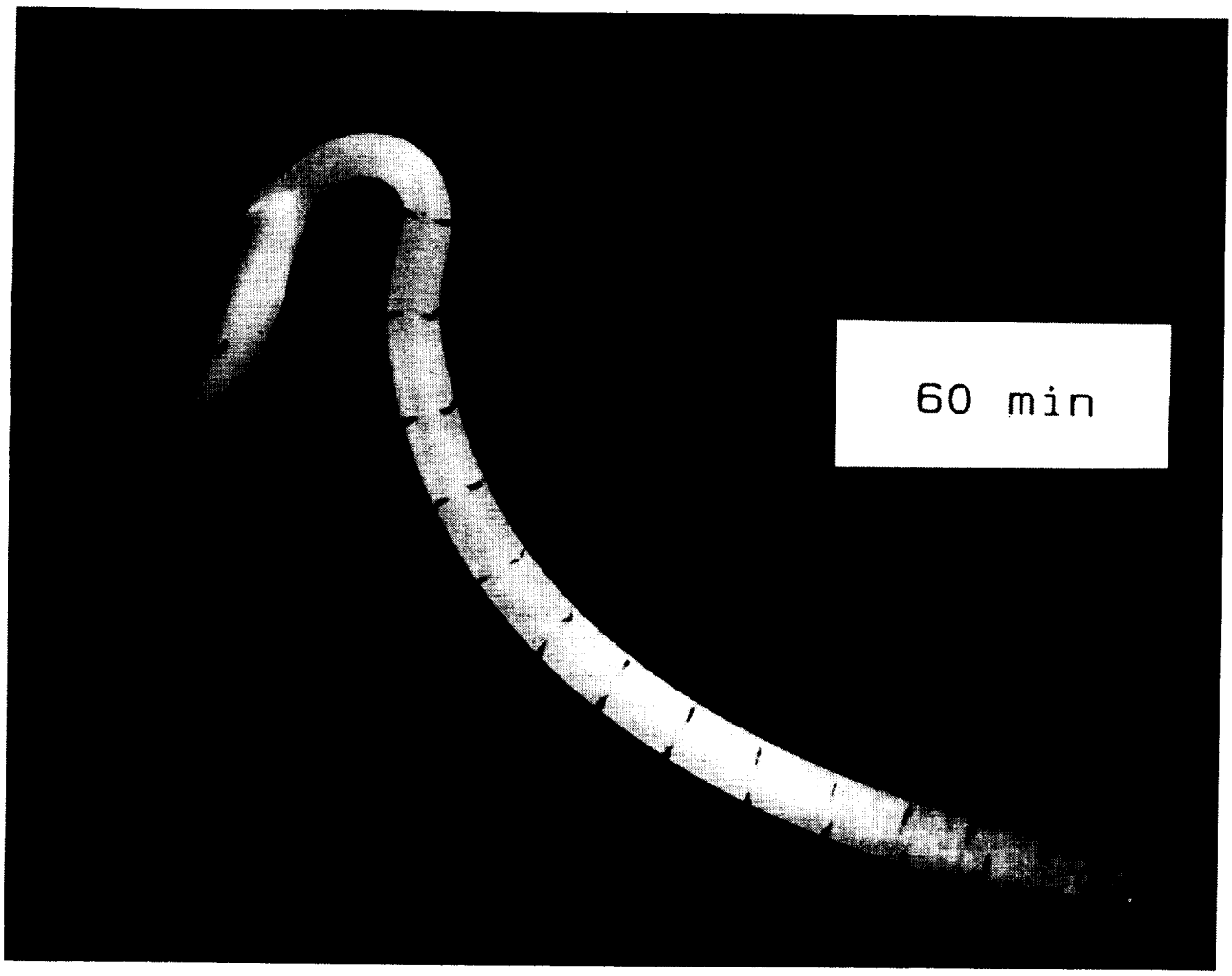


Figure 1. Gravitropism of a young, dark-grown cucumber seedling. Fine marks were placed on two sides of the stem, then the plant was placed in a horizontal position. The marks were used to measure the pattern of the growth response of the upper and lower stem surfaces, using an image analysis system. The three photographs show the plant at 0, 30, and 60 min after horizontal placement. Detailed studies show that stem growth is altered within 10 min of the start of gravitropic stimulation.

GRAVITROPIC GROWTH RESPONSE IN CUCUMBER SEEDLING



STRETCH-ACTIVATED ION CHANNELS IN PLASMA MEMBRANES OF GRAVITY-SENSING PLANT CELLS

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Theories of gravity perception in plants have yet to detail the molecular mechanism of detection. Although amyloplast settling and alteration in charge distribution across the tissue correlate with onset of gravity perception in many cases, the requirement of plasma membrane proteins and adjacent cytoskeleton in gravity detection is rationalized but undefined. The purpose of this work is to define the role of one favorable set of plasma membrane proteins, stretch-activated ion channels (SACs).

Description of Research

Stretch-activated ion channels (SACs) have been described in bacteria, fungi, animals, and plants. Evidence has firmly established their function as mechanotransducers of physical stimuli in rat neuroblastoma cells and those of the salamander choroid plexus where they serve in osmoregulation. Gravity is a physical stimulus which could conceivably affect membrane stretch and activate SACs in organisms detecting gravity. The purpose here is to identify the role of SACs in plant and fungal gravitropism and determine the molecular components contributing to detection of gravity and channel regulation.

Stretch-activated ion channels are being sought in several gravisensing systems: (1) seedling root caps, (2) shoot pulvini, and (3) sporangiophores of the fungus, *Phycomyces*. The latter system is of particular interest because mutants exist that are not gravisensing and, in addition to a gravity sensor, this organism has a distinguishable stretch receptor. Comparison of channel activity among mutants holds promise for distinguishing among the various mechanotransductive roles SACs could play in any one cell. The patch-clamp method of analysis of single ion channels requires that the plasma membrane be removed from the cell wall. This is usuallly accomplished by severe enzymatic degradation of the wall, which undoubtedly alters the membrane and frequently produces protoplasts which cannot be easily examined by patch-clamping. To improve successful examination, we are developing an assay to identify tissues/species which will release suitable protoplasts following enzymatic degradation. For these reasons, we have recently focused our attention on roots of Cucurbitaceae, cucumber, and watermelon, rather than on corn.

Efforts during the past year included moving the laboratory from Missouri to Ohio and establishing a new laboratory at Kenyon College. The new laboratory is fully operational and a data acquisition and analysis system has been installed and debugged. Two honors students (undergraduates) are presently working on the project.

Accomplishments

(1) A patch-clamp laboratory has been established at Kenyon College and a data acquisition and analysis system has been installed and debugged.

(2) Putative stretch-activated cation channels have been observed in amyloplast-containing protoplasts isolated from root caps of primary corn roots.

Significance of the Accomplishments

The existence of stretch-activated ion channels in corn root cap cells thought to have gravity-detecting capability furthers the prospects that such channels will be prominent in initiating the biochemical and biophysical sequence of events resulting in the gravitropic response of plant shoots and roots. Productivity should flourish with the now fully operational and computer-managed patch-clamp facility at Kenyon College.

Publications

Edwards, K.L. and Falke, L. Stretch-Activated Ion Channels in Cultured Tobacco Cells, A Model for the Detection of Physical Stimuli in Plants (Abstract). *ASGSB Bulletin* 1: 44, 1988.

ROLE OF CALCIUM IN SIGNAL TRANSDUCTION IN ROOT GRAVITROPISM

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Description of Research

This research is directed toward understanding the influence of gravity on plant growth — in particular, how roots become oriented with respect to gravity (gravitropism). The detection of gravity occurs at the tip of the root while adjustments in growth rate occur in the growing region about 0.5 cm behind the tip. We have accumulated evidence that gravity-induced redistribution of calcium within the tip of the root links gravidetection to asymmetric distribution of the growth-inhibiting hormone auxin in the growing region of the root.

Our research centered on the following: (1) If differential growth in gravistimulated roots is caused by asymmetric distribution of auxin, where does this auxin redistribution occur, and is there a correlation between the timing of auxin distribution and the timing of curvature? (2) We know that certain cations [especially calcium (Ca) and aluminum (Al)] induce strong curvature when applied to one side of the gravisensing root tip. Roots curve toward Ca but away from Al. Is there a correlation between the ability of these cations to induce curvature and their effects on auxin distribution? (3) Do auxin and Ca have mutually interactive effects on the uptake or efflux of the other substance, as measured in isolated root protoplasts? (4) We find that gravistimulation induces polar Ca movement to the lower side of the root cap. What is the pathway of this movement and is it obligatorily linked to gravitropism and to auxin movement across the root cap? (5) In roots of some cultivars of maize, light is required for normal gravitropic sensitivity. It has been reported that vitamin D mimics light in inducing graviresponsiveness in dark-grown roots. Is this phenomenon repeatable and, if so, how does it relate to light induction of graviresponsiveness? (6) What is the physiological basis for the lack of graviresponsiveness in roots of the light-requiring cultivars?

Accomplishments

(1) ***There is a close correlation between the kinetics of gravi-induced auxin movement across the root cap and the kinetics of gravitropic curvature.*** Auxin movement across the cap is preferentially toward the lower side in gravistimulated roots. The timing of development of this polar movement correlates closely with the time course of curvature.

(2) The curvature-inducing cations, Ca and Al, strongly modify auxin transport in the root, with Ca favoring movement from the tip toward the elongation zone and Al favoring movement toward the tip. ***Ca enhances polar auxin movement across the cap, and Ca chelators such as EGTA prevent the development of polar auxin movement.*** Neither Ca nor Al influences the curvature of roots pretreated with inhibitors of auxin transport.

(3) Auxin does not enhance the efflux of previously absorbed radioactive Ca from purified root protoplasts.

(4) In tests of the pathway of polar Ca redistribution across the cap of gravistimulated roots, we measured Ca movement in roots in which the root cap cells had been plasmolyzed (protoplast drawn away from the cell wall osmotically) and rehydrated in order to see if disruption of cell to cell cytoplasmic connections would disrupt gravi-induced polar Ca movement. We found that ***plasmolysis/deplasmolysis of root cap cells prevented subsequent gravi-induced polar Ca movement but did not prevent gravitropism or gravi-induced auxin redistribution.***

(5) Vitamin D has no consistent effect on graviresponsiveness in dark-grown roots of maize.

(6) In comparative studies of roots of dark-grown vs. light-grown seedlings of cultivars of maize that require light for normal root gravitropism, we find that ***(a) auxin sensitivity is 100-fold lower in dark-grown roots, (b) light stimulates growth in dark-grown roots and increases their auxin sensitivity 100-fold, and (c) gravistimulated roots of dark-grown seedlings do not develop polar Ca or auxin transport. Brief illumination induces both phenomena.***

Significance of the Accomplishments

Finding #1: There is a close correlation between auxin movement across the root cap and the kinetics of gravitropic curvature. This indicates that the cap is not only the site of gravity perception but also the site at which a physiological asymmetry is first established. The focus on deciphering the signal transduction mechanism should be on the cap.

Finding #2: Ca and Al effects on auxin transport parallel their effects on curvature. EGTA blocks polar auxin movement across the cap and this effect is reversible by Ca. These results indicate a cause/effect relationship between auxin movement and gravicurvature. They also suggest that the auxin transport within the cap is calcium-dependent. This provides a possible explanation of earlier observations that asymmetric Ca application can mimic gravicurvature while calcium chelators can block gravicurvature.

Finding #3: Auxin does not enhance the efflux of previously absorbed radioactive Ca from purified root protoplasts. In earlier work with crude protoplasts we found modest auxin-induced Ca efflux. The finding that this does not occur in purified protoplasts suggests either that a Ca efflux pump is lost on purification or that auxin action on root cells does not involve elevated cytoplasmic Ca levels (or at least that the putative cytoplasmic Ca elevation is not relieved by pumping to the outside of the cell).

Finding #4: Plasmolyzed/deplasmolyzed roots show normal gravitropism but no gravi-induced polar calcium movement. This is the first time we have been able to separate Ca movement and gravitropism in roots. We need to determine whether the apparent lack of polar Ca movement also applies to internal gradients of Ca. If it does, the results will indicate that gravitropism does not require gravi-induced Ca movement. This will cause us to reassess our "calcium-transduction" model.

Finding #5: Vitamin D has no consistent effect on the graviresponsiveness of dark-grown roots. This suggests that reports of vitamin D-induced calmodulin biosynthesis in roots in tissue culture may not be relevant to intact seedlings. We need to determine whether vitamin D promotes calmodulin biosynthesis in dark-grown roots. If it does, we may have a way of separating light effects on gravitropism from effects on calmodulin biosynthesis.

Finding #6: Light increases auxin sensitivity and activates the auxin transport system in dark-grown roots. These results have great potential. They suggest an explanation for the

light effect on gravitropism and provide an ideal system for studying the properties of the hormone response and transport systems in roots.

Publications

Evans, M.L. and Hasenstein, K.H. Stimulus-response Coupling in the Action of Auxin and Gravity on Roots. In: *Physiology of Cell Expansion During Plant Growth* (ed. by D.J. Cosgrove and D.P. Knievel). Rockville: American Society of Plant Physiologists, pp. 202-214, 1987.

Evans, M.L., Hasenstein, K.H., Stinemetz, C.L., and McFadden, J.J. Calcium as a Second Messenger in the Response of Roots to Auxin and Gravity. In: *Molecular Biology of Plant Growth Control*, UCLA Symposia on Molecular Biology and Cellular Biology, New Series, Vol. 44 (ed. by J.E. Fox and M. Jacobs). New York: Alan R. Liss, pp. 361-370, 1987.

Evans, M.L., Lee, J.S., and Hasenstein, K.-H. Effects of Calcium on Growth and Auxin Movement in Maize Roots (Abstract). *ASGSB Bulletin* 1: 29, 1988.

Hasenstein, K.H. and Evans, M.L. Auxin-induced Calcium Efflux from Maize Roots (Abstract). *ASGSB Bulletin* 1: 28-29, 1988.

Hasenstein, K.H. and Evans, M.L. Effects of Cations on Hormone Transport in Primary Roots of *Zea mays*. *Physiologia Plantarum* 86: 890-894, 1988.

Hasenstein, K.H. and Evans, M.L. The Influence of Calcium and pH on Growth in Primary Roots of *Zea mays*. *Plant Physiology* 72: 466-470, 1988.

Hasenstein, K.H., Evans, M.L., Stinemetz, C.L., Moore, R., Fondren, W.M., Koon, E.C., Higby, M.A., and Smucker, A.J.M. Comparative Effectiveness of Metal Ions in Inducing Curvature of Primary Roots of *Zea mays*. *Plant Physiology* 86: 885-889, 1988.

Stinemetz, C., Hasenstein, K., and Evans, M.L. The Effect of Root Curvature-inducing Ions on Calmodulin Activity (Abstract). *ASGSB Bulletin* 1: 27, 1988.

Stinemetz, C.L., Kuzmanoff, K.M., Evans, M.L., and Jarrett, H.W. Correlation Between Calmodulin Activity and Gravitropic Sensitivity in Primary Roots of Maize. *Plant Physiology* 84: 1337-1342, 1987.

Young, L.M. and Evans, M.L. Correlations Between Gravitropic Curvature and Auxin Transport Across Root Tips of *Zea mays* (Abstract). *ASGSB Bulletin* 1: 29, 1988.

PHYSIOLOGICAL, BIOCHEMICAL, AND MOLECULAR PROCESSES ASSOCIATED WITH GRAVITROPISM IN ROOTS OF MAIZE

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Description of Research

On Earth, roots typically respond to gravity by growing downward. Our research focuses on the physiological, biochemical, and molecular steps involved with translating the gravity stimulus into the downward growth shown by roots.

We know that gravity is perceived in a specialized region at the tip of the root known as the root cap. It is hypothesized that as a result of sensing gravity, a number of processes are initiated in the cap, eventually causing the root to orient so that it grows parallel to the gravity vector. We are studying these gravity-related processes. The challenge is to determine which processes within the cap are important in translating gravity.

For this work we have used a mutant of corn in which roots do not normally respond to gravity if grown in darkness. Illuminating the root causes the roots to respond to gravity and grow downward. We hypothesize that in this variety light initiates the steps necessary for gravity transduction. Earlier we showed that light markedly increases protein synthesis within the cap. If this synthesis was prevented, then the roots did not respond to gravity when they were illuminated. So protein synthesis within the cap is necessary for the root to respond to gravity. But which proteins are important, and how does light modify them? We have examined a number of proteins and documented the effects of light on these proteins. Recently we spent considerable time looking at the regulatory effects of light on one protein in particular, calmodulin. This protein is hypothesized to play a key role in gravity transduction in roots. We have investigated whether light affects this protein and the mechanism of this regulation.

A second project initiated this year involves searching for plants which are altered (mutated) in their ability to respond to gravity. In particular, we have looked for plants in which the roots respond atypically to gravity, that is, the roots do not grow straight downward. The theoretical basis for this work is that if we can find mutants, then it might be possible to locate genes, and ultimately proteins, associated with processing the gravity signal in roots.

Accomplishments

(1) Light alters the activities of a wide variety of proteins within the root cap. The effects of light occur within 30 min of illumination.

(2) The mechanism by which light alters protein activities appears to be by enhancing the levels of the messenger RNAs (mRNA) which code for the various proteins.

(3) We have found several classes of mutants in which roots respond atypically to gravity.

Significance of the Accomplishments

We have been able to demonstrate that light rapidly affects the activities of a wide variety of proteins within the root cap. This suggests that many proteins may be important for processing the gravity signal within the root cap, and further suggests that transducing gravity within the cap requires a general enhancement in the entire metabolic machinery of the root cap. How does light cause this general increase? Our recent findings suggest that light affects the activities of many proteins by stimulating an increase in a precursor of the protein, namely in the mRNA for that protein. The effects of light on the mRNAs is very rapid and occurs within 10-15 min of illuminating the roots. This is an especially interesting finding since it suggests that light does not directly affect proteins, but rather "works" by altering the levels of the precursors of these proteins. This suggests that the light-processing and gravity-processing machinery of the root share a number of common steps, and supports the idea that gravity transduction in plants is in general modified by light.

The second aspect of this research involves the search for mutant plants in which the roots do not respond in a typical fashion to gravity, that is, the roots do not grow straight downward. If we could find such mutants, then it might be possible to find genes and ultimately proteins involved with processing the gravity signal. We screened a large number of potential mutants (approximately 15,000 individual plants). From this effort we were able to find about ten individuals in which the roots grew upward (Figure 1). In addition, and much to our surprise, we discovered a second class of "mutants" in which the roots respond atypically to gravity and grow parallel to the surface of the soil (Figure 2). If the roots are reoriented, the repositioned roots respond by growing back to the parallel orientation. If the roots are reoriented several times, the roots continue to respond and can be made to grow in many directions, but they always grow back to the parallel. What we now need to do is to prove that these individual plants are indeed true "mutants." If we are lucky, we may find that one out of ten of these plants would be a true mutant. If so, then we would begin a search for the affected gene.

Publications

Feldman, L.J. and Sun, P.S. Protein and mRNA Synthesis in Gravistimulated Root Caps of Maize (Abstract). *ASGSB Bulletin* 1: 45, 1988.

MAIZE SEEDLING WITH ATYPICAL ROOT GRAVITY RESPONSE

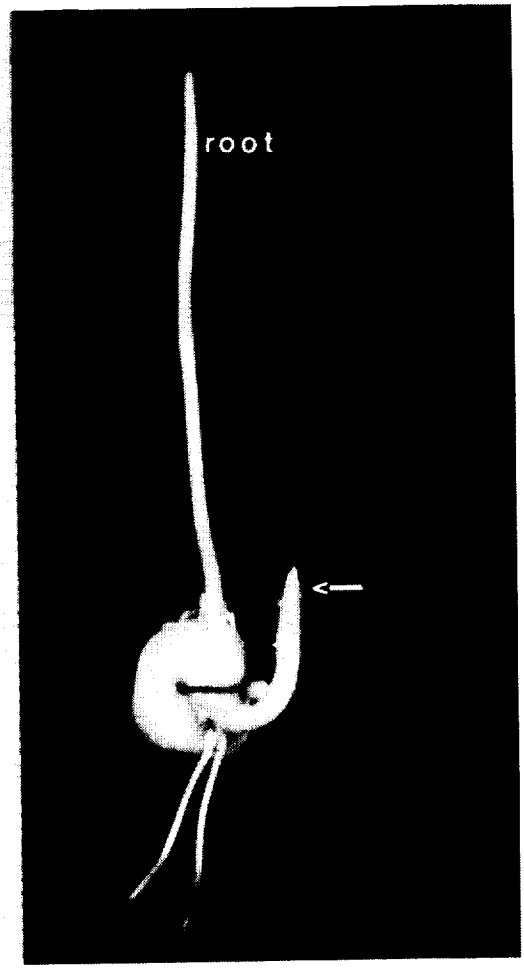
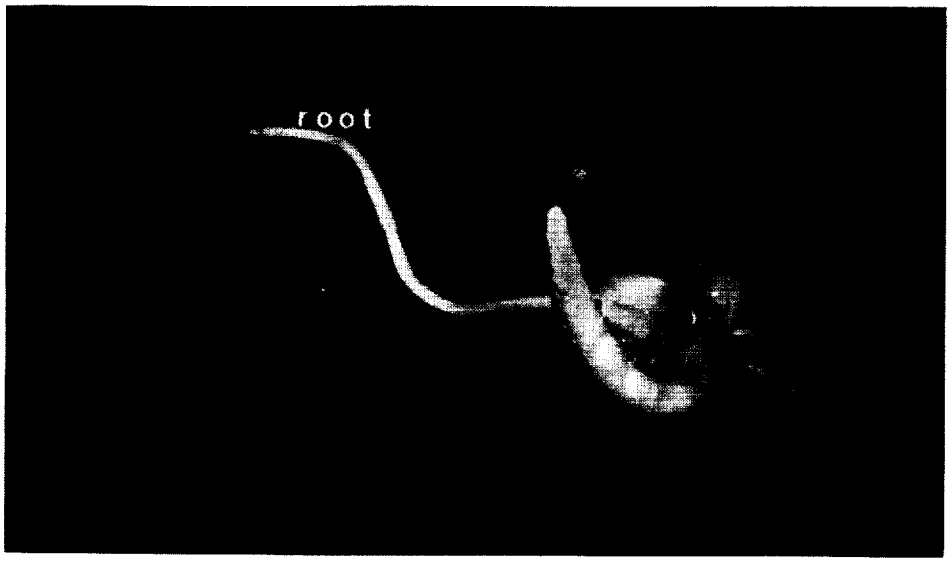


Figure 1. Maize seedling obtained from mutant screen. The root of this plant grows upward, and is not responsive to gravity. Note that the shoot (indicated by a white arrow) responds normally to gravity and grows upward.

MAIZE SEEDLINGS WITH ATYPICAL ROOT ORIENTATION TO GRAVITY



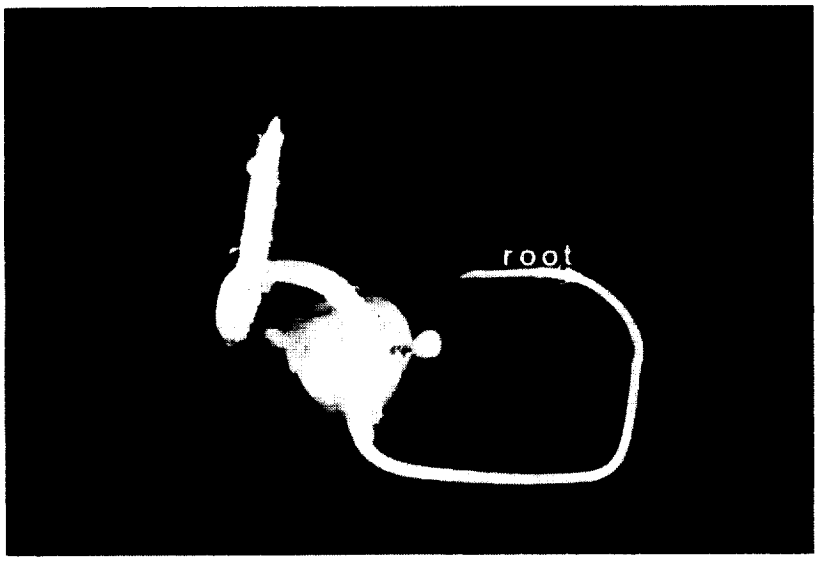


Figure 2. Maize seedlings obtained from mutant screen. These seedlings have a primary root which orients parallel to the direction of gravity. When the seedling is reoriented, the root subsequently alters its direction of growth and returns to the original parallel orientation. The seedling in the top photograph was reoriented once; the seedling in the bottom photograph was reoriented three times.

THE EFFECTS OF ALTERING THE MAGNITUDE OR VECTOR OF THE GRAVITATIONAL FORCE ON THE CIRCADIAN RHYTHM OF CONIDIATION IN *NEUROSPORA CRASSA*

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Description of Research

The research carried out in this project is designed to determine whether circadian rhythms, thought to be endogenously derived, can persist normally in the absence of all known geophysical and environmental time cues. A rhythm that persists and free-runs with a period of approximately 24 hr in constant conditions and entrains to 24-hr environmental time cues is said to be circadian. It has been proposed by some that these free-running oscillations are driven by rhythmic phenomena in geophysical cycles rather than being driven by an "internal clock." Observing the rhythmicity of an organism in an environment removed from the geophysical rhythms of Earth will suggest whether these rhythms are endogenously or exogenously derived.

The filamentous fungus *Neurosopora crassa* displays rhythmic growth patterns under specific conditions. This rhythmic growth consists of an alternating, low-growing surface mycelium and a surface mycelium with aerial hyphae which pinch off to form conidia (asexual spore formations). The mycelium that contains the aerial hyphae is clearly seen as a band when grown on media in petri dishes or in long cylindrical tubes, known as race tubes. In constant conditions the cycle of banding is repeated approximately once every 22 hr (i.e., the rhythm is circadian).

To determine whether this circadian rhythm of conidiation is endogenously derived or is driven by some geophysical time cue, an experiment was conducted on STS-9 where race tubes inoculated with growing *Neurospora* were exposed to microgravity. The results demonstrated that the rhythm can persist in space. There was an increase, however, in the period of the oscillation and the variability of the growth rate; furthermore, the rhythm of conidiation possessed a diminished amplitude and eventually damped out in 25% of the flight tubes. On day 7 of the flight, the tubes were exposed to light while their growth fronts were marked. It appears that some aspect of this marking process reinstated a robust rhythm in all the tubes which continued throughout the remainder of the flight.

Despite relocating our laboratory from the State University of New York in Binghamton to Southern Illinois University, School of Medicine at Carbondale, further tests and verifications on last year's intriguing results utilizing chronic and acute exposure to hypergravity were performed. Due to the nature of this new information, we also repeated our clinostat experiments and are currently repeating the orientation experiments, as well as developing a "centrifuge-dark inoculation" method that may alleviate our heavy reliance upon large centrifuge facilities at other institutions. The first centrifuge studies suggested that most of the aberrant effects observed on STS-9 may be explained, at least in part, by the hypergravity encountered during launch. These studies suggested procedural changes that were integrated into our flight experiment.

Accomplishments

Earth-bound simulation of microgravity is often accomplished through the use of a clinostat. Our clinostat rotates the race tubes about their axis, which evenly distributes the 1 g gravity vector in all directions, simulating a 0 g gravity vector. In the original preliminary experiments, the rhythm damping and the decreased amplitude seen in orbit on STS-9 was not simulated by the clinostat at any of the five evenly spaced circadian times. The growth rate, however, was significanlty slower on the clinostat.

Repeating this experiment, in light of the new evidence that gravitational changes can affect both the amplitude and period of the rhythm, demonstrated ***a 20% increase in arrhythmicity in BND strain Neurospora exposed to the clinostat;*** however, ***no arrhythmicity was noted in the CSP strain.*** As expected, the addition of Brij to the media slowed the growth rate from 0.92 ± 0.07 to 0.72 ± 0.01 and 1.14 ± 0.06 to 0.78 ± 0.03 mm/hr for BND and CSP strains, respectively, but had no effect on rhythmicity or period length. ***Exposure to the clinostat had mixed effects on the period of the conidiation rhythm*** while the ***BND strain on Brij media was unaffected*** by exposure to the clinostat; ***BND on regular media had a significantly shorter free-running period. Furthermore,*** while the ***CSP strain on regular media did not have a significantly altered period*** upon exposure to the clinostat, ***CSP on Brij media had a significantly shorter free-running period.***

In order to determine the effects of launch upon our system, we conducted several hypergravity studies utilizing the centrifugation facilities at the University of California, Davis, with the help of Dr. C.A. Fuller. The original study, previously reported, using chronic (7-day) and acute (10-min) exposure to a 3 g load, demonstrated that: (1) chronic exposure of *Neurospora* to a 3 g force had no damping effect; (2) acute exposure caused significant damping of the circadian rhythm of conidiation; (3) a brief light pulse given 36 hr after the acute exposure eliminated any effect of the acute 3 g exposure on damping; (4) BDN was more susceptible to the hypergravity perturbation than CSP; (5) the average free-running period increased in both strains with chronic hypergravity; (6) individual aspects of the major effects observed on the STS-9 experiment were simulated by either chronic or acute exposure to hypergravity, including the ability of a light pulse to correct the aberrations.

Since these results were so intriguing, a second study was conducted at U.C. Davis. This study was limited to the acute effects of hypergravity (10 min, 3 g exposure). The onset of the hypergravity exposure was distributed to the various groups at four different circadian times. Both CSP and BND strains were used, as were both regular and Brij media. The race tubes were then divided into three exposure groups: acute hypergravity exposure; acute hypergravity exposure with a light pulse 36 hr after the hypergravity exposure; and a "ground" control with no exposure to hypergravity or light. Figure 1 shows that ***acute exposure to a 3 g pulse causes arrhythmicity*** in 14-19% of the race tubes of the BND strain and up to 4% of the CSP strain; furthermore, ***this effect of hypergravity is eliminated by a light pulse*** in all groups. The hypergravity-light pulse group even shows less damping than the control group, at least with respect to the BND strain on Brij media (damping, or perceived damping, in this group may have been due to inadvertent mishandling; asynchronous controls demonstrated no damping in any of the groups). It is also apparent that ***the BND strain is more susceptible*** than CSP to the hypergravity effects. Holding the effects of strain and media constant, there appears to be ***no effect of hypergravity on the period of the circadian rhythm of conidiation,*** with one exception: the BND strain on regular media "ground" control had a shorter period than either the hypergravity or hypergravity and light exposed groups. This exception did not

EFFECTS OF HYPERGRAVITY AND ENVIRONMENTAL FACTORS ON CIRCADIAN RHYTHM OF CONIDIATION IN *NEUROSPORA*

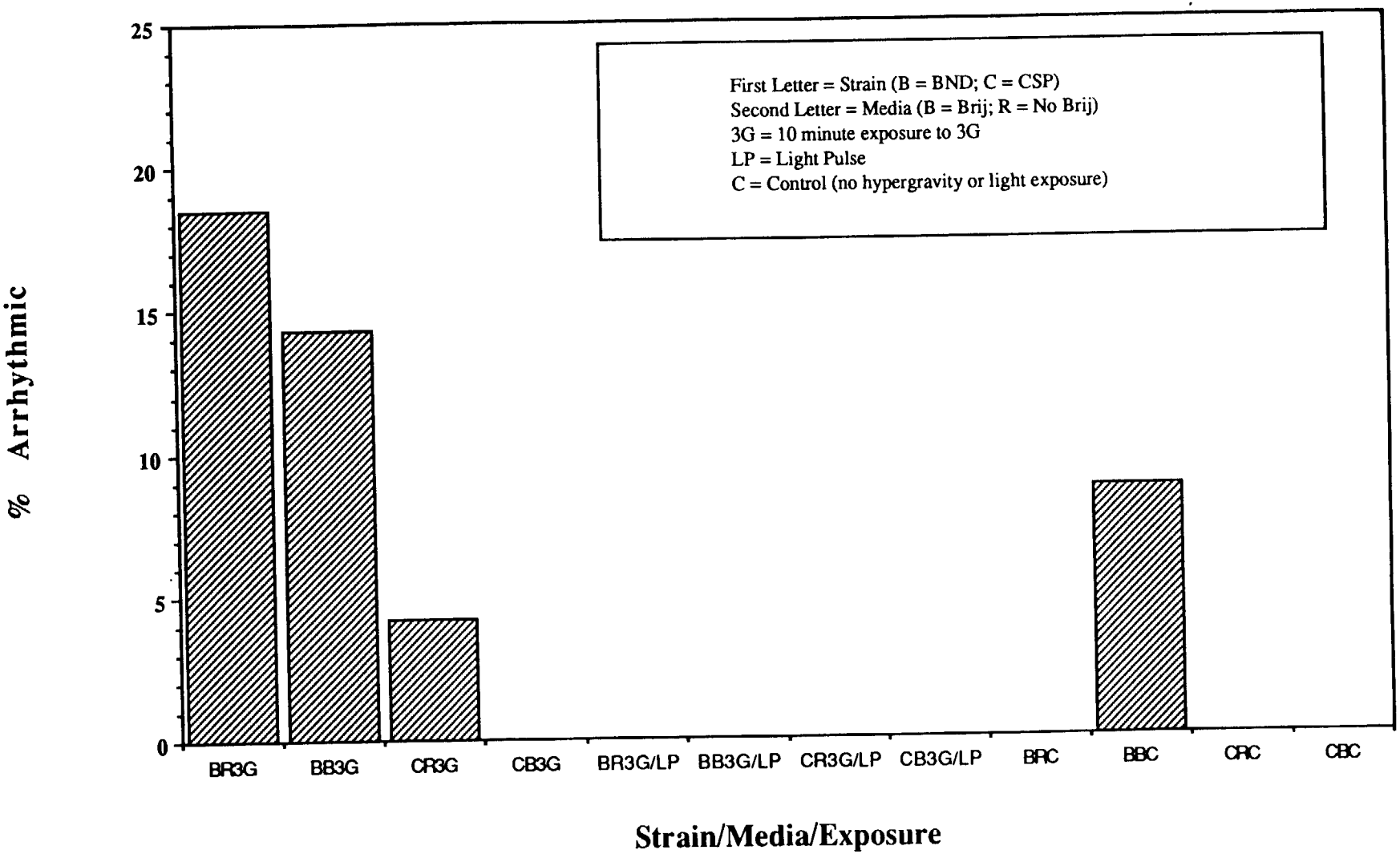

Figure 1. Effects of strain, media, exposure to hypergravity, and light pulse on arrhythmicity in the circadian rhythm of conidiation in *Neurospora crassa*.

occur in the first experiment and may be an aberrant result; however, further studies are needed to confirm this. Further analyses are also needed to determine the effects of the circadian time of hypergravity onset.

Significance of the Accomplishments

The significance of these ground-based studies becomes readily apparent when interpreting previous flight experiments and when preparing for our future *Neurospora* experiment in space. The hypergravity study confirms earlier observations that acute exposure to a "launch-like" hypergravity pulse can cause arrhythmicity; that a light pulse, similar to the one encountered during the marking procedure, can eliminate this effect; and that the strain used on STS-9 is especially susceptible to this phenomenon. While chronic exposure to hypergravity can increase the period of the circadian oscillation of conidiation in *Neurospora,* acute exposure cannot. Thus, while the acute hypergravity exposure of launch could have caused some of the aberrations on STS-9, it cannot explain all of the observed effects. From the hypergravity studies, as well as the studies using the clinostat or different orientations, it is quite clear that this system is sensitive to alterations in the magnitude and vector of a gravitational force. These results further suggest that the original experiment flown on STS-9 may not have been able to fairly examine the exogenous-endogenous question, at least prior to the marking procedure, due to the interference of the effects of launch. Flight experiment(s) in which the cultures are exposed to the microgravity environment of space for considerable durations following the marking procedure, and thus the light exposure, are needed in order to more accurately examine this question.

Publications

Antipas, A.J., Madison, D.M., and Ferraro, J.S. Circadian Rhythms in the Short-Tailed Shrew, *Blarina brevicauda* (Abstract). *American Society of Mammology* 128, 1987.

Ferraro, J.S. Syrian Hamsters With Circadian Periods Outside the Limits of Entrainment by Restricting Light Exposure to the Delay Portion of the Phase-Response Curve (Abstract). *Society of Research Biological Rhythms* 1: 76, 1988.

Ferraro, J.S., Bartke, A., Sulzman, F.M., and Krum, H.N. The Effects of Aberrant Lighting Conditions on Serum Prolactin Levels in the Syrian Hamster, *Mesocricetus auratus* (Abstract). *Abstracts of the 11th International Congress of Biometerology* 54, 1987.

Ferraro, J.S., Fuller, C.A., Sulzman, F.M., and Krum, H.N. The Effects of Hypergravity on the Circadian Rhythm of Conidiation in *Neurospora crassa* (Abstract). *ASGSB Bulletin* 1: 37, 1988.

Ferraro, J.S., Krum, H.N., Wassmer, G.T., and Sulzman, F.M. The Photoperiodic Effects of Short and Ultrashort Light-Dark Cycles on Testicular Weight and Serum Testosterone in the Syrian Hamster (Abstract). *Biology of Reproduction* 36(Suppl.1): 174, 1987.

Ferraro, J.S., Sulzman, F.M., and Krum, H.N. The Effects of Aberrant Lighting on the Circadian Rhythm of Locomotor Activity in the Syrian Hamster (Abstract). *Society for Neuroscience Abstracts* 13(1): 422, 1987.

Krum, H.N., Sulzman, F.M., and Ferraro, J.S. The Photoperiodic Effects of Normal, Reverse, and Neighbor Control Feedback Lighting on Testicular Weight and Serum

Testosterone in the Syrian Hamster (Abstract). *Biology of Reproduction* 36(Suppl. 1): 150, 1987.

Krum, H.N., Sulzman, F.M., Fuller, C.A., and Ferraro, J.S. The Effects of Hypergravity on the Circadian Rhythm of Conidiation in *Neurospora crassa* (Abstract). *Society for Neuroscience Abstracts* 13(1): 48, 1987.

Sulzman, F.M., Krum, H.N., and Ferraro, J.S. The Effects of Feedback Lighting on the Photoperiodic Response in the Mature Djungarian Hamster (Abstract). *Society for Neuroscience Abstracts* 13(2): 865, 1987.

Wagner, T., Bartke, A., Steger, R.W., and Ferraro, J.S. Transgenic Mice With a Genome for Human Growth Hormone Have Shorter Free-running Periods than Non-transgenic Siblings (Abstract). *Society Research Biological Rhythms* 1: 57, 1988.

LIGNIFICATION WITHOUT CELL DIVISION IN SINGLE CULTURED CELLS

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Description of Research

The ultimate objective of our research is to understand the mechanisms involved in the differentiation of ordinary leaf parenchyma cells into specialized tracheary elements, with concomitant deposition of lignin in cell walls that were previously mainly cellulosic. Since lignin is the major strengthening component of higher plant cell walls and its formation is known to be affected by the mass load borne by the cells, it becomes relevant to inquire into the importance of the Earth's gravitational field in inducing lignification and into the effects of hyper- and hypogravity on the intensity and nature of the lignification process.

We have chosen to work with mechanically isolated individual mesophyll cells of *Zinnia elegans*, cv. 'Envy'. When such cells are cultured aseptically on media containing an auxin (alpha-napthaleneacetic acid, NAA) and a cytokinin (benzyladenine, BA) at 0.5 μM, more than 50% of them differentiate into lignified tracheary elements within 72 hr. This roughly synchronous differentiation is inhibited or retarded when the medium is supplemented with antiauxins, anticytokinins, or inhibitors of glycosylation.

While cellular differentiation and wall lignification are observed microscopically after staining, earlier events in the cell's commitment to lignification can be visualized by colorimetric determinations of the activity of key enzymes involved in lignin formation. We have made such observations on two enzymes, 4-coumarate:CoA ligase and peroxidase. The former enzyme supplies the precursor for lignification while the latter oxidizes the precursor into unstable compounds that then polymerize into lignin. We have examined these enzymes in both the soluble and wall compartments of the cell and, where necessary, have identified and studied the behavior of individual isozymes.

Accomplishments

(1) Cells require at least 56 hr of exposure to auxin, but only 24 hr of exposure to cytokinin for optimal differentiation to occur.

(2) ***One isoperoxidase, separated by nondenaturing polyacrylamide gel electrophoresis, is differentiation-specific.*** It is detectable in soluble and wall fractions long before differentiation is apparent.

(3) ***4-coumarate:CoA ligase activity increases only as tracheary elements develop and lignification becomes apparent. It is a possible rate-controlling enzyme for the differentiation process.***

(4) ***Leaf discs of Zinnia can be used to study lignification, even without fragmentation into individual cells.*** Raising auxin and cytokinin levels a little causes differentiation into tracheary elements of cells adjacent to the veins; ***raising hormone levels even higher causes extensive lignification throughout the mesophyll and even epidermal and basal hair cells become lignified.***

Significance of the Accomplishments

These results establish a precisely controlled, rapid, and reliable system for the study of lignification. This system is now amenable for experiments on the effects of gravity on the lignification process.

Our studies also provide two biochemical benchmarks for the study of lignification even before lignin itself is apparent. 4-coumarate:CoA ligase seems a good candidate to control the rate at which lignification can occur, while a specific isoperoxidase must apparently be synthesized and then fixed in the wall, where it can act on the lignin precursor provided by the ligase enzyme. The effects of gravity on the formation and activity of these enzyme systems can now also be studied.

Publications

Church, D.L. and Galston, A.W. Lignification Without Cell Division in Cultured Single Cells (Abstract). *ASGSB Bulletin* 1: 28, 1988.

Migliaccio, F. and Galston, A.W. On the Nature and Origin of the Calcium Asymmetry Arising during Gravitropic Response in Etiolated Pea Epicotyls. *Plant Physiology* 85: 542-547, 1987.

MECHANISM OF GRAVITY RESPONSES IN CEREAL GRASS SHOOTS

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Description of Research

Our long-range goal is to elucidate the mechanism by which cereal grass shoots respond to gravity on Earth at 1 g and to understand how microgravity alters this gravitropic mechanism. In order to accomplish this goal, we have established the following objectives for our NASA-sponsored research: (1) to characterize how gravity is perceived in graviresponsive organs (pulvini) of cereal grass shoots; (2) to show how and when both free and conjugated forms of auxin (IAA) and gibberellins become unequally distributed with gravistimulation; and (3) to unravel the temporal sequence and mechanism by which a range of factors — these hormones, wall-loosening/synthesizing enzymes, invertase, Ca^{2+}, protein phosphorylation, and specific mRNAs — act to bring about an upward bending response in gravistimulated cereal grass pulvini.

Accomplishments

(1) Graviperception

(a) We have shown that ***starch grains in pulvinus chloroplasts act as the primary gravisensors in cereal grass pulvini.*** When starch disappears in dark-treated pulvini, the graviresponse is lost and, when starch reappears after sucrose treatment, the graviresponse is restored in the pulvini.

(b) ***The starch in chloroplasts of grass shoot pulvini gradually disappears during the course of upward bending in gravistimulated pulvini.*** This starch probably serves as a source of substrate (D-glucose) for cell wall synthesis in elongating cells in the lower portions of graviresponding pulvini and for glycolysis and ATP synthesis.

(2) Transduction

(a) We have demonstrated that ***in normal, upright corn, gravistimulation results in differential GA metabolism when shoots are fed a GA precursor*** (^{3}H-GA_{20}). Accumulation of putative free GA metabolites of ^{3}H-GA_{20} (especially ^{3}H-GA_1) occurs in lower halves, relative to upper halves. ***This differential metabolism of GA does not occur in the pleiogravitropic ("lazy," or la/la) recessive mutants of maize.*** In gravistimulated normal maize shoots, upper pulvinus halves consistently contain lower levels of GA-like activity, relative to lower halves. Upper halves of pulvini contain principally a GA_{20}-like substance, while lower halves contain mainly GA_1- and GA_{19} -like substances.

