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INNOVATIVE UTILIZATION OF THE SPACE STATION
PROGRAM. A REPORT TO THE NATIONAL
AERONAUTICAL AND SPACE ADMINISTRATION
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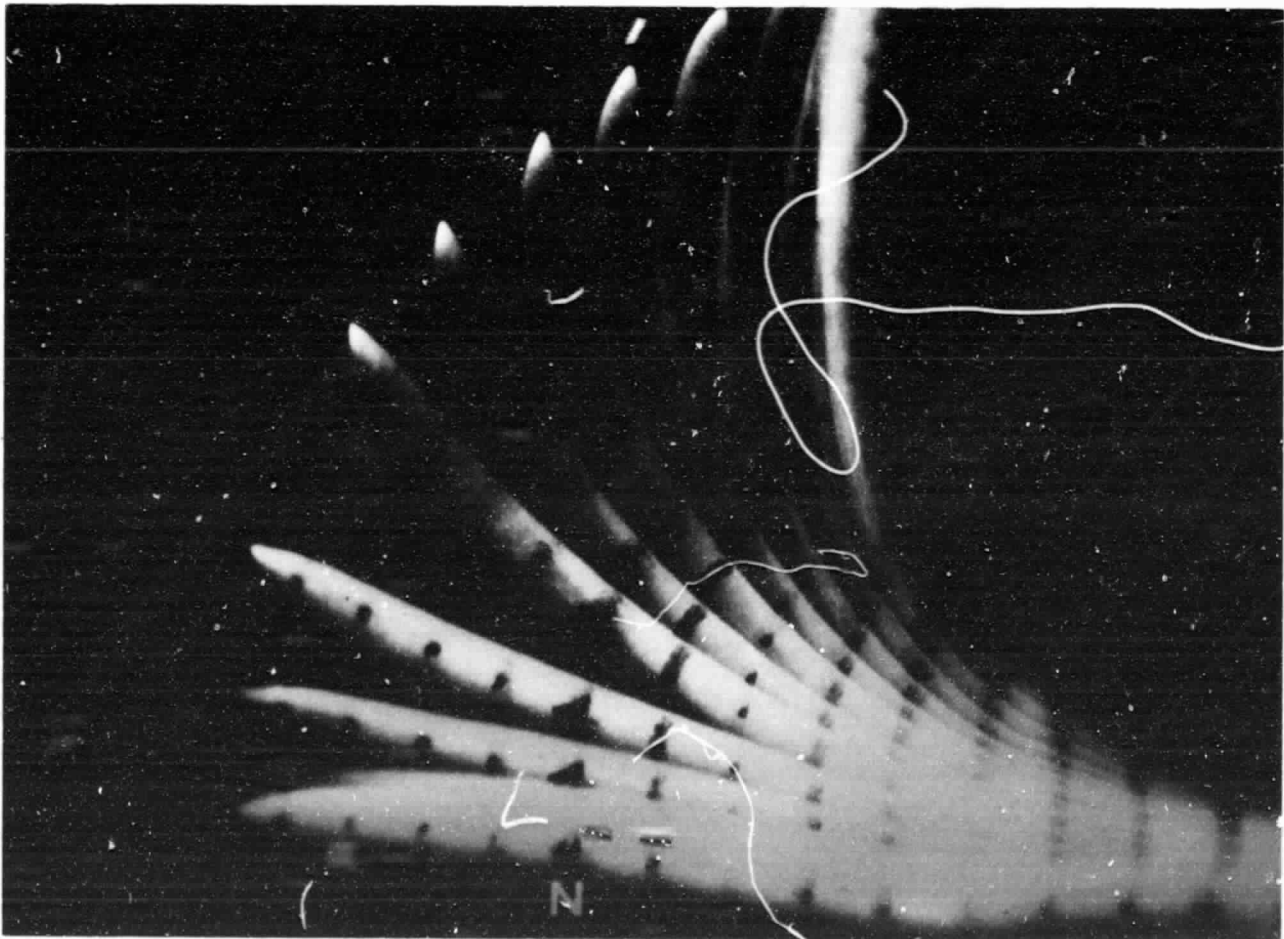


