BIOPROCESSING IN SPACE

Proceedings of the
1976 NASA Colloquium on Bioprocessing in Space
Houston, Texas, March 10-12, 1976

Compiled by
Dennis R. Morrison

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Lyndon B. Johnson Space Center

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16. Abstract

This document contains the proceedings of the 1976 NASA Colloquium on Bioprocessing in Space, held at the Lyndon B. Johnson Space Center on March 10-12. The colloquium was held to introduce this subject to academic and industrial scientists who have gravity-induced biological research problems or an interest in the possibilities of processing or manufacturing biologicals in space. The meeting was intended to establish a cooperative effort among Government and non-Government researchers to examine the potential of bioprocessing in space.

The program included general sessions and formal presentations on the following topics : NASA's Space Shuttle, Spacelab, and space-processing programs; the known unusual behavior of materials in space; space-processing experiment results; cell biology, gravity sensors in cells, space electrophoresis of living cells, new approaches to biosynthesis of biologicals from cell culture in space, and zero-g fermentation concepts; and upcoming flight opportunities and industrial application planning studies already underway.

The proceedings include the summaries of workshops held during the colloquium to allow participants to discuss new ideas, future biological research areas, and gravity-related problems in the areas of (1) industrial biosynthesis, (2) pharmaceutical research, (3) biotechnology, and (4) cell biology.

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BIOPROCESSING IN SPACE

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FOREWORD

The NASA Lyndon B. Johnson Space Center was particularly pleased to conduct this colloquium because it was the first opportunity for the life sciences elements of NASA to focus the attention of a broad spectrum of scientists on this new research area - bioprocessing. More than 170 academic scientists and industrial researchers attended the colloquium. This document is a compilation of the papers presented at the symposium and brief summaries of the discussion workshops held during the meeting.

Acknowledgment is made of the following individuals, who were directly responsible for the organization and conduct of this symposium. The colloquium committee included Dr. Wayland Hull, Bernard Mieszkuc, Dr. Dennis Morrison, and Dr. Peter Whittingham from the Johnson Space Center; and Bruce Goss of the Boeing Company.

I particularly wish to thank Dr. Whittingham for his invaluable assistance in the detailed preparation and conduct of this colloquium. Additionally, I wish to thank Dr. Merlyn Bissell and Dr. Gerald Taylor for their suggestions and assistance. Finally, I wish to thank the authors and the numerous other individuals of the participating organizations who provided active support in the preparation of this colloquium.

Lawrence F. Dietlein, M.D.
Acting Director of Life Sciences
NASA Lyndon B. Johnson Space Center

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SESSION LEADERS

General Sessions

  Space Shuttle and Space Processing

    Chairman: Dr. Dennis R. Morrison, Lyndon B. Johnson Space Center, Houston, Texas

  Materials Behavior and Space Processing

    Chairman: Dr. Peter Whittingham, Lyndon B. Johnson Space Center, Houston, Texas

  Cellular Research and Biological Processing

    Chairman: Mr. Bernard J. Mieszkuc, Lyndon B. Johnson Space Center, Houston, Texas

  Biosynthesis

    Chairman: Dr. Wayland E. Hull, Lyndon B. Johnson Space Center, Houston, Texas

  Flight Opportunities and Potential Programs

    Chairman: Dr. Dennis R. Morrison, Lyndon B. Johnson Space Center, Houston, Texas

Workshops

  Biotechnology

    Cochairmen: Mr. John M. Walsh, Beckman Instruments, Inc., Anaheim, California; and
    Dr. Robert E. Allen, George C. Marshall Space Flight Center, Huntsville, Alabama

  Cell Biology

    Cochairmen: Dr. Jerry V. Mayeux, Bio Innovar, Inc., Storm Lake, Iowa; and
    Dr. Gerald R. Taylor, Lyndon B. Johnson Space Center, Houston, Texas

  Industrial Biosynthesis

    Cochairmen: Dr. W. E. Brown, Squibb Institute, Princeton, New Jersey; and
    Dr. R. E. Sparks, Washington University, St. Louis, Missouri

  Pharmaceuticals

    Cochairmen: Dr. E. John Staba, University of Minnesota, Minneapolis, Minnesota;
    and Dr. Takeru Higuchi, University of Kansas, Lawrence, Kansas

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BIOPROCESSING IN SPACE

By Dennis R. Morrison
Lyndon B. Johnson Space Center

INTRODUCTION

Experiments conducted in space onboard Apollo, Skylab, and the recent Apollo-Soyuz Test Project flight have established that many materials exhibit unique properties in virtual weightlessness, a condition impossible to duplicate on Earth. These results have led to new, Earth-based research and development efforts in areas of fluid mechanics, metallurgy, crystal growth technology, acoustic levitation and manipulation of materials without containers, silicon refining for semiconductors, and several innovations in electrophoretic separation of different types of biological cells.