(3) Response Mechanism

(a) Gravistimulation results in ***greatly enhanced invertase activity in lower halves of upward-bending pulvini, relative to upper halves.*** Invertase exists as five principal isozymes, two associated with cell walls and three that are soluble. All invertase isozymes are increased to the same relative extent by gravistimulation.

(b) ***Gravistimulation brings about an altered (enhanced) responsiveness of pulvini of cereal grass shoots to exogenously applied IAA and GA_3.*** Pulvini do not respond to GA_3 until they are gravistimulated, indicating the existence of an inductive process leading to responsiveness to this hormone. Gravistimulation appears to decrease the responsiveness of pulvini to moderate to high levels of exogenous IAA.

(c) Cell wall analyses of upright vs. gravistimulated oat pulvini show that ***proportions of beta-glucan and arabinan in the bottom halves of gravistimulated pulvini increase, relative to top halves, whereas the percentage of cellulose decreases.*** The decrease in cellulose may reflect the intercalation of other cell wall components, mostly arabinoxylan, between annular bands of cellulose. ***Gravistimulation also causes starch content to decrease in lower halves of pulvini.***

Significance of the Accomplishments

Since starch-containing chloroplasts are the primary gravisensors (statoliths) in cereal grass pulvini, we shall now analyze their roles in the graviperception process. These starch statoliths could function in several ways: (1) they could serve as a source of substrate (D-glucose) for cell wall synthesis and ATP synthesis and respiratory metabolism, (2) they could serve as pressure probes to open membrane channels that allow for flux of ions (e.g., Ca^{2+}, K^{+}) or hormones (IAA, GAs) through plasma membrane channels, and (3) they could serve as information carriers (e.g., bringing IAA or GA deconjugating enzymes or of the conjugated forms of IAA or GAs to the plasma membrane).

Development of hormone asymmetry for free IAA and for free GAs and their glycosyl conjugates as a result of gravistimulation of cereal grass pulvini is one of the early key events in the upward bending response of gravistimulated cereal pulvini. The free GAs and IAA accumulate in the lower halves. We can now test how this hormone asymmetry arises. One especially attractive hypothesis is that gravistimulation brings about differential release of free IAA or GAs from their respective conjugates. We are now in a position to test this idea as well as to determine more precisely when and where the asymmetry develops for free IAA and GAs and their conjugates after the initiation of gravistimulation of pulvini.

Gravistimulation has profound effects on growth metabolism in cereal grass shoots: in lower halves, where most cell elongation takes place, levels of starch decrease and invertase activity increases. Both result in elevated levels of cytosolic D-glucose. This glucose is thus made available for synthesis of new wall polysaccharides such as arabinoxylan, beta-glucan, and arabinan.

The fact that gravistimulation alters tissue responsiveness to exogenous IAA and GA_3 suggests that gravistimulation may increase the number of hormone receptor sites for binding the free hormone moieties. Since hormone binding (e.g., to a macromolecule such as a protein) should be a prerequisite to hormone action (e.g., stimulated cell wall loosening and cell wall synthesis), the results we have obtained on gravity-altered tissue responsiveness may provide strong support for the idea of increased number of hormone receptor sites.

Publications

Brock, T.G., Bright, J., Burg, J., and Kaufman, P.B. Gravistimulation Alters the Sensitivity of Oat Pulvini to Exogenous Indole-3-Acetic Acid and Gibberellic Acid (Abstract). *ASGSB Bulletin* 1: 45, 1988.

Brock, T.G., Cameron, M.J., Karuppiah, N., Ghosheh, N.S., and Kaufman, P.B. Sensitivity vs. Responsiveness in Hormone Action: A Case Study Involving the Effect of Gravistimulation on IAA and GA_3 Action (Abstract). *Plant Physiology* 86 (Suppl.): 112, 1988.

Brock, T.G. and Kaufman, P.B. Altered Growth Response to Exogenous Auxin and Gibberellic Acid by Gravistimulation in Pulvini of *Avena sativa*. *Plant Physiology* 87: 130-133, 1988.

Brock, T.G. and Kaufman, P.B. The Onset and Development of Competency for Graviresponsiveness in the Leaf-Sheath Pulvinus of Oat (*Avena sativa* L.) (Abstract). *Plant Physiology* 83(Suppl.): 20, 1987.

Gibeaut, D.M., Carpita, N.C., Song, I., Ghosheh, N., and Kaufman, P.B. Starch and Cell Wall Metabolism of *Avena sativa*: Gravistimulation-induced Growth of Leaf-Sheath Pulvini and Gibberellin-induced Growth of Excised Internode Segments (Abstract). *Plant Physiology* 86(Suppl.): 28, 1988.

Karuppiah, N., Hester, K., and Kaufman, P.B. Purification and Characterization of Barley Invertases (Abstract). *Plant Physiology* 86(Suppl.): 62, 1988.

Kaufman, P.B., Brock, T.G., Song, I., Rho, Y.B., and Ghosheh, N.S. How Cereal Grass Shoots Perceive and Respond to Gravity. *American Journal of Botany* 74: 1446-1457, 1987.

Kaufman, P.B. and Song, I. Hormones and the Orientation of Growth. In: *Plant Hormones and Their Role in Plant Growth and Development*, (ed. by P.J. Davis). Dordrecht, The Netherlands: Martinus Nijhoff, pp. 375-392, 1987.

Kaufman, P.B., Song, I., and Lu, C. Do Starch Statoliths Act as the Gravisensors in Cereal Grass Pulvini? (Abstract). *ASGSB Bulletin* 1: 19, 1988.

Rood, S.B., Kaufman, P.B., Abe, H., and Pharis, R.P. Gibberellins and Gravitropism in Maize Shoots: Endogenous Gibberellin-like Substances and Movement and Metabolism of [^{3}H] Gibberellin A_{20}. *Plant Physiology* 83: 645-651, 1987.

Song, I., Lu, C.R., Brock, T.G., and Kaufman, P.B. Do Starch Statoliths Act as the Gravisensors in Cereal Grass Pulvini? *Plant Physiology* 86: 1155-1162, 1988.

Song, I., Lu, C., and Kaufman, P.B. Starch Statoliths as the Graviperceptors in Cereal Grass Pulvini (Abstract). *Plant Physiology* 83(Suppl.): 19, 1987.

CELLS, EMBRYOS AND DEVELOPMENT IN SPACE/MORPHOLOGY OF PLANT CELLS IN SPACE

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Description of Research

It is now generally recognized that the problems of development constitute a major part of the objectives of modern biology. The ultimate aim of our research is to furnish systems at different levels of initial organization that will enable us to test the effect of microgravity in the space environment on the behavior of plants in contrast or comparison with their performance at 1 g and in ground controls.

While the main focus is aimed towards the broad effects of near zero gravity that operate on systems as they grow and develop, the systems are or will be adaptable to a variety of more specific tests. For example, any one of our test systems is capable of being used to ascertain whether there might be differences in the normal rate, frequency, and patterning of cell division, or in the fidelity of partitioning of the chromosomes during or after exposure to spaceflight.

The thrust of the investigations deals with:

(1) The induction of active growth, cell proliferation, and metabolism in otherwise mature quiescent cells as they exist *in situ*. This is a problem that has involved and still involves the identity and mode of action of relatively simple growth-regulating substances of low molecular weight, their synergists and cofactors as well as nutrient elements.

(2) The obtaining and multiplication in culture of free cells and their contrasted development into unorganized callus masses, and as somatic (non-zygotic) embryos into plantlets.

(3) The growth, morphogenesis, and metabolism of intact plantlets and tissue-culture derived propagules with their established growing regions of shoot and root, in response to interacting factors which are both environmental (i.e., different regimes of photoperiodicity and changing temperatures) and nutritional.

(4) The development of protocols which have a high level of reliability for establishing growth and chromosomal characteristics and profiles for the plant species we are working with, while at the same time seeking to extend the principles so gained to a still broader range of species.

(5) The management of cultured systems from the perspective of being able to use them effectively and with a minimum of human intervention in the space environment.

Accomplishments

A carrot system has been developed that is much simpler than our cell suspension system. Callus can be used to direct, controllably, the cyclical production of secondary embryos. The system is quite simple to manage, can grow in darkness, and tolerates a wide range of temperatures. It has the added advantages of being compact and

amenable to conventional postflight analysis, and any recovered materials can be "grown out" to maturity. This means we can obtain more information on (a) whether proembryos can be initiated *de novo* in space; (b) if they do form, the exact extent to which they form; (c) the rate at which they progress from proembryos to later stages; and (d) the extent to which they can show any instability of the differentiated state by giving rise to secondary embryos.

The entire system has been induced and sustained in the absence of exogenous hormones. Hormones in culture media may induce mutations that are undesirable in experimental situations and during micropropagation. This research points to the elimination of hormones, which is very desirable. ***The work has screened the need for various nutritional additives, and this had led to much simpler media.*** The new carrot system can be initiated from zygotic or seed-derived embryos as well as somatic, tissue culture-derived embryos and provides the means to evaluate the basis of initiation and modulation of embryo development. The relationships of media components for initiation and development have been studied. A simple medium which permits step-wise analysis has been initiated. *Haplopappus gracilis*, a small Composite with the lowest known chromosome number (2n = 4) in dicotyledonous plants, has been placed in tissue culture. Aseptic clonal multiplication systems have been put in place. Karyotype analysis has been carried out and correlated with culture procedures.

Significance of the Accomplishments

The continued refinements and understanding of the complexities of control mechanisms active in developing and differentiating plant systems renders ever more effective our ability to provide these systems for space experimentation. Mineral nutritional controls of differentiation patterns and regeneration have scarcely been recognized, and the fact that controls normally effected by hormones can be effected by cations and anions has opened up a full new capability of identifying key points in the sequences of decision-making metabolic pathways.

Publications

Cronauer-Mitra, S.S. and Krikorian, A.D. Adventitious Shoot Production from Calloid Cultures of Banana. *Plant Cell Reports* 6: 443-445, 1988.

Cronauer-Mitra, S.S. and Krikorian, A.D. Determinate Floral Buds of Plantain (*Musa* AAB) as a Site of Adventitious Shoot Formation. *Annals of Botany* 61: 507-512, 1988.

Cronauer-Mitra, S.S. and Krikorian, A.D. Plant Regeneration via Somatic Embryogenesis in the Seeded Diploid Banana *Musa ornata* Roxb. *Plant Cell Reports* 7: 23-25, 1988.

Krikorian, A.D. Callus and Cell Culture, Somatic Embryogenesis, Androgenesis and Related Techniques for *Musa* Improvement. In: *Proceedings of an International Workshop, Cairns, Australia 13-17 October, 1986* (ed. by G.T. Persley and E. de Langhe). Canberra Australian: Centre for International Agricultural Research, pp. 128-135, 1987.

Krikorian, A.D. Chromosomes and Plant Cell Division in Space (Abstract). *ASGSB Bulletin* 1: 13, 1988.

Krikorian, A.D. Concepts, Strategies and Potentials Using Hypo-g and the Space Environment for Studying Higher Plant Development: Synopsis of a Lecture Presented in Tokyo, Japan, January 31, 1987 at Institute of Space and Aeronautical Science (ISAS).

Krikorian, A.D. Plants and Extended Duration Spaceflight (Abstract). In: *Space Life Sciences Symposium: Three Decades of Life Science Research in Space,* Washington, D.C., June 21-26, p. 243, 1987.

Magnuson, J.M., Williams, K.M., Magnuson, C.E., Fares, Y., Scheld, H.W., and Krikorian, A.D. Flight Hardware for Cell Culture Experiments (Abstract). *ASGSB Bulletin* 1: 39, 1988.

PERCEPTION AND TRANSDUCTION OF GRAVITROPISM IN PLANTS

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Description of Research

The physiological mechanisms by which organisms respond to gravity can be assumed to be complex, both physically and biochemically. For more than half a century, plant biologists have tended to consider the responses of plants to gravity as involving two components: a sensing step which involves the sedimentation of amyloplasts, and a growth response which results from the redistribution of a growth hormone and consequent gravitropic curvature. Recent discovery of a role for calcium in plant gravitropism has created a broadening of the biochemical concepts. Studies in this laboratory have been aimed at a more detailed separation of the possible components of the gravitropic system in roots, in hopes of being able to identify some of the partial processes in gravity responses, and especially to establish some evidence concerning the nature of the calcium participation.

If we consider the gravitropic system to involve three components: sensing, transductions, and then motor response, we can attempt to discriminate physical and biochemical events associated with each component. Our experiments have been carried out to try such a discrimination, and we have utilized red light as a regulator of root gravitropism in corn seedlings.

Accomplishments

(1) We have previously shown that the sensing step in gravitropism is associated with a shift in electric current patterns around the rootcap.

(2) New experiments concerning the transduction step in gravitropism show that ***red light can shift the gravitropic response from a diagravitropic one (seeking a horizontal orientation) to an orthogravitropic one (seeking the direction of the Earth)***. This ability to change the transductive mode with application of red light has permitted several new findings.

(a) The ***diagravitropic transduction state is apparently independent of calcium depletion or calcium inhibitors.***

(b) The ***orthogravitropic transduction state is strongly inhibited by depletion of calcium, or application of calcium or calmodulin inhibitors.***

(c) ***Preliminary evidence indicates that the calcium requirement is related to the operation of a second messenger system: the phosphatidylinositol messenger system,*** which is well known in animal hormone systems but is just being found in plant systems.

(3) The motor phase of gravitropism is sensitive to inhibitors of auxin transport.

Significance of the Accomplishments

These experiments establish for the first time some identifiable biophysical and biochemical components of each of the steps in gravity responses: sensing, transduction, and motor. The sensing step is ordinarily accomplished through the sedimentation of starch-filled

organelles in the rootcap. As these sediment, some unknown events occur which bring about a shift in electric currents around the root tip. Then, the transduction step occurs in the rootcap. This appears to occur in two possible modes: one which leads to a horizontal root orientation (this mode is relatively independent of calcium) and another (activated by red light) which leads to the familiar downward orientation of roots (and is strongly dependent upon the presence of calcium). The calcium involvement of this step appears to involve a second messenger system, which is a relatively new item in plant biochemistry. Finally, the motor step in gravitropism can be identified by a sensitivity to inhibitors of auxin transport.

Gradual progress is thus being made in unraveling some of the component parts of the responses of plants to gravity.

Publications

Björkman, T. and Leopold, A.C. An Electric Current Associated With Gravity Sensing in Maize Roots. *Plant Physiology* 84: 841-846, 1987.

Björkman, T. and Leopold, A.C. Effect of Inhibitors of Auxin Transport and of Calmodulin on a Gravisensing-dependent Current in Maize Roots. *Plant Physiology* 84: 847-850, 1987.

Leopold, A.C. Plagiotropism in Corn Roots and Its Relation to Calcium (Abstract). *ASGSB Bulletin* 1: 27, 1988.

Perdue, D.O., LaFavre, A.K., and Leopold, A.C. Calcium in the Regulation of Gravitropism by Light. *Plant Physiology* 86: 1276-1280, 1988.

Perdue, D.O., LaFavre, A.K., and Leopold, A.C. Evidence for Phosphatidylinositol Turnover in Gravitropic Transduction (Abstract). *ASGSB Bulletin* 1: 27, 1988.

ANALYSIS OF COMPONENTS OF THE GRAVITROPIC RESPONSE IN PLANTS USING PURIFIED PLASMA MEMBRANE VESICLES

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Description of Research

In 1984, the Life Sciences Division of NASA asked the Committee on Space Biology and Medicine (National Research Council) and the Workshop on Plant Gravitational and Space Research to set priorities for research. Each group emphasized the need for subcellular investigations into the mode of action and regulation of the transport of the plant growth regulator auxin and of calcium, especially with respect to their interactions with each other. The main objective of the studies outlined here is to determine the relationship between auxin and Ca^{2+} fluxes across the plasma membrane (PM), and the role of those fluxes in gravitropic curvature in plant stems.

Gravicurvature is thought to be the result of lateral movement of specific chemical or electrical signals which leads to differential growth. Movement of the plant growth hormone auxin (indole-3-acetic acid, IAA) has long been considered to be a primary signal involved in the gravitropic response. In recent years, many studies have also indicated that redistribution of Ca^{2+} is somehow involved in and necessary for curvature. However, *in vivo* studies of the transport and localization of IAA and Ca^{2+}, using either intact tissue or stem segments, have been complicated by the intra- and extracellular compartmentalization of IAA and Ca^{2+}. For this reason, definitive conclusions have not yet been reached as to either the exact role of IAA or Ca^{2+} in gravitropism or their interactions with each other. The work outlined here will address this issue by using purified plasma membranes instead of intact tissue, so that the interactions between IAA and Ca^{2+} fluxes can be measured directly.

As a simple approach to a complicated problem, we are examining only those components of the gravitropic response which involve the plasma membrane of dicot shoot seedlings. Several essential steps involved in the gravitropic response are associated with the PM. IAA efflux carriers are thought to be the controlling factor in polar auxin transport, and it has been proposed that the efflux of auxin may be linked to transient Ca^{2+} fluxes. While specific systems involved in Ca^{2+} movement across the PM have not been definitively demonstrated in plants, they are thought to exist, and changes in cytoplasmic Ca^{2+} concentration as well as the interaction of Ca^{2+} with PM proteins are known to regulate many cellular responses. It has been suggested that the gravitropic response may be regulated at the level of IAA and Ca^{2+} carriers located at the PM, either via the Ca^{2+}-binding protein calmodulin (CaM), and/or via phosphorylation of the carriers by a Ca^{2+}-stimulated protein kinase.

Specific and saturable IAA uptake and efflux carriers have been described in PM from zucchini (*Cucurbita pepo* L.) hypocotyls, so that the basic mechanism for polar IAA transport is known. However, compared to what is understood about animal hormone secretion, very little is known about how IAA fluxes are modulated by the environment. We do not have an understanding of how gravity modifies either the rate or direction of

IAA transport. While Ca^{2+} may play a role, its effect on IAA uptake and efflux has not been investigated. Nothing is known in plants about PM Ca^{2+} transport mechanisms, since earlier studies have been carried out with microsomal rather than purified PM preparations. Furthermore, in previous membrane studies, the entire seedling segment was used, so that both the graviperceiving and graviresponding tissues were present. With those problems in mind, we are proceeding with the following investigations:

(1) Establishment of a system for *in vitro* studies of membrane transport components.

(2) Analysis of the interactions between Ca^{2+} and the transport of IAA.

(3) Examination of the role of protein kinases in modifying transport activity.

(4) Definition of the Ca^{2+} transport mechanisms in dicot shoot plasma membranes.

(5) Comparison of IAA and Ca^{2+} transport in graviperceptive vs. graviresponsive tissue.

Accomplishments

As mentioned above, an essential component of this study is the use of purified plasma membranes for the transport studies. In the past, good separation of PM from the membranes of other plant organelles has been difficult to achieve. The technical breakthrough which now makes this study possible is the development of a method to obtain large quantities of highly purified PM from a variety of plant tissues. The method involves phase separation of microsomal vesicles between defined concentrations of the polymers polyethylene glycol (PEG) and Dextran. Different species of membrane vesicles are separated on the basis of both surface charge and density, and plasma membrane vesicles of extremely high purity can be prepared.

We have now adapted this technique to membrane preparations from zucchini (*Cucurbita pepo*) hypocotyls, which are known to yield vesicles that will stay tightly sealed for many hours. In the six months we have been working on this project, we have demonstrated that the vesicles that we prepare:

(1) are at least 95% of plasma membrane origin, as indicated by enzyme marker assays;

(2) have a primarily right-side out orientation;

(3) are osmotically sealed and able to maintain a pH gradient for many hours;

(4) ***contain all of the necessary components of the polar auxin transport system; an electrogenic $IAA^-/2H^+$ symport for uptake and a phytotropin-sensitive IAA^-/H^+ efflux carrier;***

(5) ***have a native protein kinase that is dependent upon Ca^{2+} for activity and autophosphorylation.***

Significance of the Accomplishments

The characteristics of the zucchini PM vesicles will now allow the study of specific membrane transport with vesicles of known origin and sidedness, which is necessary to reach a definitive answer as to the directionality of transport, something that has rarely been considered in plant membrane transport investigations. The presence of the protein kinase also will allow us to assess *in vitro* the role of phosphorylation in modifying the activity of the transport components. These attributes taken together make the vesicles ideal for the transport studies described above. We now plan to determine the influence of Ca^{2+} on IAA uptake and efflux, define the basic mechanisms of PM Ca^{2+} fluxes, the effect of IAA on Ca^{2+} transport, and finally to ascertain at which step in gravitropism — perception, signal transduction, or response — each component is involved.

REGULATION OF PLANT GROWTH AND DEVELOPMENT BY MECHANICAL STRESS

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Description of Research

This laboratory investigates the physiolgoical basis whereby plants respond to dynamic physical forces in the growth environment. Dynamic physical forces at the Earth's surface generally result from the action of wind and precipitation. These forces cause exposed plants to experience mechanical stress. Mechanical stresses are classified according to the manner in which they are applied to plants, e.g., seismic (shaking), thigmic (contact rubbing), or vibrational. Terrestrial plants respond to mechanical stress by acquiring a more compact growth habit. Stems usually become shorter, thicker, and stronger. Leaves are smaller but thicker, and water is conserved by closure of stomates and reduced leaf area. The compact growth habit permits affected plants to better resist extreme environmental conditions, such as high wind velocities or reduced water availability. However, there are negative effects associated with mechanical stress. Although stomatal closure limits transpirational water loss, it also reduces the amount of carbon dioxide absorbed by the leaf. Reduced carbon dioxide uptake inhibits photosynthesis and subsequent growth. Mechanical stress also may delay the onset of reproductive growth as well as the number of reproductive structures initiated and retained by the plant. Harvestable yield may also be reduced by exposure to mechanical stress.

The growth environment within future spacecraft (e.g., Space Station) will not be typical of that found in the terrestrial biosphere. Light intensity will be constant unless orbital photoperiod is used, but also will be substantially reduced relative to that of plants grown outdoors. Roots likely will grow in or on some artificial medium rather than in soil, and nutrients probably will be supplied hydroponically. The dynamic physical forces experienced by spacecraft-borne vegetation also will be exceedingly different. Acute shaking, vibration, and G-force signals will be perceived by these plants during the launch phase. Once in orbit, plants suddenly will be exposed to microgravity conditions. Low-level vibration and intermittent shaking will result from machine operation and astronaut activity. The questions arises, how will plants that develop in an otherwise microgravity environment respond to the dynamic physical forces that will occur during spacecraft launch or in orbit?

The objective of our ground-based research program is to characterize and determine the physiological and biochemical basis whereby plants respond to brief, periodic episodes of dynamic physical agitation.

Accomplishments

Our investigations make use of both dark-grown and light-grown plant tissues. Significant findings resulting from these studies are as follows:

(1) ***Mechanical stress-induced inhibition of straight growth appears to be associated with an accumulation of calcium in the zone of maximum stem***

elongation. In experiments using ^{45}Ca in nutrient solution, twice as much calcium accumulated in the growth zones of mechanically stressed, dark-grown soybean seedlings as in those that were nonstressed. There also were 2-3-fold increases in calcium accumulation in the cotyledons, plumules, and apical hooks of stressed seedlings. However, in time-course experiments in which elongation and fresh weight accumulation were correlated with ^{45}Ca uptake, growth inhibition occurred much more rapidly than calcium accumulation.

(2) ***Mechanical stress affects plant reproductive growth at discrete periods of development.*** Application of seismic stress to greenhouse-grown soybeans during any stage of reproductive growth reduced harvestable seed mass. The greatest seed mass reductions occurred when stress was applied during the R6 (full pod) stage. Seed mass reductions resulted mainly from a reduction in total seed number. Individual seed dry weights were not significantly affected. A smaller number of two- and three-seeded pods on stressed plants accounted for the reduced seed number.

Collectively, these experiments represent efforts to better understand and characterize plant mechanical stress responses at both cellular and whole-plant levels.

Significance of the Accomplishments

Previous investigations in our laboratory demonstrated evidence for the involvement of calcium in mechanical stress-induced growth inhibition by use of calcium chelators and calmodulin activity inhibitors. The observation that growth inhibition occurs much more rapidly than radiolabeled calcium accumulation implies that calcium assimilated during growth is not a primary factor in mechanical stress-induced inhibition of straight growth. A more likely scenario is that the calcium involved in mechanical stress-induced growth reduction is derived from calcium pools already present within cells of the growth zone at the time of stress application. Present results do not rule out an early role for this calcium in mechanical stress growth inhibition.

Yield reductions are more likely to occur when soybean plants are subjected to mechanical stress during reproductive development than during vegetative growth. Knowledge of the timing of periods during reproductive growth that are most sensitive to mechanical stress may prove useful in negating or preventing undesired effects of mechanical agitation. This information may prove useful in designing systems for plant growth to be utilized in future Controlled Ecological Life Support Systems (CELSS) aboard orbiting spacecraft.

Publications

Cuellar, M.D. and Mitchell, C.A. Effects of Static vs. Flowing Atmospheres on Plant Growth in the Space Shuttle Plant Growth Unit (Abstract). In: *Space Life Sciences Symposium: Three Decades of Life Science Research in Space,* Washington, D.C., June 21-26, pp. 11-12, 1987.

Jones, R.S. and Mitchell, C.A. Calcium-45 Accumulation in the Growth Zone of Mechanically-stressed Soybean (Abstract). *Plant Physiology* 86(Suppl.): 28, 1988.

Jones, R.S. and Mitchell, C.A. Calcium Influence on Mechanical Stress Growth Inhibition of Dark-grown Soybean Seedlings (Abstract). *ASGSB Bulletin* 1: 19, 1988.

Jones, R.S. and Mitchell, C.A. Evidence for the Involvement of Calcium and Calmodulin Activity in Mechanically-induced Growth Inhibition (Abstract). *Plant Physiology* 83(Suppl.): 84, 1987.

HOW GRAVITY AFFECTS PLANT GROWTH

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Description of Research

The purpose of our research is to determine how gravity affects plant growth and development. We have taken several approaches to studying this problem:

(1) We have studied how plants grow in space by analyzing plants grown aboard flight 61-C of the Space Shuttle *Columbia*.

(2) We have studied how calcium affects growth and gravitropism by quantifying and localizing endogenous calcium in graviresponding roots.

(3) We have studied how roots respond to gravity by determining where gradients of gravitropic effectors form in graviresponding roots.

(4) We have studied the influence of other ions in gravicurvature by studying how asymmetries of ions such as aluminum influence root growth.

Accomplishments

(1) ***Microgravity strongly affects the structure and function of plant cells.*** For example, microgravity significantly inhibits accumulation of starch and stimulates accumulation of lipid bodies.

(2) Asymmetries of calcium and several ions induce gravitropiclike curvature.

(3) ***A gradient of gravitropic effectors occurs in the mucigel surrounding horizontally oriented roots.***

(4) ***A gradient of calcium occurs in the mucigel and epidermal cells of horizontally oriented roots.*** Interestingly, there appears to be a gradient of calcium across epidermal cells, with the largest concentration nearest the mucigel. A corresponding gradient of calcium does not appear in the root's cortex.

Significance of the Accomplishments

Our results indicate that gravity exerts specific effects on the structure and function of plant cells. These effects are similar throughout a plant and in different types of cells. For example, plants grown in microgravity contain much less starch than those grown on Earth. If our conclusions hold true for long-term studies, then the caloric value of starch-rich plants grown in space will probably be less than that for plants grown on Earth.

Several studies have shown that asymmetries of exogenously applied calcium induce gravitropiclike curvature. Our studies indicate that other ions induce curvature. For example, roots curve away from aluminum, barium, and cadmium. These results indicate that ions other than calcium may affect how roots respond to gravity.

Asymmetries of gravitropic effectors accumulate in mucigel and epidermal (i.e., outermost) cells but apparently not across the interior of graviresponding roots. These results suggest that (1) the signals affecting curvature may move outside of cells rather than directly from cell to cell, and (2) the epidermis may be the "target" for signals inducing gravicurvature.

Indeed, an intact epidermis is necessary for roots to respond to gravity. These results are important because they identify a target tissue with which we can more effectively study the effects of gravity.

Publications

Fondren, W.M. and Moore, R. Collection of Gravitropic Effectors from Mucilage of Electrotropically-stimulated Roots of *Zea mays* L. *Annals of Botany* 59: 657-659, 1987.

Hasenstein, K.H., Evans, M.L., Stinemetz, C.L., Moore, R., Fondren, W.M., Koon, E.C., Higby, M.A., and Smucker, A.J.M. Comparative Effectiveness of Metal Ions in Inducing Curvature of Primary Roots of *Zea mays*. *Plant Physiology* 86: 885-889, 1988.

Mauseth, J.D., Moore, R., Montenegro, G. Structure of Graviresponsive Capless Roots of *Tristerix aphyllus* (Abstract). *ASGSB Bulletin* 1: 19, 1988.

Moore, R. How Gravity Affects Plant Growth and Development. *Biology Digest* 14: 11-16, 1988.

Moore, R., Cameron, I.L., Hunter, K.E., Olmos, D., and Smith, N.K.R. The Locations and Amounts of Endogenous Ions and Elements in the Cap and Elongating Zone of Horizontally Oriented Roots of *Zea mays* L.: An Electron-Probe EDS Study. *Annals of Botany* 59: 667-677, 1987.

Moore, R. and Fondren, W.M. A Gradient of Endogenous Calcium Forms in Mucilage of Graviresponding Roots of *Zea mays*. *Annals of Botany* 61: 113-116, 1988.

MECHANISM OF GRAVITROPISM

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Description of Research

The long-range goal of this research is to elucidate the mechanism of plant gravitropism. In former reports I have described the model we are examining: when a plant is displaced from its position of equilibrium, stretch-activated ion channels open in receptor cells in response to the gravitational signal. There is an influx of calcium ions across the lowermost membrane of the cell. This constitutes primary energy transduction. The localized elevation of cytosolic calcium results in activation of a kinase, which phosphorylates a protein responsible for controlling auxin transport. Auxin then moves out of the lower side of each receptor cell, resulting in a decreased concentration on the upper side of the tissue and an increased concentration on the lower side. The auxin gradient leads to secondary asymmetries and ultimately to differential growth, by which the plant orientation is corrected.

The lab is examining the above model with several methodologies. Two of these are discussed below:

(1) Progress toward testing participation of mechanosensitive channels in gravity reception. One promising approach to testing the model is to evaluate, by recording electrical currents through single ion channels, whether an inhibitor of gravitropic gravity reception can also block stretch-activated channel action in receptor cells. Initially, no appropriate inhibitor was available: in spite of the large number of known inhibitors of gravitropism, none had previously been shown to block very early tropic processes. Bernard Millet, on sabbatical from the Universite de Franche-Comte, joined our group to solve this problem.

We found a relatively specific inhibitor of gravitropic reception: the gadolinium ion. Moreover, we found that a set of agents known to block a widely distributed calcium channel in plants impair gravitropic reception only nonspecifically and at high concentration.

Currently, I am on sabbatical gaining skill in the patch clamp technique of examining single ion channels. I hope that in future reports I will be able to describe the properties of mechanotransductive channels in gravity receptor cells, and to assess which if any respond to the array of inhibitors in the same manner expected of a transductor—that is, in the same manner as reception itself.

(2) Progress in isolation of a protein possibly participating in gravitropic auxin transport. We believe that we have isolated a protein which may possibly participate in the regulation of auxin movement across the cell membrane. However, we must emphasize that (1) we have not yet attained enough evidence to publish, and (2) several years of work will be necessary in order to pin down the protein function. Again, use of an inhibitor has been essential to the work—more specifically, use of several members of a group of inhibitors called phytotropins. It is well established in the literature that phytotropins block both polar auxin movement and gravitropic auxin transport. Moreover, strong evidence has been presented that the blockage results from binding to a specific protein in the cell membrane.

According to the model above, gravitropic auxin transport is a fairly immediate sequela of primary transduction, and the phytotropin receptor seemed a good first target in the plan for isolating the proteins involved in gravitropism.

Henry Slone, Terry Riehle, Dabney Dixon, and I have synthesized a special phytotropin and attached it to Sephadex beads. We have poured solubilized membrane proteins over a column of the "affinity beads" and washed off those that adhered nonspecifically. Then, we poured a solution of phytotropin through the column in an effort to displace protein specifically bound to the beads. Standard display of the eluate on gels subjected to electrophoresis indicated that a discrete band of protein was present. In some experiments an additional discrete band was seen; we do not know why its presence is erratic but it is noteworthy that two phytotropin binding sites have been described. Tests for specific phytotropin-binding activity of the eluted protein have yielded encouraging results, but the amount of protein is small and activity is too low to be reliably distinguishable from noise. We have developed an improved method to assay binding. If binding activity could be unequivocally established, it would constitute proof that the isolated protein is a receptor of the class we seek. Then, we could proceed to make antibodies in order to test whether the receptor might indeed participate in the regulation of axial and gravitropic auxin movement.

Accomplishments

(1) ***We have established an inhibitor specific for the sensory steps of gravitropism in the root of maize.*** It can now be tested whether mechanosensitive ion channels serve as gravitational signal transducers.

(2) ***We have purified small amounts of a single, discrete protein from a specially constructed affinity column.*** It can now be checked to determine if it is the sought-after phytotropin receptor that modulates gravitropic auxin transport.

Significance of the Accomplishments

Gravitropism is one of the most important ways in which plants respond to the gravitational field of the Earth, and it is essential for survival in the field. It also constitutes an excellent model system for the somewhat neglected but agriculturally essential discipline of plant sensory physiology. Characterization of the mechanism by which a newly discovered inhibitor interdicts gravitropic reception and of a protein putatively modulating gravitropic auxin transport should substantially increase our understanding of plant response to gravity.

Publications

Millet, B. and Pickard, B.G. Early Wrong-way Response Occurs in Orthogravitropism of Maize Roots Treated With Lithium. *Physiologia Plantarum* 72: 555-559, 1988.

Guinel, F.C., Pickard, B.G., Varner, J.E., and McCully, M.E. Root-cap Net in Corn (Abstract). In: *Physiology of Cell Expansion during Plant Growth.* (ed. by D.J. Cosgrove and D.P. Knievel). Rockville: American Society of Plant Physiologists, pp. 280-283, 1987.

Millet, B. and Pickard, B.G. Gadolinium Ion is an Inhibitor for Testing the Putative Role of Stretch-activated Ion Channels in Geotropism and Thigmotropism (Abstract). *Biophysical Journal* 53: 155a, 1988.

Millet, B. and Pickard, B.G. Reproducible Thigmotropic Response by Maize Roots (Abstract). *Plant Physiology* 86(Suppl.): 29, 1988.

GEOTROPISM IN *ARABIDOPSIS THALIANA*; A GENETIC APPROACH

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Description of Research

Our ultimate objective is to achieve an understanding of the mechanisms whereby a plant measures and responds to a gravitational stimulus at the molecular level. Toward that end, this project is developing and characterizing a family of mutant lines in *Arabidopsis* with genetic alterations in specific elements critical for the gravitational response(s). These lines will permit the identification of the elements in the transduction pathway and an analysis of the interrelations between the two environmental factors, gravity and light, in controlling the growth and development of this plant.

This work has been divided into several aspects. First, a family of mutant strains has been identified with altered shoot gravitropism. A second family of mutant strains has been identified with altered root gravitropism. Genetic characterization of these mutants is in progress to determine the mechanism of inheritance and the physical location of the genes within the *Arabidopsis* genome. Biophysical characterization of these strains is in progress to determine the relationship between phototropism, photomorphogenesis, shoot gravitropism, and root gravitropism. Molecular genetic techniques will be employed to physically isolate the genes associated with critical steps in the gravitropism pathway.

Accomplishments

(1) Screening procedures have been developed with which we have identified strains with altered root and shoot gravitropism.

(2) Shoot gravitropism and root gravitropism each may be altered without alterations in the other, or may be altered together. Based on these mutants, one can conclude that the pathways are relatively independent but have common elements.

Significance of the Accomplishments

Finding #1: This collection of *Arabidopsis* mutants with altered shoot gravitropism and altered root gravitropism is a unique resource for studying the interconnections between phototropism, photomorphogenesis, shoot gravitropism, and root gravitropism.

Finding #2: Mutants with alterations in genes regulating critical steps in gravitropism may be physically isolated using mutants in this collection.

Publications

Barsel, S. and Poff, K. Molecular Characterization of Genes Critical to Geotropism in *Arabidopsis* (Abstract). *ASGSB Bulletin* 1: 13, 1988.

Poff, K., Bullen, B., Best, T., Gregg, M., and Sullivan, W. Characterization of *Arabidopsis* Mutants With Altered Gravitropism (Abstract). *ASGSB Bulletin* 1: 13, 1988.

CALCIUM MESSENGER SYSTEM IN GRAVITROPIC RESPONSE IN PLANTS

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Description of Research

Our major objective is to understand how plant roots respond to gravitational stimulus. It is becoming clear that calcium plays a central role in gravity signal perception and transduction in roots. Primary signals such as gravity and light could induce changes in cytosolic calcium and initiate a cascade of biochemical events by altering calcium- and calmodulin-dependent enzymes including protein kinases leading to a physiological response. However, very little is known about the various transducers and the exact sequence of biochemical events that are involved in translating the gravity signal into a physiological response. Phosphoinositide turnover and calcium-dependent protein phosphorylation have been shown to play a vital role in signal transduction. Furthermore, amplification and diversities of the action of some signals is achieved through calcium- and calmodulin-dependent protein kinases. We have investigated various aspects of the calcium messenger system with emphasis on phosphoinositide turnover and calcium- and calmodulin- dependent protein phosphorylation and their role in the transduction of the gravity signal in corn roots.

Accomplishments

(1) Our results indicate the involvement of calcium in light-dependent gravity response. It is possible that light increases cytosolic calcium which in turn activates calcium- and calmodulin-dependent protein kinases. If this is so, one would expect changes in *in vivo* calcium-dependent protein phosphorylation when roots are exposed to light. In order to study the role of calcium-dependent protein phosphorylation in light-dependent gravitropism, *in vivo* protein phosphorylation studies are performed in dark-grown and light-treated roots of Merit corn (*Zea mays* L.). Since Merit corn roots require light to develop gravitropic sensitivity, these roots are used as a model system.

Exposure of dark-grown roots to light results in promotion of phosphorylation of specific polypeptides corresponding to 94,000, 92,000, and 48,000 kDa. ***We have been able to detect light-dependent changes in protein phosphorylation within one min. Interestingly, the light-dependent changes in protein phosphorylation are observed only in the root tips which are considered to be the site of light and gravity perception. No effect of light on the phosphoprotein pattern is observed in the root base, suggesting the specificity of the changes in light-responsive tissues and the physiological significance of these changes in light-induced gravitropism.*** Depletion of calcium by addition of EGTA and calcium ionophore (A23187) prior to light treatment has been found to decrease light-induced promotion of the phosphorylation of these polypeptides. Replenishment of calcium to depleted root tips restores the light effect on protein phosphorylation. ***These results strongly suggest light induces rapid and specific changes in protein phosphorylation and these changes are mediated by calcium.***

(2) The initial event in the gravitropic response is believed to be an increase in cytosolic calcium in the columella cells of the root cap. How light and gravity signals could bring about changes in cytosolic calcium is under investigation. Evidence obtained during the last two years indicates the existence of the phosphoinositide pathway in plants and its importance in signal transduction. Since calcium is found to have an important role in the light-dependent gravity response, we have tested the involvement of inositol phospholipid turnover in light-signal transduction in roots.

Root tips are labeled with [^{3}H]inositol, exposed to light, and the inositol phosphates (IP_1, IP_2, and IP_3) are quantified. ***The levels of inositol phosphates are found to be higher in light-treated roots than in the dark control.*** Various chemicals such as 5-hydroxytryptamine (5-HT) and carbachol have been shown to promote PIP_2 hydrolysis in animals. We tested the effect of 5-HT on gravitropic curvature and phosphoinositide turnover to further investigate the involvement of PIP_2 turnover in light-induced gravitropism. ***Application of 5-HT to roots with agar blocks showed gravitropic curvature in the dark. Furthermore, 5-HT treatment of apical root segments resulted in a higher level of inositol trisphosphate, as compared with control.*** These results indicate that light could promote the hydrolysis of PIP_2 and produce IP_3 and DG. The IP_3 thus released could raise cytosolic calcium, which in turn activates calcium- and calmodulin-dependent enzymes including protein kinases. ***We have also observed IP_3-induced calcium release from plant microsomes.***

(3) Signal-induced hydrolysis of PIP_2 is achieved through the activation of phospholipase C, which is believed to involve GTP binding proteins (G-proteins). ***We have detected the presence of phosphoinositide specific phospholipase C-I (PLC-I) by immunoblotting in both soluble and membrane proteins isolated from Merit corn roots*** (Figure 1A). PLC-I antibodies specifically reacted with a polypeptide of corn soluble and membrane proteins, indicating the presence of this enzyme in Merit corn root tips. ***We have also detected specific GTP binding proteins using [alpha-^{32}P]GTP in the membranes isolated from Merit corn root tips. Three polypeptides have been found to bind to GTP*** (Figure 1B). Binding of radiolabeled GTP to proteins is competed by cold GTP, guanosine 5'-(-thio) triphosphate (GTP [gamma s]), or GDP, whereas ATP is found to be ineffective in competing GTP binding.

Significance of the Accomplishments

Finding #1: Recent investigations on calcium, calmodulin, protein phosphorylation, and phosphoinositide turnover indicate a major role for calcium as a messenger in signal transduction.

Finding #2: Rapid changes associated with gravitropic response in roots are not completely understood. Stimulus-induced change in calcium-dependent protein phosphorylation that is observed within one min is one of the fastest responses detected and could be one of the primary events involved in signal transduction.

PATTERN OF SOLUBLE AND MEMBRANE PROTEINS FROM CORN ROOTS

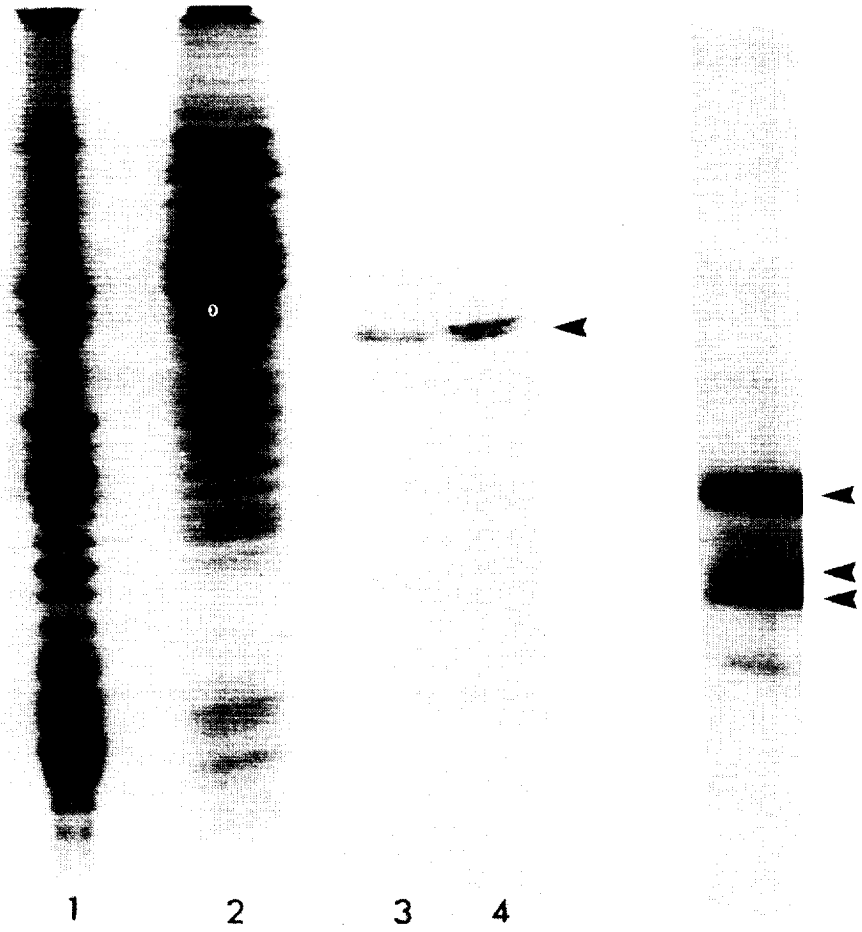

Figure 1A. Membrane (lane 1A) and soluble (lane 2A) protein pattern of Merit corn root tips (0.2 mm). Detection of phosphoinositide specific phospholipase C-I in soluble (lane 3A) and membrane (lane 4A) proteins of Merit corn root tips (0.2 mm) by immunoblotting. Soluble and membrane protein from root tips were isolated, sepa-rated on 12% SDS-PAGE gel, and electrophoretically transferred to nitro-cellulose paper. Nitrocellulose paper was first incubated with antibodies raised against phos-phoinositide specific phospholipase C-I and then with horseradish peroxidase conjugated goat antirabbit antibodies. Immunoreactive products were detected with 3-amino-9-ethyl carbazole as peroxidase substrate.