THE ALPHA-HELIX CONCEPT

Innovative Utilization of the
Space Station Program

NASA Grant No.: NASW 3748, ORD 33060
3/24/83 to 10/17/83, \$ 18,294

Robert S. Bandurski



- THE ALPHA-HELIX CONCEPT -

Innovative Utilization of the Space Station Program

A report to the National Aeronautic
and Space Administration

Requesting

Establishment of a Sensory Physiology
Laboratory on the Space Station

NASA Grant No.: NASW 3748, ORD 33060

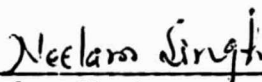
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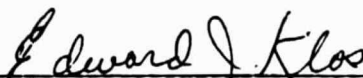
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THE ALPHA-HELIX CONCEPT

ABSTRACT

It is proposed that a major laboratory - dedicated to biological-medical research - be established on the Space Platform. The laboratory would focus on Sensory Physiology and Biochemistry since Sensory Physiology represents the first impact of the new space environment on living organisms. The objective would be to use "microgravity" and the "high radiation" environment of space to help solve the problems of prolonged sojourns in space but, more importantly, to help solve terrestrial problems of human health and agricultural productivity. The emphasis would be on experimental use of microorganisms and small plants and small animals to minimize the space and time required to use the Space Platform for maximum human betterment.

In the following document we introduce the "Alpha Helix Concept", that is, the use of the Space Platform to bring experimental bio-medicine to a new and extreme frontier - so as to better understand our worldly environment. We propose staffing and instrumenting the Space Platform Bio-Medical laboratory in a manner patterned after successful terrestrial sensory physiology laboratories.

PART I: INTRODUCTION

How does life survive at the extreme environments of our world? That, question represents the Alpha-Helix Concept. How does life cope and persist at the temperature extremes of the world where enzymatic rates drop precipitously or at the other extreme, where enzymes are literally cooked. How does life persist where salt water meets fresh and survive an osmotic challenge or where aerobic and hyperbaric conditions exist and life survives by bringing into play new metabolic pathways. This use of extremes, to better understand our mesic

conditions, is the Alpha-Helix Concept and was the life dedication of the late Professor Per M. Scholander of the Scripps Oceanographic Laboratory.

Scolander's dedication resulted in the Alpha-Helix, a floating, earth bound, biochemistry laboratory. This ship brought new meaning to many bio-medical areas. Now we can look again at the Alpha-Helix concept - not with the limitations of our worldly environment in mind - but with the expanded and new dimensions of space. All life evolved in a one "g" environment. With the advent of the Space Platform, we can, for the first time, examine the entire gamut of life processes in a "micro g" environment. Every cell and cell organelle has "weight". How has nature used weight to accomplish the objectives of life? We wish to answer that question.

What of radiation? All life has evolved under a protective screen of moisture and carbon dioxide. But what are the effects of solar radiation? Has it acted as a positive evolutionary force or placed us in danger of extinction? We wish to answer that question.

These are the dimensions presented by this new Alpha-Helix - a Space Platform that will, for the first time, enable man to study and understand the impact of gravity and solar radiation on life processes. No one can say where this study will lead. But there is one certainty: just as temperature, and salinity, just as elements such as oxygen, nitrogen, phosphorus, sulfur, etc., shaped our existence so will we find that gravity and radiation have shaped our existence. Gravity and radiation may prove to be environmental hazards - or they may prove to be life giving forces - and now these questions can be answered.

The Plan:

But, how to capitalize on this new opportunity? It is so new that no plan is certain. But there is an old-fashioned homology that can serve as a guide - "if it works - don't fix it", and so this report suggests again using the plan which served so well on earth. To wit - build a space platform - Alpha Helix! Just as the Alpha Helix carried the best instrumentation of its time to the frontiers of the world - the new Space Platform should carry a very sophisticated physiological-biochemistry laboratory to this new frontier. We can carry what is now known of the instrumentation of sensory physiology and biochemistry into space. It is towards this end that the body of this report deals with examples of sensory physiology and biochemistry. Sensory physiology is stressed because it is the first impact in the long cascade of responses of an organism to a stimulus. How do organisms sense light and gravity? How do they transduce this perception into a life-affecting response? What is the molecular basis of environmental perception? What machinery do we require to answer these questions?

The Alpha-Helix Laboratory:

To exactly plan this laboratory will require the combined efforts of the NASA laboratories; the Life Science-Space Biology Group; other physico-chemical scientists and other workers in sensory physiology. Certain needs can be outlined as follows:

Instrumentation:

First, it is fortunate that Fourier transform - Infra Red Spectrometry; High Field Nuclear Magnetic Resonance Spectrometry; Tandem Mass Spectrometry; Electron Diffraction Microscopy; capillary gas chromatography and high performance liquid chromatography and computer control of all of the above have developed - just in time. Now, for the first time, physical instrumentation is adequate for the micromolar region of the biological world. No one individual

could specify the instrumentation required, but an example of how this could be done is furnished by Dr. Robert Rabson, of the Department of Energy, who has recently convened a group under the leadership of Dr. Elijah Romanoff of the National Science Foundation to look at the interface between bio-medicine and physico-chemical instrumentation. An extension of this approach might serve as a model.

This report suggests convening our best physico-chemical instrumentalists together with our best bio-medical sensory experimentalists to exactly specify the sensitivities and capabilities of the instrumentation which must be carried on the space platform to determine how living organisms sense gravity and radiation and transduce the perception of these stimuli.

Physical Dimensions of the Alpha Helix Laboratory:

Three things are required: First, space for the necessary instrumentation, secondly, a micro "g" environment immediately adjacent to a variable, zero to one "g" centrifuge, and thirdly, a radiation shielded environment, immediately adjacent to an unshielded environment. Space should be allocated to small plants and animals - and probably - for these first adventures we must emphasize creatures which are very small and rapidly complete their life cycles - such as - fungi, nematodes, bacteria, mice and small plants.

Earth based experimentation:

No amount of space studies have relevancy unless they are based on the data provided by terrestrial studies such as provided by the Life Sciences Division of the Space Biology Program. The question must always be - What are we sensing in comparison to earth-based environments? That is the take home question.

The scope of this report:

The bulk of this report deals with the minutiae of our knowledge of the physiology and biochemistry of sensory physiology. The detailed conclusions are

not vital to the purposes of this report, - what is important is that we can use earth-based studies, the Alpha-Helix Concept, to show what physico-chemical instrumentation has helped solve terrestrial problems and how such instrumentation will solve new problems in space biology. In short, we propose using "earth bound" sensory physiology and biochemistry as a guide to the new Alpha-Helix laboratories.

PART II: MOLECULAR COMPONENTS OF THE ENVIRONMENT SENSING SYSTEM

The Bacterial Chemoreceptor System

Introduction

Most of our knowledge of this system has arisen from studies of two enteric bacteria, Escherichia coli and Salmonella typhimurium (1,2). Studies of these gram-negative bacteria have demonstrated such similar systems that the data are regarded as interchangeable. Further, studies on a gram-positive bacterium, such as Bacillus subtilis, and even on photosynthetic bacteria confirm that similar systems are operative even in these distantly related species. Also, as will be discussed below similarities between bacterial chemotaxis and higher plant and animal behavior are so striking as to indicate that we are dealing with life processes that are common to all living things. Thus, there is a clear advantage to using the reductionist approach to study the simplest things first.