Figure 1B. Binding of [alpha- ^{32} P] GTP to membrane proteins isolated from Merit corn root tips (0.5 mm). Membrane proteins were isolated, separated on 12% SDS-PAGE, and electrophoretically transferred to nitrocellulose filter. Autoradiograph shows three major protein bands that bind to labeled GTP.

Finding #3: The detection of various components of the phosphoinositide pathway such as phospholipase C-1, GTP-binding proteins, and phosphoinositide turnover in corn root tips suggests the importance of this pathway in signal transduction.

Publications

Friedmann, M. and Poovaiah, B.W. The Effect of Calcium on Protein Synthesis in Light-induced Gravitropism in Corn Roots (Abstract). *Plant Physiology* 83(Suppl.): 19, 1987.

McFadden, J.J. and Poovaiah, B.W. Rapid Changes in Protein Phosphorylation Associated with Light-induced Gravity Perception in Corn Roots. *Plant Physiology* 86: 332–334, 1988.

Poovaiah, B.W. Molecular and Cellular Aspects of Calcium Action in Plants. *HortScience* 23: 267-271, 1988.

Poovaiah, B.W., McFadden, J.J., and Reddy, A.S.N. The Role of Calcium Ions in Gravity Signal Perception and Transduction. *Physiologia Plantarum* 71: 401-407, 1987.

Poovaiah, B.W. and Reddy, A.S.N. Calcium Messenger System in Plants. *CRC Critical Reviews in Plant Science* 6: 47-103, 1987.

Poovaiah, B.W., Reddy, A.S.N., and McFadden, J.J. Calcium Messenger System: Role of Protein Phosphorylation and Inositol Bisphospholipids. *Physiologia Plantarum* 69: 569-573, 1987.

Raghothama, K.G., Reddy, A.S.N., Friedman, M., and Poovaiah, B.W. Calcium-regulated *In vivo* Protein Phosphorylation in *Zea mays* L. Root Tips. *Plant Physiology* 83: 1008-1013, 1987.

Reddy, A.S.N., Friedmann, M., McFadden, J.J., and Poovaiah, B.W. Calcium Messenger System in Light-dependent Gravitropism (Abstract). *ASGSB Bulletin* 1: 27-28, 1988.

Reddy, A.S.N., McFadden, J.J., Friedmann, M., and Poovaiah, B.W. Signal Transduction in Plants: Evidence for the Involvement of Calcium and Turnover of Inositol Phospholipids. *Biochemical and Biophysical Research Communications* 149: 334-339, 1987.

Reddy, A.S.N. and Poovaiah, B.W. Inositol Phosphates and Protein Phosphorylation (Abstract). *Plant Physiology* 83(Suppl.):158, 1987.

EFFECTS OF HYPERGRAVITY AND HYPOGRAVITY ENVIRONMENTS ON CELL CYCLE REGULATION DURING FERN SPORE GERMINATION

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Description of Research

The development of form and function in plants is accompanied by a complex interplay of cell division and expansion. Cell division is cyclic and this cycle can be divided into four component phases. The different phases of the cell cycle (G_1, S, G_2, and M) are influenced by the physiological activity of the cell and state of the DNA complement. The purpose of this research is to study the effect of gravitational stress on the cell cycle.

As growing plant tissue typically consists of cells at various developmental stages, it is important to employ a model system of cell division that would allow for the study of the effect of gravity on a population of cells at a similar developmental stage. Because of the need for a large number of cells at a similar developmental stage, spores of the sensitive fern *Onoclea sensibilis*, were chosen as the test material. Spores from this plant are collected in a dry dormant state and upon hydration and light activation undergo a mitotic division which leads to germination.

Accomplishments

(1) ***The application of hypogravity, as simulated by a clinostat, does not have a significant effect on the cell cycle, as evidenced by the length of time required for sensitive fern spore germination.***

(2) Spores subjected to hypergravitational forces synthesize unique species of proteins.

(3) A working model of the cell cycle of *Onoclea sensibilis* has been determined.

Significance of the Accomplishments

Several experiments were performed using a clinostat to compare germination rates of spores subjected to hypogravitational force with that of a control. Results from these experiments suggest that lowering the effective gravitational force has no effect on cell cycle length. As previous research has shown that the application of hypergravitational force has a clear effect in delaying germination or elongating the cell cycle, it appears that ***application of hypo- and hypergravitational stress affects the cell cycle differently***. When results from experiments in which spores are stressed with elevated gravitational forces are compared with information from cell cycle studies, it appears that there is a distinct sensitivity to hypergravitational stress prior to and during the period when spores are in the S (synthetic) phase of the cell cycle.

In an effort to understand the metabolic effect of gravity on the cell cycle, experiments were performed using radiolabeled amino acids to analyze protein production after spores were subjected to elevated gravitational fields. Results show that several new species of proteins

are produced after hypergravitational stress. This information when added to the previously reported work suggests that ***hypergravitational stress may be tied to changes in cell cycle length by production of stress proteins.***

Other experiments are being performed to study if stress proteins are only produced during the time periods when spores are in the synthetic phase or if this can occur during any period of the cell cycle. The RNA synthesis inhibitor 5-fluorouracil has been used to answer questions concerning production of mRNA's necessary for stress protein synthesis. Also, other work is being planned to test the effects of lower levels of hypogravitational stimuli on cell division. It is hoped that, when this work is complete, valuable information will be gained which adds to our knowledge of how gravity affects cell division and plant growth.

Publications

Folsom, M.W. and Raghavan, V. The Effect of an Elevated Gravitational Field on Spore Germination in Sensitive Fern (Abstract). *ASGSB Bulletin* 1: 21-22, 1988.

Folsom, M.W. and Raghavan, V. Effects of Hypogravity Stimuli on Spore Germination in *Onoclea sensibilis* (Abstract). *American Journal of Botany* 74: 710, 1987.

MECHANISM OF ASYMMETRIC CELL ELONGATION DURING PLANT SHOOT GRAVITROPISM

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Description of Research

We are interested in the mechanism by which plants perceive and transduce information about gravity at the cellular and molecular levels. More specifically, we want to understand the mechanism by which gravistimulation causes the asymmetric movement of growth regulators in shoots and how these agents cause asymmetric growth and ultimately reorientation of the shoot. As prior work has shown that the plant growth hormone auxin (IAA) and possibly calcium ions are involved in asymmetric growth, most of our efforts are directed toward understanding how these agents regulate cell extension.

To study the effects of auxin and/or calcium ions on cell extension, we make use of two systems. *Avena* coleoptile sections are used to investigate the mechanism of cell wall loosening, a phenomenon which ultimately controls how rapidly cells enlarge. We also use the single gene recessive tomato mutant *diageotropica* (*dgt*), a segregant of the parent variety VFN8. This gravitropically sluggish mutant is highly insensitive to auxin and thus appears to be a unique model system for studying the molecular aspects of auxin action.

Accomplishments

Studies on the mechanical properties of Avena coleoptile cell walls

(1) The extension ("creep") of frozen-thawed coleoptile sections is accelerated by the calcium chelator EGTA when applied in low molarity neutral buffers. EGTA is ineffective when high molarity buffers are used. Quin II chelates calcium from *Avena* sections as effectively as EGTA but does not stimulate extension growth. At low buffer molarities EGTA causes substantial acidification (pH 6.5 —> pH 5.2) of *Avena* coleoptile cell walls. Quin II application does not cause acidification. ***These and other data show that calcium removal per se from cell walls of Avena does not result in wall loosening.*** However, if calcium removal is coupled with acidification, proton-mediated wall loosening occurs.

(2) Instron-measured wall extensibility increases when frozen-thawed sections are incubated in acidic solutions (optimum pH 4.5). This loosening process is reversed upon transfer of sections to solutions at pH 6.5. Time course studies indicate both responses are complete in 2-4 hr. Inhibitor studies, effects of temperature, and preincubation of the sections in proteinases show that proteins are necessary and make it likely that ***enzymes are involved in both cell wall loosening and cell wall stiffening processes.***

Studies on the auxin physiology of diageotropica

(1) Prior work showed *dgt* to be highly insensitive to auxin with respect to hypocotyl elongation and ethylene production. To determine the nature of *dgt's* sluggish growth, parameters affecting auxin-induced cell elongation were measured. ***The osmotic potential of mutant and wild-type tissue is identical and both are increased by auxin treatment. Wall extensibility is increased by auxin in VFN8 but***

not increased in dgt, indicating the breakdown of an auxin-directed wall loosening mechanism possibly at the level of wall acidification.

(2) Examination of auxin transport, uptake, and efflux from VFN8 and *dgt* hypocotyls produced the following data. ***Basipetal auxin transport rates are identical in the two varieties but dgt sections demonstrate an increased capacity for transport. This capacity can be modulated downward to wild-type levels by ethylene treatment.*** Auxin uptake and efflux kinetics are nearly identical in the two varieties; however, uptake in *dgt* can be modulated well above controls by ethylene. Efflux in *dgt* can be reduced well below controls by ethylene. Collectively, these data indicate a possible auxin/ethylene feedback mechanism for the control of auxin transport.

Significance of the Accomplishments

In order to understand the control of asymmetric growth and thus gravitropism, it is important to identify the load-bearing bonds that control cell wall plasticity. Using *Avena* coleoptiles we have shown that calcium bridges are not load-bearing cell wall bonds. Their removal does not result in cell wall loosening. The addition of calcium does not result in wall stiffening. Instead, the physical properties of *Avena* walls appear to be regulated by wall pH, which in turn modulates the activity of wall loosening and wall stiffening enzymes. Studies on the nature of these enzymes may lead to the identification of the load-bearing cell wall bonds.

Mutant organisms have proved valuable in the elucidation of behavioral and biochemical pathways. Our studies on the auxin physiology of *diageotropica* may allow us to better understand the molecular mechanism of auxin action. Current data suggest a reduced polar transport rate, and therefore an altered efflux carrier does ***not*** result from the *dgt* mutation. Rather, an altered building site at some other site of action or sequestration seems likely.

Publications

Daniel, S. and Rayle, D.L. Studies on a Tomato Mutant With Altered Gravitropic Behavior (Abstract). *ASGSB Bulletin* 1: 21, 1988.

REGULATION OF ENZYMES DURING LIGHT- STIMULATED GRAVITROPISM

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Description of Research

Light greatly accelerates the gravitropic response of roots, coleoptiles, and stems in certain plants. This indicates that some cellular response initiated by light is the same as, or affects, one of the gravity-induced cellular responses necessary for gravitropism. The objective of this research is to identify the specific cellular processes that are altered by gravity and light during the induction of gravitropic growth in plants.

The best characterized photoreceptor for light-regulated gravitropism is the pigment phytochrome. The photoconversion of this pigment to an activated form, called Pfr, has rapid and dramatic effects on the straight growth of coleoptiles and stems, and accelerates the gravitropic curvature of coleoptiles and some shoots and roots. Understanding the mechanisms by which Pfr regulates growth should enlighten how light alters gravitropic growth. Recent research has supported the hypothesis that phytochrome modulates growth primarily by altering wall extensibility, and that wall extensibility is at least partially controlled in many plants by wall-localized enyzmes whose activity affects the interlinking of wall structural elements.

Relevant to the enzyme hypothesis on wall extensibility control, many workers have reported that there is an inverse relationship between the activity of wall peroxidases and the growth of cell walls: those hormonal and environmental stimuli that promote growth inhibit wall peroxidase activity, and those stimuli that inhibit growth stimulate wall peroxidase activity. Further interest in peroxidase involvement in growth derives from the fact that Ca^{2+} ions regulate both the activity and the secretion of wall peroxidases. In corn coleoptiles, the well-known inhibitory effects of Ca^{2+} on wall extensibility appear to be mediated through the activity of one or more wall enzymes. The enhancement of both the activity and secretion of wall peroxidases by Ca^{2+} could help account for the inhibition of growth by Ca^{2+}. Such a relationship would be very relevant to hypotheses proposing a role for Ca^{2+} in mediating gravitropic growth.

Last year we reported the successful production and characterization of monoclonal antibodies against wall peroxidases present in the coleoptiles and other tissues of corn seedlings. This year we report the use of one of these antibodies to assay by immunogold cytochemistry the distribution of a specific acidic wall peroxidase and to quantitate the change in its content that occurs when coleoptiles are stimulated by a light treatment that activates phytochrome and promotes both the rate of growth and the rate of gravitropic curvature of the coleoptiles.

Accomplishments

(1) One of the monoclonal antibodies produced against wall antigens (mWP3) specifically binds to and inhibits the activity of a 98,000 dalton acidic wall peroxidase, and does not appear to cross-react with any other acidic wall peroxidase or with any of the

several basic wall peroxidases present in corn seedlings. Thus, ***mWP3 can be used to quantitate the amount of a specific peroxidase isozyme present in the walls of corn coleoptiles without the interference of cross-reactivity with other wall or cytoplasmic isoperoxidases.***

(2) An enzyme-linked immunoadsorbent assay (ELISA) using mWP3 reveals that ***within 5 min following the photoactivation of phytochrome in corn coleoptiles, the quantity of mWP3-recognized peroxidase that is extractable from the coleoptiles drops by over 40%.*** This effect of red light (R) is canceled or reversed by a far-red light (FR) irradiation, which photoconverts phytochrome back to the inactive Pr form.

(3) ***Immunogold cytochemical localization of the peroxidase recognized by mWP3 reveals that there is little if any specific staining in the cytoplasm and good, reproducible staining in the walls.*** Various tissues of corn seedlings were checked to demonstrate the normal distribution of wall peroxidase. In the coleoptile, the outermost and innermost epidermal walls did not show staining for the presence of peroxidase, whereas cell walls of the inner coleoptile cells usually demonstrated significant staining. In the leaves also, the epidermal cell walls were negative. Many, but not all mesophyll cell walls were stained, and, although no statistical analyses have been done, it appears as if the walls bordering intercellular air spaces may be the most consistently positive. In the vascular tissue, many of the walls exhibit no stain, but there is a consistent staining of the sieve element walls in the phloem. Interestingly, no immunostain was detected in any root cells in either the cap region or meristematic zone.

Significance of the Accomplishments

Finding #1: The specificity of mWP3 allows us to assay total coleoptile antigens prepared by freeze-drying of the tissue under vacuum, without being concerned about increased background noise readings due to cross-reactivity with other cellular peroxidases. The freeze-drying method, in turn, allows us to carry out assays of rapid kinetic changes in the wall content of the specific peroxidase recognized by mWP3, such as those described in Finding #2.

Finding #2: This is the first report of changes in peroxidase content in walls following a stimulus that affects gravitropic growth. The fact that these changes are R/FR reversible identifies the photoreceptor as phytochrome. This is the first report of a phytochrome-induced change in the content of a wall enzyme. The lag time between Pfr production and increased growth rate of the coleoptile has been estimated by Smith's group in Leicester to be about 8 min. The fact that significant peroxidase changes occur within 5 min is consistent with the postulate that changes in wall peroxidase could causally influence wall extensibility and growth.

Finding #3: These results revealed that mWP3 was directed specifically against a ***wall*** antigen. Thus, ELISA assays of total coleoptile antigens are not distorted by cross-reactions of mWP3 with cytoplasmic peroxidases. This is the first immunogold assay of the ultrastructural distribution of a specific wall isoperoxidase in plants, and it reveals that peroxidases that function in the walls of cells in aerial tissues (shoots, coleoptiles, leaves) may not be present at all in the walls of root cells. Thus, enzymatic wall components that regulate growth may be significantly different in root and shoot cells.

Publications

Clark, G., Dauwalder, M., and Roux, S.J. Partial Purification and Characterization of a Calcimedin-like Protein in Peas (Abstract). *Plant Physiology* 86(Suppl.): 114, 1988.

Dauwalder, M. and Roux, S.J. Distribution of Calmodulin in Corn Seedlings: Immunocytochemical Localization in Coleoptiles and Root Apices. *Advances in Space Research* 6: 67-70, 1986.

Kim, S.-H. and Roux, S.J. Immunochemical Detection of Rapid Red-light Induced Changes in Wall-localized Peroxidase Content in Corn Coleoptiles (Abstract). *Plant Physiology* 86(Suppl.): 92, 1988.

Kim, S.-H., Terry, M.E., Dauwalder, M., Clark, G., and Roux, S.J. Production and Characterization of Monoclonal Antibodies Against Wall Proteins of Corn (Abstract). *Plant Physiology* 83(Suppl.): 42, 1987.

Kim, S.-H., Terry, M.E., and Roux, S.J. Production of Monoclonal Antibodies to Cell Wall Peroxidase and Cellulase. In: *Physiology of Cell Expansion During Plant Growth* (ed. by D.J. Cosgrove and D.P Knievel). Rockville: American Society of Plant Physiologists, pp. 289-291, 1987.

Roux, S.J. Regulation of Nuclear Enzymes in Plants. In: *Physiology of Cell Expansion During Plant Growth* (ed. by D.J. Cosgrove and D.P. Knievel). Rockville: American Society of Plant Physiologists, pp. 145-155, 1987.

CELLULAR POLARITY AND INTERACTIONS IN PLANT GRAVIPERCEPTION

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Description of Research

Our work is centered around determining whether amyloplasts — heavy, starch-filled organelles — are the primary structure upon which gravity acts to trigger perception. If amyloplasts are involved, then the next question is to identify the cellular structure(s) with which they interact such as endoplasmic reticulum (ER) or cytoskeleton.

Recently, a starchless mutant of *Arabidopsis* has been isolated which shows "vigorous" gravicurvature without significant plastid sedimentation (these plastids are amyloplasts without starch) (Caspar, Somerville, and Pickard, 1988, in press). These researchers attribute the slightly reduced graviresponsiveness of the mutant to its lower growth rate, and they therefore reason that it is as sensitive as the wild type (WT) and that plastids play no role in graviperception.

We examined the ultrastructure and the kinetics of curvature of the mutant and WT *Arabidopsis* to determine whether their sensitivity and structure were truly comparable.

Accomplishments

(1) ***The mutant root is much less sensitive than the WT to repeated, short periods of gravistimulation*** (horizontal placement). For example, if both are placed horizontally for 1 min, then clinostated for 9 min, and this pattern is repeated for 2 hr, then the WT curves about 20^{o} and the mutant curves about 2^{o}. Longer doses, e.g., one 10 min period horizontal followed by clinostating, result in the mutant curving only about 50-70% as much as the WT.

(2) The growth rates of roots of both genotypes are identical.

(3) ***The mutant plastids are the heaviest and most movable components of the columella cells.*** Only the plastids move to the bottom of these cells in mutant roots centrifuged at low gravity for short periods.

(4) ***Plastids in both strains contact ER*** (Figure 1). Impregnation of the ER with osmium ferricyanide demonstrates this definitively in columella cells of both genotypes.

(5) The surface density of the ER is higher in the base compared with the top of columella cells in vertical roots of both genotypes. However, this is only true for columella cells that are in an intermediate stage of development. Furthermore, the relative density of ER near the lower wall stays the same throughout development.

GRAVITY SENSING CELLS IN WILD TYPE AND MUTANT STRAINS OF *ARABIDOPSIS*

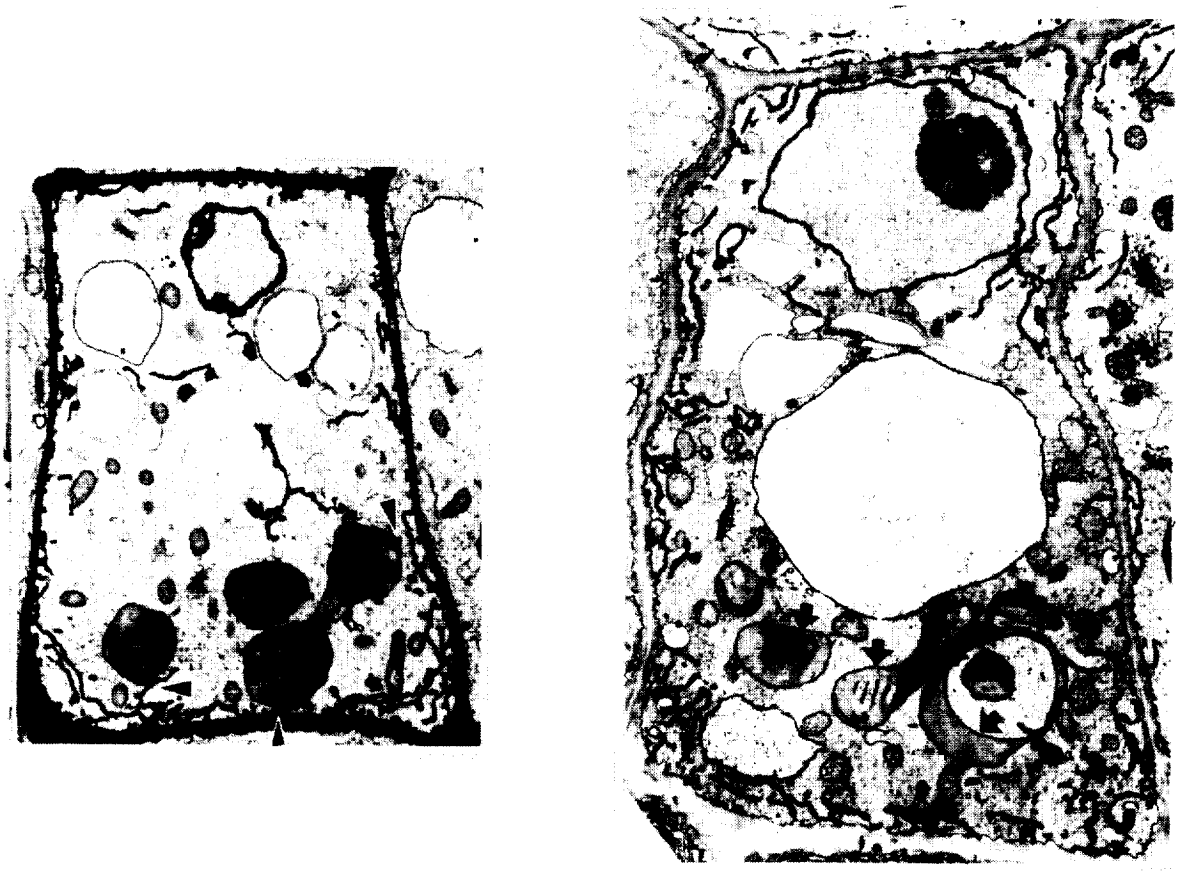

Figure 1. Electron micrographs of the rootcap cells thought responsible for gravity perception. The cells are specially stained for endoplasmic reticulum (ER) which appear as dark lines. Left, wild type *Arabidopsis* with starch-containing amyloplasts at bottom of cell and in contact with ER in several places (arrowheads). Right, cell from mutant rootcap showing starchless plastids (arrows). In this cell, the plastids are at the base of the cell, but sedimentation is not as consistent in the mutant, compared with the WT.

Significance of the Accomplishments

Findings #1-#3: Differences in sensitivity are best measured at threshold levels of stimulus. Presumably, the reception mechanism is closer to saturation at longer than at shorter doses. The use of repeated (intermittent) stimulation helps discriminate by reducing noise while retaining a level of stimulus that is closer to the threshold.

The fact that the mutant is considerably ***less*** sensitive than the WT demonstrates that at least the mass of the starch is necessary for full sensitivity. The differences in response cannot be explained by differences in growth rate. Thus, contrary to the claims of its developers, this mutant provides significant evidence ***for*** the role of amyloplasts.

The fact that the mutant is capable of substantial gravicurvature indicates that it is still remarkably sensitive despite the absence of starch. It is logically possible that perception in both genotypes is occurring by an unknown system that is independent of plastids and that the plastid-based system is only present in the WT. However, our data indicate very strongly that plastids are the primary component in perception in both genotypes.

Certainly, the fact that the mutant plastids are the heaviest and most movable component in columella cells is relevant. While it is difficult to calculate directly the relative buoyant weights of plastids with and without starch, if the mutant plastids were 1/10th as dense as amyloplasts, then this difference could account for the up-to-10-fold difference in sensitivity.

Findings #4 & #5: This mutant now becomes an extremely useful tool for studying what are the minimum requirements for graviperception. For example, the fact that the mutant can perceive and respond to gravity without significant plastid sedimentation suggests that this is not essential, but it also suggests that sedimentation could contribute to full sensitivity. In this regard, our finding that plastids of both genotypes contact ER — the first such definitive demonstration of plastid-ER contact (Finding #4, above) in statocytes — is consistent with the working hypothesis that plastid pressure upon the ER somehow triggers perception.

Furthermore, given that the ER is polarly distributed in some *Arabidopsis* columella cells (Finding #5), it becomes tempting to speculate that the heightened sensitivity of the WT is conferred not just by greater mass and hence greater pressure, but by sedimentation bringing the amyloplasts down to regions enriched in ER where more contact and amplification are possible.

Publications

Sack, F.D. Structural Polarity in Rootcap Cells of Wild Type and Starchless TC7 *Arabidopsis* (Abstract). *ASGSB Bulletin* 1: 44, 1988.

Sack, F.D. The Structure of the Stem Endodermis in Etiolated Pea Seedlings. *Canadian Journal of Botany* 65: 1514-1519, 1987.

Sack, F.D. and Kiss, J.Z. Structural Asymmetry in Rootcap Cells of Wild Type and Starchless Mutant *Arabidopsis* (Abstract). *Plant Physiology* 86 (Suppl.): 172, 1988.

GRAVITROPISM IN DICOT STEMS

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Description of Research

Our broad goal is to understand the mechanisms by which a plant stem, turned to the horizontal, curves upward in response to gravity. We began by examining mechanisms of response at the tissue and cellular levels: how differential growth on the top and bottom of stems leads to bending. More recently, we have studied the role played by plant hormones in gravitropic bending. We have published evidence implicating ethylene (a gaseous hormone that occurs in much higher quantities in bottom tissues of a horizontal stem), although the exact role of ethylene remains an enigma. Currently, we are attempting to understand the role of auxin (indoleacetic acid, IAA), which is known to be essential for the elongation of stem cells that is necessary for gravitropic bending. For six decades, most research was guided by the assumption that changing auxin concentrations account for bending; i.e., auxin concentrations increase in bottom tissues. But measured changes seem insufficient to account for the differential growth (or changes may not occur at all). An alternative to control by auxin concentration is control via changing sensitivity of the tissue to the auxin already present. We measure sensitivity by varying the stimulus over a wide range and observing the response.

In our basic system, we use a tank that is subdivided into eight compartments, each containing a different solution (one with buffer but no auxin; the others with auxin concentrations ranging from 10^{-8} to 10^{-2} M IAA). Stem sections from sunflower or soybean seedlings are immersed in the solutions in a vertical or horizontal position and photographed at half-hour intervals. Negatives are projected from below onto a transparent digitizer, which is used to measure angles of bending and changes in lengths of the top and bottom surfaces of horizontal stem sections or lengths of vertical stem sections. Results are interpreted according to Michaelis-Menten enzyme kinetics. In our system, V_{max} is considered to be equivalent to the maximum growth induced by the optimum auxin concentration. A decreased V_{max} indicates a decreased sensitivity to applied auxin. K_m is the auxin concentration that produces growth equal to half of V_{max}. An increased K_m equates with a decreased sensitivity to applied auxin.

Accomplishments

(1) Considerable effort was spent during the period of this report to conceive of, purchase, develop, and finally utilize the digitizer/computer system described above.

(2) The standard experiment was repeated about a dozen times with both sunflower and soybean stem sections. As auxin concentrations increase, gravitropic bending decreases until finally at a high enough auxin concentration, the segments bend down instead of up (Figure 1). Measurement of surface growth shows that this occurs because increasing auxin decreases growth of the bottom surface (which is very high to begin with in response to the endogenous auxin in the tissue) and increases growth of the top surface until an optimum concentration is reached, after which some decrease in growth occurs. Typically, maximum growth (V_{max}) of the bottom surface exceeds that of the top surface,

and both surfaces exceed maximum growth of vertical control segments. Thus, V_{max} sensitivity is increased somewhat for both upper and lower surfaces compared with vertical stems. The lowest concentration required to cause half-maximum growth of the bottom surface must be very low: lower than the endogenous auxin in the tissue and certainly much lower than 10^{-8} M IAA, the lowest concentration used in our tanks. The K_m concentration for upper surfaces, on the other hand, is higher, on the order of 10^{-7} M IAA. Thus, K_m sensitivity is much lower for upper surfaces than it is for bottom surfaces. K_m sensitivity for vertical controls is difficult to determine but appears to be intermediate between that for upper and for lower surfaces.

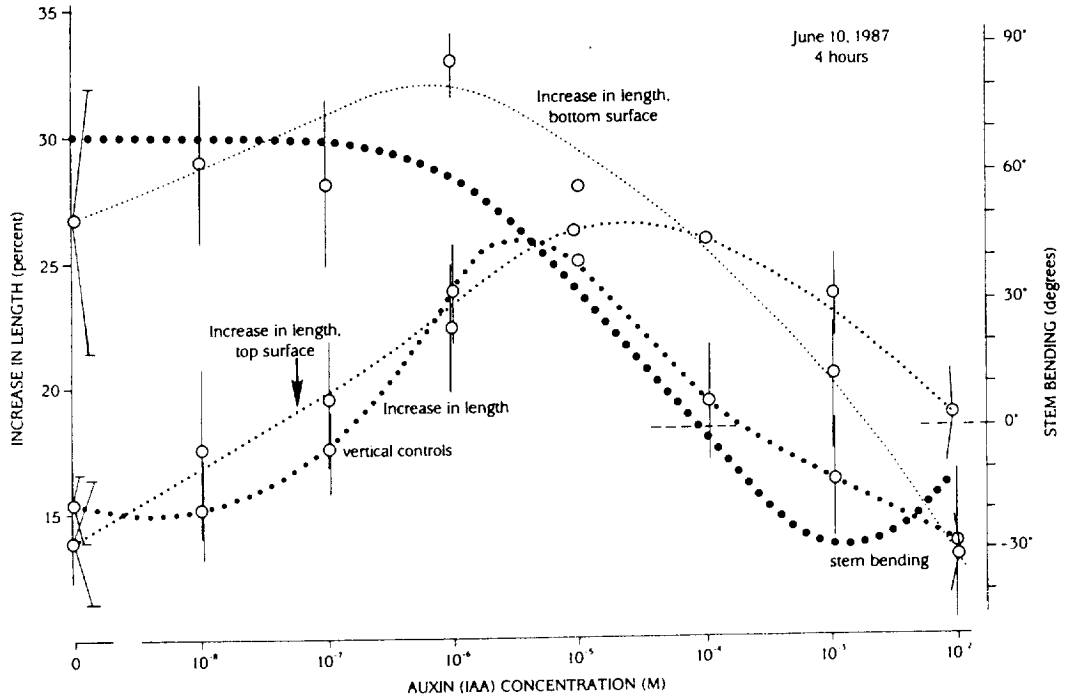

Figure 1. Effect of different auxin concentrations on angles of stem bending and changes in length of top and bottom stem sections in horizontal or vertical positions.

These results clearly show that sensitivity to applied auxin is markedly different for upper and for lower tissues in a gravistimulated stem, with sensitivity (both V_{max} and K_m) being greatly reduced in upper compared with lower tissues. There is little reason to doubt that sensitivity to endogenous auxin is changing in the same way. We also note changing sensitivity from experiment to experiment, presumably in response to slightly different environments and observably in response tc plants of different ages.

(3) In separate experiments we have shown that tissue sensitivity changes as a function of age.

(4) ***Using our system, we have successfully confirmed the auxin depletion and "gravitropic memory" experiments of Brauner and Hager, first published in 1958.*** If sunflower seedlings are decapitated and left in the dark for 4 days, they do not bend up when gravistimulated for up to 3 hr. If they are returned to the vertical after such a gravistimulation and immersed in auxin at the right concentration, bending occurs in the expected direction. When stems not treated this way are turned to the horizontal, there is a lag (about 1 hr) before bending begins. The depleted stems, vertical or horizontal, show no lag after being immersed in auxin. Thus, the lag must be a matter of perception rather than response to auxin. These are key observations that separate gravity perception from the growth response. There are several implications for future avenues of research.

(5) We have initiated studies of calcium interactions with auxin. So far, results have been disappointing (i.e., calcium has had little effect).

(6) Patricia Rorabaugh, a graduate student on the project, has repeated her early soybean experiments with the new, more reliable equipment that allows a much wider range of auxin concentrations than were used previously.

(7) Rorabaugh spent 3 weeks in the laboratory of Robert S. Bandurski analyzing tissue auxin from stem segments used in our standard experiments. ***She found only slight differences in auxin in upper and lower stem halves, and the differences that did appear suggest higher concentrations in the upper tissue.*** The theory that suggests that gravitropic bending is under the control of auxin concentration would predict just the opposite.

Significance of the Accomplishments

Finding #1: Completion of our system opens many new avenues of research.

Findings #2 & #3: The sensitivity concept is a radical departure from the thinking that has directed research in gravitropism for over six decades. It provides a whole new way of looking at gravitropism in stems and suggests new experimental approaches. Other workers are now investigating sensitivity.

Finding #4: Because others have failed to repeat the Brauner-Hager experiments and because these experiments are extremely significant for studies in gravitropism, this is an important accomplishment. Combined with our other data, it suggests that the "gravitropic memory" is a change in sensitivity to auxin.

Finding #5: Studies with calcium represent the first of many similar studies we have planned with several growth regulators and other compounds.

Finding #6: Our conclusions are on a firmer footing because of the repetition of earlier experiments.

Finding #7: Those who have questioned the role of changing sensitivity in gravitropic stem bending have always pointed out that endogenous auxin levels were not known. While this seemed relatively unimportant to us because of the wide range and high concentrations of auxins used in our experiments, it was nevertheless essential to measure tissue auxin with the most sophisticated modern approaches and equipment. The results of these studies are therefore highly significant to the overall interpretation of our results.

As to the significance of research on gravitropism in stems: Use of plants in microgravity environments could depend on knowledge about stem gravitropism. Recently, another potential application of such understanding has been encountered: Cut flowers, especially snapdragons, bend gravitropically while being shipped in a horizontal position. Understanding the gravitropic response might suggest ways to solve this interesting problem.

Publications

Bugbee, B.G. and Salisbury, F.B. Exploring the Limits of Crop Productivity: Photosynthetic and Carbon Partitioning Efficiency in Optimizing Environments for a CELSS (Abstract). *ASGSB Bulletin* 1: 30-31, 1988.

Bugbee, B. and Salisbury, F.B. Physiological and Genetic Studies on Wheat for the Controlled Environments of Space (Abstract). In: *Space Life Sciences Symposium: Three Decades of Life Science Research in Space,* Washington, D.C., June 21-26, p. 246-248, 1987.

Rorabaugh, P.A. and Salisbury, F.B. Changing Sensitivity to Auxin During Gravitropic Stem Bending of Soybean Hypocotyls (Abstract). *ASGSB Bulletin* 1: 45-46, 1988.

Rorabaugh, P.A. and Salisbury, F.B. Gravitropic Bending in Dicot Stems: A Role for Changes in Cell Sensitivity to Preexisting Auxin (Abstract). *Plant Physiology* 83(Suppl.): 101, 1987.

Salisbury, F.B. USU Research Helps Agriculture Enter the Space Age. *Utah Science* 48: 147-152, 1987.

Salisbury, F.B. and Bugbee, B. Plant Productivity in Controlled Environments. *HortScience* 23: 293-299, 1988.

Salisbury, F.B., Gillespie, L., and Rorabaugh, P. Changing Sensitivity to Auxin During Gravitropic Stem Bending of Sunflower Hypocotyls (Abstract). *ASGSB Bulletin* 1: 28, 1988.

Salisbury, F.B., Rorabaugh, P., and Gillespie, L. The Role of Changing Sensitivity to Auxin in Gravitropic Stem Bending (Abstract). In: *Abstracts of XIV International Botanical Congress,* p. 117, 1987.

AMINE OXIDASE AND CA^{2+}-DEPENDENT PEROXIDASE IN DIFFERENTIAL GROWTH RESPONSES OF GRAVISTIMULATED PLANTS

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Description of Research

Our long-term goals are in line with those of the Space Biology Progam in that we would like to understand how living organisms, plants in particular, are influenced by gravity with respect to their ability to adapt to and survive in the space environment. The biochemical and physiological processes by which plants regulate growth in response to altered gravity vectors or changes in acceleration within a gravitational field are poorly understood. An elucidation of such mechanisms will facilitate our understanding and prediction of plant growth responses in both the normal Earth (1 g) environment and the hypogravity environments that would be encountered in space.

Our specific goal is to determine the extent to which peroxidase-mediated cross-linking mechanisms, and the expected inhibition of cell wall extensibility and cell growth resulting from such cross-linking, contribute to short-term, largely reversible tropistic growth responses in plants (i.e., differential growth, or bending of a plant organ in response to an altered gravity vector). More permanent, irreversible cross-linking of this type is thought to be responsible for lignification processes, which provide long-term structural support to plants, as there is evidence that these processes are influenced by gravity.

Our research examines: (1) the dependence of these peroxidase activities on wall-localized amine oxidases, which could provide the H_2O_2 (hydrogen peroxide) substrate for peroxidase-catalyzed reactions, and (2) the possible regulation of cell wall peroxidase activities by asymmetric gradients of calcium (Ca^{2+}) and auxin, which have been observed across gravistimulated plant organs. Initially we plan to investigate the cellular and tissue-level distribution of amine oxidase and peroxidase activities in normal and gravistimulated plant organs, in order to determine whether they are correlated with changes in growth rates across the organ. These experiments will employ both cytochemical and biochemical methods. In later experiments, we will examine whether changes in enzyme activities and growth can be modulated by exogenous applications of putative "transduction" signals, such as Ca^{2+} or auxin.

Accomplishments

(1) We have unambiguously demonstrated, for the first time, that ***amine oxidase activity is localized in the plant cell wall, using an electron microscopic cytochemical method.***

(2) We have shown that the cytochemical distribution of amine oxidase activity in the tissue is highly correlated with known sites of lignification (e.g., vascular parenchyma).

(3) We have completed biochemical studies of the distribution of amine oxidase activity. These studies show that ***there is no lateral asymmetry in the activity of***

POLYAMINE OXIDASE ACTIVITY IN CORN COLEOPTILE TISSUES

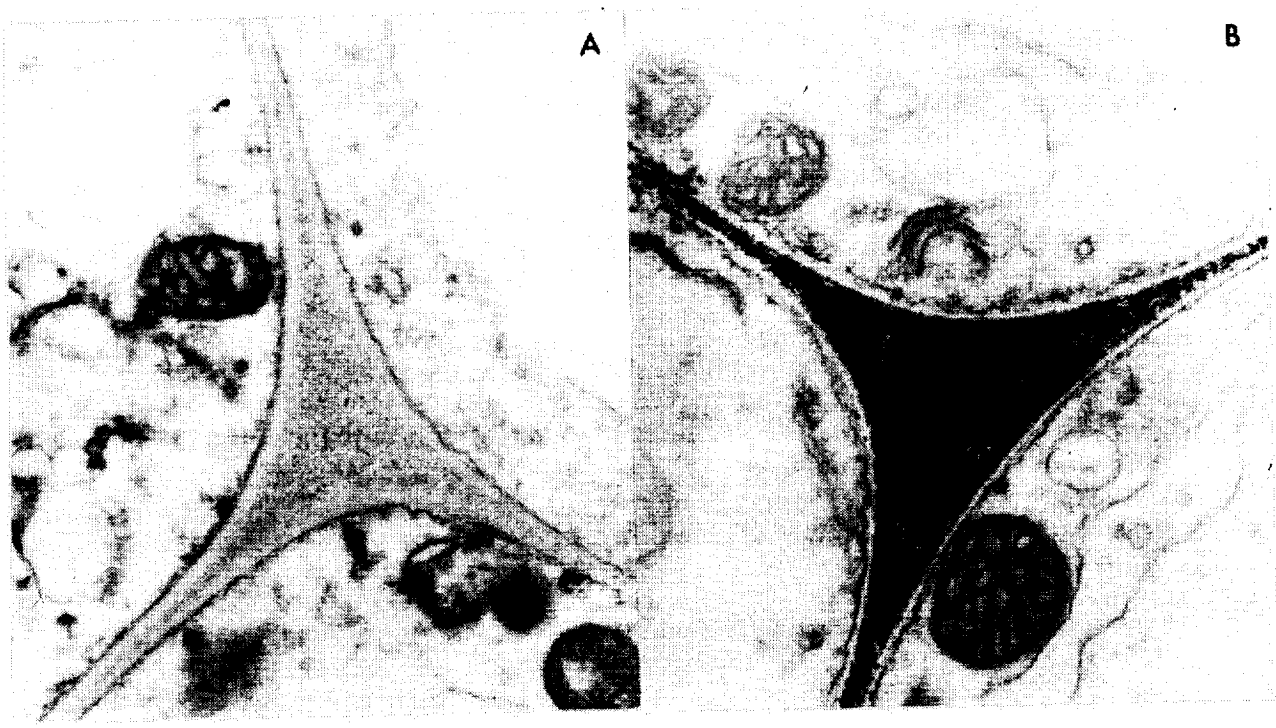

Figure 1. Electron microscopic cytochemical localization of polyamine oxidase activity in corn coleoptile tissues, based on detection of H_2O_2 using $CeCl_2$. (A) A typical parenchyma cell showing weak staining of the cell wall middle lamellar region. Magnification x 20,540. (B) A vascular parenchyma cell showing marked staining of the cell wall. No intracellular activity staining is seen. x 25,410.

this enzyme across gravistimulated organs. There are, however, marked asymmetries in cell wall-bound peroxidase activities and polyamine titers.

Significance of the Accomplishments

Finding #1: The unambiguous demonstration of amine oxidase activity in the cell wall is essential for the proposed model, in that this enzyme could provide the H_2O_2 substrate needed by wall-bound peroxidases to catalyze the various biochemical cross-linking reactions that are thought to be operative in the tropistic growth response. Since the polyamine substrates for amine oxidases are also localized in the wall, all prerequisites for the model have been met.

Finding #2: The structural support provided by lignified tissues enables plants to grow upright, with respect to Earth's gravity vector. In the hypogravity environment of the Space Shuttle, it has been shown that lignification of tissues is decreased significantly, although it is not clear what mechanisms control lignification processes in a gravity-dependent manner. Lignification is the result of covalent cross-linking of cell wall phenolic constituents into rigid polymers by wall-bound peroxidases. Our model presupposes that this cross-linking is dependent upon amine oxidase-generated H_2O_2, thus the strong correlation between amine oxidase activity and sites of lignification in the tissue support the model (see Figure 1A and 1B). Further studies will be required to determine whether gravity-mediated changes in lignification result from changes in the activities of amine oxidase, peroxidase, or both.