Now to briefly describe the system. The bacterium has slender thread-like appendages scattered over the cell surface. When these cilia- appendages - beat in an uncoordinated manner the bacterium tumbles in a random, Brownian-motion-type manner. The bacterium is just as likely to tumble away from a food source as towards it. Now comes the magic! If the bacterium encounters a dissolved nutrient then the cilia fuse into an oar-like flagellum and the bacterium swims in a straight line. If the nutrient concentration increases -

indicating the bacterium is swimming towards the nutrient source - the bacterium continues to swim in that straight-line direction. If, however, the nutrient concentration decreases then the flagella dissociate into dispersed cilia and the bacterium resumes random tumbling. Thus, moving away from the nutrient source causes random tumbling, whereas, if by chance the bacterium starts on the right course - it continues on that course.

The analogy to things even so complex as human behavior is obvious. We tumble until we reach the right course and then that behavior is reinforced and we move in the correct direction. Even memory is involved since the bacterium must "remember" whether the nutrient concentration is increasing or decreasing.

The following description of microbial chemotaxis is given in the hope that a) it will guide us in laboratory design, and b) guide us in studies of more complex systems.

The Sensory Receptor

First, the organism must perceive the environment. The best understood environment receptors are the chemoreceptors of the enteric bacteria - that is the receptors which recognize a favorable chemical and - ultimately - enable the bacterium to swim towards that nutrient chemical and/or away from toxic substances. Thus, most of this section will be devoted to the kinds of experimentation and equipment which led to our present knowledge of bacterial chemoreceptors.

Many other kinds of receptors have, however, been studied. A few examples are receptors for: light, sound, heat, temperature, touch, moisture, osmolarity, and gravity. Unfortunately gravity perception is among the input perceptions that is least well understood and as to radiation, other than a narrow band in the visual region, our knowledge is very limited.

Nonetheless, as will be seen from these few examples there is a certain pattern to the experimentation and one can try to seek out experimental patterns and something of the equipment and facilities needed.

The bacterial chemoreceptor:

A bacterium must swim towards a favorable nutrient and away from a toxic chemical. To do so requires, of course, the motility apparatus, but also a means of receiving the chemical signal - and, most importantly - a signal memory device. The bacterium must measure the nutrient concentration at point "A" and "B" and remember whether A is larger or smaller than B. This is the only way a bacterium can swim towards a nutrient and away from a toxicant.

The bacterial chemoreceptor

To date, all chemoreceptors have been found to be proteins. They are highly specific, usually recognizing only 1 or 2 compounds, and they have a high affinity for that particular compound with a dissociation constant (K_D) of the order of 10^{-7} M. For example the galactose-glucose receptor of S. typhimurium has a K_D for galactose of 2×10^{-7} M, for glucose, 10^{-7} M, for arabinose, 10^{-5} M, for lactose, 10^{-4} M, for fucose, 10^{-3} M and no binding for ribose. The specificity and affinity of a chemoreceptor protein is thus comparable to that of an enzyme and, indeed, the similarity to an enzyme is great except that enzymes catalyze chemical changes in their substrate whereas chemoreceptors, usually, release their substrate unchanged.

The specificity of chemoreceptors can be studied very easily in vivo simply by placing a capillary pipette filled with the attractant into a beaker containing the bacteria with or without the competitive attractant. If there is no competition, the bacteria concentrate in the capillary. If there is competition, the bacteria remain distributed in the beaker.

For detailed studies, however, the chemoreceptor must be isolated, purified to homogeneity - if possible - and then K_D 's be determined using equilibrium dialysis or related techniques.

Once the purified chemoreceptor is available it also becomes possible to determine the amino acid composition and hopefully ultimately to even determine tertiary structure of the folded protein. Thus, with effort, we can "see" the pocket in the protein into which the chemical fits.

This then must become a major objective - to identify the environmental acceptors and to see if there is a commonality of amino acid composition in the composition of these receptors.

Analogies to other systems:

The analogies to the chemotactic system of bacteria are the photo and gravity-responsive systems of higher plants and animals. For example, if in plants and animals the stimulus of light or gravity caused the release of a chemical messenger, and this chemical messenger then came to rest on a chemoreceptor - much as in the bacterial chemoreceptor system - then there would emerge an exact analogy between bacterial chemotropism and higher organism behavior and a unity of how organisms respond to their environment would emerge.