Finding #3: The fact that amine oxidase activity remains unchanged across the bending organ suggests that asymmetric distribution of amine oxidase activity per se is not the cause of differential growth. However, marked changes in the concentration of the polyamine substrates for this enzyme have been observed across gravistimulated organs and might support the asymmetric distribution of amine oxidase-generated H_2O_2. This H_2O_2 asymmetry, even if there were no asymmetry in the distribution of peroxidase activities, could support different degrees of peroxidase-mediated cross-linking of wall constituents, locally influencing cell growth.

Even if there were no involvement of amine oxidases and peroxidases, ionic "bridging" of cell wall anionic groups by strongly basic polyamines could inhibit wall extensibility and growth directly. We have shown that higher polyamine titers and wall-bound peroxidase activities are associated with slower-growing tissues of the gravistimulated plant organ, but at present we do not know the relative contribution of ionic vs. covalent cross-linking mechanisms described here to the overall growth response.

Publications

Slocum, R.D. Role for Diamine Oxidase in Differential Growth Response Accompanying Tropistic Curvature in Pea Epicotyls (Abstract). *ASGSB Bulletin* 1: 20, 1988.

ANIMAL PROJECTS

EFFECT OF SKELETAL UNLOADING ON BONE FORMATION

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Description of Research

The long-range goal of our research program is to understand the effects of gravity on skeletal development and bone metabolism. Our present objectives are to define the effects of gravity or mechanical stress on bone formation and resorption and to determine the mechanisms (hormonal or paracrine) by which mechanical stress is coupled to bone cell activity.

Bone is a dynamic, living tissue. It is continually undergoing change, or remodeling, which involves a delicate balance between bone formation and bone resorption. This balance is influenced by systemic hormones such as parathyroid hormone, glucocorticoid hormones, growth hormone, and the vitamin D metabolites, as well as local factors such as blood flow, neuromuscular activity, and mechanical stress. Recently, a number of cytokines have been observed to stimulate or inhibit bone formation and resorption. Some of these cytokines, such as insulin-like growth factor-1 (IGF-1), transforming growth factors, and fibroblast growth factors, have been identified in bone. IGF-1 production by bone appears to be regulated by growth hormone. Such cytokines may participate in coupling mechanical stress to bone cell activity, or the activity of one type of cell to the activity of another type of cell (for example, osteoblast activity to osteoclast activity). Our research over the past year was directed toward elucidation of the role that the systemic hormones play in coupling the mechanical stress of weight bearing to the cellular response of bone formation, as well as beginning assessment of the role of locally produced cytokines such as IGF-1.

We have continued to use the hindlimb unloading model to produce skeletal unloading of the hindlimbs of a growing rat. Bone formation was assessed by histomorphometry, ^{45}Ca and ^{3}H-proline incorporation, as well as change in fat-free weight and calcium content. Bone maturation was assessed by separating different fractions of powdered bone using toluene:bromoform density gradient centrifugation and evaluating the fractions for bone weight, total calcium, and ^{45}Ca and ^{3}H-proline incorporation. The effect of the active vitamin D metabolite $1,25(OH)_2D$ on bone formation and mineralization was determined by infusing the $1,25(OH)_2D$ into the rats with osmotic minipumps. Similar studies have been performed with growth hormone. The role of glucocorticoid hormones in mediating changes in bone formation with skeletal unloading was determined using surgical ablation techniques. To initiate studies of locally produced cytokines, we established cultures of mouse calvarial cells and long bone chondrocytes. We plan to use such cultures to assess directly the effects of cytokines on bone cell function.

Accomplishments

(1) Demonstrated that ***high physiological doses of $1,25(OH)_2D$ cause a mineralization defect*** as detected by density gradient analysis.

(2) Demonstrated that ***hindlimb unloading blunts the ability of growth hormone infusion to sustain growth of bone (tibia) in hypophysectomized rats***.

(3) Demonstrated a ***decrease in somatomedin C (IGF-1) concentration in the tibial growth plates from hindlimb unloaded animals*** compared with pair-fed controls.

(4) Demonstrated that ***hindlimb unloading does not result in a change in serum corticosterone level or its circadian rhythm.***

(5) Demonstrated that ***adrenalectomy does not prevent the inhibition of bone growth that occurs in the tibiae of the hindlimb unloaded rat.*** Orchiectomy in combination with adrenalectomy was more effective than adrenalectomy alone in eliminating corticosterone production in the rats. This combination inhibited bone formation to such a degree that the additional effect of hindlimb unloading could not be discerned.

(6) Demonstrated that ***bone formation is accelerated when rats are allowed to recover after hindlimb unloading such that the deficit in bone mass is nearly completely reversed*** in two weeks.

(7) Demonstrated that fetal osteoblast cultures can be grown on collagen-coated beads in the NASA Bioreactor. Under these conditions the cells lay down a collagen matrix and produce alkaline phosphatase.

Significance of the Accomplishments

Our current hypothesis is that the inhibition of bone formation that occurs transiently after skeletal unloading is due to a combination of systemic factors such as $1,25(OH)_2D$ and locally produced factors such as somatomedin C (IGF-1). Our observation that $1,25(OH)_2D$ infusion can lead to a mineralization defect encourages us to explore factors in bone under the control of $1,25(OH)_2D$ that regulate mineralization. Osteocalcin is the most obvious candidate. This bone matrix protein is increased by $1,25(OH)_2D$, and has been postulated to regulate bone crystal formation. In previous studies we have demonstrated that the concentration of osteocalcin in bone and blood falls during hindlimb unloading.

The decrease in somatomedin C levels in the growth plates of unweighted tibiae, combined with the inability of growth hormone to reverse the inhibition of bone formation caused by unweighting, suggests that the unloaded bone may have an abnormal response to growth hormone. This could be the key to understanding why bone formation is inhibited by unloading, since somatomedin C stimulates bone formation and its production by bone is thought to be under growth hormone control.

Our results indicating that adrenalectomy does not protect against the inhibition of bone formation by hindlimb unloading, combined with our observations that corticosterone production is not increased by hindlimb unloading, indicate that increased glucocorticoid production is not the reason bone formation is inhibited by hindlimb unloading.

The preliminary studies with osteoblasts in the Bioreactor point to future flight and ground-based opportunities to assess the effects of gravity on bone cell function.

Publications

Bikle, D.D., Halloran, B.P., Cone, C.M., Globus, R.K., and Morey-Holton, E. The Effects of Simulated Weightlessness on Bone Maturation (Abstract). *ASGSB Bulletin* 1: 34, 1988.

Wood, H.B., Roberts, W.E., Bikle, D.D., Halloran, B.P., Chambers, D.E., Morey, E.R. Perivascular Distribution of Osteoblast Precursor Cells in Periodontal Ligament of Rats Exposed to Simulated Weightlessness (Abstract). *ASGSB Bulletin* 1: 35, 1988.

PHYSIOLOGY OF DEVELOPING GRAVITY RECEPTORS AND OTOLITH-OCULAR REFLEXES IN RAT

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Description of Research

The long-term objective of this research is to examine the effects of microgravity on the physiology of the adult and developing mammalian vestibular gravity receptor system. Ground-based experiments include: (1) study of the physiological responses of otolith afferents in the adult rat and during postnatal development, and (2) determination of the otolith organ contribution to the rat vertical vestibulo-ocular reflex.

Progress has been made in two areas: (1) we have continued software development for acquisition and analysis of spike train data from first-order otolith afferents, and (2) we have continued to characterize the physiological responses of otolith afferents in adult rats. These efforts provide needed background data on peripheral otolith responses in the adult rat, which can then be compared with those in neonates to study the developing otolith system, and with those in the CNS to examine central information processing in the otolith-ocular pathways. Data on these developmental questions and issues regarding the processing of otolith information will permit an analysis of the effects of microgravity on the adult and developing mammalian gravity receptors.

Accomplishments

Our research accomplishments can be divided into two areas:

(1) Software development.

(2) Study of physiological responses of adult rat otolith receptors.

Significance of the Accomplishments

Software Development: Large amounts of spike train data must be processed to analyze the response of otolith afferents during natural stimulation. Software development has continued in order to complete the routines for data collection, interspike interval histogramming, and other display modes.

Otolith Receptors: In studies to characterize the physiological responses of otolith afferents in the adult rat, otolith units were isolated and subjected to several linear acceleration profiles: sinusoidal accelerations (0.025 to 0.5 Hz) in roll and/or constant velocity (1-10 deg/sec), and ramp changes of head position in roll.

In response to ramp changes in head position, ***the vast majority of otolith units show predominantly "tonic" responses in which the discharge rate is proportional to head position and is independent of the velocity of transition. The remaining otolith units have "phasic-tonic" responses characterized by an overshoot (or undershoot) during transition followed by a return, within 4-12 sec, to a new steady-state rate.*** "Tonic" units show no

dynamics, whereas the responses of "phasic-tonic" units are proportional to both head position and the velocity of head transition.

The behavior of "tonic" and "phasic-tonic" otolith afferents, also seen in approximately the same proportions in other mammalian species (e.g., cat, squirrel monkey), are best examined in the frequency domain. The "tonic" units show a small phase lead (0-20^{o} re angular displacement) and approximately flat gain curves over the frequency range tested. On the other hand, "phasic-tonic" units show larger phase leads (20-55^{o}) and 2-5 fold gain increases over the same frequency range. These data from first-order otolith afferents will permit a quantitative comparison between peripheral and central parts of the otolith-ocular pathways, and will allow us to chart the postnatal stages in the physiological development of the peripheral otolith system.

Publications

Blanks, R.H.I. Physiological Studies on Semicircular Canal and Otolith Afferents in Rat (Abstract). In: *Space Life Sciences Symposium: Three Decades of Life Science Research in Space,* Washington, D.C., June 21-26, p. 193, 1987.

Blanks, R.H.I. Response Dynamics of First Order Gravity Receptor Afferents in Rat (Abstract). *ASGSB Bulletin* 1: 17, 1988.

BASIC GRAVITATIONAL REFLEXES IN THE LARVAL FROG

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Description of Research

The purpose of this study is to understand the mechanisms by which a simple vertebrate (the larval frog) is able to detect, process, and respond to alterations in its spatial orientation with respect to the gravitational field of the Earth. Since the structure of the peripheral vestibular system is similar in all vertebrates, it is likely that the cellular components responsible for mediating the gravitationo-ocular reflexes are similar, but more simply organized in the tadpole than in the adult or "higher" vertebrates such as mammals. It is my intent to determine this elementary organizational framework in order to further uncover what changes may occur in this framework as the tadpole metamorphoses into frog and what changes may occur in development of the vestibular reflexes in altered gravitational environments, such as in orbit.

This research involves electrophysiological and morphological investigations of the gravitationo-ocular reflexes generated by the isolated tadpole head. The tadpole shows a prominent ocular-counterrolling in response to static head tilt. The isolated head also will exhibit ocular-counterrolling for several days *in vitro*. The head also exhibits a number of apparently spontaneous behaviors such as rhythmic alterations of the neck, suggesting intended swimming activity, and contractions of the jaw musculature, suggesting intended feeding/respiratory activity.

The goal of the present study is to identify the neuronal elements in the vestibular periphery and in the brain that are responsible for generating these gravitational reflexes. Initial investigations have concentrated on morphological and electrophysiological studies of the vestibular labyrinth in order to determine its similarity to that of the adult.

Accomplishments

(1) Morphological investigations of the inner ear reveal that all of the internal structures present in the adult are differentiated in the premetamorphic tadpole. These structures include the amphibian papilla, the lagena, the sacculus, the utriculus, and the three canals. The general appearance of the labyrinth is that it is the same as that of the adult, only smaller.

(2) Electron microscopic observations indicate that there are well developed synaptic contacts within the labyrinthine structures. These synaptic specializations are also similar to those found in the adult: (a) synapses consisting of spherical dense bodies surrounded by vesicles within the hair cells form Gray Type I contacts with VIIIth nerve afferents; and (b) efferent boutons contact hair cells, often with an apposed subsynaptic cistern within the hair cells. No contacts were detected between hair cells or between efferents and VIIIth nerve afferents.

(3) Electrophysiological investigations of VIIIth nerve afferent activity suggest that the afferents function similarly to those of the adult. Intracellular recordings from these afferents reveal the presence of small depolarizing potentials, indicating that the hair cell synapses are functional. The pharmacological sensitivities of the hair cells and afferents are

also qualitatively similar to those of the adult. Receptive field properties of these afferents are also similar to those found in the frog in that some fibers respond phasically to rotation (probably canal afferents) while others respond to vibration (probably saccular), and still others respond to static tilt of the preparation (probably utricular and lagenal).

(4) Electrical stimulation of the VIIIth nerve fibers within the labyrinth (or glutamate superfusion into the labyrinthine cavity) elicits excitation of Purkinje cells within the cerebellum, confirming that there is a strong vestibular input to the Purkinje cells.

(5) Initial observations of the oculomotor neurons indicate that they respond vigorously to static tilt of the preparation, unlike those of the adult. Electron microscopic observations of these neurons reveal that they are studded with Gray Type I (probably excitatory) and Gray Type II (probably inhibitory) synaptic contacts. These cells appear devoid of "mixed" contacts (i.e., Gray Type I and gap junctions), suggesting that inputs to these cells are through chemically mediated synaptic contacts exclusively. This observation is in contrast to observations of the central vestibular neurons, which can show "mixed" synapses from VIIIth nerve afferents, indicating that some transmission occurs electrically as well as chemically.

Significance of the Accomplishments

As the tadpole metamorphoses into frog, it grows limbs and develops a neck and relies more heavily on these appendages to stabilize its gaze in space. Accompanying these postural adjustments, the gravitationo-ocular reflex gain decreases. The accomplishments mentioned above help to define the components responsible for mediating the gravitationo-ocular reflexes in the premetamorphic tadpole and help to delineate the possible stages in the reflex pathway that change with metamorphosis. Findings #1, 2, and 3 suggest that the basic organization of the tadpole labyrinth is similar to that of the adult. ***It is then unlikely that the decrease in gain of the gravitationo-ocular reflex is due to a major reorganization of the vestibular periphery during metamorphosis.*** That the response sensitivity of the oculomotor neurons is high in the tadpole (Finding #5) as compared with the adult frog indicates that the decrease in gain accompanying metamorphosis is not due principally to change in the synaptic efficacy between oculomotor neuron terminals and eye muscle fibers. ***Rather, it is probable that the reduction in this gain is due to rearrangement of neuronal pathways central to the VIIIth nerve input, but prior to the nerve-muscle synapse.***

That Pukinje cells can be activated by labyrinthine stimulation (Finding #4) indicates that parallel vestibular pathways can be invoked in the isolated head, attesting to its viability. Future efforts will be directed toward localizing which central neurons receive gravity information and how they influence oculomotor neuron activity.

Publications

Cochran, S.L. and Hackett, J.T. The Inner Ear of the Bullfrog Tadpole (Abstract). *ASGSB Bulletin* 1: 16, 1988.

Cochran, S.L. and Hackett, J.T. Sensory Coding by the Vestibular Labyrinth of the Larval Frog (Abstract). *Society for Neuroscience Abstracts* 13: 1223, 1987.

STUDIES OF INTERCELLULAR COMMUNICATION AND INTRACELLULAR RESPONSES BY BONE CELLS TO SIMULATED WEIGHTLESSNESS

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Description of Research

In the microgravity environment of space, the skeleton adapts to a reduction in mechanical force exerted on the bone by reducing the rate of new bone formation. Using an Earth-bound model, it has been shown by placing long bones into a non-weight-bearing position that there is also a reduction in new bone formation. In both these conditions (i.e., microgravity and non-weight-bearing), a continued normal resorption of bone coupled with a reduced replacement by new bone results in an osteoporotic condition. Our objectives therefore are to study skeletal tissues following spaceflight or non-weight-bearing to determine the mechanism(s) responsible for these skeletal adaptations that result in reduced bone formation.

Because the spaceflight studies do not presently permit "real time" manipulation of the flown animals, we have spent significant time and effort to develop techniques which can be applied to tissues at some time period following the flight. We presently use a combination of light and electron microscopy, combined with histochemistry, immunocytochemistry, and quantitative measurements of morphological changes. This includes the ability to study cell membrane-associated enzyme activities, Golgi activity and function, intracellular lysosomal activities, and secretory activity of the bone-forming cells. In addition, results from non-weight-bearing bones (e.g., calvaria) are being compared with the weight-bearing bones (e.g., tibia and femur).

The model used for non-weight-bearing has been designed by Dr. Emily Holton at NASA Ames Research Center. Following various periods of unloading, samples of bone are collected and the studies are carried out in the same manner as for the flight rats.

In addition to the animal non-weight-bearing model studies, Ames Research Center has been successfully growing bone cells in culture, and we have been active in the morphological study of these cells. These cultures will permit more direct studies of cellular response to mechanical forces and permit us to delineate purely mechanical effects on bone cells, compared with other physiological effects seen in the whole animal.

Accomplishments

(1) Analysis of samples of skeleton from Cosmos 1887 are still in progress. This mission was in collaboration with Soviet scientists and involved a study of adult rat skeletons following a 12.5-day spaceflight.

(2) Analysis of all our collected spaceflight data (Cosmos 1129 and 1887; Spacelab-3) indicates that ***blood vessels in bone are affected by the microgravity environment*** (Figure 1).

EFFECTS OF MICROGRAVITY ON BLOOD VESSELS IN BONE

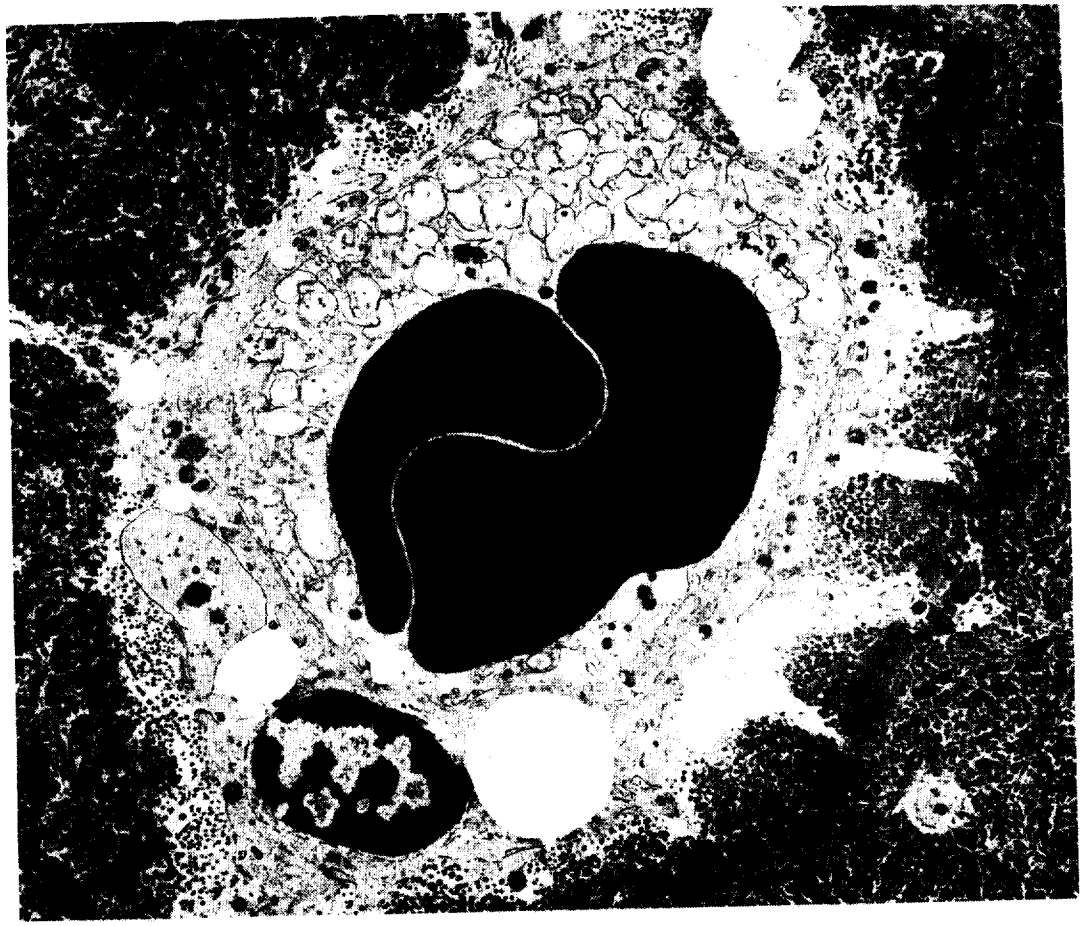

Figure 1. An electron micrograph illustrating that the blood vessels in bone following spaceflight are filled with debris, and there is degeneration of the cells associated with the vessels.

(3) Tracer studies in suspended, non-weight-bearing animals indicate that ***blood flow in bone is reduced during the first 48 hours of non-weight-bearing.*** This reduction is brought back to normal within 5-7 days, even in the presence of continuous non-weight-bearing.

(4) Bone cells grown in culture have many characteristics of bone-forming cells *in vivo,* based on morphological criteria. With prolonged culture, a mineralizable collagen matrix is formed.

(5) Histochemical measurements indicate that ***osteoblasts adjacent to vessels in bone are affected by non-weight-bearing.*** These cells may be responsible for the reduction in new bone formation and may be the most sensitive to microgravity and non-weight-bearing conditions.

Significance of the Accomplishments

The reduction in new bone formation resulting from microgravity (spaceflight) or non-weight-bearing is a modification by the skeleton to reduce its mass. This reduction has been thought to occur because less mechanical stress is applied to the skeleton during non-weight-bearing. However, this may not be the only adaptation which the skeleton experiences. We noted that the vasculature in bone was altered with spaceflight (Finding # 2) and that a transient reduction in blood flow occurred during non-weight-bearing (Finding # 3). These changes can alter the bone-forming cells that are associated with the vasculature of bone (Finding #5) and thus alter the rate of new bone formation. These results suggest that normal maintenance of the skeleton requires mechanical stress and also an active blood flow through a patent microvasculature in bone. The extent of contribution of each of these factors to the maintenance of the skeleton is presently unknown but microgravity certainly seems to influence both.

Publications

Doty, S.B. The Role of the Cytoskeleton in Bone Matrix Formation (Abstract). *ASGSB Bulletin* 1: 41, 1988.

Jones, D., Scholuebbern, G., Becker, M., and Doty, S.B. Evidence for More Than One Type of Bone Forming Cell. *Calcified Tissue International* 41(Suppl.): 47, 1987.

GROWTH AND DIFFERENTIATION OF MAMMALIAN TISSUES EXPOSED TO HYPERGRAVITY *IN VIVO* AND *IN VITRO*

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Description of Research

A basic question of the Space Biology Program is how gravitational changes affect developing systems. Effects of gravitational changes on postimplantation mammalian development can be studied on Earth only by using excess gravity produced by centrifugation, a system routinely used in the Soviet Union for predictions of microgravity effects. This laboratory has been studying the effects of altered gravity on development of the mammalian skeletal system, which is known to be responsive to gravitational changes *in vivo* and *in vitro*. Mice were chosen for this study because their small size makes them less susceptible to Δg, thus lessening any effects on development due to effects on the mother. Also, the mouse limb bud is a well-characterized system that can be maintained *in vitro*.

For *in vivo* studies, 5-week-old male and female mice were placed on a small animal centrifuge. For this experiment, cages were redesigned to allow division into one to four compartments, each with its own food and water supply. Mice were allowed to adapt to g forces between 2.3 and 3.5 g for 8 weeks prior to breeding. Mice were weighed weekly and estrus studies were used to determine females appropriate for pairing. Two types of overnight pairings were done: with a male at the same g level, or with a control male (at 1 g). Skeletal development was assessed using 18-day alizarin red/alcian blue-stained fetuses.

Accomplishments

(1) After 51 weeks at excess g, weights of centrifuged males did not differ significantly from weights of control males. ***Weights of centrifuged females remained less than controls for the duration of the experiment.***

(2) Cephalometric studies showed that ***elongation and compression of skulls of centrifuged animals were more likely to be significant in females.***

(3) A reliable method was developed for providing timed pregnant mice in hypergravity conditions, but pregnancy rates were less in centrifuged animals and no pregnancies occurred at 3.5 g.

(4) Centrifuged fetuses (2.6 and 2.9 g) weighed significantly less than control fetuses and had smaller crown rump lengths.

(5) ***Morphometric analysis of long bones of 18-day centrifuged fetuses showed them to be smaller and shaped differently from bones of control fetuses.***

(6) The ratio of the cube root of fetal weight to the longest dimension of the tibia, a scaling relationship, was greater in centrifuged fetuses.

(7) ***Effects of centrifugation on fetus size and bone size and shape were mitigated in fetuses resulting from pairings of centrifuged females with 1 g males.***

(8) ***Con A-induced thymidine uptake in lymphocytes was increased several fold in cells from spleens of centrifuged animals.***

Significance of the Accomplishments

Findings #1 and #2: These gender-related differences indicate that females adapt more readily to gravitational changes, and dictate that the data base on females (both rodents and humans) be increased.

Finding #3: Our mating method insures a steady supply of timed pregnancies for future studies of the effects of centrifugation on various growth factors involved in fetal development.

Finding #4: Other studies, including our own, have taken one or the other of these measurements. By having both measurements available, a complete analysis of scaling effects can be carried out.

Finding #5: While the smaller size of centrifuged bones may be in part due to nutritional factors, the different bone shapes resulted from increased mechanical forces *in utero*, a direct gravitational effect.

Finding #6: This finding indicates that reduction in tibial length is greater than that predicted from the fetal size and is probably a scaling effect related to excess gravity.

Finding #7: This serendipitous finding indicates that some event regulating fetal skeletal growth occurs during early reproductive stages (spermatogenesis, fertilization, and/or early cleavage stages) and that this regulatory event can be altered by alterations in g-force.

Finding #8: This finding is highly significant in view of the decrease in con A reactivity seen in microgravity and supports A. Cogoli's *in vitro* centrifugation work.

Publications

Duke, J., Moore, J., and Montufar-Solis, D. The "Cells" Experiment: Tests of Prototype Hardware (Abstract). *ASGSB Bulletin* 1: 39, 1988.

Monufar-Solis, D. and Duke, J. Effect of Simulated Spaceflight on Tibial Epiphyseal Plates (Abstract). *ASGSB Bulletin* 1: 34, 1988.

Moore, J. and Duke, J. Effect of Chronic Centrifugation on Mouse Breeding Pairs and Their Offspring. *Physiologist* 31 (Suppl.): S120-S123, 1988.

Moore, J., Duke, J., Leifeste, W.S., and Leifeste, N.P. Cephalometric Evidence of Skull Changes in Excess Gravity (Abstract). *ASGSB Bulletin* 1: 35, 1988.

Moore, J., Leifeste, W.S., Leifeste, N.P., and Duke, J. Effects of Hypergravity on Craniofacial Morphology in Mice. In: *Space Life Sciences Symposium: Three Decades of Life Science Research in Space,* Washington, D.C., June 21-26, pp. 50-52, 1987.

OTOCONIA CALCIFICATION PROCESS: A CHICK EMBRYO MODEL

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Description of Research

The objectives of this project are: (1) to establish the genesis of statoconial units (gravity-sensing structures), (2) to analyze the subcellular arrangements of the subunits making up statoconia units, and (3) to correlate biochemical properties of statoconia constituents with their ultrastructural appearance.

The development modifications that occur to the statoconial membrane and statoconia during embryonic stages need elucidation if one is to understand the properties of this gravity-sensing structure of the inner ear. In this laboratory, standard transmission electron microscopy has been complemented by immunocytochemical and histochemical techniques and biochemical assays to examine some of the properties of the statoconial membrane during development and its relationship to the underlying supporting epithelia, which is made up of sensory hair cells, nerve terminals, and supporting cells.

Accomplishments

Results obtained thus far have allowed the investigator to conclude that the ***organic matrix of the statoconial membrane of chick embryos reacted with monoclonal antibodies known to be present in cell coats.*** The integral components of most cell coats have been well characterized and thus provide a good system for comparison. Most cell coats contain glycoproteins and glycosaminoglycans; both families of macromolecules are also found in different proportions in bone, teeth, and the statoconial membrane, in which an organic template is gradually mineralized.

In embryonic chicks, the statoconial membrane reacts with stains known in rodents to precipitate glycoproteins and glycosaminoglycans (Figures 1-5). This may indicate that macromolecules in the statoconial membrane include glycoproteins and glycosaminoglycans. Monoclonal antibodies to proteins of known molecular weights and properties and usually seen in cell coats were used. They included the glycoproteins fibronectin and laminin and the glycosaminoglycan keratan sulfate. Fibronectin and laminin were demonstrated in the statoconial membrane and to a lesser degree in statoconia units of hatchlings. Keratan sulfate yielded a very strong immunoprecipitate. ***When all three antibodies were reacted in the same age embryo, keratan sulfate gave an approximately 5 times stronger reaction than the glycoproteins used, probably because the statoconial membrane and statoconia units contained large quantities of glycosaminoglycans.*** Furthermore, the immunoprecipitate resulting from keratan sulfate reaction changed during development. ***At 7 days of incubation (stage 31) the reaction was scanty and its appearance was slightly grainy (Figure 1). At 11 days (stage 37), the appearance of the reaction was smoother and denser (Figure 2). Finally, at hatching (stage 46) the precipitate was the strongest and very smooth throughout the statoconial membrane (Figure 3).***

REACTIONS AND BIOCHEMICAL PROPERTIES OF CHICK STATOCONIAL MEMBRANE

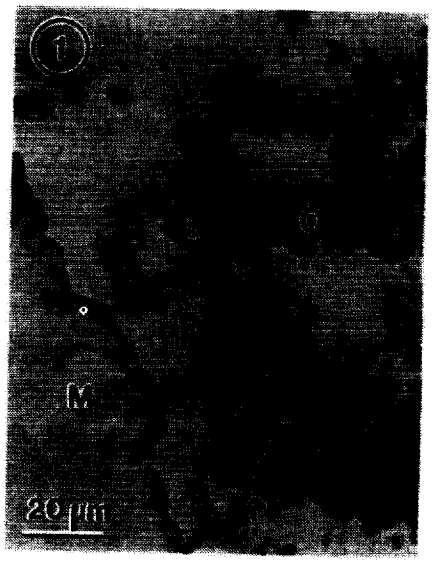

Figure 1. Keratan sulfate immuno-like reaction (a glycosaminoglycan) at 7 days (stage 31), seen to be abundant in statoconia (O), but not over the macula (M). The reaction product is not very intense and is somewhat granular, probably because at this stage the keratan sulfate macromolecules secreted into the statoconial membrane are scarce.

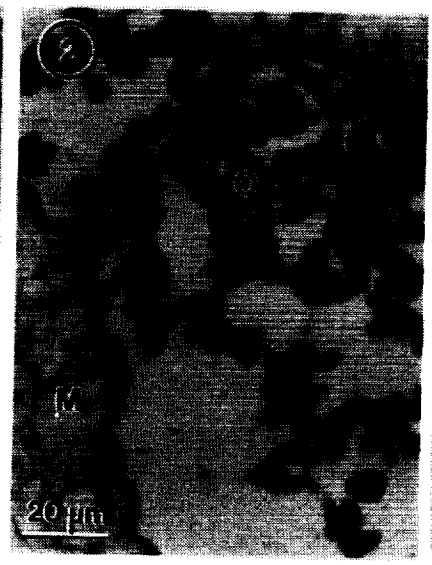

Figure 2. Area similar to that shown in Figure 1, but at 11 days (stage 37). Note increased density of reaction, which indicates that more keratan sulfate is made a week later. In addition, the smoother appearance of the precipitate may indicate that more macromolecules are being combined as the statoconial membrane grows.

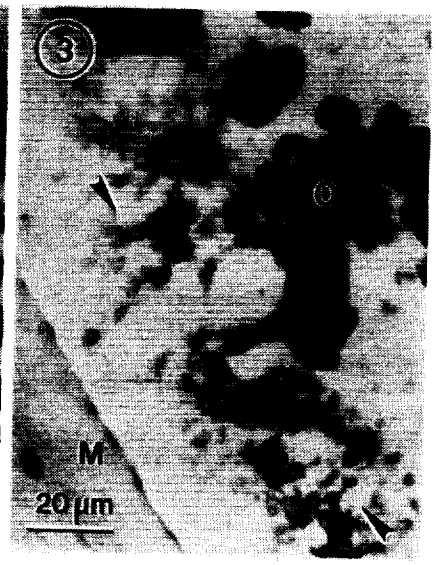

Figure 3. Similar to Fig. 2, but at hatching (stage 46). Note that the precipitate is now smoother and denser, probably because keratan sulfate becomes orderly arranged between other protein macromolecules, and because it is maximally precipitated at this age during chick embryonic development. The changing intensity and appearance of the reaction products yielded by the glycosaminoglycans and glycoproteins studied under identical experimental conditions may indicate that indeed glycosaminoglycans are deposited at a faster rate than glycoproteins, but this requires further study.

Figure 4. Control section for keratan sulfate antibody used is seen with phase contrast light microscopy (standard bright field microscopy failed to show the unstained tissues). This control and others confirmed that under the present experimental conditions, glycosaminoglycans form the main bulk of the chick statoconial membrane.

Figure 5. Keratan sulfate immunofluorescence over saccular statoconia (5) showing the specificity of reaction product obtained. Note that the epithelia and cartilage (below arrowheads) did not have fluorescence, indicating that glycosaminoglycans are mainly confined to the statoconial membrane area.

Significance of the Accomplishments

The composition of the chick's statoconial membrane may be partially related to that of rodents. Because of this and because chick development is not dependent on its mother, the chick embryo remains a very good model in which to study gravity-related issues.

Glycoproteins and glycosaminoglycans, both shown in the past to be part of the adult rodent statoconial membrane and found in cell coats, are also integral parts of the chick's statoconial membrane.

It is possible that the differential distribution of glycoproteins and glycosaminoglycans at different stages of development and in different inner ear structures may determine their fate as far as the mineralized vs. nonmineralized state.

The immunohistochemical precipitate obtained with monoclonal antibodies to proteins of known molecular weight and properties confirmed previous results that emphasized that certain histochemical stains hinted of the presence of the above-mentioned substances.

Publications

Fermin, C.D., Crawford, J., and Igarashi, M. ^{3}H-Thymidine Study of the Chick Embryo Inner Ear (Abstract). *Midwinter Meeting Association Research of Otolaryngology* 11: 123, 1987.

Fermin, C.D., Dunner, K., Igarashi, M., and Lovett, A.E. Cytodifferentiation of the Chick Vestibular Epithelia and Histochemistry of the Otolithic Membrane (Abstract). *Texas Society of Electron Microscopy Journal* 18: 28, 1987.

Fermin, C.D., Enge, A., Igarashi, M., and Crawford, J. Terminal Mitosis in the Sensory Epithelia of the Chick Inner Ear (Abstract). *Texas Society of Electron Microscopy Journal* 18: 27, 1987.

Fermin, C.D. and Igarashi, M. Morphometry and Ultrastructure of the Squirrel Monkey *(Saimiri sciureus)* Vestibular Nerve. *Acta Anatomica* 129: 188-199, 1987.

Fermin, C.D., Igarashi, M., and Yoshihara, T. Ultrastructural Changes of Statoconia after Segmentation of the Otolithic Membrane. *Hearing Research* 28: 23-34, 1987.

Fermin, C.D., Lovett, A.E., Igarashi, M., and Dunner, K. Immunocytochemical and Biochemical Properties of the Otolithic Membrane (Abstract). *ASGSB Bulletin* 1: 15, 1988.

Fermin, C.D., Lovett, A.E., Igarashi, M., and Dunner, K. Monoclonal Antibodies and the Otolithic Membrane of the Chick Embryo (Abstract). *Texas Society of Electron Microscopy Journal* 18: 27, 1987.

Yoshihara, T., Fermin, C., and Igarashi, M. Localization of Na^{+}-K^{+}-ATPase in the Tegmentum Vasculosum of the Chick. *Archives of Otorhinolaryngology* 243: 401-402, 1987.

HOMEOSTASIS IN PRIMATES IN HYPERACCELERATION FIELDS

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Description of Research

The ultimate goal of this research program is to understand the role of gravity in influencing the physiology of living organisms. Of particular interest are the physiological mechanisms leading to adaptation of an organism to an altered gravitational environment. Included in these responses are identification of receptors, pathways of information transfer (neural and endocrine), mechanisms of integration of information, and pathways and mechanisms affecting the organism's response to alterations in gravity.

The adaptation of homeostatic systems to changes in gravitational loading are poorly understood. Such changes in centrifuged animals include depressed body temperature, alterations in the circadian timekeeping system, and changes in the level of arousal. To date, research interests in this laboratory have focused on the sensitivity of these and other homeostatic systems to alterations in gravity. This research has demonstrated the gravitational responsiveness of these systems and elucidated the underlying mechanisms of the responses. Further, this program has focused on the responses of the whole organism to further understand the interaction between the various physiological systems of interest. This research has required the ability to alter the dynamic environment of the organism. At the Chronic Acceleration Research Unit at the University of California at Davis, four centrifuges (8 to 18 ft in diameter) are available. These facilities provide for acute and chronic exposures of animals to gravitational fields ranging from 1 to 20 g.

The research accomplished in the past year has examined the responses of rodents to such altered fields (2 g) for up to 40 days exposure duration. Additionally, control studies at 1 g have begun to examine the neural control mechanisms integrating the circadian timekeeping system with those of body temperature utilizing the head-down (antiorthostatic) rat unloading model.

Accomplishments

(1) The presence of a ***hyperdynamic environment depresses rodent body temperature for an extended period*** (more than 20 days).

(2) ***Exposure to prolonged (60 days) 2 g field leads to a reduction on the average amplitude of the circadian temperature*** rhythm, which persists for up to 20 days prior to the animal's recovery to baseline.

(3) Animals in constant light at 2 g do not recover their temperature rhythms as fast as animals in a light-dark cycle (18 vs. 10 days).

(4) Preliminary data in unloaded rats (antiorthostatic position) ***suggest a depression in body temperature and a change in the normal entrainment by the 24-hr light-dark cycle.***

Significance of the Accomplishments

In general, Findings #1-3 demonstrate the response of mammals to increased levels of gravitational loading. The response underscores the significant delay (i.e., days) in the adaptation of these organisms to changes in the dynamic environment. For example, Finding #1 demonstrates that body temperature is depressed for an extended period of time as a result of exposure to hyperdynamic environment. Similarly, exposure to hyperdynamic fields also leads to multiple-day recovery periods for the temperature rhythms of these animals. Finding #3 also demonstrates an apparent change of the utilization of photic information by animals. The homeostatic regulation of body temperature was depressed for 6 days on average, compared to the depression of the circadian regulation of body temperature which lasted 2-3 times as long. This suggests that the effects of gravity on temperature regulation are multiple and distinct.

That rodent body temperature rhythms may not synchronize to the 24-hr light/dark cycle is a novel finding (Finding #4). Historically, such observations have only been made in primates and rodents exposed to the microgravity environment. That such a change in photic response only occurs in the temperature rhythm further underscores our understanding of the circadian timekeeping system and photic entrainment.

Publications

Ferraro, J.S., Fuller, C.A., Sulzman, F.M., and Krum, H.N. The Effects of Hypergravity on the Circadian Rhythm of Conidiation in *Neurospora crassa* (Abstract). *ASGSB Bulletin* 1: 37, 1988.

Fuller, C.A. Applications for Artificial Gravity in Biology. In: *Space Life Sciences Symposium: Three Decades of Life Science Research in Space,* Washington, D.C., June 21-26, p. 240-241, 1987.

Fuller, C.A. and Murakami, D.M. Gravity and the Circadian Timekeeping System (Abstract). *ASGSB Bulletin* 1: 38, 1988.

NEURAL MECHANISMS BY WHICH GRAVITATIONAL STIMULI AND STRESS AFFECT THE SECRETION OF RENIN AND OTHER HORMONES

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Description of Research

The long-term goal of this research is delineation of the neural pathways and transmitters that mediate changes in the secretion of renin and other hormones concerned with regulation of salt and water balance in response to gravitational and other stimuli. Evidence from this laboratory indicates that stimulation of certain serotonergic neurons in the dorsal raphe nucleus of the midbrain increases renin secretion, and that these neurons project to the mediobasal hypothalamus. One goal has been determination of specific parts of the hypothalamus that affect renin secretion and the importance of this region in the physiological control of renin secretion. Another has been determination of the pathway from the nervous system to the renin-secreting cells in the kidney. To study the role of the hypothalamus and other parts of the brain in the regulation of renin secretion, we have used stimuli which increase renin secretion in diverse ways. Emphasis has been placed on the postural stress of 45° head-up tilt. Other standard tests include: (1) administration of the serotonin-releasing drug p-chloroamphetamine (PCA); (2) the psychological stress of immobilization; (3) the volume depletion stress of a low sodium diet; and (4) the acute volume stress of nonhypotensive hemorrhage.

Accomplishments

(1) We demonstrated that, like the renin response to PCA, the renin responses to immobilization and head-up tilt are blocked by the ß adrenergic blocking drug propranolol. This is good evidence that in all three situations the ***final common pathway from the spinal cord to the renin-secreting cells in the kidney is sympathetic.***

(2) In another set of experiments, head-up tilt was found to produce a reproducible increase in plasma vasopressin concentration. This response was only slightly reduced by paraventricular lesions, indicating that postural information also reaches the supraoptic nuclei. Blood pressure and heart rate responses to tilting were unaffected. Vagotomy had little effect on the vasopressin response to tilting, but it was abolished by sinoaortic denervation. Experiments with vasopressin and renin antagonists suggested that the ***renin-angiotensin system plays a bigger role in maintaining blood pressure during head-up tilt than vasopressin.***

(3) As noted before, we have discovered that lesions of the paraventricular nuclei of the hypothalamus depress circulating angiotensinogen, the substrate on which renin acts to produce the vasoconstrictor peptide angiotensin II. Since the paraventricular nuclei play an important role in the regulation of anterior pituitary secretion, we have begun a detailed study comparing the effects of hypophysectomy and paraventricular lesions, measuring pituitary hormones as well as the components of the renin-angiotensin system. The decrease in plasma angiotensinogen produced by paraventricular lesions appeared to correlate well with a decline in circulating thyroid hormones, due in all probability to decreased secretion of thyroid hormone. Hypothyroidism is known to cause decreased

secretion of angiotensinogen. In preliminary experiments, however, lesions just anterior to the paraventricular nucleus also reduced circulating angiotensinogen, and these lesions did not produce a change in circulating thyroid hormones. This suggests that ***there may be direct neural as well as neuroendocrine control of angiotensinogen secretion.***

(4) Lesions of the ventromedial nuclei of the hypothalamus, like lesions of the paraventricular nuclei, were found to reduce the renin responses to PCA, immobilization, head-up tilt, and a low sodium diet. However, these lesions did not reduce circulating angiotensinogen.

(5) Exploring the possibility that ***vasopressin-secreting nerve fibers from the hypothalamus to the lower brain stem are involved in mediating brain-controlled renin responses,*** we confirmed the observation of others that renin responses were supernormal in Brattleboro rats. These rats have a genetic defect which makes it impossible to secrete vasopressin in the posterior pituitary or in the peptidergic pathways to medulla and spinal cord. Since vasopressin does not cross the blood-brain barrier, the supernormal responses could be due to vasopressin deficiency in the brain or vasopressin deficiency in the circulation. We infused vasopressin peripherally in Brattleboro rats and showed that the renin responses stayed supernormal. This is good evidence that the defect causing excessive secretion of renin is central rather than peripheral.