The important point is that a study of environment-receptor proteins must be planned for on the space platform.

Photoreceptors for fungi and higher plants (4,5):

The best studied photoreceptor is that of the fungus Phycomyces blaksleeanus and even those studies are based primarily on action spectra - that is - the response of the organism to light is measured as a function of wavelength. Obviously action spectra can be misleading owing to distorting factors such as a possible dependency of quantum yield on wavelength and, most importantly, the presence of screening pigments. Nonetheless, for both Phycomyces and higher plants there is reasonable certainty that the absorbing pigment is either a flavin or carotenoid.

Despite the uncertainties concerning the photoreceptor pigment, it is possible, as pointed out above and by Delbruck, that we deal with a special case of chemoreception - "the chemical in question being a photochemical product"

(4). Delbruck believed that the amount of photoproduct would be proportional to the number of incident quanta and their cross-sectional capture area. Cross sectional capture area should be proportional to the molecular extinction coefficient of the light capturing pigment. Thus, the flux of quanta at each wavelength to give a standard effect should provide an absorption spectrum of the photoreceptive pigment. Similar reasoning has been applied to many photo effects in higher plants.

Here again we introduce a positive note. Possibly the photo- and geo-act releases a product which binds to a receptor - and thus chemo, photo- and geo-perception are exactly analogous. The point is that once the receptor and its effector are united - they can depolarize a membrane and initiate all the processes of growth.

It is obvious that there is an analogy between terrestrial chemoreception of bacteria, and photoreception of plants and fungi and the problem of georeception by higher organisms, plants and microorganisms.

The action spectra (efficacy as function of wavelength) was measured by Delbruck and Shropshire (5) by interposing on a *Phycomyces* sporangiophore - an upright, hair-like strand of the fungus - a standard light of broad wavelength a test light of variable and narrow wavelength. In this manner they then could measure the bending of the sporangiophore and they found an action spectrum with clear maximum at 485, 455, 385 and 280 nm. This action spectrum fits the absorption spectrum of a flavin - although, unfortunately, it could also be a carotenoid. Thus we must leave this terrestrial problem with the ambiguous

conclusion that for fungi, as well as higher plants, there is uncertainty as to whether the photoreceptor for blue light is a flavin or carotenoid.

The georeceptor:

The problem of the georeceptor introduces even more uncertainties. The prevalent notion is that some heavy cytoplasmic body falls to the base of the cell and there - in an unspecified manner - initiates appropriate growth responses such that a vertical orientation is again attained (6,7).

The problem is that there are no real controls. That a cellular microbody denser than the surrounding cytoplasm falls to the bottom of the cell is no surprise. But is this any more a message of gravity than Newton's falling apple? Indeed, Newton could have concluded that the apple tree sensed the direction of gravity by the direction of the fall of the apple.

Clearly, this is a problem for the space platform laboratory. We have definite and refined knowledge of the chemoreceptors of bacteria. These are characterized and sequenced proteins with a high affinity for the appropriate chemotactic chemical.

We have no comparable knowledge for the photo and gravity reception systems. Does the light or gravity release a chemical that then binds to a "chemoreceptor" protein - or do light and gravity circumvent this initial step of chemoreceptor complex and go immediately to a transduction signal for the appropriate biological response?

This is a major problem of terrestrial - as well as space biology. How do organisms transduce an environmental signal into an appropriate biological response?

Phycomyces sensor mutants that are deficient in both photo and geo responses

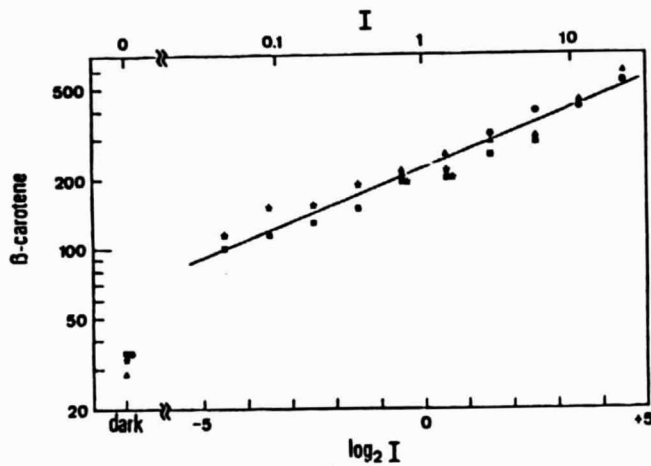
(8):

Many mutants of *Phycomyces* with abnormal phototropism have been prepared and studied. Of the greatest interest is the fact that these mutants are very often also deficient in their response to gravity as well as response to other tropic stimuli.

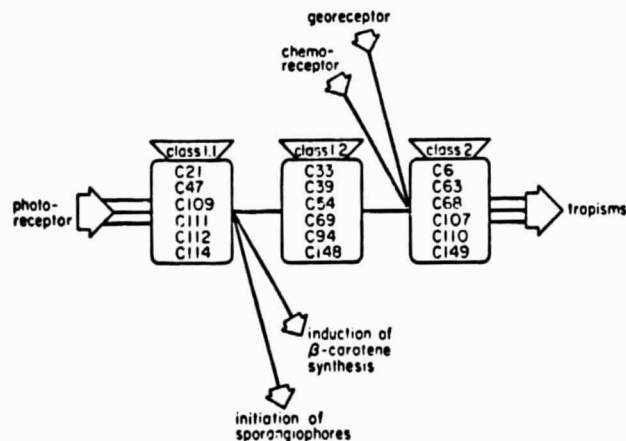
Briefly, the system is as follows: *Phycomyces* has a giant spore-bearing aerial hyphae called a sporangiophore which will readily attain a height of 10 or more centimeters. The sporangiosphere is really one giant multi-nucleated cell with one central vacuole. It shows the following tropic responses: First it is phototropically sensitive and will respond to a stimulus of about 500 quanta (Shropshire) of 440 nm light; second, it responds to gravity and will grow again into an upright position if placed horizontally; thirdly, it can be photoinduced by blue light to synthesize beta-carotene; and lastly, and most strikingly, it evidences a phenomenon called "autochemotropism". Autochemotropism means that the sporangiophore will grow away from an object placed in its growth path, for example, a glass rod, without even touching the obstructing object. How this avoidance phenomenon is accomplished is completely veiled in mystery. It has been postulated that the sporangiophore releases a gas - identity unknown - and reflectance of the gas causes the avoidance response. Nonetheless there are then at least the 4 types of tropic responses and these can be studied in mutant strains.

Mutagenesis was accomplished with nitrosoguanidine and then a most ingenious method was used to select for the mutants. The mutated spores were grown in closed containers illuminated with a horizontal beam of light. Normal, non-mutant sporangiophores do not grow horizontally towards the light because of the gravity response and instead grow at an angle of 30°. This 30° angle is

attained over a factor of 10^9 variation in the intensity of light. Thus the sporangiophore is attracted to the light but cannot grow directly towards the light owing to the corrective gravity response which would cause the sporangiophore to grow vertically upright. Thus the sporangiophores could be scored for their phototropic response, their geotropic response; also their beta-carotene content and their autochemotropic response. An example of the autochemotropic response of the wild type (NRRL1555) and two mutant strains is shown in the figure below (taken from Bergman et al., p. 10).



By this means it was possible to arrange the mutants into 3 phenotypic classes as shown in the figure below (8).