Significance of the Accomplishments

The experiments described above and experiments conducted in previous years have done much to map out the pathways and transmitters involved in brain regulation of renin secretion. This has appreciable significance for NASA because both postural changes and stressful stimuli affect renin secretion and renin plays a vital role in salt and water balance and the maintenance of blood pressure. In addition, our demonstration that paraventricular lesions lower circulating angiotensinogen is important because it demonstrates for the first time that there is a neuroendocrine and possibly an additional neural control of the circulating level of this important component of the renin angiotensinogen system. The discovery that the ventromedial nuclei affect renin secretion without affecting the concentration of angiotensinogen indicates that an additional hypothalamic mechanism has an important role in regulating salt and water homeostasis and maintaining blood pressure.

Publications

Golin, R., Keil, L., and Ganong, W.F. Effect of Head-up Tilt on Plasma Vasopressin, Heart Rate, and Blood Pressure in Anesthetized Rats (Abstract). *Society for Neuroscience Abstracts* 13: 1368, 1987.

Gotoh, E., Golin, R.M.A., and Ganong, W.F. Relation of the Ventromedial Nuclei of the Hypothalamus to the Regulation of Renin Secretion. *Neuroendocrinology* 47: 518-522, 1988.

Gotoh, E., Golin, R.M.A., and Ganong, W.F. Role of the Dorsal Raphe Nucleus and the Hypothalamic Ventromedial Nuclei in the Regulation of Renin Secretion (Abstract). *ASGSB Bulletin* 1: 24, 1988.

Gotoh, E., Murakami, K., Bahnson, T.D., and Ganong, W.F. Role of Brain Serotonergic Pathways and the Hypothalamus in the Regulation of Renin Secretion. *American Journal of Physiology* 253: R179-R185, 1987.

EFFECTS OF MICROGRAVITY ON SYNAPTOGENESIS IN CELL CULTURE

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Description of Research

To support life in space, embryonic development must occur normally under microgravity. Gravity is the only evolutionarily constant variable under which all terrestrial life has evolved. It is therefore essential to know (a) if biosystems require gravity for normal development; (b) how the microgravity affect environment of space will development; and (c) if deleterious, how microgravity can be altered (gravitational substitution) to permit normal development in space.

Our objective is to determine whether vertebrate cell development is altered, first, under simulated microgravity (clinorotation), with specific emphasis on neuronal communications representing the development of the nervous system, and eventually by subjecting a well-defined developing synapse system to actual microgravity during spaceflight.

Accomplishments

We completed an extensive morphologic study (Figures A-F) which shows that under clinorotation of 1-100 rpm, embryonic nerve and muscle cells developing in culture exhibit reproducible changes resulting from the averaging of the gravitational vector (simulated microgravity). The specific changes we found are reversed at high speeds (due to centrifugal forces which neutralize the simulated microgravitational field), and they are not produced due to vibrations or rotation alone (motional controls revolving around the vector of gravity are unaffected).

The most profound changes, which appear as early as 12 hr after rotation, are: abnormal increases in muscle cells (Figure C) and in nuclear and nucleolar size (Figure D), formation of large and frequent aneurysms along the shafts of neuronal extensions (Figures E&F), and the specific inhibition of accumulation of acetylcholine receptors to the synaptic junction between nerve and muscle cells. These findings, repeated for verification of statistical significance over 1,000 times per measurement, strongly suggest that ***simulated microgravity exerts profound changes on the ability of embryonic cells to mature and to communicate with each other.***

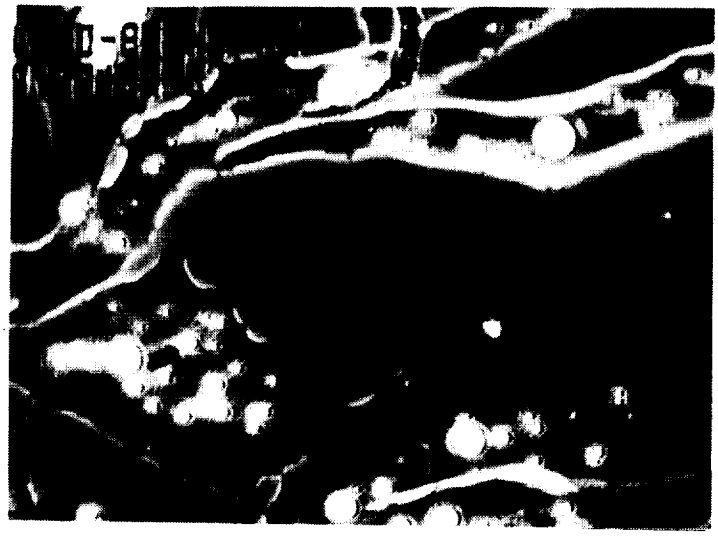
If this simulation is a faithful reproduction of space microgravity, it implies that ***embryonic nervous system development will be substantially altered during spaceflight.*** Just as interestingly, our studies suggest that gravity on Earth has been an important environmental factor under whose influence all cells have evolved. Furthermore, it suggests that cells have "gravisensing" mechanisms (possibly stretch-activated membrane channels or intracellular organelles like the centrosome) which may be responsible for sensing gravity during development. We ***hypothesize that the cytoskeleton is the cellular structure that transduces gravitational changes resulting in our findings.***

Publications

Gruener, R. and Hoeger, G. Does Vector-free Gravity Simulate Microgravity? Functional amd Morphologic Attributes of Clinorotated Nerve and Muscle Grown in Cell Culture. *Physiologist* 31 (Suppl.): S48-S49, 1988.

Gruener, R. and Hoeger, G. Simulated Microgravity Interferes With Synaptogenesis (Abstract). *ASGSB Bulletin* 1: 41-42, 1988.

MORPHOLOGIC ABNORMALITIES OF NERVE AND MUSCLE CELLS INDUCED BY CLINOROTATION

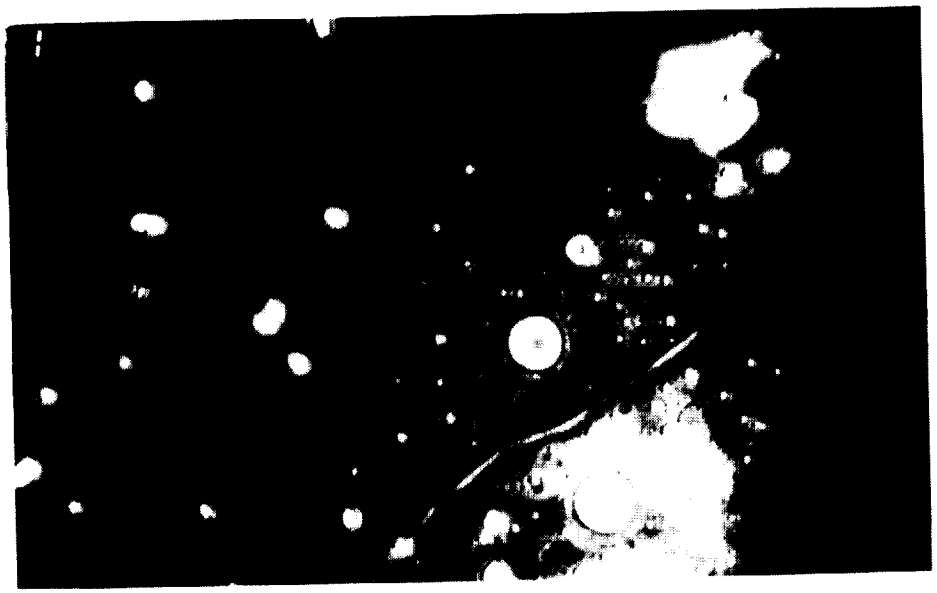


A. CONTROL: Innervated Myocyte
Note distinct striations and the straight neuritic shafts.

B. CONTROL: Neurites
Note thin neurites with smooth shafts and triangular adhesion plaques.

MORPHOLOGIC ABNORMALITIES OF NERVE AND MUSCLE CELLS INDUCED BY CLINOROTATION

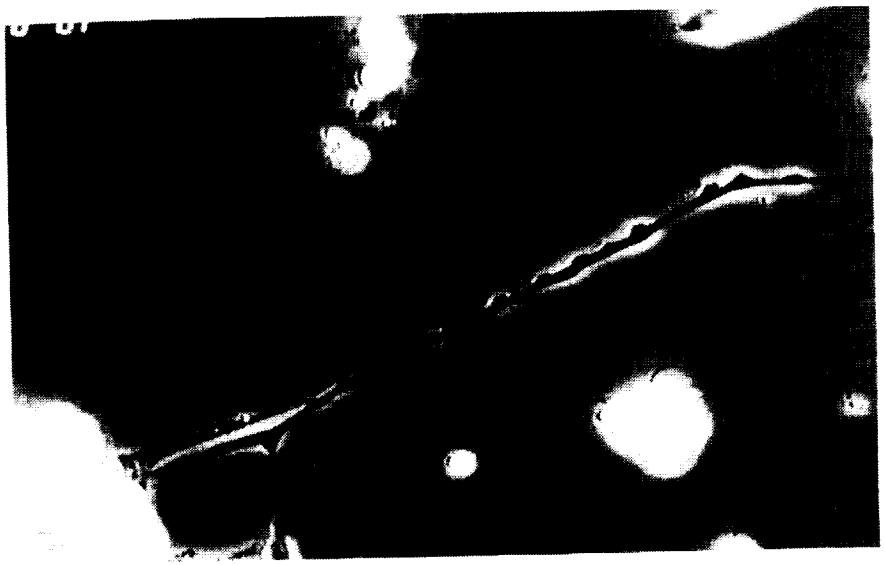
C. 1 RPM: Myocyte
Muscle cell rotated for 48 hr; note large cell size and lack of striations at arrows.



D. 5 RPM: Myocytes
Cells rotated for 24 hr; note very large nucleus and nucleoli and enlarged cell. Compare with Figures A & C.

MORPHOLOGIC ABNORMALITIES OF NERVE AND MUSCLE CELLS INDUCED BY CLINOROTATION

E. 5 RPM: Neurites
Note extensive aneurysms at arrows and thickened neuritic diameter. Compare with Figure B.



F. 10 RPM: Neurites
Culture rotated for 24 hr; note enlarged aneurysms at arrows along neuritic shafts.

THE EFFECTS OF GRAVITATIONAL FIELDS ON NEURAL SIGNALING IN THE HIPPOCAMPUS

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Description of Research

The long-range goal of this research is the elucidation of the effects of altered gravitational fields on neuromodulatory mechanisms. We have initiated a series of experiments on a region of the central nervous system recently shown to be modified by both hypogravic and hypergravic fields. Rats flown on Spacelab-3 showed an increased number of receptors for the neuromodulator serotonin after 7 days in space. This striking result associates a neuromodulator with a cellular change in neurons in specific structure in the central nervous system, a change evoked by a microgravity environment. Moreover, rats exposed to a hypergravic field of 2 g for 7 days showed decreased receptors for serotonin. Thus, as the gravitational field increased from microgravity levels to Earth gravity (1 g) and then to 2 g levels, the number of serotonergic receptors decreased. Serotonin is a well-studied neuromodulator involved in many neural systems, including sleep and temperature regulation. The hippocampus has been associated with memory and learning. Our experiments on hippocampal serotonergic mechanisms are designed to further characterize the effects of altered gravity on neural networks. The experiments are designed to build on the structural/biochemical data presently available by measuring electrical signaling in the hippocampus.

More specifically, this past year we have continued development of a neural model of hippocampal neurons and related experiments on the rat hippocampus under 1 g conditions. A neural model was augmented to simulate further properties of pyramidal cell activity in the hippocampus. In experimental studies on rats, the electrical activity of the same population of neurons has been further characterized. These studies at 1 g (on the effects of temperature, stimulation parameters, acid-base state) provide baseline data for physiological experiments on rats in altered gravitational fields.

Accomplishments

(1) A model to predict the electrical activity of a hippocampal pyramidal cell (including the effects of temperature) was developed. Four populations of membrane channels in the pyramidal cell were simulated. The model includes fast Na^+ and K^+ channels (voltage- dependent channels similar in basic respects to channels for action potentials over axons), a Ca^{2+} channel, and a calcium-activated K^+ channel. ***Model simulations correspond to experimental data over a range of temperature from 40°C to 35°C.***

(2) Neural activity was recorded in an *in vitro* hamster hippocampal preparation while the temperature of the Ringer's solution bathing the slice was controlled at selected levels. The amplitude and duration of the population spike (action potentials from a group of pyramidal cells) was measured as pH of the bathing solution was changed from 7.5 to 7.1 and as temperature was lowered from 35°C to temperatures where a response could not be evoked. ***Plots of population spike amplitude vs. temperature have bell-shaped curves. The population spikes increased in amplitude as***

temperature was lowered from 35°C, reached a peak between 25 and 20° C, and then decreased until a response could not be evoked.

(3) Poststimulus time (PST) histograms of rat hippocampal cells were recorded *in vivo* following single-shock stimulation of the fornix. ***The PST histograms in rats displayed a series of peaks of decreasing amplitude, similar to damped oscillatory responses previously recorded in cats and rabbits.*** The effect of increased neural background activity was investigated by recording histograms with concurrent pulse train stimulation of the contralateral hippocampus. The histograms showed a decreased latency to the onset of the second peak. Damped oscillatory activity seen in this *in vivo* rat preparation could not be elicited in the *in vitro* rat slice preparations, indicating that the level of background activity is one factor contributing to the genesis of multiple peaks in histograms in the *in vivo* preparation.

Significance of the Accomplishments

Finding #1: Demonstated that a computer model can simulate many of the electrical properties of pyramidal cell neurons. For example, the pyramidal cell typically fires either a single action potential or a burst of action potentials, and these responses can be simulated by varying stimulus intensity.

Finding #2: Characterized acid-base and thermal properties of the *in vitro* slice preparation. The advantage of the slice preparation over an *in vivo* preparation is that electrodes can be inserted relatively easily into cells in the slice to measure transmembrane potentials and thus monitor the effect of serotonin. By varying pH, HCO_3^-, and temperature, the response of the slice was determined for a range of variables, allowing the choice of optimal recording conditions.

Finding #3: Complemented other studies showing that in major respects responses recorded in the *in vitro* slice preparation are similar to those observed in the *in vivo* preparation. One difference between the preparations can be related to the level of neural background activity — signals arriving from other neural structures in the brain. For our studies, the slice has the advantage that fluctuations in signals from outside the hippocampus are absent. In addition, changes evoked by serotonin must be due to its effects on the hippocampus and not some other structure.

Publications

Giacchino, J.L., Thomas, M.P., and Horowitz, J.M. Repetitive Excitation of Bursts of Action Potentials in the Rat Hippocampus Following Single Shock Stimulation. *Comparative Biochemistry and Physiology* 89A: 37-44, 1988.

Horowitz, J.M. and Thomas, M.P. Serotonergic Mechanisms and the Hippocampal Slice Preparation (Abstract). *ASGSB Bulletin* 1: 24-25, 1988.

Horowitz, J.M., Thomas, M.P., and Eckerman, P. Thermal Dependence of Neural Activity in the Hamster Hippocampal Slice Preparation. *Journal of Thermal Biology* 12: 97-101, 1987.

Lindley, M.A., Thomas, M.P., and Horowitz, J.M. A Simulation of the Effects of Temperature on Hippocampal Neurons. *Computer Methods and Programs in Biomedicine* 25: 3-12, 1987.

MECHANISM OF CONTROL OF BONE GROWTH BY PROSTAGLANDINS

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Description of Research

The long-range goal of this research program is to understand the effects of physiological changes seen in spaceflight on skeletal development and bone metabolism. Biomedical studies of manned spaceflight have shown a loss of weight-bearing bone. The physiological regulation of bone growth and remodeling in space as well as on Earth is not understood. Growth and remodeling are influenced by hormones such as calcitonin, parathyroid hormone, vitamin D, and glucocorticoids. Cortisol, a glucocorticoid, has been shown to be increased in spaceflight.

It is possible that there are other locally produced growth factors that may play a role in bone growth. Infants treated with prostaglandin have a remarkable increase in new bone formation. Prostaglandins are produced by bone cells. Recently, Webb Jee demonstrated that PGE_2 stimulates trabecular bone formation *in vivo* by 120% over a 21-day period. Prostaglandins are known to stimulate collagen and noncollagen protein synthesis and DNA synthesis.

We recently acquired a new osteoblastic cell line, MC3T3. This line produces prostaglandin E_2 in culture, responds to exogenous prostaglandin E_2 stimulation, and will mineralize after the cells reach confluency. To evaluate the roles of glucocorticoids and prostaglandins on bone growth, we have synchronized the bone cells and have studied the effects of glucocorticoids on cell cycle and endogenous prostaglandin production. Cell cycle was accessed by ^3H thymidine incorporation into DNA and flow cytometry. Prostaglandin E_2 was measured by RIA. In addition to growth studies, we have mineralized the culture and have begun our evaluation of mineralization by histological stain of calcium, ^{45}Ca incorporation, visualization of the collagen matrix in the mineralizing bone, and analysis of protein synthesis by gel electrophoresis.

Accomplishments

(1) Demonstrated that ***dexamethasone slows the entry of the cell into S-phase DNA synthesis which causes an overall 30% decrease in cell growth.*** Dexamethasone caused a 50% decrease in prostaglandin production in synchronized cells as they enter S-phase.

(2) Synthesis of PGE_2 is associated with the interphase stage of the cell cycle. Prostaglandin E_2 levels increase during late G_1 and then again in G_2, causing an overall increase in both intracellular and extracellular prostaglandin concentrations.

(3) Found that dexamethasone decreased endogenous prostaglandin levels by 50% in nonsynchronous cells. DNA synthesis was decreased by 18% accompanied by 30% decrease in ^{14}C proline incorporation into protein synthesis in these cells. Total protein synthesis was only decreased by 10% by dexamethasone.

(4) ***In both synchronous and nonsynchronous cells, dexamethasone caused a decrease of ^{14}C proline incorporation into protein.*** This decrease of protein synthesis was more pronounced than the incorporation of ^{3}H lysine, ***suggesting a preferential effect on collagen synthesis.***

(5) Successfully established a mineralizing culture of osteoblast and developed the method of visualizing osteocalcin and the collagen matrix in these cultures.

Significance of the Accomplishments

Findings #1 and #2: Dexamethasone is known to slow the rate of growth of cells in culture. From these studies, it is possible that entry to S-phase is the location of the dexamethasone action. In addition, the fall of prostaglandin synthesis caused by the dexamethasone is most affected in late G_1 at the entry of S-phase, suggesting a possible causal relationship between prostaglandin E_2 and cell growth.

Findings #3 and #4: The dexamethasone-caused 50% decrease in prostaglandin synthesis in nonsynchronous cells was accompanied by an 18% decrease in thymidine incorporation and a 30% decrease in ^{14}C proline incorporation into proteins, while total protein synthesis was only inhibited by 10%. Further studies showed that the decrease in ^{14}C proline incorporation was approximately twice that of ^{3}H lysine, suggesting specific effects of dexamethasone on growth and on collagen synthesis.

Finding #5: Our ability to grow bone cells and mineralize them in culture provides model systems that we can use to further test the effect of dexamethasone and prostaglandins on the cell cycle and on mineralization. We plan to show the specific effects of dexamethasone on growth and mineralization of bone using microscopy, gel electrophoresis, and radioisotope tracers.

Publications

Hughes-Fulford, M. Cell Cycle Arrest Is Associated With Induction of Synthesis of Three 79k Proteins (Abstract). *FASEB Journal* 2: A1689, 1988.

Hughes-Fulford, M. Mechanisms of Action of Prostaglandin Inhibition of the Cell Cycle. In: *Proceedings of the International Conference on Prostaglandins and Cancer* (ed. by M. Santoro, R. Paoletti, and E. Garaci). Berlin/Heidelberg: Springer-Verlag, pp. 115-128, 1987.

Meehan, R., Duncan, U., Neale, L., Alexander, M., and Hughes-Fulford, M. Effect of Prostaglandin A_1 on Human Mononuclear Cells *In vitro* Activation (Abstract). *Federation Proceedings* 46: 453, 1987.

MICROGRAVITY-INDUCED EFFECTS ON PITUITARY GROWTH HORMONE CELL FUNCTION

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Description of Research

One of the major hormones made and released from the anterior pituitary gland is growth hormone (GH). This hormone is probably misnamed because it does many things in addition to being required for long bone growth. For example, it regulates metabolism of fat and carbohydrate and also controls proper functioning of the immune system. There are also indications that it participates in the complicated processes involved in wound healing, kidney cell function, and muscle metabolism. Since many of these GH target systems are affected by spaceflight, it is important to determine the effects of spaceflight on GH release from the pituitary.

Our laboratory has participated in three spaceflight experiments that address the issue of how microgravity affects GH release. In two experiments (SL-3; Cosmos 1887), pituitary GH cells were prepared from rats flown 7-14 days. In another experiment (STS-8), dispersed pituitary cells were flown in a middeck locker at 37°C in closed tubes containing culture medium. On return to Earth, the GH-secreting capacity of the flight cells was compared with that from ground-based controls. The results from each of these experiments has shown that:

- *In vitro* release of GH is reduced by ~ 50% after flight.
- *In vivo* release of GH is also attenuated by the same amount (transplantation approach).
- The biological, but not necessarily the immunological, activity of the hormone is most affected by flight.
- Flight may preferentially affect release of high molecular weight forms of the hormone.
- The flight effect may be fairly specific since prolactin, another pituitary hormone, is not affected.

The issue of whether or not exposure of cells directly to microgravity can affect cell function is one of current debate. Our STS-8 result, while preliminary, nevertheless offers important positive evidence for this concept. Indeed, it becomes stronger when coupled with the data obtained from flight rats. There is an urgent need to "refly" pituitary GH cells under conditions where replication of cell culture vials will substantiate the preliminary results of STS-8. In addition, experimental approaches to probe the nature of the cellular secretory defects are required. The goals of our flight experiment are therefore: (a) to establish and validate a closed vial cell culture system suitable for flight, and (b) to develop

morphologic and functional tests that probe the mechanism(s) underlying the secretory defect.

Accomplishments

(1) ***A closed vial cell culture system (holding 165 vials) has been developed and found to be suitable for maintenance of primary rat pituitary cells for 9 days at 37°C.***

(2) ***The secretory output of GH from cells in the vial compares favorably with that from cells cultured under usual laboratory conditions.***

(3) Cells attach to the glass surface within 24 hr of seeding and remain attached when exposed to vibrations equivalent to a 1981 Shuttle launch profile.

(4) Numerous experimental results establish the optimal numbers of cells/vial; the effects of different serum types on GH secretion; the performance of GH cell subpopulations in the vials; the long-term responses of GH synthesis on hydrocortisone addition; and, finally, postflight responsiveness of the cells to the hypothalamic peptide GRF (growth hormone releasing factor).

(5) A unique single cell secretion assay has been developed (Figure 1).

Figure 1. Dark brown growth hormone (GH) cells on the Immobilon membrane showing the zones of GH secretion surrounding the cells. Note the various amounts of GH secreted by the different cells.

Significance of the Accomplishments

The fact that pituitary GH cells can be kept alive for 9 days in the closed vial system and remain functional (i.e., release GH) as well as they do obviously means that we are in a position to determine if GH cell function is modified directly by spaceflight. Moreover, our experimental design should allow us to make statements regarding the nature of the suspected GH secretory lesion (cell membrane receptor defect; microtubule organization; steroid receptor involvement). Postflight testing of the cells is still required to optimize responses.

Publications

Farrington, M. and Hymer, W.C. Characterization of High Molecular Weight Aggregates of Rat GH (Abstract). In: *The Endocrine Society: 70th Annual Meeting Abstracts,* p. 80, 1988.

Howland, D.S., Farrington, M.A., Taylor, W.D., and Hymer, W.C. Alternate Splicing Model for the Synthesis and Secretion of the 20 Kilodalton Form of Rat Growth Hormone. *Biochemical and Biophysical Research Communications* 147: 2, 1987.

Kendall, M.E. and Hymer, W.C. Cell Blotting: A New Approach to Quantify Hormone Secretion from Individual Rat Pituitary Cells. *Endocrinology* 121: 2260-2262, 1987.

Morrison, D.R., Hymer, W.C., Todd, P., and Grindeland, R.E. Mammalian Cell Culture Methods in Microgravity (Abstract). *ASGSB Bulletin* 1: 41, 1988.

Motter, K., Vasques, M., Hayes, C., Kendall, M., Tietjen, G., Hymer, W.C., and Grindeland, R. Effects of Microgravity on Rat Pituitary Growth Hormone (GH) Cell Function: Comparison of Results of the SL-3 Mission with Rat "Unloading" on Earth (Abstract). In: *Space Life Sciences Symposium: Three Decades of Life Science Research in Space,* Washington, D.C., June 21-26, p. 52-53, 1987.

Todd, P., Hymer, W.C., Morrison, D.R., Goolsby, C.L., Hatfield, J. M., Kunze, M.E., and Motter, K. Cell Bioprocessing in Space: Applications of Analytical Cytology. *Physiologist* 31 (Suppl.): S52-S55, 1988.

MECHANOCHEMICAL TRANSDUCTION ACROSS THE CELL SURFACE

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Description of Research

The process of gravity sensation is based upon a mechanism in which mechanical signals are transduced into alterations of cell biochemistry. Our specific aim is to develop an electromagnet system that can be used in conjunction with magnetic microparticles to apply mechanical tension to the surfaces of cultured cells. In this manner, we hope to be able to begin to study the effects of physical forces on cellular metabolism. From a more practical standpoint, a compact direct-current electromagnet could also provide a new means for cell and molecular separations in space, as well as a novel method for providing mechanical orientation in a gravity-free environment. This system could eventually be used for studies in the Space Shuttle or within an orbiting Space Station.

Many previous studies have shown that cell metabolism and gene expression can be altered by perturbing cell shape. For example, anchorage-dependent cells must adhere and spread on culture substrata in order to grow. Our working hypothesis is that cell shape may convey regulatory information through modulation of physical forces distributions. This is based upon the observation that cell shape results from the action of tensile forces generated by cytoskeletal microfilaments and resisted by extracellular matrix (ECM) attachment points. Recently, cell surface receptors have been identified that span the membrane and physically interconnect ECM molecules with specific intracellular cytoskeletal elements. Thus, as spreading cells form increasing numbers of interconnections between cell surface receptors and ECM molecules, they experience an increase in the magnitude of force that is exerted on their cytoskeleton.

We propose that these mechanical forces may be transduced into biochemical information based upon changes of local thermodynamic parameters that alter cytoskeletal filament assembly; the function of many molecules involved in regulation of growth and metabolism depends upon the state of cytoskeletal integrity. In this manner, previously described "anchorage-dependence" and "shape-dependence" may actually represent "tension-dependence." We believe that this mechanochemical transduction system may also mediate gravity sensation in anchorage-dependent cells.

Our main goal is to devise a new methodology that can be used to test this hypothesis. We require a system in which mechanical tension can be applied directly to specific ECM receptors on the surfaces of cells that are free of attachment. We have set out to develop a controllable electromagnet system that can be used for these types of studies. This system requires use of magnetic microparticles (approximately 1-5 μm diameter) that are first coupled to purified ECM molecules and then allowed to bind cell surfaces in the presence or absence of a controlled magnetic field. Once the feasibility of this system is demonstrated, we will use this system to analyze the process by which mechanical forces can alter cell growth as well as other aspects of cell metabolism (e.g., growth, protein synthesis, gene expression).

Accomplishments

(1) ***Development of a controllable direct-current electromagnet:*** We have adapted a direct-current electromagnet (controllable from 0 to 100 amperes) that was previously used to target magnetically labeled drugs. When energized to the maximum, this cylindrical magnet (height = 30 cm; diameter = 25 cm) produces a ***central field of approximately 7500 gauss*** at 0.5 cm from its pole tip ***without any associated electric field.*** The controllable electromagnet ***maintains fields of constant intensity over areas large enough to include standard tissue culture plates.***

(2) ***Computerized field analysis:*** We have used computerized analysis to map out the precise distribution of both magnetic field strength and field gradient at various distances above the magnet pole tip.

(3) ***Fabrication of a tissue culture chamber:*** We have adapted a standard carbon dioxide, controlled-temperature chamber for use with the electromagnet system. The magnet required installation of high voltage power lines as well as a high flow water supply. Our major problem centered around the effects of the magnet's cold-water-cooling system on temperature control within the tissue culture chamber. We have recently circumvented this problem by using 37°C water maintained at high flow rates for magnet cooling.

(4) ***Characterization of magnetic microparticles:*** We have compared magnetic microparticles from a variety of suppliers. We now use magnetic microspheres (4.5 vs. 0.5 μm) that are perfectly spherical in form and extremely consistent in size. ***Application of a magnetic field results in magnetic microsphere alignment and formation of "chains" in a force-dependent fashion.*** The microsphere chains also ***spontaneously disassemble when the magnetic field is removed.***

Over the past year we have also been able to develop: (a) a system for quantitating the number of magnetic particles within unknown solutions utilizing an ELISA reader (based upon optical absorbance) or a simple hemacytometer; (b) protein adsorption methods using a high pH borate buffer for coating magnetic microparticles with purified fibronectin; (c) a method for measuring the efficiency of protein adsorption using ^{125}I fibronectin tracers; and (d) a rapid method for quantitating adhesive interactions between beads and suspended cells.

(5) ***Studies with attached cells:*** We have devised a ***chemically defined, serum-free medium*** for our studies designed ***to characterize specific cell-matrix interactions.*** In studies with attached cells grown in this medium, we have been able to show that ***fibronectin can modulate cell proliferation by promoting cell spreading.*** We have also been able to induce cell retraction and inhibit DNA synthesis in a parallel, dose-dependent fashion by specifically interfering with binding of fibronectin to its cell surface receptors using soluble fibronectin peptides (i.e., from the cell binding region). These findings confirm that ***the growth-modulating effects of matrix components depend upon their ability to provide a physical anchoring substratum that can resist cell-generated tensile forces and support cell extension.***

Significance of the Accomplishments

Finding #1: We believe that we now have a controllable direct-current electromagnet that will be suitable for our biological studies. The use of a direct-current electromagnet rather

than one based upon alternating current is extremely important: direct-current magnets do not generate associated electric fields that can have potent and confusing effects on cells. Our prototype can also be easily adapted if different field strength-gradient relationships are needed by configuring new pole tips with different geometries.

Finding #2: The field strength and gradient values generated by computerized field analysis will be crucial for demonstrating field-dependent effects on cell metabolism.

Finding #3: Adaptation of the magnet system for use with cultured cells has been our most difficult task to date. However, based upon preliminary studies, we have been able to devise a functional tissue culture arrangement in which temperature control is now not a problem.

Finding #4: The regularity of the size and shape of the microspheres that we are using should facilitate mathematical calculations of the effects of magnetic forces on cells as well as particle-particle interactions in future studies. We also expect that these magnetically stabilized chains will serve as attachment fibrils for suspended cells, resist cell-generated tensile forces, and support cellular DNA synthesis only in the presence of applied magnetic fields based upon the observation that they spontaneously disassemble when the field is removed.

Finding #5: Having characterized cell-fibronectin interactions within attached cells (fibronectin is a major ECM component), we feel that we are ready to assess the significance and specificity of effects of fibronectin-coated magnetic microparticles on suspended cells. During the next year, we must unequivocally demonstrate that DNA synthesis can be stimulated within suspended, anchorage-dependent cells using magnetic microparticles in conjunction with controlled electromagnetic fields. These studies are just beginning to get underway.

Publications

Ingber, D.E. and Folkman, J. Regulation of Endothelial Growth Factor Action: Solid State Control by Extracellular Matrix. *Progress in Clinical and Biological Research* 279: 273-282, 1987.

Ingber, D.E., Madri, J.A., and Folkman, J. Endothelial Growth Factors and Extracellular Matrix Regulate DNA Synthesis Through Modulation of Cell and Nuclear Expansion. *In Vitro Cellular and Developmental Biology* 23: 387-394, 1987.

THE STUDY OF DEVELOPING VESTIBULAR FUNCTION USING RECORDINGS OF PERIPHERAL AND BRAINSTEM VESTIBULAR ACTIVITY

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Description of Research

The principal aim of the research is to examine the role played by gravity in controlling or influencing the ontogeny of peripheral and central vestibular function. Ultimately an in-depth comparison will be made of how vestibular function develops and matures under the influence of gravitational fields having strengths less than (< 1.0 g, hypodynamic), equal to (1.0 g), and more than (> 1.0 g, hyperdynamic) the natural gravitational field strength of Earth. Studies will be undertaken to examine how the gravitational vector strength and direction may modulate or influence normal responses to transient stimuli. Efforts will also be directed to investigate how vestibular function may adapt to changes in gravitational fields and to evaluate the vestibular systems' ability to adapt as a function of the organism's age. Through these kinds of studies we may begin to appreciate the limits to, and determinants of, physiological adaptation in the vestibular system under a variety of gravitational environments.

The first year of research was undertaken to develop and evaluate a direct and noninvasive electrophysiological method capable of measuring the collective activity of afferent neurons in the vestibular system. The approach chosen was analogous to procedures commonly used to record peripheral and brainstem auditory neurons noninvasively. In the latter case, transient sound stimuli (e.g., clicks) are used to collectively activate auditory neurons. The collective activity of the auditory nerve (i.e., compound action potential) can then be detected and studied from the surface of the skull. The current research tested the hypothesis that pulsed cranial acceleration may be used in a similar manner to collectively activate vestibular afferent neurons in the bird. The use of pulsed rotational acceleration stimuli to elicit vestibular responses in mammals has been described by Elidan and co-workers (1982-1987) and by Hoffman & Horowitz (1978-1984). In the present study, pulsed linear acceleration stimuli were used for the first time in an attempt to collectively recruit otolith afferents such that the resulting compound action potential could be detected from the surface of the skull.

An avian animal model was chosen for the current studies in part because it provides an excellent opportunity to access and study embryos at any stage of development. The surface-recorded evoked potential was chosen because it promised to be a simple, noninvasive quantitative measure of various aspects of vestibular function including response thresholds. In theory, the approach will also provide a means of measuring the timing of peripheral neural activation and subsequent central neural transmission. The dynamics of the vestibular system may be tested further using this technique since response amplitudes and latencies may be characterized as a function of stimulus intensity and rise time. Ultimately these measures may be used to provide an overview of changes in vestibular function during normal development and in turn used to identify critical periods of development that display a sensitivity to gravitational field strength.

Accomplishments

(1) Methodology developed: A method was developed to deliver precisely defined pulsed linear acceleration stimuli to the cranium. Systematic variation of stimulus amplitude and rise time were key design features of the system.

(2) Short-latency vestibular responses to pulsed linear cranial acceleration were described for the first time. Responses were recorded noninvasively, consisted of four to seven dominant peaks and occurred within the first 8 msec following stimulus onset. Mean thresholds for responses were detemined (0.12 ± 0.45 g). Response peaks did not invert upon stimulus inversion, were present in response to cranial but not trunk acceleration, were not attenuated by intense (98.5 dBSPL) broad-band auditory masking nor affected by ambient light conditions, and disappeared with complete destruction of the labyrinth. ***Responses, therefore, are not dependent upon the auditory, somatosensory, or visual modalities but are dependent on the activity of vestibular neurons bilaterally.***

The very short latency of the response onset (1.59 msec), as well as the degree of temperature sensitivity for the earliest response peaks (P1, N1), suggest that the first positive and negative response peaks are produced by neurons of the vestibular nerve ($VIII_V$). Peaks having longer latencies may, in part, reflect the activity of brainstem neurons (i.e., second order or higher).

(3) The onset of embryonic vestibular function is an important variable and may be influenced by the ambient gravitational field. ***Vestibular responses were successfully recorded in embryos as young as embryonic day 15.*** These data have not been reported elsewhere and were collected in pilot studies. Subsequent in-depth study will likely find vestibular responses occurring at embryonic days 8 through 12 under normal conditions.

Significance of the Accomplishments

The research demonstrates that direct, noninvasive electrophysiological recordings of the collective action of vestibular afferents can be made in birds, including embryos. Because pulsed linear acceleration stimuli were used, it is also likely that the vestibular responses are, at least in part, derived from afferents innervating the otolith maculae (gravity receptors). The exact contribution made by otolith vs. canal afferents to response waveforms remains to be determined. To my knowledge, the pilot studies in the embryo represent the first observations of embryonic electrophysiological activity in the vestibular portion of the eighth nerve.

The experiments have set the stage for a detailed survey of vestibular development as it may be reflected by the collective action of primary vestibular afferents in the embryo at 1 g. This will lead to studies designed to evaluate the effects of hyper- and hypodynamic environments on vestibular development.

The new approach differs significantly from traditional single unit studies in that a rapid assessment of functional events can be made noninvasively, and by comparison, quite simply. The approach will provide a means to estimate when collective vestibular function first begins in the embryo as well as a means to express the sensitivity of emerging function as a response threshold. It will also enable quantitation of vestibular response dynamics (the timing of neural activation and transmission) throughout development.

The simplicity of the recording technique should allow individuals with only minimal training to carry out recordings during spaceflight. As already suggested, the method will be used to identify particular major functional events of development. Where appropriate, we may then focus on these events and investigate the underlying cellular mechanisms in great detail using single unit electrophysiological techniques.

Publications

Jones, T.A. Responses to Pulsed Linear Acceleration Recorded from the Surface of the Skull (Abstract). *ASGSB Bulletin* 1: 18, 1988.

MECHANISMS OF VESTIBULAR ION TRANSPORT

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Description of Research

The two classes of vestibular sensory organs, the maculae and ampullae, differ by virtue of the accessory structures which mediate their respective mechanical sensitivities to linear or angular acceleration. In contrast, the mechanoreceptive hair cells associated with each type of sensory organ show many similarities of organization and function within the sensory epithelia. The hairs of the receptor cells in each type of structure extend toward the lumen of a fluid compartment filled with endolymph. This specialized extracellular fluid is distinguished by its unique low sodium/high potassium ionic composition. Vestibular transduction is thought to commence when mechanical displacement of the sensory hairs results in the opening of "transduction channels" at the apical cell surface, with augmented flow of ionic current from endolymph into the hair cell. This "transduction current," which is carried in mammalian hair cells almost entirely by endolymphatic potassium ions, exits the hair cell by way of ion channels situated in the basolateral membrane.

Given the importance of potassium movements for vestibular transduction, and the documented losses of body fluid and electrolytes which occur upon exposure to microgravity, the long-term objective of this project is the characterization of molecular mechanisms mediating active and passive ion fluxes in vestibular endolymph and sensory epithelia. As an additional goal, we seek to determine whether these mechanisms participate in possible adaptive responses to changes in fluid and electrolyte balance, and to altered patterns of vestibular stimulation experienced in microgravity.

We have established the distribution of presumptive muscarinic acetylcholine receptor sites (MAChR) in sensory structures of the mammalian vestibular apparatus. Vestibular sensory epithelia are known to receive a complement of cholinergic nerve endings distinct from the afferent innervation, and it is thought that these cholinergic terminals exert a regulatory influence on transduction and/or afferent transmission. Although recent electrophysiological studies of anuran ampulla provide evidence that nicotinic acetylcholine receptors mediate rapid changes in afferent activity, there have also been indications that MAChR may be present on hair cells and may mediate a slow modulation of afferent discharge. In other systems, muscarinic responses are marked by long latency and prolonged duration; the MAChR often exert their actions by altering the conductance of various membrane ion channels, including voltage-sensitive potassium channels and calcium-dependent potassium channels. Until now, there has been no direct demonstration of muscarinic receptors in mammalian vestibular organs. In the present studies, therefore, vestibular organs were isolated from gerbil inner ear by microdissection, then incubated with [^{3}H]quinuclidinyl benzilate (^{3}H-QNB) at 1 nM concentration. QNB is a specific antagonist with high affinity for muscarinic-type acetylcholine receptor sites.

Accomplishments

(1) Binding of ^{3}H-QNB was measured by a microscale filtration assay utilizing liquid scintillation spectrometry, and was normalized to tissue dry weight. ***Specific binding (displaceable by unlabeled atropine) was higher in vestibular organs than in cochlear structures assayed for comparative purposes, and higher in the ampullae than in the macular organs.*** The ratio of specific to nonspecific binding was 5:1 or greater.

(2) Vestibular tissues, incubated with ^{3}H-QNB, were prepared for light-microscopic autoradiography. As autoradiographic exposures progressed, the first structure to display marked autoradiographic labeling was the sensory epithelium of the crista ampullaris (Figure 1). At all exposure durations, autoradiographic observations paralleled the quantitative results, in that ***highest grain densities were always associated with the sensory epithelium of the ampulla*** (Figures 2 and 3).

(3) Few silver grains were found over connective tissue regions or nonspecialized epithelium of the vestibular organs (Figures 1-3). Control preparations, incubated in medium containing ^{3}H-QNB together with excess unlabeled atropine, showed a marked reduction in grain density.

(4) ***In autoradiographs of both the ampullae and the macular organs, silver grains, corresponding to ^{3}H-QNB binding sites, were most numerous over the sensory epithelium. Labeling within the sensory epithelium was concentrated about midway between the luminal surface and the basal lamina, at a level corresponding to the synaptic poles of the hair cells*** (Insert, Figure 1).

(5) In all vestibular sensory organs, labeling was also associated with nerve fibers passing through the underlying connective tissue (Figures 1-3).

Significance of the Accomplishments

Findings #1 and 2: Our quantitative assays and autoradiographic results indicate that differences exist, with respect to the densities of presumptive muscarinic receptor sites, in the various hair-cell sensory organs of the inner ear. Binding sites for ^{3}H-QNB were more numerous in the cristae ampullares than in other organs, raising the possibility that muscarinic effects may be most pronounced in the sensory epithelia of the semicircular canals.

Finding #3: The fact that few ^{3}H-QNB binding sites were associated with connective tissue or with nonspecialized epithelium in autoradiographs of vestibular tissues suggests the specificity of labeling observed within the sensory epithelium. Connective tissue regions and nonspecialized epithelium do not receive cholinergic innervation. Similarly, specific binding of ^{3}H-QNB should be displaceable by the muscarinic antagonist atropine.

Finding #4: The most important aspect of this work is the autoradiographic demonstration of presumptive muscarinic receptor sites, within vestibular sensory epithelia, at a locus corresponding to the synaptic poles of the hair cells. In a previous quantitative study of the cochlea, other investigators reported that muscarinic receptor sites were most abundant in nonsensory tissues, and in the auditory nerve. The present results, however, provide clear-cut ***evidence of muscarinic acetylcholine receptors in vestibular sensory***

EFFECTS OF BRIEF AUTORADIOGRAPHIC EXPOSURE ON MAMMALIAN VESTIBULAR TISSUES

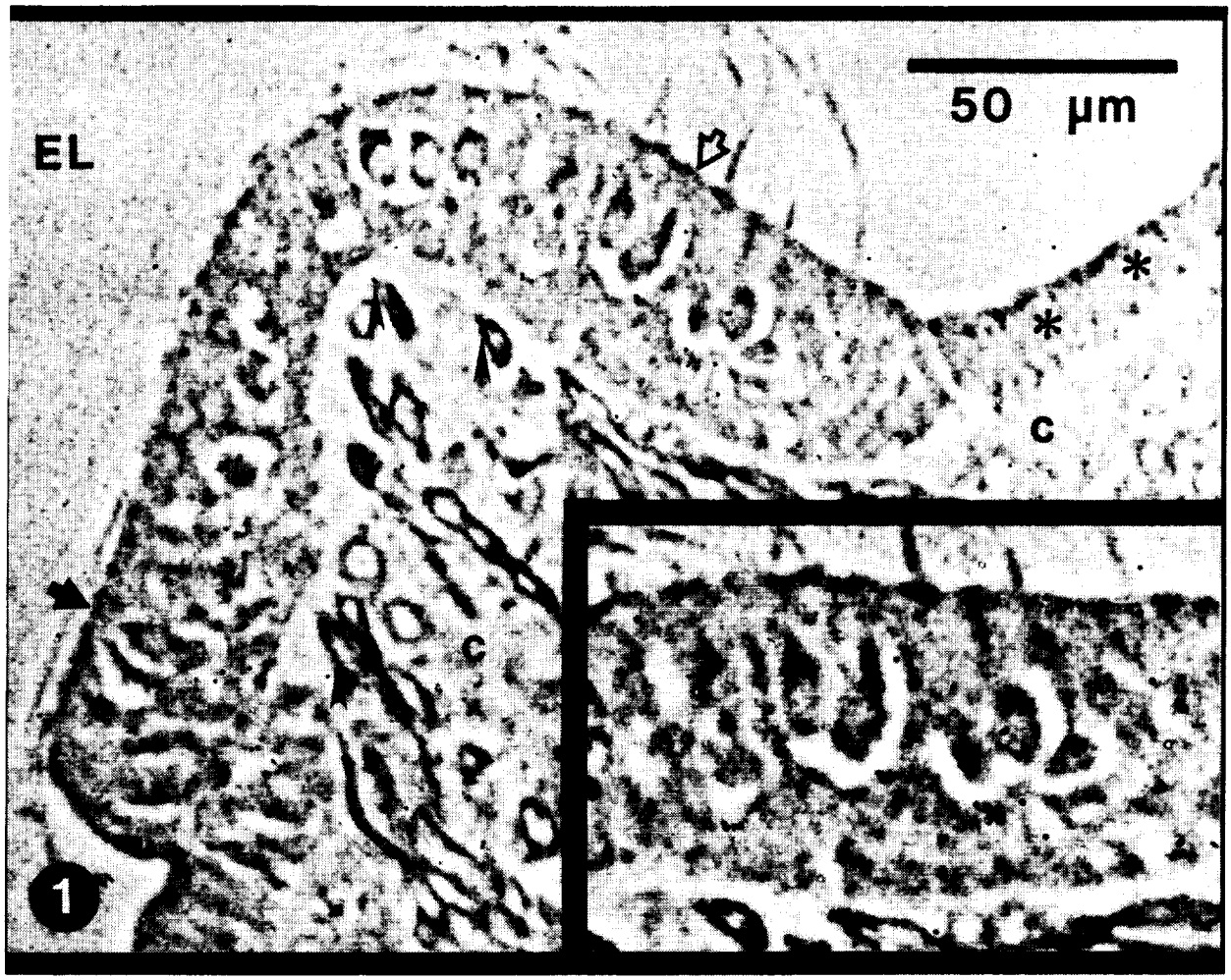

Figure 1. This phase-contrast light micrograph shows an autoradiograph of the crista ampullaris, incubated *in vitro* with ^{3}H-QNB. After a relatively brief (17-day) autoradiographic exposure, the first regions to display distinct labeling are the areas of sensory epithelium located on the "shoulders" of the crista (indicated by open and filled arrows).

The region of sensory epithelium beneath the ***open*** arrow is shown at higher magnification (inset, lower right). Notice that most silver grains are located over the mid-portion of the sensory epithelium, at a level corresponding to the synaptic poles of the hair cells.

A few silver grains are also associated with nerve fibers (e.g., at arrowheads) passing through the connective tissue (c) beneath the sensory epithelium. Little or no labeling is associated with the connective tissue *per se*, or with transitional epithelium (*). At this brief exposure time, autoradiographic "background" remains low, as indicated by the near-absence of silver grains over the endolymphatic compartment (EL).

PROLONGED AUTORADIOGRAPHIC EXPOSURE DISTRIBUTION ON MAMMALIAN VESTIBULAR TISSUES

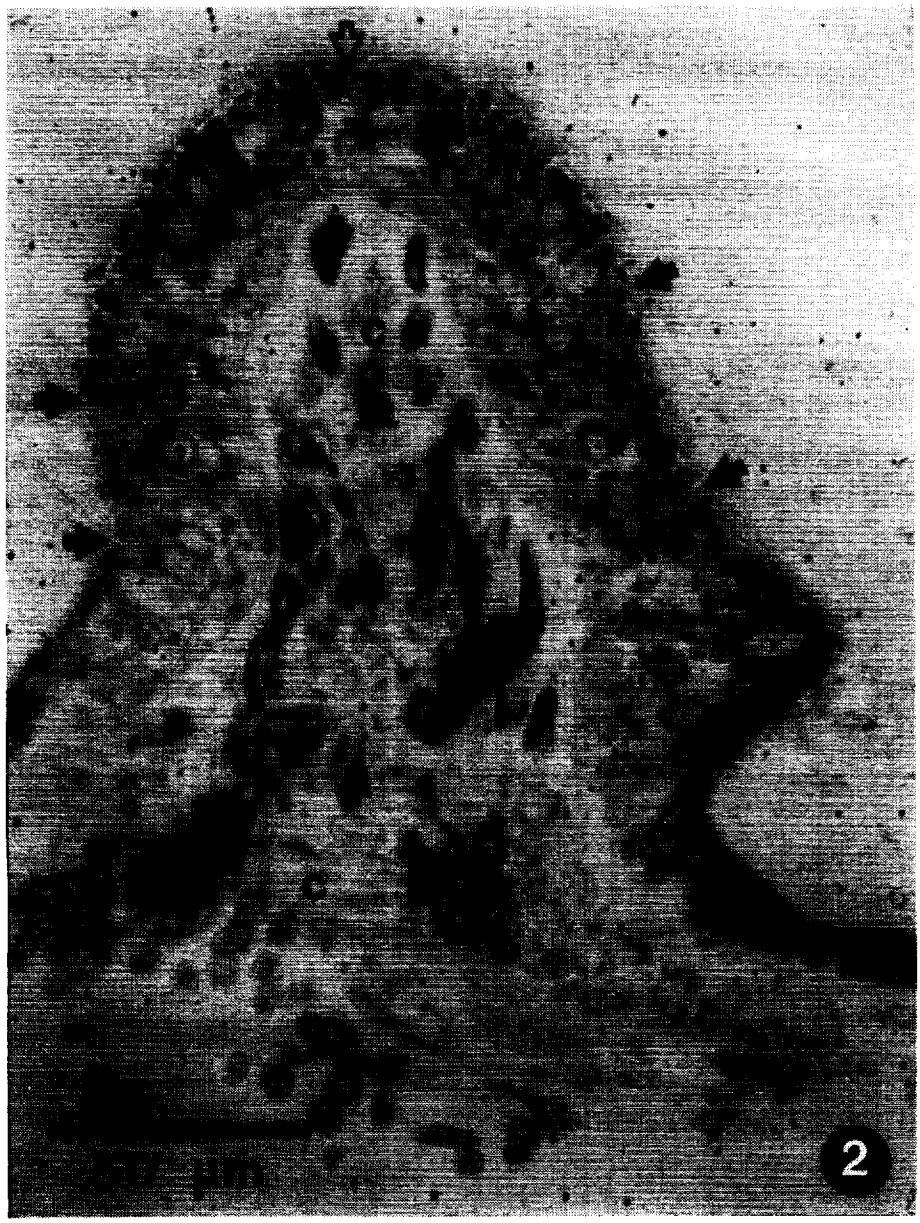

Figure 2. This brightfield light micrograph shows another autoradiograph of the crista ampullaris, incubated *in vitro* with ^{3}H-QNB, and given a prolonged (78-day) autoradiographic exposure. Although the regional pattern of label distribution is the same as depicted in the previous micrograph, label density is higher overall because of the longer autoradiographic exposure. Once again, highest grain densities are associated with sensory epithelium along the "shoulders" of the crista (filled arrows), while a lower density of labeling occurs in sensory epithelum at the apex of the crista (open arrow). Nerve fibers beneath the sensory epithelum (e.g., at arrowheads) show a lesser degree of labeling, while connective tissue (c) and transitional epithelium (*) are not labeled above the background level observed in the endolymphatic compartment (EL). Methylene blue stain.

PROLONGED AUTORADIOGRAPHIC EXPOSURE DISTRIBUTION ON MAMMALIAN VESTIBULAR TISSUES

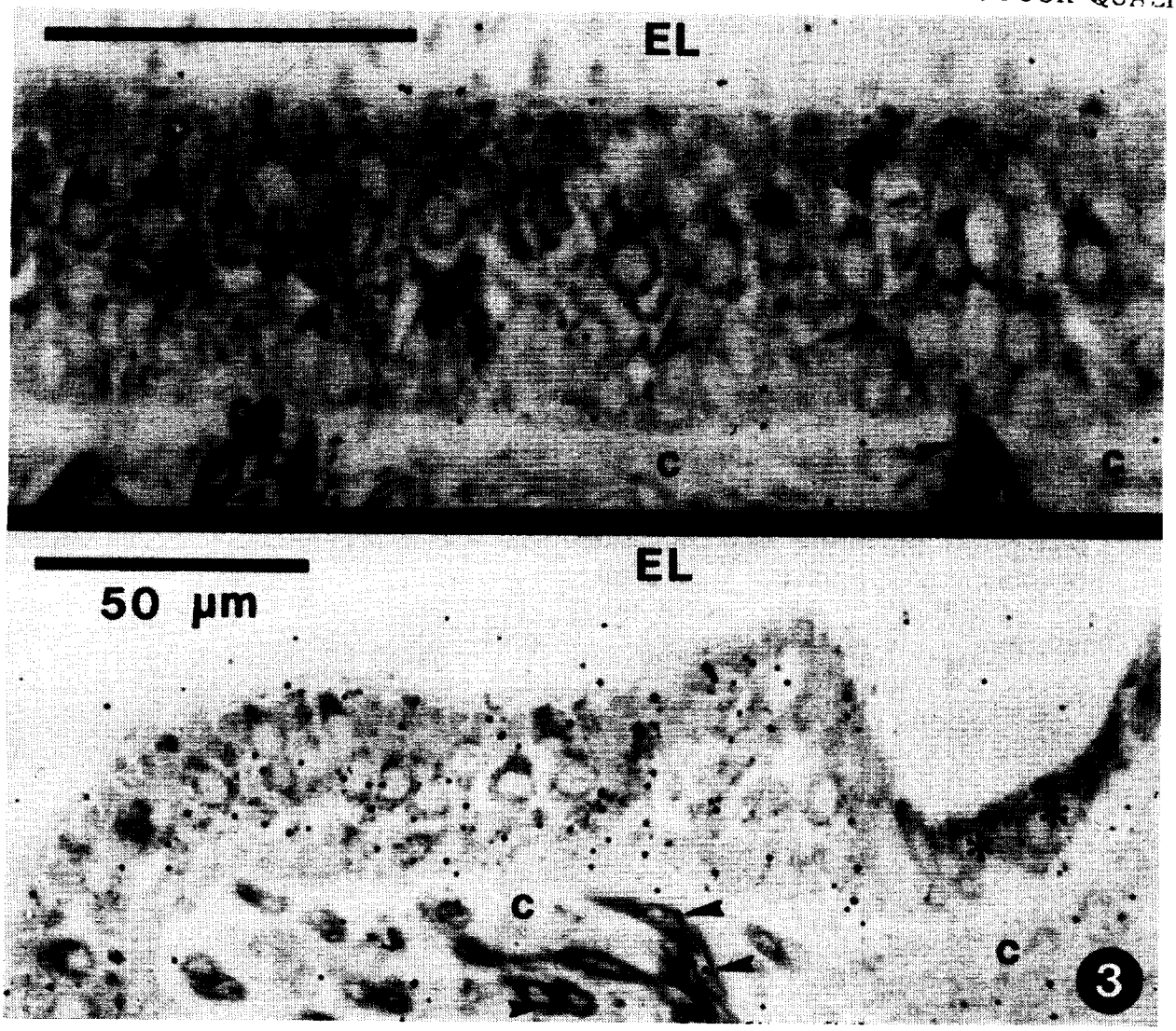

Figure 3. These brightfield light micrographs show sensory epithelium of the utricle (above) and crista ampullaris (below), incubated with 1 nM ^{3}H-QNB and processed identically.

In both structures, highest label density is found over the sensory epithelium, although some silver grains are also associated with nerve fibers (arrowheads). Connective tissue (c) is not labeled above the background level observed over the endolymphatic compartment (EL). Similarly, transitional epithelium of the crista ampullaris (*) shows little, if any, labeling.

Although these specimens were processed identically in every respect, label density in sensory epithelium of the utricle is considerably lower than in sensory epithelium of the crista. Within the sensory epithelium of the crista, label density is highest on the "shoulder," (which extends horizontally in this micrograph), and is relatively low at the apex (lower left). 78-day autoradiographic exposures, methylene blue stain.

epithelia. It is known that cholinergic nerve endings make synapse, within vestibular sensory epithelia, both upon hair cells and upon afferent nerve endings. Since vestibular synaptic detail is not readily resolved by light microscopy, further study will be required to differentiate receptor sites on hair cells vs. afferent nerve endings.

Finding #5: The presence of ^{3}H-QNB binding sites on vestibular nerve fibers beneath the sensory epithelium suggests the presence of muscarinic receptor sites on these fibers, or at least upon their myelin sheaths.

To our knowledge, the present results comprise the first direct demonstration of a presumptive neurotransmitter or neuromodulator receptor site in mammalian vestibular sensory organs. These receptors may well be involved in the modulation of potassium flux across the plasma membranes of vestibular hair cells and afferent nerve fibers.

Publications

Kerr, T.P. and Drescher, D.G. Low NA^{+}, K^{+}-ATPase Activity in Epithelial Wall of the Saccule (Abstract). *ASGSB Bulletin* 1: 18, 1988.

Kerr, T.P. and Drescher, D.G. Quantitative and Autoradiographic Demonstration of Presumptive Muscarinic Acetylcholine Receptor Sites in Mammalian Vestibular Organs (Abstract). *ASGSB Bulletin* 1: 17, 1988.

MICROMOTIONAL STUDIES OF UTRICULAR AND CANAL AFFERENTS

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Description of Research

The long-range goal of this research is to refine our understanding of the sensitivity of the ear to very-low-amplitude motion, especially the role of gravity in this sensitivity.

It is known that the human vestibulo-ocular reflex operates quite well for head rotations with amplitudes that are a small fraction of a degree. Our preliminary data from an experimental animal, the American bullfrog, suggested that the sensory inputs for this reflex might be provided not only by the semicircular canals, but also by the otoconial vestibular organs (e.g., the utricle). Traditionally, the otoconial organs of mammals have been considered sensors of lineal motion and of head orientation relative to the gravity vector. The evidence for this role lies in the tonic responsiveness to acceleration that is commonly observed in afferent nerve fibers from the otoconial organs. However, in a large subpopulation of those fibers, one also finds phasic responsiveness to acceleration. On the basis of our observations of that responsiveness in the bullfrog, we propose another role for the otoconial organ, namely, a sensor of vertical orientational motion of the head (i.e., head rotational motion in the vertical planes). That sensitivity traditionally has been assigned to the vertical semicircular canals.

To test our hypothesis, we elected to stimulate the vestibular sensors with very-small-amplitude sinusoids and to observe the corresponding responses of the afferent axons under steady-state conditions. This choice was based on the fact that sinusoidal steady-state analysis presently is the most powerful tool available for identification of physical or biophysical systems. For this purpose, we set out to design and construct a stimulating device that could provide vertical rotational sinusoids at frequencies down to approximately 0.01 Hz, amplitudes down to approximately 0.01 deg peak-to-peak, and a minimum of harmonic distortion.

Continuing to use the bullfrog as our experimental animal, we studied carefully the phasic responsiveness of afferent fibers from the utricle and compared them with the response of axons from the anterior vertical semicircular canal.

Accomplishments

The desired stimulating apparatus was designed and constructed to include refinements of existing conventional apparatus. The newly designed tilt stimulator now consistently delivers sinusoidal tilts with less than 1% distortion for amplitudes from 0.02 deg (peak-to-peak) to 1.0 deg peak-to-peak and frequencies ranging from less than 0.01 Hz to approximately 20 Hz.

Employing this system, we found that the ***phasic sense of the bullfrog utricle is remarkable in two respects:*** (1) ***its sinusoidal steady-state response over the four octaves from 1/8 Hz to 2 Hz is directly proportional to the rate of***

change of acceleration (i.e., to "jerk"); (2) it is ***extraordinarily acute,*** with sensitivities varying from one nerve fiber to another and ranging from 0.3 to 2.5 spikes/sec per cm/sec^3.

Over the same four octaves of head-rotation frequencies (1/8 to 2 Hz), we found that ***the response of the anterior semicircular canal was approximately proportional to rotational velocity*** (with a slight rotational displacement component), and that ***the sensitivity varied from one fiber to another,*** with most fibers falling in the range from 5 to 40 spikes/sec per deg/sec. A few anterior canal nerve fibers exhibited extraordinary sensitivities, ranging up to several hundred spikes/sec per deg/sec.

Significance of the Accomplishments

In a gravity field, the jerk sensitivities that we observed from the bullfrog utricle translate directly into velocity sensitivity for head rotation in a vertical plane. For small head rotations about any horizontal axis, 1.0 deg/sec of rotational velocity in a 1.0 g field yields 17.1 cm/sec^3 of equivalent jerk. Without the gravity field, the jerk produced by our rotational sinusoids on the utricle would be more than three orders of magnitude smaller than this. Thus the gravity vector acts as a lever to amplify tremendously the jerk on the utricle during vertical head rotation. Remarkably, the observed sensitivity of the utricle (0.3 to 2.5 spikes/sec per cm/sec^3) translates (in a 1.0 g gravity field) to the same range of rotational velocity sensitivities (5 to 40 spikes/sec per deg/sec) that we found in the majority of anterior canal fibers. Thus, in a 1.0 g gravity field, the phasic sense of the bullfrog utricle overlaps almost precisely with that of the bullfrog anterior semicircular canal. The intriguing difference is that the utricle (lacking the rotational position component seen in the canal response) provides a much more precise rotational velocity measure than does the canal. We do not know the role of this utricular input in compensatory eye movements or other vestibular reflexes; but we do know that the signal will be absent in a microgravity environment, such as exists in space.

The sensitivities that we have observed in the bullfrog utricle and anterior canal are at least an order of magnitude greater than those reported for mammals. There are at least three possible explanations for this: (1) the mammalian ear is not as sensitive as the ear of the bullfrog, (2) the phasic sensitivity of the mammalian utricle has not been studied extensively (investigators tend to focus on the tonic sensitivity), and (3) studies of the mammalian vestibular system have been carried out with macromotions (sinusoidal rotations typically of the order of 10 deg peak-to-peak). Regardless of the explanation, the fact itself leads mammalian vestibular researchers to discount the importance of our observations on the bullfrog. Therefore, our next step will be to extend our micromotional studies to the mammalian ear.

Publications

Lewis, E.R., Yu, X.-L., and Myers, S.F. A Micromotion Stimulator for Inner Ear Gravity Receptors (Abstract). *ASGSB Bulletin* 1: 16-17, 1988.

Myers, S.F. and Lewis, E.R. Utricular Striolar Afferents Are Very Good Gravity Sensors! (Abstract). *Association for Research in Otolaryngology Abstracts,* pp. 57-58, 1988.

HIGH RESOLUTION ANALYSIS OF GRAVITY ORIENTATION OF AMPHIBIAN EGG CYTOPLASM

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Description of Research

The amphibian egg displays a dramatic gravity orientation following activation by a fertilizing sperm. That gravity orientation is easily visualized because of pigmentation differences between regions of the egg surface. Internally, relatively large yolk platelets provide a key marker for the cytoplasmic rearrangements that accompany gravity orientation. Studies under microgravity simulation (clinostat), egg inversion, or spaceflight can be employed to understand the basic cell biology of the organization of the egg cytoplasm. The focus of recent experimentation has been directed towards understanding the forces and mechanisms that are responsible for maintaining the structural integrity of the various compartments (see below). Also, the mechanisms that are responsible for rearranging the compartments are being studied, and the localization of mRNA is being tracked.

Our goal is to develop a coherent view of the manner in which the egg cytoplasm is organized, as well as the way in which that organization is altered when the egg is challenged by novel gravity orientation (e.g., egg inversion or clinostat rotation).

We are testing the so-called "density compartment" model for egg organization. It proposes that the egg cytoplasm is arranged into a series of compartments (or domains), which shift their topography when the egg is activated by the fertilizing sperm.

Accomplishments

(1) mRNA localization: In order to extend the range of cytoplasmic components that are tracked in "novel gravity orientation" eggs, ***we have begun to track a specific mRNA. It is localized in the vegetal hemisphere of fertile eggs.*** We have brought contemporary recombinant DNA technology methods to this project; the "Veg-1 cDNA" was transcribed into an antisense mRNA (radioactive). Embedded and sectioned eggs were then bathed in a solution of that antisense mRNA, and RNA:RNA hybridization was permitted to occur. Using radioautography, silver grains localized the endogenous mRNA to the egg's vegetal hemisphere. That observation begins a new chapter in the "density compartment story." During the next year, we plan to track Veg-1 mRNA in clinostated, inverted, and rotated eggs. The goal is to establish whether mRNAs are included in their own cytoplasmic compartments, or whether they track with compartments belonging to either yolk platelets or soluble proteins.

(2) Yolk platelet packing density: The structural integrity of various yolk platelet compartments was investigated at the level of packing density. The following hypothesis was tested: There is a direct relationship between high yolk platelet volume/density (fraction of total cytoplasmic volume occupied by yolk platelets) and low cytoplasmic mobility (CM) (high apparent cytoplasmic viscocity). Unfertilized and fertilized eggs from nine spawnings representing a range of CMs (16.9 to 50.0 units) were analysed by standard morphometric methods. The yolk platelet volume/density was determined for the three major cytoplasmic compartments: animal hemisphere - small yolk mass (SYM);

vegetal hemisphere - large yolk mass (LYM); central - intermediate yolk mass (IYM). There was an inverse relationship between CM and yolk volume/density in both unfertilized and fertilized eggs. For example, for low CM fertilized eggs (T=0.5) the mean yolk platelet volume-density for the egg cytoplasmic compartments was as follows: LYM=.546 $\pm$ .028; IYM=.487 $\pm$ .038; SYM=.216 $\pm$ .048. For high CM eggs the yolk platelet volume/density was LYM=.612 $\pm$ .030; IYM=.534 $\pm$ .050; SYM=.256 $\pm$ .048. This study rules out the yolk platelet volume/density as a major contributing factor to the variation in CM.

(3) Pre-symmetrized eggs: Occasionally, *Xenopus* (frog) eggs are spawned which, prior to fertilization, appear to be bilaterally symmetrical. Those eggs have a "tilted" appearance. When carefully observed, they polarize according to the direction of tilt, rather than in line with the sperm entrance site (as is the case for normal eggs).

(4) Egg cytoskeleton: The cytoplasmic viscosity of amphibian eggs can be diminished substantially by a brief cold shock. That observation supports the hypothesis that the ***cytoskeleton*** (e.g., microtubules) ***plays a role in stabilizing the density compartments.***

Each of the above achievements serves as a stepping stone for further studies. In steady increments, our research program is developing resolution techniques that are providing insights into the molecular biology of egg organization.

Publications

Malacinski, G.M. and Neff, A.W. The Consequences of Developing Upside Down (Abstract). *ASGSB Bulletin* 1: 14, 1988.

STRUCTURAL DEVELOPMENT AND GRAVITY

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Description of Research

The goal of this research is to understand the role of gravity in skeletal growth and development. To achieve this goal, we must first learn what turns bone cells on and off, if/how these cells communicate with each other and with their environment, if/how secretory products are altered by different gravity levels and how alterations in organic matrix might affect mineralization and strength, and the role of local and systemic factors (including endocrine, blood flow, and fluid shifts) in these responses. To accomplish these studies, both flight simulations and flight experiments are essential.

Gravity is a major factor determining the amount of structural support required by Earth organisms. The hypothesis of this research effort is that skeletal support structures will change during spaceflight, and that the magnitude and duration of change will be dependent upon the modeling or remodeling activity in each bone and the length of exposure to spaceflight; changes in both quantity and quality of bone will occur. Most ground-based research is done in rats exposed to simulated spaceflight; three flight experiments have been approved and will allow gathering of more information to support or negate the hypothesis.

Accomplishments

(1) Completion of a simulation of Spacelab-3 younger rat study using the unloaded hindlimb rat model showed that ***unloading duplicates many, but not all, of the effects of the spaceflight experiment. The muscle and bone changes were qualitatively similar, but testicular atrophy was much greater with the model.***

(2) A ***long-duration hindlimb unloading study (40 days) showed both no major bone changes and the transient nature of the muscle atrophy.***

(3) ***A short-duration hindlimb unloading study (1, 2, 5 days) showed focal occlusion of subperiosteal vessels*** within 1 day, which was at least normal by 5 days (done in collaboration with Dr. Steve Doty).

Significance of the Accomplishments

Finding #1: Suggests that the ground-based rat model is excellent for studying certain aspects of spaceflight such as muscle loss and acute bone changes, i.e., increase in bone mass with no increase in bone strength. However, in growing male rats, testicular atrophy is a potential problem and is more pronounced on the model than in flight.

Finding #2: Suggests that both muscle and bone alterations in the unloaded rat hindlimbs resume growth during chronic unloading so that changes in both organ systems appear to be transients. Whether the same phenomena occurs during spaceflight is not known.

Finding #3: Suggests that focal changes in blood distribution may trigger the bone changes seen during unloading, or at least play a role in the changes. The vascular occlusion may be due to extraosseous debris.

Publications

Arnaud, S.B., Holton, E.M., Buckendahl, P.P., and Tietjen, G. Early Effects of Weightlessness on Growing Bone (Abstract). In: *Space Life Sciences Symposium: Three Decades of Life Science Research in Space*, Washington, D.C., June 21-26, p. 226-227, 1987.

Bikle, D.D., Halloran, B.P., Cone, C.M., Globus, R.K., and Morey-Holton, E. The Effects of Simulated Weightlessness on Bone Maturation. *Endocrinology* 120: 678-684, 1987.

Bikle, D.D., Halloran, B.P., Cone, C.M., Globus, R.K., and Morey-Holton, E. The Effects of Simulated Weightlessness on Bone Maturation (Abstract). *ASGSB Bulletin* 1: 34, 1988.

De Gennaro, M., Morey-Holton, E., Maese, C., and Cone, C. Role of Muscle Tension at the Tibiofibular Junction on the Rate of Periosteal Bone Formation during Simulated Near Weightlessness. *Transactions of the 33rd Annual Meeting of the Orthopaedic Research Society*, p. 460, 1987.

Jackson, C.B., Roberts, W.E., and Morey, E.R. Growth Alterations of the Mandibular Condyle in Spacelab-3 Rats (Abstract). *ASGSB Bulletin* 1: 33, 1988.

Morey-Holton, E.R., Maese, A.C., and Fast, T.N. Effect of 40 Day Suspension on Growth and Development of Young Rats (Abstract). *ASGSB Bulletin* 1: 33, 1988.

Morey-Holton, E.R., Maese, A.C., Kraft, L.M., and Fast, T.N. Spacelab 3 Simulation (Abstract). *ASGSB Bulletin* 1: 38-39, 1988.

Morey-Holton, E. and Tischler, M. (Eds.). *NASA Workshop on Biological Adaptation*. Moffett Field: NASA Ames Research Center, 103 pp., 1988. (NASA TM-89468)

Roberts, W.E., Fielder, P.J., Rosenoer, L.M.L., Maese, A.C., Gonsalves, M.R., and Morey, E.R. Nuclear Morphometric Analysis of Osteoblast Precursor Cells in Periodontal Ligament, SL3 Rats. *American Journal of Physiology* 252: R247-R251, 1987.

Schwartzkopf, S.H., Givens, J.J., and Morey-Holton, E.M. LifeSat: An Unmanned Life Sciences Flight Program (Abstract). *ASGSB Bulletin* 1: 36, 1988.

Wood, H.B., Roberts, W.E., Bikle, D.D., Halloran, B.P., Chambers, D.E., and Morey, E.R. Perivascular Distribution of Osteoblast Precursor Cells in Periodontal Ligament of Rats Exposed to Simulated Weightlessness (Abstract). *ASGSB Bulletin* 1: 35, 1988.

SKELETAL COLLAGEN TURNOVER BY THE OSTEOBLAST

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Description of Research

Among the most overt negative changes experienced by man and experimental animals under conditions of weightlessness are the loss of skeletal mass and attendant hypercalciuria. These clearly result from some disruption in the balance between bone formation and bone resorption (i.e., remodeling), but precisely what this disruption is and how it might occur have not been established. Recently, a body of information has accrued indicating that the osteoblast plays an important role in bone resorption as well as bone formation and may hold the cellular "key" to understanding the response of the skeleton to conditions of weightlessness. In the present study, the clonal osteoblast cell line UMR-106-01 has been used to investigate the regulation of several proteins whose expression is central to both bone formation and bone resorption; these are Type I collagen (the major organic constituent of bone), collagenase, and collagenase inhibitor. Expression was monitored initially at the protein level using a combination of techniques, including radiolabeling of the proteins, gel electrophoresis and ELISA assays, and subsequently at the levels of RNA, principally by Northern blot analysis. This project will shed some light on the comprehensive role of the osteoblast in the remodeling process, and in so doing provide some insight into how the process might be disrupted under conditions of zero gravity.

Specific aspects of our research include:

Hormonal Regulation of Collagen Synthesis

This section has been completed with the conclusion that parathyroid hormone (PTH) causes approximately 40% decrease in collagen synthesis by UMR 106-01 cells. This decline can first be observed 12 hr after treatment with the hormone, with the greatest inhibition seen at 24 hr. The effect of PTH is dose-dependent with a concentration of 5×10^{-10} M PTH eliciting a half-maximal inhibition.

Regulation of Collagenase Synthesis and Turnover

We have previously shown that PTH causes the transient and dramatic secretion of collagenase by UMR cells. Experiments documenting that the turnover of collagenase in the media of UMR cells is cell-mediated have been repeated and confirmed and have extended the previous findings. In particular, it has become very clear that control cells have a greater ability to remove the enzyme from the extracellular environment than PTH-treated cells. We will carry on with this work, e.g., testing whether the mechanism is saturable and identifying a cell surface receptor.

Analysis of Collagenase mRNA

Northern blot analysis was conducted on mRNA from UMR 106-01 cells cultured with PTH for various times. The cells were grown in roller bottles since we have observed far

greater expression of procollagen mRNA under these conditions than in stationary flasks. This may indicate the responsiveness of the osteoblast to stress and gravity-induced forces. We undertook the present experiments to determine the time course of expression of the collagenase gene, as well as to ascertain the size of the specific transcript.

Using oligonucleotide probes, we determined that the mRNA of the rat collagenase gene in UMR cells is approximately 2.95 kilobases in size. Maximal expression occurs about 4 hr after treatment of the cells with PTH. No collagenase message is detectable in the 0 hr control (before addition of PTH). After 8 hr of incubation of the cells with PTH, expression of the gene falls to less than half of the 4 hr level. Thereafter, the collagenase message abundance continues to decrease more gradually.

Library Construction

Based on the Northern blot analysis data, poly (A^+) mRNA from UMR cells treated with PTH for 4 hr was used to construct a cDNA library in the EcoRI site of Lambda ZAP. A total of 2.2×10^7 recombinant phage were obtained. Screening of this library is now in progress.

We will also start screening a rat genomic library to find the regulatory region of the collagenase gene. Having this regulatory region cloned will allow us to determine the role of transcriptional regulation in collagenase gene expression.

Studies on Collagenase Inhibitor

In collaboration with Dr. John Jeffrey at Washington University, we have purified the rat collagenase inhibitor from UMR-conditioned media and determined the amino-terminal sequence up to residue 25. The protein reveals highest homology to the bovine inhibitor and considerable divergence from the mouse and human proteins. However, all four inhibitor proteins contain a homologous region which we are presently subcloning from a human genomic clone (pEH5.2, given to us by Dr. Judith Gasson, UCLA) of the collagenase inhibitor. It will be used to screen the UMR cDNA library for this gene.

Other ongoing work with the human osteogenic sarcoma cell line, SaOS-2, has demonstrated that epidermal growth factor (EGF) and the tumor promoter, phorbol myristate acetate (PMA), cause an increase in the synthesis and secretion of collagenase inhibitor which is paralleled by increases in the mRNA for this protein. However, like our observations on procollagen mRNA, culture of the cells in roller bottles rather than in stationary flasks yields comparatively greater numbers of transcripts for the inhibitor.

Accomplishments

(1) ***Demonstrated that PTH inhibits collagen synthesis in the osteoblastic cell line, UMR 106-01.***

(2) ***Provided evidence that the neutral metalloprotease, collagenase, is not only secreted by osteoblastic cells but also removed from the media by a cell-mediated event.***

(3) Used oligonucleotide probes with procollagenase to show that the mRNA for the enzyme is 2.95 kilobases in size and is inducible by PTH.

(4) ***Established a cDNA library from mRNA from PTH-treated UMR cells, which can be used to clone both collagenase and collagenase inhibitor.***

(5) Purified rat collagenase inhibitor and obtained N-terminal peptide sequence.

(6) ***Demonstrated that EGF and PMA induce collagenase inhibitor in SaOS-2 cells and that the mRNA abundance is greater in roller bottle culture.***

Significance of the Accomplishments

Finding #1: These data support the notion that the UMR cell line is a good model for both ground-based and spaceflight research on osteoblastic cells.

Finding #2: Treatment with PTH appears to change the phenotype of the osteoblast from a matrix synthesizing cell to one actively involved in the resorption process. Collagen synthesis declines while production of enzymes associated with matrix removal increases. This situation may resemble that seen in situations of weightlessness where bone formation is perturbed. Nevertheless, the cell appears to exert tight control over the amount of collagenase in the extracellular medium by rapidly reextracting it.

Findings #3-5: The ability to use oligonucleotide probes to detect an inducible transcript for collagenase and the construction of a cDNA library from UMR cells means that we expect to have probes for both collagenase and its inhibitor in the near future. Once these are obtained the experiments that can be conducted are numerous.

Finding #6: Human osteoblastic cells as well as rat cells produce collagenase inhibitor, although the agents causing its stimulation are different. However, both cell lines show effects of roller bottle culture, compared with stationary flasks, which indicate their responsiveness to motion and/or gravity.

Publications

Civitelli, R., Strege, D.W., Kahn, A.J., Avioli, L.V., Hruska, K.A., and Partridge, N.C. PTH Stimulation of Collagenase in Osteoblast-like Cells: Role of Second Messengers. In: *Osteoporosis, 1987,* (ed. by J. Jensen, B. Riis, and C. Christiansen). Denmark: Norhaven, Viborg, pp. 222-226, 1987.

Kahn, A.J. and Partridge, N.C. New Concepts in Bone Remodeling: An Expanding Role for the Osteoblast. *American Journal of Otolaryngology* 8: 258-264, 1987.

Partridge, N.C., Jeffrey, J.J., Ehlich, L.S., Teitelbaum, S.L., Fliszar, C., Welgus, H.G., and Kahn, A.J. Hormonal Regulation of the Production of Collagenase and a Collagenase Inhibitor Activity by Rat Osteogenic Sarcoma Cells. *Endocrinology* 120: 1956-1962, 1987.

THE EFFECTS OF GRAVITY ON ORGANIZING PATTERNS OF CELL DETERMINATION IN EARLY AMPHIBIAN EMBRYOS

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Description of Research

The purpose of this research is to study the effects of terrestrial and non-terrestrial gravitational-like forces on early embryonic development. We are concentrating on gravitational influences affecting pattern formation of the amphibian nervous system. The approach employed in this study is to prepare monoclonal antibodies to early cell surface antigens, specific for either the neural or epidermal ectoderm (primary tissue which can make either nervous tissue or skin). The monoclonal antibodies are being used as probes to show which ectoderm cells have made decisions as to whether they will become nerve cells or skin cells, long before they show any distinct morphological differences. We can now use the antibody probes to begin an analysis of how these ectoderm cells make the choice between skin or nervous tissue and how altered gravitational environments might affect the decision-making process.

Accomplishments

Previous studies have documented that the vegetal hemisphere cytoplasm (presumptive gut region) has the ability to induce the formation of chordamesoderm, which in turn induces the overlying ectoderm to become neural tissue instead of skin. We have shown that there are at least three events involved in establishing these patterning events.

(1) Cytoplasm is distributed differentially between the future dorsal (neural) and future ventral (epidermal) blastomeres. The differences in cytoplasmic content normally occur soon after fertilization and are highly susceptible to gravitational influences. The existence of these cytoplasmic differences can be monitored by the ability of isolated blastomeres to synthesize specific cell surface components which match the tissue specific monoclonal antibody probes we use as markers for neural or epidermal differentiation. Tipping (1 g alteration) or centrifugation (up to 30 g alterations) of the embryo significantly changes the position of cells that will express the cell specific markers.

(2) A second event involved in formation of the nervous system occurs at the beginning of gastrulation (the period of cell rearrangement prior to the formation of the nervous tissue). A set of cells at the mid-line of the future neural plate sends a chemical signal through the ectoderm which effectively sets the pattern for future brain and spinal cord development. We have developed an assay for this chemical signal and have begun to characterize the signal and how it operates. The source of cells that ultimately sends the signal, thus establishing the pattern for future brain development, is easily affected by gravitational forces earlier in development.

(3) We have found that ***embryos subjected to higher gravitational stresses,*** sufficient to alter the position of the nervous system, ***have the ability to regulate under some situations, producing a normal neural pattern as assayed at the molecular level.*** We are examining the ability of embryos to compensate for environmentally induced stress in greater detail. To date, we have determined when neural patterns may be altered by changes in the gravitational environment

and when regulation occurs to restore the normal pattern of neural development. We have also begun to study what part of the embryo controls this mechanism.

Significance of the Accomplishments

The experiments discussed above help us to understand how neural development normally ocurrs within the confines of Earth's gravitational influences. Once we have the necessary probes and have characterized the normal process, we will be in a position to study how animal development outside of the Earth's influence might occur differently. We already know that altered gravity (position or magnitude) influences the position of the final neural pattern, but until molecular probes are used, we will not know which of the many early steps essential for final patterns are being affected. We have also shown that embryos have the ability to monitor their own developmental progress and can, in some instances, correct for mistakes due to adverse environmental conditions, such as altered gravity. This finding would not have been possible without the use of these early tissue-specific monoclonal antibody probes. Therefore, we are now in a position to study the built-in mechanisms embryos have evolved to correct for developmental mistakes due to environmental stress. The corollary to this idea is that we will also begin to understand how much environmental stress embryos can be subjected to and exactly where the correction mechanisms are failing. This will become especially important as we venture further into space for longer periods of time.

Publications

Phillips, C.R. Molecular Analyses of the Gravitational Effects on Positioning the *Xenopus* Nervous System (Abstract). *ASGSB Bulletin* 1: 15, 1988.

BONE CELL KINETICS OF SIMULATED WEIGHTLESSNESS

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Description of Research

The osteogenic (bone-forming) components of bone modeling (change in size and/or shape) and probably remodeling (turnover of preexisting bone) are inhibited by decreased skeletal loading and/or microgravity. The long-range goals and objectives for this research are: (1) to define the cellular mechanism of osteoblast (bone- forming cell) production, and (2) determine how this process is suppressed in microgravity. Spaceflight is the only experimental means known for probing the gravity dependence of osteoblast production.

The present cell kinetics research utilizes DNA labeling (^{3}H-thymidine), mitotic activity, and nuclear size as indices of the proliferation and differentiation aspects of osteoblast histogenesis (production of bone-forming cells). The central thrust of these studies is to determine the relative influence of gravity, mechanical loading, and physiological stress on osteoblast histogenesis in weight-bearing bones (tibia, ulna, etc.), a non-weight-bearing bone (maxillary molar periodontal ligament) and an antigravity postured bone (mandibular condyle).

Previous research in periodontal ligament (PDL), the osteogenic interface between tooth and bone, reveals that there are three kinetically and/or morphometrically distinguishable cell types in the osteoblast histogensis sequence: (1) self-perpetuating, less differentiated precursor cells (A type), (2) committed osteoprogenitor cells (A' type), and (3) preosteoblasts (C/D cells). The osteoblast (Ob) histogenesis sequence is A -> A' ==>C->D->Ob (Figure 1). The rate-limiting step (A'==>C) in differentiation of an osteoblast is associated with an increase in nuclear volume, immediately prior to the last proliferation event (each D cell divides and forms two osteoblasts). This morphological manifestation of change in genomic expression (differentiation) is an effective tool for assessing inhibition of bone formation during spaceflight and simulated weightlessness (SW). The nuclear volume assay of osteogenic activity and/or potential is applicable to all skeletal sites tested, i.e., PDL, tibial metaphysis, mandibular condyle, and mandibular periosteum.

Research conducted in the past year focused on further definition of the differentiation mechanism for osteoblast production at three skeletal sites: maxillary molar PDL, mandibular condyle (major growth site of the lower jaw bone), and secondary spongiosa of lumbar vertebrae. With respect to preosteoblast differentiation, the following principal questions were addressed: (1) Effect of 3 days of SW with the most recent version of the Morey-Holton unloading model in maxillary molar PDL? (2) Circadian rhythm of PDL preosteoblast cells following 3 days of SW? (3) Influence of 7 days of spaceflight on the osseous morphology and cell kinetics of osteogenic cells in the mandibular condyle of Spacelab-3 (SL-3) rats? (4) Morphological changes in lumbar vertebral secondary spongiosa of SL-3 rats? (5) Influence of 1 month of hypofunction (loss of opposing tooth) in rat PDL? (6) Influence of 1 month of hyperfunction (unilateral mastication) in rat molar PDL?

OSTEOBLAST HISTOGENESIS PATHWAY

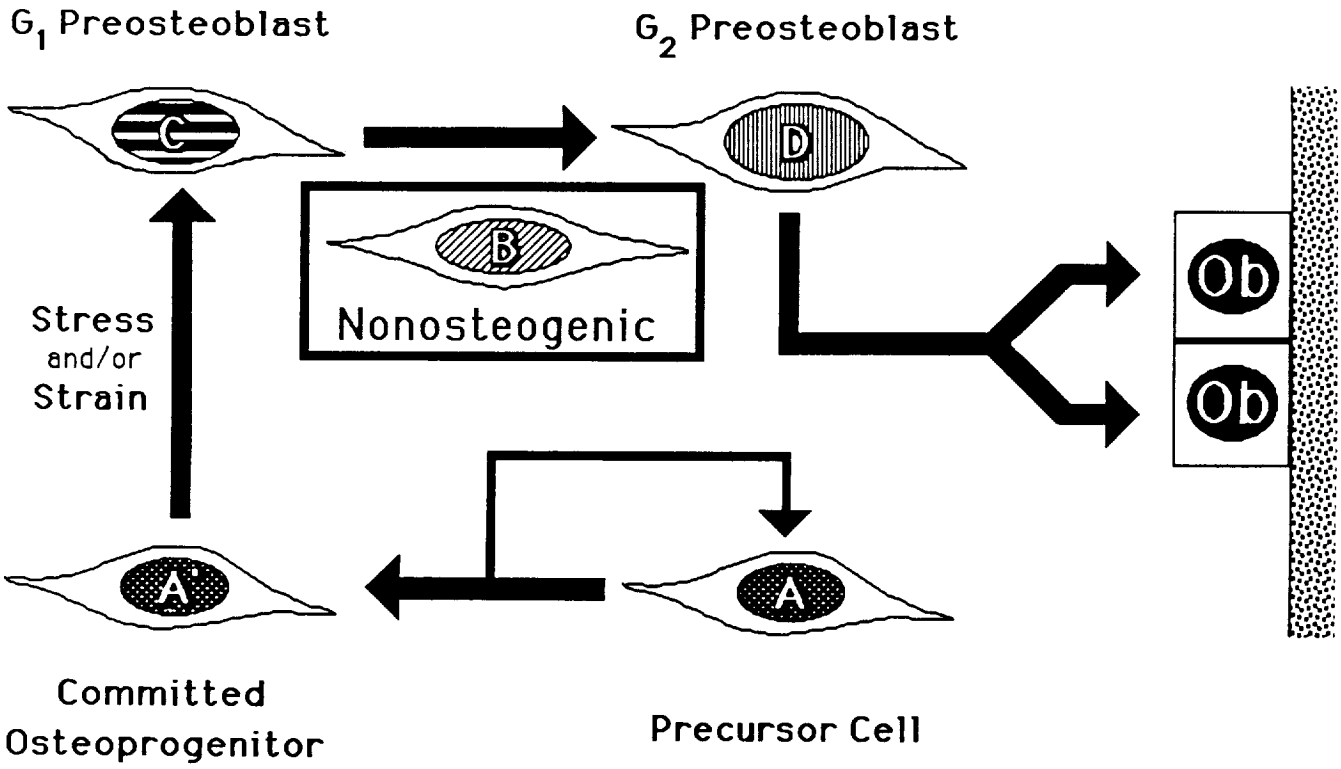

Figure 1. Schematic diagram of the osteoblast (Ob) histogenesis pathway, demonstrating the sequence of progressively more differentiated osteogenic cells (A, A', C, D). B cells are present in the same area as osteogenic cells but do not participate in osteoblast production.