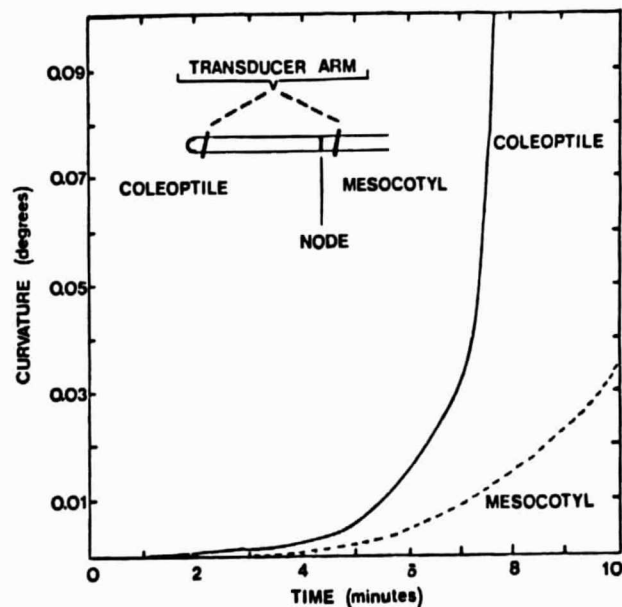


The remarkable thing is that the mutants do not fall into the full 16 classes expected if the 4 criteria were independent. Instead they fall into 3 general classes.

These remarkable experiments again indicate that somehow environmental receptors are plugged into only a few transduction systems which then give the appropriate growth response. This is a strong argument towards using a "micro g" and high radiation environment to better learn how small organisms perceive and then transduce an environmental stimulus. Knowledge gained from these experimentally advantageous organisms will certainly have general applicability to mammalian physiology.

The tropic systems of plants

Plants respond both to light and to gravity. Further, as to light, they measure the length of the light (and, or dark) photoperiod and the wavelength of the light. These light responses have been thoroughly reviewed elsewhere (9). Concerning the gravity sensing component of plants - there is much less certainty (7,8). As shown below, the plant can begin to respond to gravity within minutes - probably instantaneously (10).



The time period for the response seems so short as to preclude the settling of a heavy organelle to the bottom of the plant cell. Further, in the case of young plant shoots, the shoot responds uniformly - to the gravitational stimulus along its entire length - governed only by its intrinsic growth rate.

Thus, for gravity, we do not know what the gravity sensing system is. There is much literature concerning "statoliths" and, indeed, there are even studies of mutants and of starved plants indicating that "starch-free" plants no longer respond to gravity. Thus a case can be made indicating that the plant senses gravity by means of a heavy, starch-like organelle. Still, the time course of the response - and the similarity to other tropic responses seems to argue against this simple mechanical explanation.

Conclusions concerning receptors:

Any laboratory dealing with receptors for gravity and for radiation sensing elements must be equipped as would a modern protein chemistry laboratory on earth. There is no way one can "freeze" a protein in its intricate tertiary configuration, and bring it back to earth for detailed study. The "Alpha Helix" Space Platform laboratory must contain the accoutrements of an excellent protein structure laboratory - as defined by current practitioners of the art.

The equipment required would include that necessary for sequencing both proteins and nucleic acids together with the ancillary equipment required for preparing the pure proteins.

PART III: TRANSDUCTION OF THE RECEPTOR RESPONSE (2,11,12)

Once the stimulus is perceived - whether it be light falling on a photoreceptor, gravity perceived by some unknown apparatus, or a chemical attractant combining with an appropriate receptor site - there arises the problem of the transduction of this [receptor-stimulus] complex to some meaningful physiological response.

Again, it is the bacterial chemoreceptor system that is best understood. Mutations in three genes, abolish chemotaxis (8). The bacterium is still actively motile but it doesn't swim towards an attractant or away from a toxic substance. It can still bind the attractant molecule. Thus, it is believed that these transducer proteins are transmembrane proteins that relay information to the flagella concerning the degree of occupancy of the chemoreceptors (Boyd & Simon).

Somehow the receptor protein reacts with the attractant - with a certain rate constant and as a function of the concentration of the attractant. There is also a rate constant for the rate of dissociation of receptor and attractant. It is this rate of formation and rate of breakdown of [receptor-attractant] that governs the degree of receptor occupancy. Thus the transducer protein must "tell" the flagellar apparatus what the degree of occupancy of the receptor is.

This then represents the simplest form of memory - the transducer protein measures at two times, t_1 and t_2 , how many receptors are occupied. One cannot help but wonder whether human short term memory - in one form or another - also measures "occupancy" of a neurotransmitter site.

The nature of the signal from transducer protein to the flagellar motor apparatus - prolonged effects:

The transducer then has 3 domains, an external one which measures receptor occupancy, an internal domain where chemical changes in the transducer must occur and lastly, a domain where the transducer causes disperse cilia to form into a swimming-effective, flagella.

What changes occur in the transducer? There appears to be an immediate effect followed by a more gradual prolonged effect (2). The prolonged effect involves transducer methylation and sensory adaptation is an overall measure of the activities of a methyltransferase and a methyl esterase. A certain level of

stimulus can be correlated with a certain steady-state level of methylation of the transducer (2). Thus, when the organism is moving toward the attractant, there is a block to methyl esterase activity, thus causing a transient rise in the level of methylated transducers.

Of the very rapid short range effects we know much less. In higher plants (13), in the case of mammalian neurotransmitters (14), in the case of ciliated protozoa (11) as well as in the enteric bacteria one sees rapid changes in membrane potential. Possibly the occupied receptor protein opens a gated pore which changes the cellular Ca^{++} , Na^+ and K^+ concentrations.

How the stimulated receptor protein transfers its knowledge to a transducer and then how that transducer affects a motor apparatus represent weak points in our knowledge. These points, however, are absolutely central to any understanding of behavioral psychology and of how the environment "instructs" both plants and animals to behave. The over-all importance of this knowledge to our terrestrial well-being cannot be overemphasized.

PART IV: Summary and specific recommendations for a sensory physiology laboratory on the space platform:

There is attached to this a small list of references which concern specific statements in this report. In addition, there is attached a list of references that will serve as an introduction to these aspects of sensory physiology. References to mammals, in general, and human physiology, in particular are left to other reports in this program. Here we have focused only upon a few of the best studied microbial, protozoan and seedling plant systems.

We regret these omissions and restrictions. The field of sensory physiology is so huge as to require a multi-disciplinary approach by many workers. With that caveat in mind, we make the following recommendations:

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- 1) It is apparent that the medical problems of space physiology are closely related to the problems of sensory physiology in lower plants, animals and bacteria.
 - 2) It is clear that the response to an environmental signal, terrestrial or "micro-g", is mediated by: a) the signal; b) a receptor protein; c) a transducer and; d) a response apparatus.
 - 3) There is great similarity in the manner in which sensory physiology research is conducted, including, a) use of mutant organisms; b) the techniques of protein and nucleic acid isolation and characterization; c) the methods of small molecule chemical characterization; d) electrophysiological techniques and, e) microscopy.
 - 4) As stated in the Introduction, there is need for side by side comparisons of a "micro-g" environmental with the environment of a 0 to 1 g centrifuge.
 - 5) There is need for a heavily radiation shielded environment - preferably a "layered" environment adjacent to a region with minimal shielding.
 - 6) To the best of our current knowledge - based on terrestrial studies of sensory physiology - it would be best to equip the laboratory exactly as one would equip a sophisticated chemistry-biochemistry laboratory. The equipment would include: a 0°C working area - that is a small "walk-in" cold room; the centrifuges and chromatography and electrophoresis apparatus required for protein and nucleic acid research; a sterile hood and chemical fume hood; a wet lab area; and the standard IR, NMR, and mass spectral equipment.
 - 7) Owing to the complexities of the above described studies, it would be best to use teams of perhaps 3 scientists with orientations in biology, genetics and chemistry-biochemistry.
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PART VIII: GRAVITY AND GEOTROPISM (PLANT)

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PART IX: PHOTOTAXIS AND PHOTOTROPISM

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