Experiments were: (1) fifty-six 47-day-old male rats (half experimental and half control) were placed in simulated weightlessness using the Morey-Holton model and then examined at 9 am, 3 pm, 9 pm, and 3 am; (2) histomorphometric and nuclear volume analysis of the mandibular condyles and lumbar vertebrae from SL-3 rats (7 days of spaceflight) were conducted; and (3) twelve 49-day-old male rats were anesthetized and all right mandibular first molars extracted, rendering the maxillary right first molar hypofunctional and the maxillary left first molar hyperfunctional.

Accomplishments

(1) At both 9 am and 9 pm following 3 days of SW, A/A' cells (less differentiated precursors) were increased 16.5% ($p<.001$), while C/D cells (preosteoblasts) were decreased 22.0% ($p<.001$).

(2) At 3 am and 3 pm ***following 3 days of SW, there were no significant differences between SW and control animals.***

(3) Decreased cell density in zone 3 (21-30 μm from the nearest major blood vessel) was noted at 9 pm.

(4) Both ***SW and control groups demonstrated a circadian rhythm favoring increased numbers of preosteoblasts at 9 pm.***

(5) SL-3 vertebrae were not significantly different from controls with regard to percent trabecular bone, bone apposition following a preflight label, or percent labeled surface.

(6) ***First molars of all groups drifted distally and erupted occlusally:*** hypofunction > control > hyperfunction. Hypofunctional teeth also rotated medially.

(7) ***Unopposed, hypofunctional molars showed a 61% increase in physiological extrusion associated with an accelerated formation of apical bone, apical cementum, and interradicular bone.***

Significance of the Accomplishments

Finding #1: A relatively uniform inhibition of osteoblast production both in the morning (9 am) and at night (9 pm) is suggested. This effect appears to be mediated by suppression in the rate-limiting step (A'==>C) from a committed osteoprogenitor cell (A') to a G1 stage preosteoblast (C cell). These results are consistent with previous biochemical and morphometric data, indicating that 3 days of SW is sufficient for a suppression of preosteoblast formation.

Finding #2: No significant differences between SW and control animals are consistent with the circadian maxima and minima for preosteoblasts occurring at 9 pm and 9 am, respectively.

Finding #3: Decrease in cell density enhances differentiation to a preosteoblast. These data support the hypothesis that ***decreased preosteoblast production may result from a relative increase in cell density due to loss of extracellular fluid during spaceflight.***

Finding #4: Preosteoblast production is suppressed uniformly both day and night. Disturbance of the circadian rhythm does ***not*** appear to be the mechanism by which SW decreases the number of preosteoblasts.

Finding #5: A 7-day spaceflight is insufficient to elicit a significant change in gross morphology of lumbar secondary spongiosa.

Finding #6: The physiological drift pattern of maxillary molars within alveolar bone under a variety of mechanical conditions is documented. Knowledge of this process is ***important for distinguishing flight effects associated with microgravity, posture, dental attrition, and/or diet changes.***

Finding #7: The remarkable bone adaptation capability of the PDL which allows teeth to maintain a functional occlusion despite growth and mechanical challenges is demonstrated. ***The bone-labeling method used to document physiological drift over time will be useful for extended spaceflight studies*** (weeks to months).

Publications

Burk, D.R., Rosenoer, L.M.L., and Roberts, W.E. Aging and Circadian Rhythm of Cell Proliferation in Rat Molar Periodontal Ligament. *Anatomical Record* 218(1): 19-20A, 1987.

Noorda, C.B., Gonsalves, M.R., and Roberts, W.E. Temporally Related Modeling and Remodeling in Growing Cortical Bone of Adolescent Rabbits. In: *Program and Abstracts, 33rd Annual Meeting of Orthopaedic Research Society,* p. 188, 1987.

Roberts, W.E., Fielder, P.J., Rosenoer, L.M.L., Maese, A.C., Gonsalves, M.R., and Morey, E.R. Nuclear Morphometric Analysis of Osteoblast Precursor Cells in Periodontal Ligament, SL3 Rats. *American Journal of Physiology* 252: R247-R251, 1987.

Roberts, W.E., Fielder, P.J., Rosenoer, L., and Morey, E.R. Osteoblast Histogenesis of Weightbearing and Nonweightbearing Bones Exposed to Simulated Weightlessness. In: *Program and Abstracts, 33rd Annual Meeting of Orthopaedic Research Society,* p. 459, 1987.

Roberts, W.E., Jackson, C.J., Weinreb, M.M., and Morey, E.R. Gravitational Effects on Bone Cells (Abstract). In: *Abstracts, 9th Annual IUPS Commission on Gravitational Physiology,* Nitra, Czechoslovakia, p. 26, 1987.

Roberts, W.E., Turley, P.K., Brezniak, N., and Fielder, P.J. Bone Physiology and Metabolism: Implications in Dental Implantology. *California Dental Association Journal* 15(10): 54-61, 1987.

Roberts, W.E., Wood, H.B., Chambers, D.W., and Burk, D.T. Vascularly Oriented Differentiation Gradient of Osteoblast Precursor Cells in Rat Periodontal Ligament: Implications for Osteoblast Histogenesis and Periodontal Bone Loss. *Journal of Periodontal Research* 22: 461-467, 1987.

MAMMALIAN GRAVITY RECEPTORS: STRUCTURE AND FUNCTION

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Description of Research

The long-term goal of our research is to learn whether changes in otolith organs will occur in space and to determine the degree to which any changes remain static, advance, or decline over a 1-week period following return to Earth. Spaceflight offers us the only empirical opportunity to examine this system without its primary stimulus, gravitational acceleration. However, ground-based research has already begun, using transmission electron microscopy and computer assisted reconstructions, that will lead to development of computer models of functioning maculas. The results of such a combined effort should lead to further insights into how the system adapts to a new environmental stimulus in space and on Earth.

Currently, 3-D reconstructions are based upon sections that are photographed in the transmission electron microscope. The photographs are put together into montages that display each section in its entirety. Tracings of nerves of interest, together with the hair cells synapsing with their calyces (a receptive field), are made on acetate and then are digitized with the aid of a bit pad into a PC computer. The hair cell receptive fields and nerves are reconstructed with the aid of software developed at the University of Colorado (Kinnamon, 1987). The data are transferred from the PC to a high-performance graphics workstation, which has been programmed for reconstruction of the images as solids. From these reconstructions we have been able to produce motion pictures and video recordings of animated receptive fields.

Accomplishments

Previous reconstructions have shown that three major types of receptive fields occur from the striola to the borders of the anterior portion of the utricular macula. No two fields are identical, but the differences in receptive field architectures are continuous and range from compact and rounded near the striola to nearly linear arrangements at the borders. The receptive fields are linked together by type II hair cells to form a complex neural network, a part of which is shown symbolically in Figure 1. The type II hair cells distribute information to neighboring calyces. Figure 2 shows four U-type nerves near the macular border that are not only linked, but intertwined.

The ***results described above support the concept that the maculas are organized for parallel distributed processing of information.*** The findings, taken together with others obtained from maps of more than 125 hair cells of the network, show that ***no two receptive fields are identical.*** It appears that both randomness and redundancy may characterize the macular neural network, making it a robust and highly adaptive system. The results can be interpreted in light of known physiology, and stimulate thinking in terms of how messages are coded by nerve arrays in the network.

Macular research in space and on the ground, then, is particularly important to NASA because the bioaccelerometers appear to be plastic and highly adaptive to new environments such as microgravity. Current research suggests that ***as the macula matures, certain circuits begin to emerge so that there are identifiable, largely repeatable responses to specific kinds of input. Weighting within the neural network***

changes between the non-functional and functional stages to permit this kind of self-organization. The circuits continue to respond in their learned way until a perturbation occurs, such as microgravity. ***Then, after initial disarray, the neural elements readjust their weights, and the new circuitry can be said to have adapted to the new situation.*** This means that maculas are excellent candidates for study of neural adaptation to altered gravitational environments.

A further part of our research is the ***development of a new computer-based procedure that will generate 3-dimensional reconstructions from serial sections imaged in the electron microscope.*** Software is being developed that will remove the need for the time-consuming production of montages and of acetate tracings, and will ease the digitizing of tracings. When complete, the system will allow a user to capture images on a PC-AT clone directly off an electron microscope and store them either digitally on magnetic medium or in analog fashion on an optical disk. From there, the images will be assembled into montages on a more sophisticated image-processing computer, at which a user will trace features on the digital equivalent of an acetate sheet. The resulting data will be transferred to a high-performance graphics workstation on which solid reconstructions and animation scripts will be produced. This system of distributed computers will be the target of on-going development, to bring it to the point that it can generate not only a geometric, but a functional model of the macula on which simulations can be run.

Publications

Meyers, S.F. and Ross, M.D. Morphological Evidence of Vestibular Pathology in Long-term Experimental Diabetes Mellitus. II. Connective Tissue and Neuroepithelial Pathology. *Acta Otolaryngology* 104: 40-49, 1987.

Pote, K.G. and Ross, M.D. Ultrastructural Morphology and Protein Content of the Internal Organic Material of Rat Otoconia. *Journal of Ultrastructural Research* 95: 61-70, 1987.

Ross, M.D., Cutler, L., Meyer, G., Vaziri, P., and Lam, T. 3-D Imaging of Neural Networks in Preparation for Space Flight (Abstract). *ASGSB Bulletin* 1: 17, 1988.

Ross, M.D., Donovan, K., and Rogers, C. Peripheral Sensory Processing in Mammalian Gravity Receptors: Observations of Ciliary Tuft Configurations. In: *The Vestibular System: Neurophysiologic and Clinical Research* (ed. by M.D. Graham and J.L. Kemink). New York: Raven Press, 1987.

Ross, M.D., Komorowski, T.E., Rogers, C.M., Pote, K.G., and Donovan, K.M. Macular Suprastructure, Stereociliary Bonding and Kinociliary/Stereociliary Coupling in Rat Utricular Macula. *Acta Otolaryngology* 104: 56-65, 1987.

NEURAL NETWORK ARRANGEMENT OF RAT UTRICULAR MACULA

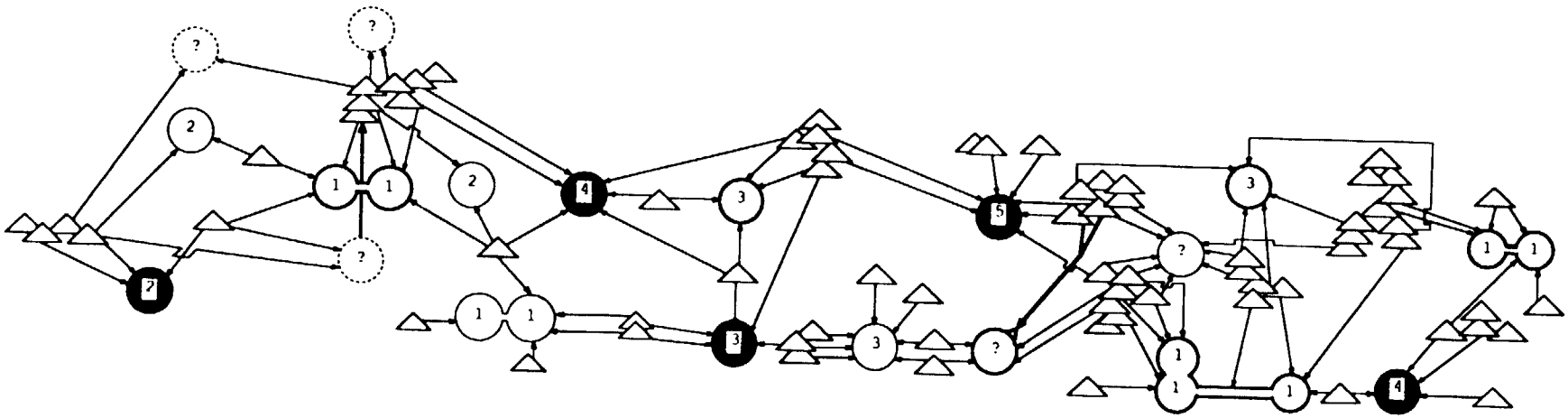

Figure 1. Symbolic diagram of the information flow and cell grouping in one part of the rat utricular macula.

NERVE CELLS OF MACULAR RECEPTIVE FIELDS

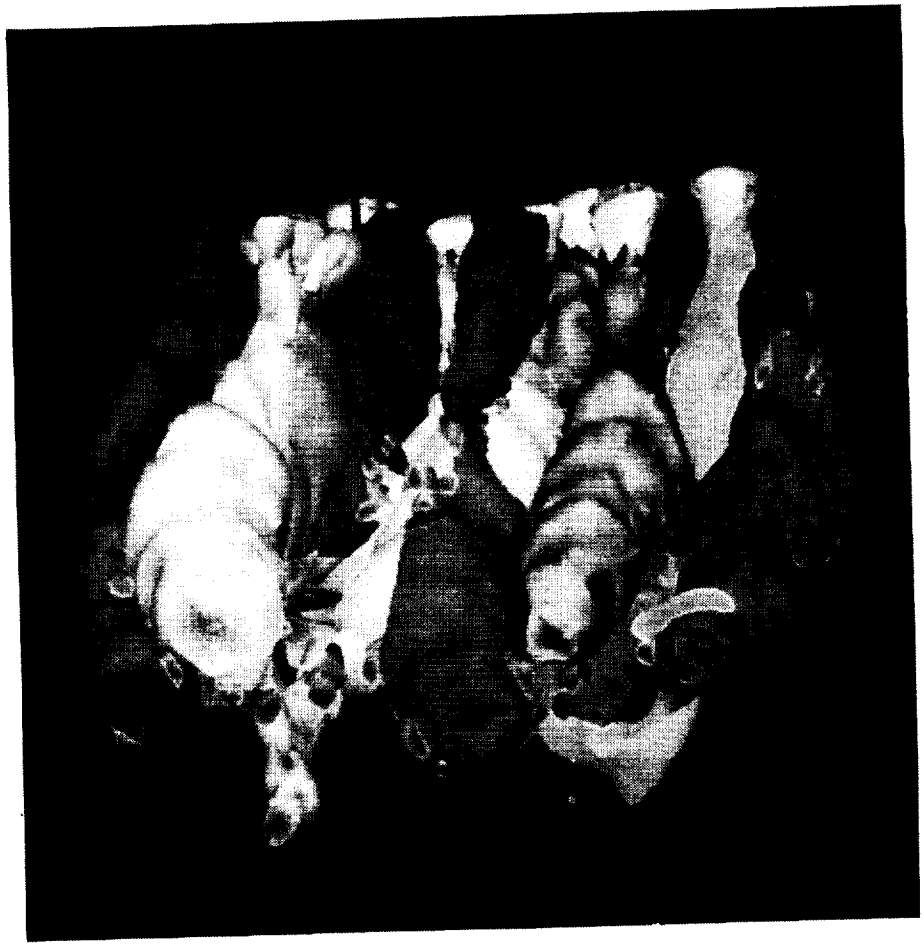

Figure 2. Solid reconstruction of the calyces and type I cells of linked macular receptive fields.

EFFECTS OF WEIGHTLESSNESS ON *AURELIA* EPHYRAE DIFFERENTIATION AND STATOLITH SYNTHESIS

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Description of Research

The long-range goals of the research are: (1) to discover the role(s) of gravity on the behavior and the development of *Aurelia* ephyrae (tiny jellyfish) and on their graviceptor structures (rhopalia), and (2) to discover the effects of microgravity on ephyrae and rhopalia development after the short-term (7-day Space Shuttle flight) and long-term (Space Station and Biosatellite) exposure to a microgravity environment.

Specific objectives are: (1) To determine whether the microgravity of space will modify: the development of ephyrae from polyps; the development of the graviceptors of ephyrae; the formation or demineralization of statoliths of rhopalia; and the swimming/pulsing behavior of ephyrae; (2) To compare the features listed above in ephyrae that developed in space with those that developed on Earth; to discover the role that gravity plays in the development of ephyrae, their graviceptors, and their behavior; and (3) To prepare the Jellyfish Experiment for a 7-day Space Shuttle flight (SLS-1).

Our research included the following:

(1) ***SEM studies of Aurelia rhopalia:*** A detailed study of *Aurelia* graviceptor development was undertaken using the scanning electron microscope (SEM) to determine whether very early surface changes in developing rhopalia could be discerned. Tiny polyps were induced to metamorphose (strobilate) using iodine, giving rise to ephyrae (young medusea) with graviceptors. Distal segments of strobilae and nonstrobilating polyps were viewed oral side up with the SEM. General morphology and cilia length were compared between segments at 24, 48, 72, and 120 hr (mature ephyrae) following metamorphosis induction. Emphasis was placed on tracing the location and quantity of sensory cilia believed to be associated with mechanosensory cells (which resemble hair cells in higher animals). Cilia of these cells are shorter than are the cilia of the epidermal cells of the polyps. A few of these sensory cilia were found among the polyp cilia (even before rhopalia formation), as early as 24 hr after iodine treatment. Sensory cilia develop in increasing number as the ephyrae rhopalia mature. Rhopalia begin as round protuberances at the base of the swollen tentacles and elongate as they mature. Surface morphology of the rhopalia differs from the morphology of the tentacle base from which they arise.

(2) ***Cloning of polyps for normal arm numbers:*** Several years ago, selected polyps were cloned (through isolation from the rest of the cultures) because they had given rise to ephyrae with normal (8) arm numbers. These polyps budded and multiplied into clones of sufficient number to be periodically tested to determine whether the arm number of eight is persistently maintained. We found that these animals continue to have a higher percentage of eight arms than polyps from the general population.

(3) ***Chemical Delivery System:*** The testing of the Chemical Delivery System (see *1986-87 NASA Space/Gravitational Biology Accomplishments*) was completed this year.

Accomplishments

(1) The surface morphology of the developing rhopalia of ephyrae was determined and it was found that ***cilia length can be used to trace the location and time of formation of the mechanosensory (hair) cells of the rhopalia of the jellyfish.*** Sensory cell cilia were found to induce metamorphosis as early as 24 hr after iodine administration and increased in number as the rhopalia formed. Rhopalia surfaces and shapes were very different from polyp tentacle surface morphology and shape.

(2) Periodic testing of animals cloned for normal arm number revealed that this feature persists over a period of several years in the asexual progeny.

(3) The testing of the Chemical Delivery System for delivery of chemicals to polyps during the SLS-1 Space Shuttle flight was completed.

Significance of the Accomplishments

Finding #1: An understanding of the surface morphology of developing rhopalia will enable rhopalia that are immature to be distinguished from those that may be abnormal following ephyra development in the microgravity environment of the SLS-1 Space Shuttle flight. In addition, this study enhances our understanding of the time period in which the mechanosensory cells develop and hence the time period of their greatest vulnerability to environmental stress.

Finding #2: Jellyfish polyps have never before been cloned to give rise to ephyrae with specific morphological features. The continuing high yield of ephyrae with eight arms in cloned organisms establishes that this approach is practical for developing normal animals for the flight experiment and indicates that this method could be used to clone for other features of ephyrae for future research purposes. This response of the animals also establishes the fact that the animals are capable of maintaining a high level of morphological integrity over a long period of time through asexual reproduction.

Finding #3: The successful completion of the testing of the Chemical Delivery System for biocompatibility insures the use of this system for the jellyfish experiment on SLS-1 and will possibly lead to its use in future experiments involving other small aquatic organisms.

Publications

Oman, C. and Spangenberg, D. Gravity Dependent Behavior of *Aurelia aurita* Ephyrae (Abstract). In: *Space Life Sciences Symposium: Three Decades of Life Science Research in Space,* Washington, D.C., June 21-26, p. 55-56, 1987.

Rossberg, K. and Spangenberg, D. A Self-contained Inducement/Fixation System for Small Aquatic Organisms (Abstract). In: *Space Life Sciences Symposium: Three Decades of Life Science Research In Space,* Washington, D.C., June 21-26, p. 57, 1987.

Spangenberg, D.B. and Davis, S. Stem Studies of *Aurelia* Rhopalium Development During Ephyra Formation (Abstract). *ASGSB Bulletin* 1: 16, 1988.

SKELETAL MUSCLE METABOLISM IN HYPOKINETIC RATS

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Description of Research

Our research concerns the mechanisms of atrophy and metabolic alterations associated with lack of load-bearing (unloading), as may occur with prolonged bedrest or weightlessness. To further understand this model of muscle wasting, comparisons are made with measurements made for a different model of muscle wasting, denervation, which is characterized by an interrupted nerve supply to the muscle. Our work has dealt with two aspects of muscle metabolism, carbohydrate metabolism and protein metabolism. For both systems the research has focused on the time course for the changes in these processes with unloading and/or reloading.

For carbohydrate metabolism, we conducted a thorough study of recovery of glycogen synthase and glycogen phosphorylase for 15 min to 3 days of reloading after 3 days of unloading. Not only have we measured activity ratios (i.e., activity in the absence or presence of activator) but also the physiological activity (i.e., activity at the physiological concentration of the activator). Protein metabolism studies included measurement of *in vivo* protein synthesis and protein degradation at 24 hr intervals from 1 to 6 days of unloading, with or without stretch, and of denervation.

Accomplishments

(1) **Protein metabolism** ***in vivo***

(a) ***A decline in protein synthesis contributes more to atrophy of the unloaded soleus than does acceleration of protein degradation in the first 48 hr.***

(b) ***Accelerated protein degradation is a major contributor to unloading atrophy between 48 and 72 hr.***

(c) ***The rate of atrophy due to unloading declines markedly after 72 hr because protein degradation falls to 70% of the control rate.***

(d) ***Stretching does not appear to prevent unloading atrophy per se.*** Instead, during the initial 48 hr when atrophy is relatively slow, the muscle undergoes significant hypertrophy. Thereafter unloading prevents continued hypertrophy, with the muscle undergoing a marked growth reduction. ***Hence, unloading can still influence growth of the stretched muscle.***

(e) Denervation atrophy exceeds unloading atrophy and shows its biggest response from 24 to 48 hr.

(f) Accelerated protein degradation may be a bigger contributor to denervation atrophy than to unloading atrophy.

(2) **Carbohydrate metabolism**

(a) ***With reloading, changes in glucose-6-phosphate parallel the variations in glycogen content.*** Glycogen declines within only 15 min after reloading and reaches a minimum concentration at 2 hr of reloading a 3-day unloaded soleus. Glucose-6-phosphate concentration doubles by 15 min after reloading and thereafter declines to normal levels at 4 hr. It then increases again between 12 to 48 hr and subsequently returns to normal by 72 hr of reloading.

(b) Reloading has little effect on the concentration of AMP, an important activator of glycogen phosphorylase.

(c) Total activity of glycogen synthase is not affected by unloading, but the "physiological" activity varies with concentration of glucose-6-phosphate, an allosteric activator.

(3) **Growth**

Feeding rats an antagonist of glucocorticoid (stress hormone) binding abolishes the small difference in growth between unloaded and control animals.

Significance of the Accomplishments

Finding #1: Finding 1c suggests that the unloaded muscle and possibly denervated muscle slows its rate of atrophy by reducing protein degradation. It will be important to determine this mechanism so that we can understand how the muscle may protect itself. However, to completely prevent unloading atrophy it is necessary to understand the mechanism causing slowing of protein synthesis (Finding #1a). The data with stretched muscle (Finding #1d) showed that stretch alone can prevent only some effects of unloading.

Finding #2: The primary response to reloading likely occurs at glycogen phosphorylase (experiments in progress), since changes in glycogen synthase seem to be secondary. Altered concentrations of its activator, glucose-6-phosphate, due to prior changes in glycogen turnover seem to be a primary factor determining its activity.

Finding #3: As we have suggested before, the slightly slower growth of the unloaded animals is likely a stress effect due to increased plasma glucocorticoids.

Publications

Henriksen, E.J. and Tischler, M.E. Recovery of Glycogen Following Short-term Unloading of the Rat Soleus Muscle (Abstract). *ASGSB Bulletin* 1: 23, 1988.

Henriksen, E.J. and Tischler, M.E. Time Course of the Response of Carbohydrate Metabolism to Unloading of the Soleus. *Metabolism* 37: 201-208, 1988.

Jaspers, S.R. and Tischler, M.E. Insulin Effect on Amino Acid Uptake by Unloaded Rat Hindlimb Muscles. *Hormone and Metabolic Research* 20: 125-126, 1988.

Kirby, C.R., Henriksen, E.J., Cook, P., and Tischler, M.E. Metabolic Response of Skeletal Muscle to Isoproterenol Following Unloading or Denervation (Abstract). *ASGSB Bulletin* 1: 23-24, 1988.

Morey-Holton, E. and Tischler, M. (Eds.). *NASA Workshop on Biological Adaptation.* Moffett Field: NASA Ames Research Center, 103 pp., 1988. (NASA TM-89468)

Satarug, S., Cook, P., and Tischler, M.E. Time Course of *In vivo* Protein Metabolism of the Unloaded Soleus and the Effect of Stretch (Abstract). *ASGSB Bulletin* 1: 23, 1988.

Tischler, M.E. Muscle Protein Degradation and Its Regulation. In: *Nutrition 87* (ed. by O.A. Levander). Beltsville: American Institute of Nutrition, pp. 135-140, 1987.

Tischler, M.E., Henriksen, E.J., Jacob, S., Satarug, S., and Cook, P. Problems in Analysis of Data from Muscles of Rats Flown in Space. *Physiologist* 31(Suppl.): S10-S13, 1988.

Tischler, M.E., Satarug, S., Cook, P., and Henriksen, E.J. Comparisons of *In vitro* Metabolic Responses in Unloaded Soleus (US) or Denervated Soleus (DS) of Rats (Abstract). *ASGSB Bulletin* 1: 23, 1988.

EFFECTS OF MICROGRAVITY ON THE STATOCYST OF *APLYSIA CALIFORNICA*

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Description of Research

The statocysts of molluscs are simple gravity receptors that offer advantages over the more complicated homologous organs in mammals for both physiological and anatomical study. In all gravity receptors, gravitational forces cause a dense test mass ("otoliths" or "statoconia") to deflect sensors (usually hairs or cilia) on sensory-receptor cells. In the mammalian gravity receptors, there are several thousand receptor cells and many thousand small calcium carbonate stones which make up the test mass. These organs are encased in the temporal bone, the densest bone in the body, making it very difficult to approach the organs without damaging them. In the statocysts of gastropod molluscs, including the sea hare *Aplysia,* there are only 13 receptor cells, each of which is much larger than the mammalian gravity-receptor cells. The number of stones ranges from 1, in newly hatched animals, to a maximum of 1000 in adults. The number of stones, their form and composition, as well as changes in these parameters during development, can be studied quantitatively in the molluscs.

We know very little about the development of the otoliths in mammals or about how their development might be affected by the absence of gravity. Due to the relative simplicity and accessibility of the molluscan statocysts, we are using this preparation to study the development of gravity receptors. The *Aplysia* statocyst is basically a sphere whose wall is made up of the 13 receptor cells, with small supporting cells separating them. The center of the sphere is filled with fluid and the statoconia (Figure 1). As the statoconia fall, they interact with the cilia on the surface of those receptor cells at the bottom of the cyst. The receptor cells then generate action potentials which are propagated along their nerve fibers to the central ganglia. These signals tell the animal which direction is "down." The statoconia appear to be generated in either the receptor cells or the supporting cells, and are then ejected into the fluid-filled center of the cyst. In adult animals, the statoconia have a complicated structure, made up of successive layers of membrane and calcification. It is not known whether additional layers are added after the statoconia are in the cyst lumen. Our earlier data suggest that the calcium carbonate is in the aragonite form, rather than the calcite form found in mammalian otoconia.

The fact that the statoconia are generated either within the receptor cells or the adjacent supporting cells suggests that the metabolic activity in the receptor cells could influence the production of stones. Thus, in the weightless condition of space, where there would be no gravitational stimulus, the production of stones could be either accelerated, in an attempt to make up for the lack of stimulus, or could be turned off due to a lack of interpretable neural signals, or could remain unchanged, independent of gravitational stimulus. If the rate of generation of stones or their form is significantly altered in space, this would have profound implications for the ability of specimens, or perhaps even humans, reared in space to function when later brought back to the normal gravitational environment of Earth. Thus, our plan is to first study the normal development of the *Aplysia* statocyst on Earth, and then see how this development is altered by periods in the weightlessness of space at different stages of maturation.

PROPERTIES OF MOLLUSCAN STATOCYST

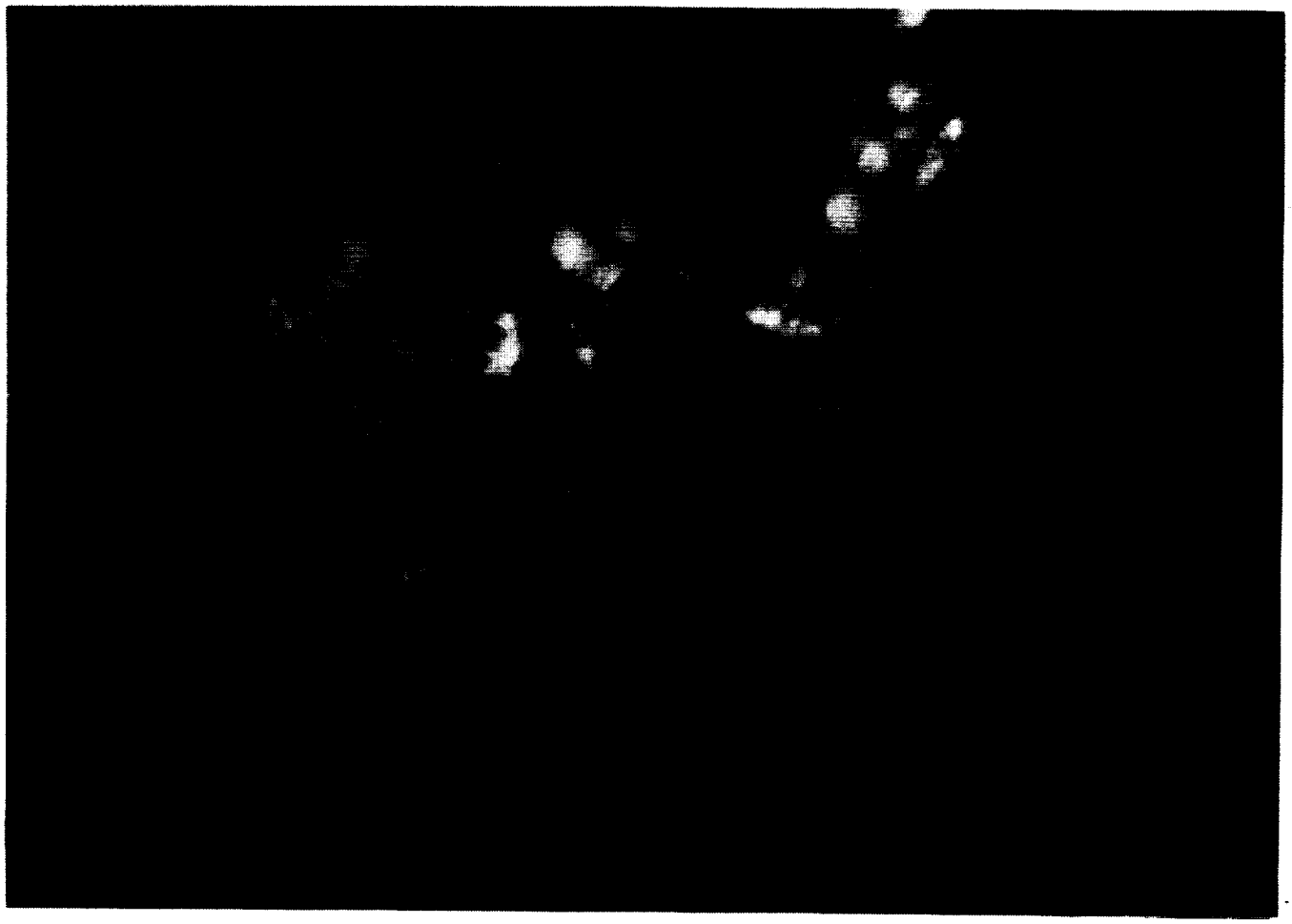

Figure 1. Photomicrograph of an *Aplysia* statocyst, using combined Nomarski and polarized-light microscope (1 micron section). Portions of two receptor cells, with their cilia projecting into the cyst lumen, are seen at the bottom of the figure. The statoconia appear brightly colored due to the birefringence of the calcium carbonate mineral phase. The full width shown in the micrograph is 150 μm.

Accomplishments

(1) We have established a refrigerated marine aquarium system in which we can maintain specimens of *Aplysia* ranging from 5 to several hundred grams weight. A separate room-temperature sea-water system is used for growing red kelp, on which the animals feed.

(2) Techniques have been established to flatten a statocyst preparation on a microscope slide to facilitate counting the statoconia.

(3) ***In our initial series of 20 animals, in which we are confident of the statoconia counts, the number of stones appears to grow linearly with the animal's weight.***

(4) ***In a subset of animals, in which the date of hatching from eggs is documented, no correlation is seen between the number of statoconia and the age of the animal.***

Significance of the Accomplishments

Our preliminary results, showing that the number of statoconia increases with animal weight, but not with age, per se, has important implications for the normal development of the statocyst. When maintained in an aquarium, *Aplysia* will eat more than their full weight of kelp every day, if given the opportunity. Since our specimens were fed different amounts, there is not a direct relationship between their age and their weight. The finding that the number of statoconia increases with weight indicates that stone production is related to whole-body metabolism; the number of stones produced is proportional to the amount of food the animals metabolize. This is consistent with the hypothesis that statoconia production in animals maintained in space will be different from that in ground-based controls, since the rate of metabolism in the gravity-receptor cells of the statocyst should be significantly modified in a weightless environment.

Publications

Wiederhold, M.L., Sheridan, C.E., and Smith, N.K.R. Statoconia Formation in Molluscan Statocysts. *Scanning Electron Microscopy* 2: 781-792, 1986.

EFFECTS OF SIMULATED MICROGRAVITY ON MAMMALIAN DEVELOPMENT AND EXPRESSION OF CALCIUM BINDING PROTEINS

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Description of Research

The long-range goal of our research has been to assess the effects of altered gravitational environments on mammalian development and differentiation. Our research has focused on examining the effects of a simulated microgravity environment on the development and differentiation of mammalian germ cells and early embryos. We previously showed that mouse oocytes rotated on a clinostat exhibited anomalies of the meiotic maturation process. Oocytes rotated at 100 RPM in an axis perpendicular to the gravity vector (the experimental rotation axis) were inhibited in the rate with which they achieved the metaphase II stage of meiosis, whereas oocytes rotated in the control orientation were not inhibited. In subsequent series of experiments, ova were rotated in the presence of sperm, thereby addressing the question of whether or not fertilization was sensitive to the effects of simulated microgravity. No alterations in the efficiency of fertilization were noted. Although the zygotes appeared normal, as judged by gross morphological criteria, the developmental potential of such rotated zygotes needs to be investigated. We have recently extended these studies to include examination of very early embryos. A recent modification of the experimental system involves embedding the embryos in low melting point agarose in order to immobilize them at the axis of rotation. This obviates the effects of the movements of rotating cells in the liquid suspension that might be elicited in this system. We have now begun to use sensitive molecular markers of normal development to evaluate the effects of our experimental system on early development. This will be useful not only for studying directly the effects of altered gravitational fields on mammalian development, but also for evaluating biological effects on cells flown in space. In particular, our initial studies have focused on examining the expression of heat shock or cellular stress genes.

Accomplishments

(1) ***Subcellular events in mammalian cells may be affected by simulated microgravity.*** That is, reorientation of mammalian oocytes relative to the gravitational vector through the use of a clinostat, affects meiotic maturation. Oocytes rotated at 100 RPM in the experimental axis revealed an inhibition of achieving metaphase II of meisois, whereas oocytes rotated in the control orientation did not. This may reflect inhibition of normal chromosome orientation and movement.

(2) ***No abnormalities in the appearance of fertilized ova or in the efficiency of fertilization have been observed in ova which were rotated at 100 RPM at the time of fertilization.*** In these experiments, ova which had undergone meiotic maturation *in vivo* were placed with capacitated sperm in the clinostat rotation system. The ova were rotated at 100 RPM (the speed at which meiotic abnormalities had been observed) for 8 hr and were examined for the presence of pronuclei and for any morphological abnormalities. Although the ova appeared normal and fertilization rates were similar between the experimental and control systems, it will be of

interest to evaluate more fully the developmental potential of such rotated ova. We are currently developing the protocols for reimplanting *in vitro* fertilized zygotes into pseudopregnant foster mothers.

(3) A modification of the culture system has been incorporated in our recent studies on ***the effect of simulated microgravity on early embryogenesis.*** In these experiments, embryos are embedded on low melting point agarose and immobilized at the center of the axis of rotation. With these conditions and rotation at 100 RPM, ***development up to the blastocyst state has been achieved.*** These embryos will now be removed from the culture system and allowed to implant in the uteri of foster mothers in order to assess normal development, as noted above for the *in vitro* fertilization experiments.

(4) An effort has been initiated to ***develop sensitive molecular markers for normal development.*** This will ***enhance the ability to analyze experiments using the clinostat and,*** most importantly, will greatly enhance the ***potential analysis of space-flown tissues.*** The molecular markers we have considered are ***the genes for the cellular stress proteins (also called heat shock proteins), using detection methods at the level of RNA and protein.*** Specifically, individual embryos can be processed and RNA can be detected using *in situ* hybridization.

Significance of the Accomplishments

Finding #1: The observation of an effect on a division process of cells under conditions of reorientation relative to the gravity vector is significant at several levels. First, an inhibition of normal chromosome disjunction would affect the developmental potential of the ova. Second, these observations are of particular interest because of the observations of other investigators on the effects on mitotic divisions in cells which have been flown in space.

Finding #2: The observation of apparently normal fertilization under clinostat rotation suggests that formation of the male and female pronuclei is not sensitive to reorientation relative to the gravity vector.

Finding #3: Our use of the cells in the immobilized situation has considerably strengthened the arguments in favor of using the clinostat as a model for simulating microgravity. That is, we are able to reduce the motion of the rotating cells as a factor in interpreting our results.

Finding #4: Sensitive indicators of normal development and differentiation are badly needed for assessing the effects of altered gravitational environments on cells. Our studies on the expression of the cellular stress protein genes will be very useful for analyzing both ground-based and flight studies. In particular, we will be able to make maximum use of the very precious flight tissue to examine the possible effects of altered gravity or other biological stresses.

Publications

Mutter, G.L., Grills, G.S., and Wolgemuth, D.J. Evidence for the Involvement of the Proto-oncogene c-*mos* in Mammalian Meiotic Maturation and Possibly Very Early Embryogenesis. *The EMBO Journal* 7: 683-689, 1988.

Wolgemuth, D.J. and Grills, G.S. Early Mouse Development Using Experimental Conditions Immobilizing Cells and Rotating Them Relative to Gravity Vector (Abstract). *ASGSB Bulletin* 1: 14, 1988.

Zakeri, Z.F., Welch, W., and Wolgemuth, D.J. Expression of Heat Shock Proteins during Mammalian Development (Abstract). In: *UCLA Symposium on Cellular Stress Proteins*, 1988.

Zakeri, Z.F. and Wolgemuth, D.J. Developmental-Stage-Specific Expression of the hsp70 Gene Family during Differentiation of the Mammalian Male Germ Line. *Molecular Cell Biology* 7: 1791-1796, 1987.

SPECIAL ACTIVITIES

SPACE BIOLOGY RESEARCH ASSOCIATES PROGRAM

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The Space Biology Research Associates Program provides a unique opportunity to train individuals to conduct biological research in areas relevant to NASA's interest. To maximize the potential for Space Biology as an emerging discipline, there is a need to develop a cadre of scientists interested in working in this area. This grant was developed to train biologists by offering Research Associate Awards to young scientists. These grants provide opportunities to work on projects directly related to Space Biology and in laboratories that provide the necessary facilities and a suitable research environment. It is anticipated that these scientists will develop research careers in the evolving discipline of gravitational biology, a focused area of Space Biology. The field of gravitational biology is rapidly growing and its future will reflect the quality and training of its scientific personnel.

The program began on June 1, 1980, with funding to support several Research Associates each year. To date (April 1988), 61 annual awards have been made. There have been 37 awardees, of whom 24 have received a second year of funding. On April 23, 1988, the Review Panel recommended funds for eight more awards. The subsequent list of awardees illustrates the variety of projects, laboratories, and institutions to which the 37 Research Associates have been assigned. These scientists represent different disciplines including zoology, developmental biology, botany, and physiology (animal and plant). In June 1980 there were 19 laboratories participating. Presently (April 1988) there are 51 laboratories in the program.

Many of the Research Associates have been asked to participate in NASA panels, national workshops, and national meetings. There have been 102 publications in refereed journals and as many abstracts of papers presented at national and international meetings. Each year, in the fall, the Research Associates are requested to attend the annual meeting of the American Society for Gravitational and Space Biology (ASGSB). The Research Associates are an integral part of that meeting, presenting papers and posters along with their senior colleagues. All of the current Research Associates and many of the former Research Associates are members of ASGSB. Twelve Research Associates were present at the 1987 meeting held in Logan, Utah. Research Associates are also encouraged to participate in other national meetings in their own disciplines.

RESEARCH ASSOCIATE AWARDEES

The awardees are listed alphabetically, including their award term in parentheses, their host laboratory, research title, and current location.

DR. SARA-ELLEN BARSEL (6/1/87-5/30/88) is working on "Molecular and Genetic Phototropism in *Arabidopsis thaliana*" in Dr. Kenneth Poff's laboratory at Michigan State University, East Lansing, Michigan.

DR. MICHAEL BINDER (1/1/83-12/30/83) worked on "Congenital Heart Malformations and Situs Inversus" in Dr. W.M. Layton, Jr.'s laboratory at Dartmouth Medical School. He is now on a research fellowship in the Pathology Department at Brown University, Providence, Rhode Island.

DR. THOMAS BJÖRKMAN(10/1/86-9/30/88) is working on "The Mechanism of Gravity Sensing in Plants" in Dr. Robert Cleland's laboratory at the University of Washington, Seattle, Washington.

DR. STEVEN BLACK (7/1/82-6/30/84) worked on "Determination by Gravitational and Centrifugal Force of the Amphibian Dorsal-Ventral Axis" in Dr. Raymond Keller's laboratory at the University of California, Berkeley. He is continuing research with Dr. Keller and is also working with Dr. Kenneth Souza at NASA Ames Research Center, Moffett Field, California.

DR. HARRY BLAIR (7/1/84-6/30/86) worked on "Cellular Mechanisms of Bone Degradation" in Dr. Steven Teitelbaum's laboratory at The Jewish Hospital/Washington University Medical Center, St. Louis, Missouri. He is continuing to work in Dr. Teitelbaum's laboratory funded by an NIH Physician Scientist Training Grant.

DR. THOMAS BROCK (8/1/86-7/30/88) is working on "Comparison of Changes in Protein Synthesis Induced by Gravity and Auxin Treatment in Pulvini and Coleoptiles of Oat (*Avena sativa* L.)" in Dr. Peter Kaufman's laboratory at the University of Michigan, Ann Arbor, Michigan.

DR. JAY BUCKEY, JR. (7/1/82-6/30/84) worked on "2-D Echocardiography as an Accurate Means for Measuring Left Ventricular Volume and Central Venous Pressure during Zero-gravity" in Dr. C. Gunnar Blomqvist's laboratory at the University of Texas Health Sciences Center, Dallas. At the present time he is the project manager for the cardiovascular experiment scheduled on Spacelab-4 and a Research Assistant Professor/Instructor in Clinical Medicine at the University of Texas Health Sciences Center, Dallas, Texas.

DR. GEORGE H. BURROWS (7/1/81-6/30/83) worked on "Studies of Synaptogenesis" in Dr. Marshall Nirenberg's laboratory at NIH, Bethesda, Maryland. He is now on the staff of the National Heart, Lung, and Blood Institute, Bethesda, Maryland.

DR. DENIS CLOHISY (7/1/86-6/30/87) worked on "Mechanisms of Osteoclast Precursor Differentiation" in Dr. Steven Teitelbaum's laboratory at The Jewish Hospital/Washington University Medical Center, St. Louis, Missouri. He is now completing his clinical training in Orthopaedic Surgery at the University of Minnesota, St. Paul, Minnesota.

DR. MARK COOPER (1/1/85-12/30/86) worked on "Osteoporosis of Weightlessness and the Electrophysiology of Bone" in Dr. John Miller's laboratory at the University of California at Berkeley, California. He is now a Research Associate in the Department of Molecular Neurobiology at Yale Medical School, New Haven, Connecticut.

DR. MARK DESROSIERS (7/1/86-6/30/88) is working on "A Search for Voltage-gating of Plant Hormone Transport Channels" in Dr. Robert Bandurski's laboratory at Michigan State University, East Lansing, Michigan.

DR. J. DAVID DICKMAN (6/1/87-5/30/88) is working on "High Frequency Response Properties of Semicircular Canal Fibers" in Dr. Manning Correia's laboratory at the University of Texas, Galveston, Texas.

DR. JOHN S. GARAVELLI (1/1/82-4/30/82) worked on "Chemical Characterization of Volatile Products of Algal Cell Cultures" in Dr. Franklin Fong's laboratory at Texas A&M University. He is now working for the Extraterrestrial Research Division at NASA Ames Research Center, Moffett Field, California.

DR. JOHN GAYNOR (1/1/81-12/30/82) worked on "Purification and Characterization of Amyloplasts from *Pisum sativum*" in Dr. Arthur Galston's laboratory at Yale University. He is now an Assistant Professor and Henry Rutger's Scholar in the Botany Department at Rutgers University, Newark, New Jersey.

DR. STEVEN GLOTZBACH (1/1/84-12/30/84) worked on "Neurophysiological Studies of Circadian Rhythm Control Mechanisms" with Dr. H. Craig Heller at Stanford University and Dr. Charles A. Fuller at the University of California, Riverside. He is continuing to work in Dr. Heller's laboratory funded by NIH-NIRA, Palo Alto, California.

DR. CHERYL GOULD (7/1/84-8/30/85) worked on "Effect of Weightlessness on Various Immunological Functions Using a Murine Simulated Space Flight Model" in Dr. Gerald Sonnenfeld's laboratory at the University of Louisville, Louisville, Kentucky. She is now an Assistant Professor at the University of Kentucky, Lexington, Kentucky.

DR. MARTHA GRAY (7/1/86-6/30/87) worked on "The Correlation of Applied Strain Distributions to the Location of New Bone Formation: A Rigorous Mechanical Analysis of an *In-vivo* Bone Preparation" in Dr. Clinton Rubin's laboratory at Tufts University School of Veterinary Medicine, North Grafton, Massachusetts. She is now an Assistant Professor at the Massachusetts Institute of Technology, Boston, Massachusetts.

DR. MARCIA HARRISON (71/83-8/30/85) worked on "Participation of Ethylene in Two Modes of Gravitropism of Shoots" with Dr. Barbara Pickard at Washington University, St. Louis. She is now an Assistant Professor in the Biology Department at Marshall University, Huntington, West Virginia.

DR. GARY JAHNS (1/1/83-4/30/84) worked on "Interactions of Light and Gravity on the Growth, Orientation, and Lignin Biosynthesis in Mung Beans" in Dr. Joe Cowles' laboratory at the University of Houston. He is continuing to work with Dr. Cowles, Houston, Texas.

DR. TIMOTHY JONES (1/1/81-12/30/82) worked on "The Effects of Hypergravic Fields on Brainstem Auditory-Evoked Potentials" in Dr. John Horowitz' laboratory at the University of California, Davis. He is now an Assistant Professor at the University of Nebraska, Lincoln, Nebraska.

DR. THOMAS KERR (1/1/83-12/30/84) worked on "Cellular Localization of Na^+, K^+-ATPase in the Mammalian Vestibular System"; the first year in Dr. Muriel Ross' laboratory at the University of Michigan and the second year in Dr. Dennis Drescher's laboratory at Wayne State University. He is now an Assistant Professor at Wayne State University, Detroit, Michigan.

DR. DOUGLAS KLIGMAN (7/1/82-6/30/84) worked on "The Role of Neurite Extension Factor Nerve and Muscle Tissue Response to Stress or Injury" in Dr. David Jacobowitz' laboratory at the National Institute of Mental Health, Bethesda, Maryland. He is now on the staff at NIMH, Bethesda, Maryland.

DR. KONRAD KUZMANOFF (7/1/83-7/30/85) worked on "Isolation and Identification of ß-glucan Synthetase: A Potential Biochemical Regulator of Gravistimulated Differential Cell Wall Loosening" in Dr. Peter Ray's laboratory at Stanford University. He is now a Research Associate working with Dr. Craig Beattie at the University of Illinois, Chicago, Illinois.

DR. MICHAEL MATILSKY (1/1/81-12/30/82) worked on "Gravity Perception in the Algal Coenocyte *Caulerpa prolifera*" in Dr. William Jacobs' laboratory at Princeton University. He is now a Senior Research Scientist with Plant Biotech Industries, Ashrat, Israel.

DR. KENNETH MCLEOD (11/1/87-10/30/88) is working on "*In-vivo* Measurement of Strain Generated Potentials in Bone During Controlled Mechanical Loading" in Dr. Clinton Rubin's laboratory at the State University of New York, Stony Brook, New York.

DR. DEWEY MEYERS (7/1/81-6/30/83) worked on "Response, Adaptation and Gravitational Perception in a Parthenogenic Freshwater Microcrustacean, *Daphnia galeata mendotae* " in Dr. Allan Brown's laboratory at the University of Pennsylvania. He was the Science and Curriculum Coordinator in the Space Life Sciences Training Program at Kennedy Space Center, Florida. Recently he became an Adjunct Associate Professor at the West Virginia School of Osteopathic Medicine, Lewisburg, West Virginia.

DR. LLOYD MINOR (7/1/87-6/30/88) is working on "Primary Vestibular Afferent Inputs to Central Pathways Mediating the Vestibulo-ocular Reflex" in Dr. Jay Goldberg's laboratory at the University of Chicago, Chicago, Illinois.

DR. DEAN MURAKAMI (1/1/85-12/30/86) worked on "Influences of the Hyperdynamic Environment on the Development of the Visual System in the Rat" in Dr. Charles Fuller's laboratory at the University of California at Davis. He is continuing to work with Dr. Fuller at the University of California, Davis, California.

DR. MARY MUSGRAVE (6/1/86-10/30/88) worked on "Studies of Respiratory Metabolism" in Dr. Boyd Strain's laboratory at Duke University, Durham, North Carolina. She is now an Assistant Professor at Louisiana State University, Baton Rouge, Louisiana.

DR. GARY RADICE (7/1/81-6/30/83) worked on "Control of Gravity-sensing Mechanism in Amphibian Eggs" in Dr. George Malacinski's laboratory at Indiana University. He is continuing to work with Dr. Malacinski, Bloomington, Indiana.

DR. FARREL R. ROBINSON, JR. (7/1/84-6/30/86) worked on "Sensory Motor Properties of the Uvula and Nodulus" in Dr. David Tomko's laboratory at the University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania. He is now working as a Research Associate with Dr. Albert Fuchs in the Physiology Department of the University of Washington School of Medicine, Seattle, Washington.

DR. BRUCE SERLIN (7/1/84-6/30/85) worked on "Differential Wall Growth in Gravistimulated Corn Roots: Its Timing and Regulation" in Dr. Stanley Roux' laboratory at the University of Texas at Austin. He is now an Assistant Professor at DePauw University, Greencastle, Indiana.

DR. ROBERT SLOCUM (1/1/81-12/30/83) worked on "Studies on the Localization and Functional Role of Calcium in Gravistimulated Plant Organs"; the first year in Dr. Stanley Roux' laboratory at the University of Texas at Austin and the second year in Dr. Arthur Galston's laboratory at Yale University. He is now an Assistant Professor at Williams College, Williamstown, Massachusetts.

DR. J. HENRY SLONE (7/1/85-6/30/87) worked on "Characterization of the Protein Responsible for the Lateral Transport of Auxin During Gravitropism of Pea Shoots and Determination Whether Phosphorylation Participates in Gravitropic Activation" in Dr.

Barbara Pickard's laboratory at Washington University in St. Louis, Missouri. He is continuing to work with Dr. Pickard in St. Louis, Missouri.

DR. JOSEPH STEFFEN (7/1/81-6/30/83) worked on "Glucocorticoid Receptor Levels in Hindlimb Skeletal Muscles and Diaphragm During Prolonged (2 Week) Antiorthostatic Hypokinesia and Recovery" in Dr. X.J. Musacchia's laboratory at the University of Louisville. He is now an Assistant Professor at the University of Louisville, Louisville, Kentucky.

DR. JULIANNA SZILAGYI (7/1/81-12/30/81) worked on "Progressive Hemodynamic Changes in Simulated Weightlessness" in Dr. Carlos Ferrario's laboratory at the Cleveland Clinic. She is now an Assistant Professor at the University of Houston, Houston, Texas.

DR. YASUHIRO TORIGOE (1/1/84-12/30/85) worked on "Anatomical Correlated Underlying Vestibulo-autonomic Outflow to the Gut" with Dr. Robert H.I. Blanks at the University of California, Irvine. He is continuing to work with Dr. Blanks at the University of California, Irvine, California.

THE MECHANISM OF GRAVITY SENSING IN PLANTS

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Description of Research

This research is intended to discern events associated with the initial sensing of gravity and the differential signal produced by the sensing cells.

To help identify the response of the sensing cells, it would be useful to know the nature of the signal transmitted from it. One clue is the site of its target and the location of the cells through which it is transported. The target in roots has long been assumed to be the epidermis of the growing zone as it is in shoots; my experiments do not support that assumption.

Accomplishments

(1) The ***gravitropic response of roots with the epidermis and cortex removed from two flanks in the responding region is identical whether the remaining cortex and epidermis are on the sides or on the top and bottom.***

(2) ***The amount of cortex remaining does not affect gravitropism, but damage to the endodermis eliminates gravitropism.***

(3) Roots with the entire epidermis removed from the elongating zone respond (grow slower) to auxin.

Significance of the Accomplishments

Finding #1: The epidermis does not control growth in corn roots; removing those epidermal cells that would produce the differential growth does not affect gravitropism.

Findings #1 and #2: The signal does not move through the cortex of corn roots; removing that part of the cortex through which the largest gradient of signal would move does not affect gravitropism. The endodermis appears to be important in the transport of or the response to the signal.

Finding #3: Auxin, a proposed signal substance, could act as a gravitropic mediator in tissues internal to the epidermis.

Publications

Björkman, T. Calmodulin Activity in Gravity Sensing. In: *Proceedings of the International Botanical Congress*, p. 2-20, 1987.

Björkman, T. Recovery of Gravisensitivity in Decapped Corn Roots (Abstract). *ASGSB Bulletin* 1: 44, 1988.

Björkman, T. and Cleland, R.E. Maize Root Gravitropism Is Not Regulated by the Epidermis (Abstract). *Plant Physiology* 86(Suppl.): 67, 1988.

Björkman, T. and Leopold, A.C. An Electric Current Associated With Gravity Sensing in Maize Roots. *Plant Physiology* 84: 841-846, 1987.

Björkman, T. and Leopold, A.C. Effect of Inhibitors of Auxin Transport and of Calmodulin on a Gravisensing-dependent Current in Maize Roots. *Plant Physiology* 84: 847-850, 1987.

MECHANISM OF RESPONSE FOLLOWING GRAVISTIMULATION IN CEREAL SHOOTS

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Description of Research

This research program is directed at understanding the cellular basis of growth regulation in plants, using the oat pulvinus as a model system. Both gravity and exogenous hormones induce growth in this system. Our approach involves using gravity as a noninvasive growth promoter to identify physiological components of growth regulation. We then compare these effects with those directed by exogenous hormones.

The primary goals of this year's research were: (1) to characterize the development of the graviresponsive system from onset to loss of response to the two stimuli, gravity and hormones; (2) to identify the cellular basis for perception of the stimulus by the system; (3) to detail the apparent link between perception and hormonal response; and (4) to separate the gross response to gravity from that to hormones. This research forms the essential framework for ongoing studies at the cellular level.

Accomplishments

(1) The development of the pulvinus, in terms of its ability to show a growth response following gravistimulation, was described from the onset of competency to the loss of ability to respond. During this study, we also detailed the kinetics of response (lag time, half-time, and maximum rate) to both the gravitational and the hormonal stimuli, differentiated response completion from loss of competency, and correlated the loss of competency at one site with the onset at a second.

(2) ***Pulvini which are competent to respond to a gravistimulus were found to lose this ability when pretreated in the dark for five days. This phenomenon was linked to loss of starch from sedimentable plastids. These pulvini could still respond to applied hormones, suggesting that the dark pretreatment affected a stimulus perception element rather than a response component.*** The subsequent addition of sucrose led to starch reappearance, and this preceded reappearance of a graviresponse.

(3) Further studies with the above protocol linked the perception element with the assumed response component, hormones. Over the course of the 5-day dark pretreatment, the lag to response following gravistimulation gradually increased, but the rate of response, once initiated, did not vary. In contrast, the lag to response following hormone stimulation in similarly treated tissues did not change while the rate of response declined.

(4) ***Gravistimulation was found to alter the response of pulvinus tissue to applied hormones. Gravistimulation altered the effect produced by IAA at moderate to high levels of hormone. This change, an inhibition of response, was opposite to theoretical expectations of stimulated response at low hormone levels. In contrast, pulvini were not responsive to gibberellic acid until they were gravistimulated. This suggests that gravity induced responsiveness to this hormone.***

Significance of the Accomplishments

These results add essential details to the conceptual framework provided by the Cholodny-Went model for response to gravity, as applied to the oat pulvinus. The Cholodny-Went model suggests that gravitropism is a three-component process, involving perception of the gravistimulus, reformulation (***"transduction"***) of perception into a hormonal signal, which leads to the ***response***. As a result of this year's work, we know that a critical element in perception is the presence of starch in pulvinus plastids, and that the starch is used to drive plastid sedimentation and supply substrate during ensuing cell expansion growth. We know when, developmentally, the individual capacities to both perceive and respond appear and when they are lost. We also have established the differences in timing of response to the two types of stimuli, gravitational vs. hormonal. This last point is particularly useful since the gravitational response time must include all three components of the Cholodny-Went model, while the hormonal response time should involve only the final one.

Perhaps one of the most interesting sets of findings relates to the difference in response to applied hormones triggered by a gravistimulus. Several important implications arise from these experiments. For example, there appear to be gravity-specific effects at the cell response level that are distinct from hormonal effects. This finding is not predicted by the Cholodny-Went model, although it does not reject the model in any way. Also, a mechanism limits the magnitude of possible response. This process requires a sufficient stimulus to be initiated, and it may involve hormone removal by either chemical or physical means.

As a result of this year's work, a series of studies related to growth as induced by both gravitational and hormonal stimuli are in progress. In particular, we are comparing the kinetics of changes in specific proteins as induced by the different stimuli and cellular parameters that affect these changes. We will be particularly interested in differentiating which changes are gravity-specific or hormone-specific, and which changes are critical for growth promotion.

Publications

Brock, T.G., Bright, V., Burg, J., and Kaufman, P.B. Gravistimulation Alters the Sensitivity of Oat Pulvini to Exogenous Indole-3-Acetic Acid and Gibberellic Acid (Abstract). *ASGSB Bulletin* 1: 45, 1988.

Brock, T.G., Cameron, M.J., Karuppiah, N., Ghosheh, N.S., and Kaufman, P.B. Sensitivity vs. Responsiveness in Hormone Action: A Case Study Involving the Effect of Gravistimulation on IAA and GA3 Action (Abstract). *Plant Physiology* 86(Suppl.): 112, 1988.

Brock, T.G. and Kaufman, P.B. Altered Growth Response to Exogenous Auxin and Gibberellic Acid by Gravistimulation in Pulvini of *Avena sativa*. *Plant Physiology* 87: 130-133, 1988.

Brock, T.G. and Kaufman, P.B. The Onset and Development of Competency for Graviresponsiveness in the Leaf-sheath Pulvinus of Oat (*Avena sativa* L.) (Abstract). *Plant Physiology* 83(Suppl.): 20, 1987.

Kaufman, P.B., Brock, T.G., Song, I., Rho, Y.B., and Ghosheh, N.S. How Cereal Grass Shoots Perceive and Respond to Gravity. *American Journal of Botany* 74: 1446-1457, 1987.

Song, I., Lu, C.R., Brock, T.G., and Kaufman, P.B. Do Starch Statoliths Act as the Gravisensors in Cereal Grass Pulvini? *Plant Physiology* 86: 1155-1162, 1988.

VOLTAGE GATING OF PLANT HORMONE TRANSPORT CHANNELS

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Description of Research

The objective of this research is to understand the vectorial growth response of plants to gravity. We postulate that the mechanism of this response involves the interaction between the plant's internal electrical gradients and the transport of the plant growth hormone indole-3-acetic acid (IAA) within the plant. Changes in a plant's orientation with respect to gravity are followed by a realignment of the plant's internal bioelectric field. We have developed a working theory which proposes that this realignment opens and/or closes voltage-gated, hormone-transporting channels between the plant's vascular tissue (stele) and the surrounding cortical tissue (cortex), leading to accumulation of the hormone on the lower side of the shoot. This asymmetric hormone concentration results in lateral asymmetric growth until the plant's axis is again vertical.

We are testing this theory by measuring how the plant's growth rate is altered by the application of an external electric potential and comparing the change in growth rate with the corresponding change in endogenous IAA concentrations in actively growing parts of the plant. We are also examining the effect of the applied potential upon the transport of radiolabeled IAA and other compounds from the seed to the actively growing sections of the plant. Previous work from this laboratory indicates a close correlation between a plant's growth rate and endogenous free IAA so that changes in the growth rate may suggest changes in the concentration of endogenous IAA.

Accomplishments

We previously developed an apparatus for applying a steady electrical potential along the entire length of vertically oriented 4- day-old corn seedlings. We used that apparatus to determine the electrical parameters that would influence the shoot's growth without causing damage to the plants. We found that ***the growth rate of the shoot at low potentials was dependent only on the polarity of the applied potential.*** The magnitude of the current did not depend on the polarity. The growth rate decreased 90% when the tip of the plant was held electrically positive, whereas the reverse polarity had no effect on the growth rate.

We determined the effect of the applied potential upon the concentrations of free and ester forms of IAA in the actively growing tissues. ***The effect of the applied potential upon the endogenous IAA concentrations in the plant was found to be dependent on the polarity of the applied potential.*** The tip-negative polarity-treated plants exhibited no difference in their endogenous IAA concentrations in the growing tissues, whereas the concentration of the ester form of IAA in the vascular stele of the tip-positive polarity-treated plants doubled.

We determined the effect of the applied potential upon the transport of radiolabeled glucose, IAA, and calcium from the endosperm of the seed to the actively growing regions of the shoot. There was no effect of the applied potential upon the transport of the radiolabeled free form of IAA from the endosperm to the shoot. ***The applied potential decreased the amount of radiolabeled glucose transported. The tip-positive treated***

plants showed a greater decrease in the amount of transported IAA than the tip-negative treated plants. The tip-negative treated plants exhibited no difference in the amount of radioactive calcium transported, compared with the controls. The tip-positive treated plants showed a 30% decrease in the amount of radioactive calcium transported in the vascular stele.

Significance of the Accomplishments

This is the first time that the endogenous IAA concentrations of electrically stimulated plant tissue were examined using physiocochemical methods that specifically identified IAA.

This is the first attempted correlation between the electrically induced growth inhibition and the endogenous IAA levels.

We found that the polarity-dependent growth inhibition was correlated with an accumulation of ester IAA in the vascular stele. The theory predicts that IAA in the stele would accumulate due to a block in the voltage-gated channels between the stele and cortex. These results support the theory, but do not prove it.

Publications

Bandurski, R.S., Schulze, A., Desrosiers, M., and Epel, B. Transduction of the Gravitational Stimulus (Abstract). *ASGSB Bulletin* 1: 28, 1988.

Bandurksi, R.S., Schulze, A., Leznicki, A., Reinecke, D.M., Jensen, P., Desrosiers, M., and Epel, B. Regulation of the Amount of IAA in Seedling Plants. In: *International Symposium on Physiology and Biochemistry of Auxins in Plants*. (ed. by M. Kutacek), Prague, 1988.

Desrosiers, M. and Bandurski, R.S. Effect of an Applied Voltage Upon the Growth Rate of *Zea mays* Seedlings (Abstract). *Plant Physiology* 83(Suppl.): 19, 1987.

Desrosiers, M. and Bandurski, R.S. Effect of an Applied Voltage Upon IAA Transport (Abstract). *ASGSB Bulletin* 1: 21, 1988.

HIGH FREQUENCY RESPONSE PROPERTIES OF SEMICIRCULAR CANAL FIBERS

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Description of Research

A number of investigators have studied the dynamic responses of semicircular canal afferents in various species since Ross (1936) first recorded frog vestibular nerve responses. The current project has both theoretical and practical significance when viewed in the context of the study of semicircular canal system dynamics. Theoretically, the investigation addresses the issue of whether the semicircular canals function as a band pass, high pass, or low pass filter in their response to the natural range of physiological head motions. Practically, a mechanical stimulation technique for the semicircular canals has been developed that allows the animal preparation to remain stationary while simulating natural rotational stimulation. This mechanical stimulation method should prove to be quite advantageous in both the current and future anatomical and physiological studies of the vestibular system.

Research conducted during the past year has focused on the development of the mechanical stimulation technique, the comparison of mechanical and rotational stimulation methods for producing vestibular responses, and the recording of semicircular canal primary afferent fiber responses to high frequency stimulation. In these experiments, recordings from isolated semicircular canal afferent fibers were obtained in anesthetized pigeons (*Columba livia*), using standard electrophysiological techniques. The semicircular duct of the horizontal canal was exposed for mechanical and rotational stimulation. Afferent fiber responses were recorded while delivering independent mechanical or rotational sinusoidal stimuli across a frequency bandwidth ranging from 0.01-200 Hz. The magnitude and phase of the response to each stimulus was quantified and then compared as to type of stimulation (i.e., mechanical or rotational) and frequency.

Accomplishments

(1) An *in vivo* method was developed for mechanical stimulation of the semicircular canals. Small amplitude displacements ($\pm$ 0.5-7.5μ) of the exposed semicircular duct can be reliably produced and monitored, using a variety of stimulus waveforms.

(2) Responses from afferent fiber to mechanical stimulation were shown to be sensitive, discrete, and reproducible. Stimulation of one semicircular duct (e.g., the horizontal) was shown to elicit responses only from fibers that innervate that canal and not from afferent fibers innervating the other vestibular organs.

(3) Intensity functions that relate response magnitude to stimulus intensity were obtained with mechanical stimulation and were found to be directly related to responses elicited by rotational stimulation for individual afferent fibers.

(4) Semicircular canal afferent fiber responses produced by high frequency mechanical and rotational stimulation (i.e., 5-200 Hz) are currently being obtained and analyzed.

Significance of the Accomplishments

The development of the mechanical stimulation technique is important for present and future studies of the vestibular system. As compared with other nonmotion types of stimuli that have been used to study the vestibular system, mechanical stimulation produces endolymph movement and consequent cupula deflections in a precise, controlled manner, with a number of stimulus waveforms and a broad frequency range. The use of this technique *in vivo* allows the study of vestibular system dynamics in the peripheral as well as the central nervous system pathways. In addition, mechanical stimulation will provide the capability of intracellular recordings and dye injection for neuroanatomical studies, a technique that is quite difficult using rotational stimulation.

Intensity functions relating mechanical and rotational stimulation have shown that the two methods will elicit afferent responses that are comparable in magnitude. If these response comparisons can be continued into the high frequency range (i.e., 20-200 Hz) of stimulation, where recordings become quite difficult using traditional rotational stimulation methods, the high frequency vestibular system dynamics can be more routinely studied. In order to determine the vestibular system filter characteristics of the semicircular canals in different species having different natural regions of physiological head movements, responses to a broad frequency range of stimulation will be desired. The question becomes, how do the environmental requirements for head movements (e.g., low frequency motions in amphibians or fish as compared with high frequency motions in birds) translate to the vestibular system response characteristics? Further, in a low gravity environment, do the semicircular canal system dynamics change or adapt in order to accommodate a new range of head motions? The current studies will provide the beginnings of the answers to these questions by obtaining the response characteristics of semicircular canals in pigeons throughout their entire response bandwidth.

Publications

Dickman, J.D. and Correia, M.J. High Frequency Response of Pigeon Semicircular Canal Afferent Fibers (Abstract). *ASGSB Bulletin* 1: 25, 1988.

IN VIVO MEASUREMENT OF STRAIN-GENERATED POTENTIALS IN BONE DURING CONTROLLED MECHANICAL LOADING

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Description of Research

The goals of this research are to both develop an exercise regimen appropriate for maintaining bone mass during spaceflight and to improve our understanding of the processes by which bone responds to environmental stimuli. Our studies on the distribution of strain energy in functionally loaded bone *in vivo,* and on the efficacy of electrical currents of physiological magnitudes to modulate bone remodeling activity, have led us to believe that an efficient mechanical loading protocol, based on the endogenous induction of stress-generated potentials (SGPs), can be developed to minimize, if not prevent, microgravity bone loss.

The specific objectives of this investigaton are to develop the techniques necessary to record stress-generated potentials, *in vivo,* under controlled loading conditions, propose an optimal loading paradigm for the maintenance of bone mass based on maximizing stress-generated potential energy while minimizing mechanical input energy, and test the proposed loading paradigm against an alternative controlled loading regimen.

This research is centered around the avian disuse osteoporosis experimental model developed by Rubin and Lanyon. In this model, the ulna of adult male turkeys is isolated such that the bone is unloaded. Through the use of a specially modified Instron mechanical test apparatus, loading can be applied to the bone in a controlled manner. This preparation has been used to demonstrate that dynamic loading is responsible for the maintenance of bone mass and that both load magnitude and loading duration are factors in the maintenance of bone mass. In order to investigate the role of the electric currents that arise in bone during dynamic loading, electrodes are placed into the cortex of the isolated ulna, and electrophysiological recordings are obtained during a controlled dynamic loading session. The characteristics of the recorded electric signals are then correlated with the efficacy of the specific loading regimen to maintain bone mass.

Accomplishments

(1) Techniques have been developed for the recording of stress-generated potentials during controlled dynamic loading of the isolated avian ulna.

(2) We have established that for physiologic strain magnitudes, peak induced electric field intensities in the cortex of the bone are on the same order of magnitude as the exogenously induced electric fields that are capable of inhibiting bone remodeling activity.

(3) We have demonstrated a ***correlation between the decay of stress-generated potentials during cyclic loading and the role of increased loading duration in the control of bone remodeling activity.***

(4) We have demonstrated a correlation between the induced electrical energy of stress-generated potentials as a function of peak mechanical load and the ***effectiveness of increased mechanical load in modulating bone remodeling activity.***

(5) We have shown that fluid flow within cortical bone is not localized but reflects the generalized stress patterns in the bone, consistent with the fact that sites of greatest strain gradient, and not strain magnitude, are those that undergo the most active bone remodeling.

Significance of the Accomplishments

A causal relationship between endogenously produced electrical currents and bone maintenance has been suggested by many investigators. Yet, a direct link between physical strain, the electrical currents engendered by this strain, and bone remodeling activity has not previously been established. This study demonstrates a strong correlation between the energetics of the induced electrical fields caused by functional loading and the remodeling response of bone to those same functional loading patterns. Specifically, we have shown that: (1) the decay of strain-generated potential magnitude with increased load cycling is predictive of the *in vivo* remodeling response to increased loading duration; (2) the variation of SGP energy under various loading patterns is consistent with the variation in effectiveness of the different loading patterns; and (3) the magnitude and spatial distribution of the induced currents is consistent with sites of remodeling activity and with results we have obtained through the exogenous induction of electric fields into the *in vivo* model.

The ability to predict the remodeling response from the SGP energetics will lead to the design of a mechanical loading regimen that can maximize the stimulus to the bone while minimizing the time and physical energy required to maintain bone mass.

Publications

McLeod, K.J. and Rubin, C.T. Correlation of *In-vitro* Bone Surface Potentials With Remodeling Activity in the Isolated Avian Ulna Model. *Transactions Bioelectrical Repair and Growth Society* 7: 39, 1987.

McLeod, K.J. and Rubin, C.T. Stimulation of New Bone Formation by Low Frequency Sinusoidal Electric Fields. *Transactions of Orthopedic Research Society* 34: 123, 1988.

CENTRAL VESTIBULAR MECHANISMS

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Description of Research

The long-term goal of this research is to understand the functional organization of the central vestibular pathways. Research during this past year has concentrated on three projects. The first of these is directed toward determining the profile of primary vestibular afferent inputs to the vestibulo-ocular reflex (VOR). The VOR functions to maintain the stability of images on the retina during head movements. The peripheral vestibular system transduces information about head velocity into changes in the discharge frequency of primary vestibular afferents. These primary vestibular afferents innervate secondary vestibular neurons in the vestibular nuclei that in turn project to the oculomotor nuclei. Oculomotor neurons innervate the extraocular muscle, and the VOR produces compensatory eye movements in the same plane as the plane of head rotation but in an opposite direction. In order to understand the characteristics of the signal transformations that occur along the brainstem pathways involved in control of the VOR, it is first essential that we define the physiological properties of the primary vestibular afferents that provide input to these pathways.

We have developed a paradigm that permits us to selectively and reversibly ablate one physiological class of primary vestibular afferents. The method is based on differences in the electrical sensitivity of vestibular afferents as a function of discharge regularity. Irregularly discharging afferents are, on average, 10 times more sensitive to dc currents than are regular afferents. The technique consists of presenting an anodal (inhibitory) current of 100 μA to the middle ears of squirrel monkeys. Our single unit recordings from primary vestibular afferents in the alert squirrel monkey have shown that this current silences most irregular afferents to the extent that they no longer respond to rotational stimuli. The background discharge of regular afferents is reduced by the current, but the rotational sensitivity of regular afferents is decreased by less than 5%. In short, the polarizing currents produce a selective and reversible ablation of most irregular afferents, thereby making it possible for us to study the physiology of vestibular reflexes in the presence and absence of inputs from irregular afferents. The results indicate that the VOR receives input mainly, if not exclusively, from regular afferents.

The VOR has been studied in animals from which eye movements are recorded in response to passive head rotations. Under most behavioral circumstances, active head movements and eye movements interact in the control of gaze. The second project pursued during this period is an initial step toward understanding the mechanisms controlling these interactions. We have studied a group of secondary vestibular neurons whose axons project to the extraocular motor nuclei and to the spinal cord. These experiments have morpho-physiologically characterized these secondary neurons in terms of their rostral and caudal terminations, their collateral projections in the brainstem, and their inputs from vestibular afferents innervating the contralateral labyrinth.

In the third project, we have investigated the position-dependent asymmetry present in the horizontal nystagmus evoked by caloric stimuli. The motivation for this study came, in

part, from the observation that a caloric nystagmus can be evoked during the microgravity conditions of orbital spaceflight (von Baumgarten, et al., 1984). Barany (1906) suggested that the nystagmus resulting from irrigation of the external auditory canal with warm or cold water is due to the convective movement of endolymph in the horizontal semicircular canal. The convective flow mechanism accounts for many of the features of caloric nystagmus, including its directionality and its dependence upon the temperature of water infused into the external canal relative to body temperature. The convective flow mechanism cannot, however, account for the caloric response evoked in microgravity nor can it account for the observation that the responses to caloric stimuli given with the subject's head in nose-up positions are consistently larger than those with the subject's head in complementary nose-down positions. We studied caloric and rotational responses in squirrel monkeys. The results indicate that a similar position-dependent asymmetry is present in the responses to low-frequency rotational stimuli. An otolith signal related to the static position of the head is responsible for modulating the horizontal nystagmus produced by stimuli that engage velocity storage mechanisms.

Accomplishments

Primary vestibular afferent inputs to the VOR

(1) ***The horizontal and vertical VORs in response to sinusoidal head rotations were not changed in gain or phase when the irregular afferents were ablated with the anodal currents.*** Single unit recordings from primary vestibular afferents and field potential studies establish that the currents are acting to selectively ablate irregular afferents while having little effect on the rotational sensitivity of regular afferents.

(2) A rapid change in horizontal head velocity stimulus was used to study the transient response properties of the VOR. The stimulus consisted of a short duration (50 msec) acceleration ramp (600 deg/sec^2) followed by a velocity plateau. The VOR in response to this stimulus began after a 12-14 msec latency and reached a peak in eye velocity at the same time as the peak in head velocity. Neither the latency nor the profile of this response was changed by ablation of the irregular afferents.

Secondary vestibular neurons projecting to the extraocular motor nuclei and to the spinal cord

(1) Intracellular recordings from the axons of secondary vestibular neurons were made in the medial longitudinal fasciculus (MLF) at the level of the trochlear nucleus and caudal to abducens nucleus. Of the 169 secondary axons characterized in the caudal MLF, 62 were antidromically activated only from the spinal cord (SP), 47 only from the oculomotor nucleus (OC), and 60 from both SP and OC.

(2) Intra-axonal injections of horseradish peroxidase were made in 47 secondary vestibular axons that projected in the MLF to OC and SP. Histological examination of the brainstem following these experiments revealed that the axons passed through the ipsilateral abducens nucleus, crossed the midline at the same level, and branched immediately. The branch projecting to the rostral MLF was thinner than the branch projecting in the caudal MLF to the spinal cord. Rostral collaterals were observed to terminate in either the abducens or the oculomotor nucleus. The caudal collaterals coursed ventrally in the MLF.

Position-dependent asymmetries in the nystagmus evoked by caloric and rotational stimuli

(1) Caloric responses to warm or cold stimuli were, on average, 3.9 times greater in the supine (90° nose-up) position than in the prone (90° nose-down) position. A similar, although not as large, position-dependent asymmetry was also present in the responses to velocity trapezoid rotations with long-duration acceleration ramps. The mean acceleration gain (G_a) calculated from responses to velocity trapezoid rotations given in the 45° nose-up position was 20.9 deg·sec^{-1}/deg·sec^{-2} whereas G_a in the 45° nose-down position was 10.7 deg·sec^{-1}/deg·sec^{-2}. This asymmetry between nose-up and nose-down responses was also seen in the time constants of the VOR and of optokinetic after nystagmus. The asymmetry persisted following inactivation of the vertical canals, indicating that ***an otolith signal indicating the static position of the head relative to gravity is most likely responsible for this modulation of horizontal nystagmus.***

(2) The results of the caloric and low frequency rotational experiments were shown to be consistent with the notion that the normal caloric response is composed of a thermal convective component, a position-dependent component arising from a static otolith signal, and a direct temperature effect on hair cells and/or afferent nerve fibers.

Significance of the Accomplishments

Primary vestibular afferent inputs to the VOR. The experiments evaluating the VOR without and with the ablation of irregular afferents indicate that this reflex is receiving inputs mainly, if not exclusively, from regular afferents. This study provides direct evidence that the VOR receives a selective afferent input and not an indiscriminate mixture of inputs from afferents with varying physiological properties. Irregular afferents have more phasic response dynamics and high sensitivities to activation of the vestibular efferents than do regular afferents. This experiment establishes the physiological characteristics of the head velocity signal transmitted by the primary vestibular afferents projecting to pathways mediating the VOR. The VOR is receiving input from afferents with low gains to rotational head movements and tonic response dynamics. Solution of the transfer functions describing the primary vestibular afferents and the oculomotor plant for inputs from regular and irregular afferents reveals that the dynamics of the irregular afferents are inappropriately matched to the dynamics of the oculomotor plant. The results of these experiments are, therefore, consistent with our theoretical understanding of the properties of this system.

Secondary vestibular neurons projecting to the extraocular motor nuclei and to the spinal cord. The findings establish the existence, in primates, of a group of secondary vestibular neurons that have axons projecting rostrally and caudally in the MLF. The recordings made in the MLF caudal to abducens nucleus indicate that more than half of the secondary neurons coursing in the medial vestibulospinal tract have collateral projections to the extraocular motor nuclei. This study has electroanatomically characterized these neurons and provides the basis for an additional study of their physiological properties when recordings are made in alert animals.

Position-dependent asymmetries in the nystagmus evoked by caloric and rotational stimuli. The results demonstrate that the position-dependent asymmetry in caloric and low frequency rotational responses is due to an influence of static pitches on velocity storage in the horizontal vestibulo-optokinetic system. The data are consistent with the predictions of a quantitative model of the caloric response (Paige, 1985). A direct effect

of temperature on vestibular hair cells and/or afferent nerve fibers is most likely responsible for the caloric response observed in the microgravity conditions of orbital spaceflight.

Publications

McCrea, R.A., Minor, L.B., and Goldberg, J.M. Collateral Projections of Medial Vestibulospinal Tract Neurons to the Extraocular Motor Nuclei in the Squirrel Monkey (Abstract). *Society for Neuroscience Abstracts* 12: 457, 1986.

Minor, L.B. and Goldberg, J.M. Determination of Primary Vestibular Afferent Inputs to the Vestibulo-ocular Reflex (Abstract). *Society for Neuroscience Abstracts* 12: 773, 1986.

Minor, L.B. and Goldberg, J.M. Primary Vestibular Afferent Inputs to the Horizontal Vestibulo-Ocular Reflex (Abstract). *ASGSB Bulletin* 1: 26, 1988.

STUDIES ON THE RESPIRATORY METABOLISM OF PLANTS UNDER SPACEFLIGHT CONDITIONS

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Description of Research

This research is directed toward understanding and counteracting the effects of microgravity on respiratory metabolism in plants. Convective air movement is a driving force of gas exchange in plants and is lacking in spaceflight conditions. In addition, because microgravity prevents directed surface water flow, waterlogging during spaceflight is an additional cause of hypoxia in plant tissue. Ultrastructural studies of plant material from short-term flight experiments showed that mitochondria may develop aberrantly, with swollen morphological features similar to those of mitochondria formed under anaerobic conditions.

My research has had two foci. The first was to quantify the effects of hypoxia on root and shoot metabolism and the consequences of hypoxia for plant energy budgets, growth, and development. This information allowed me to evaluate the possibility of growing plants at reduced pressure as a countermeasure to the limitations on gas exchange imposed by microgravity.

Because of the structural and material supply advantages which would be afforded by low pressure plant growth areas, they have been proposed as an element in a Controlled Ecological Life Support System (CELSS). Growing plants at reduced pressure would speed up diffusive processes by increasing the mean free path and could possibly compensate for the absence of convective air movement in microgravity. Little information is available about the growth of plants at low pressure and so the effects of reduced pressure (1/5 atm) on plant growth and metabolism were investigated.

Accomplishments

Facilities at the Duke University Phytotron and the F.G. Hall Environmental Laboratory at Duke were used to grow plants under low oxygen, low pressure, and waterlogged conditions. The effects of these environmental challenges on growth and metabolism were quantified and reported in detail. The information will allow space biologists to be better informed on the consequences of poor aeration associated with spaceflight conditions.

An exciting result was the finding that ***mungbean seedlings grew better at low pressure (0.2 atm) than at ambient pressure, whether the flow-through gas was air or 100% oxygen. Respiratory metabolism was found to respond to the partial pressure of oxygen regardless of total pressure, while growth was stimulated by low pressure.*** The use of cabin air at low pressure would have a potential drawback at the end of the plant's life cycle because low partial pressures of oxygen inhibit seed set. This inhibitory effect of low oxygen (5%) was overcome by simultaneous CO_2 enrichment (1000 ppm) in wheat and *Brassica campestris*.

Significance of the Accomplishments

The finding that seedlings grow better at low pressure than at ambient pressure suggests that they are diffusion limited, not by availability of oxygen, but by dissipation of contaminants (such as ethylene). Experiments on the Space Shuttle which require higher purge rates than presently available could make use of this low pressure technology to achieve an adequate purge.

The finding that respiratory metabolism and growth are normal (or enhanced) at low pressure, even when air is the flow-through gas, encourages the idea that hypobaric growth of plants could be a useful technology for space applications. Contrary to previous suggestions that oxygen would have to be supplemented for successful growth at low pressure, these results show that cabin air could be circulated in a low pressure flow-through plant growth area with no detrimental effects on respiratory metabolism.

Relative amounts of main chain and alternative respiration changed at low pressure only when the partial pressure of oxygen was different from that at ambient conditions. Since this balance plays a role in determining total plant carbon budgets, and was found to influence plant response to a waterlogging challenge, it will be important to study the response of plants which differ with regard to the amount of alternative respiration. These lines have been found in wheat, pea, and *Brassica campestris*.

At ambient pressure, sterility of plants grown at 5% oxygen can be overcome by CO_2 enrichment (1000 ppm). This suggests that at low pressure, the use of cabin air (which is enriched in CO_2) may overcome the sterility that would otherwise be expected at low oxygen partial pressures.

Publications

Musgrave, M.E., Gerth, W.A., Scheld, H.W., and Strain, B.R. Growth and Mitochondrial Respiration of Mungbeans (*Phaseolus aureus* Roxb.) Germinated at Low Pressure. *Plant Physiology* 86: 19-22, 1988.

Musgrave, M.E., Gerth, W.A., Scheld, H.W., and Strain, B.R. Improvement of Seedling Growth and Respiration by Low Pressure (Abstract). *ASGSB Bulletin* 1: 46, 1988.

Musgrave, M.E., Scheld, H.W., and Strain, B.R. Effect of CO_2 Enrichment on Growth and Reproduction of Wheat Grown Under Low Oxygen (Abstract). *Plant Physiology* 83(Suppl.): 97, 1987.

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16. Abstract

This report consists of individual technical summaries of research projects of NASA's Space/Gravitational Biology Program, for research conducted during the period January 1987 to April 1988. This Program is concerned with using the unique characteristics of the space environment, particularly microgravity, as a tool to advance knowledge in the biological sciences; understanding how gravity has shaped and affected life on Earth; and understanding how the space environment affects both plant and animal species. The summaries for each project include a description of the research, a list of the accomplishments, an explanation of the significance of the accomplishments, and a list of publications.

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