Materials processing onboard orbiting manned spacecraft can take advantage of weightlessness, readily available solar energy, and a vacuum source of unlimited capacity to purify, isolate, or manufacture materials that are too difficult, costly, or impossible to produce on Earth. The Space Shuttle and Spacelab flight in the 1980's will be used to explore manufacturing processes that benefit from convectionless behavior of weightless fluids, new techniques for production and manipulation of immiscible mixtures, and containerless processing of biological materials in liquid media, and mass transfer in liquids that is wholly controlled by diffusion. The possible utilization of the unique space environment for the manufacturing and processing of biological materials could be very significant to pharmaceutical research and manufacturing.

In March 1976, the National Aeronautics and Space Administration (NASA) sponsored a 3-day colloquium on "Bioprocessing in Space" at the Lyndon B. Johnson Space Center, Houston, Texas. The main purpose of the colloquium was to introduce this subject to academic and industrial scientists who may have gravity-induced research problems or an interest in the possibilities of processing or manufacturing biologicals in space, but who were uninformed about the behavior of materials in weightlessness and the forthcoming opportunities to fly experiments by means of NASA's sounding rocket and Space Shuttle payloads program.

The general sessions included presentations on (1) the unusual behavior of materials in a weightless environment, (2) research facilities and opportunities for flight onboard the Space Shuttle and Spacelab, (3) results of pertinent experiments conducted in space, and (4) potential research and industrial applications of the space environment. Workshops in related areas of biotechnology, cell biology, industrial biosynthesis, and pharmaceutical research also were held to generate new ideas and principles for solving biological research or manufacturing problems that are gravity dependent.

More than 170 participants, mostly from the biological- or pharmaceutical-related nonaerospace industry and academia, attended the colloquium. The interest level was quite high concerning new opportunities to perform experiments in orbit. Many participants made requests for literature indices to specific space experiment results, more payload user information, and future meetings on related space biology topics. The following proceedings include the formal papers presented at the colloquium and brief summaries of the discussions and noteworthy ideas from the different scientific workshops.

General Sessions

SPACE SHUTTLE AND LIFE SCIENCES

By John A. Mason
Chief, Bioscience Payloads Office
Life Sciences Directorate
Lyndon B. Johnson Space Center
Houston, Texas

ABSTRACT

The Space Shuttle, America's first reusable spacecraft system, will have many diverse functions: It will launch satellites geared for such tasks as communications, weather observations, pollution monitoring, Earth-resource studies, and world wide navigation. It will retrieve satellites for Shuttle-based repairs or return to Earth. And the Shuttle will carry the first laboratory dedicated to the study of life processes in space. The Spacelab, being designed and constructed by the European Space Agency, will serve as a general research facility for the exploration and eventual industrialization of space. During the 1980's, some 200 Spacelab missions will be flown in Earth-orbit. Within these 200 missions, it is planned that at least 20 will be dedicated to life sciences research, projects which are yet to be outlined by the life sciences community. Discussions within the paper cover objectives of the Life Sciences Shuttle/Spacelab Payloads Program; also discussed are major space life sciences programs including space medicine and physiology, clinical medicine, life support technology, and a variety of space biology topics. The Shuttle, Spacelab, and other life sciences payload carriers are described. Concepts for carry-on experiment packages, mini-labs, shared and dedicated spacelabs, as well as common operational research equipment (CORE) are reviewed. Current NASA planning and development includes Spacelab Mission Simulations, an Announcement of Planning Opportunity for Life Sciences, and a forth-coming Announcement of Opportunity for Flight Experiments which will together assist in forging a Life Sciences Program in space.

INTRODUCTION

It is my pleasure to present this paper: "The Space Shuttle and Life Sciences." The paper is divided into 6 parts: 1) Space Shuttle Program Description, 2) Spacelab Description, 3) Life Sciences Program, 4) Life Sciences Payload Carrier Characteristics, 5) Development of Program Planning and Operational Approaches, and 6) Summary.

1) SPACE SHUTTLE PROGRAM DESCRIPTION

The Space Shuttle is the first true blending of the manned and unmanned space programs. The Shuttle will fly approximately 20 years after the United States first entered space in January 1958. The Shuttle is designed to support Spacelabs (which will be discussed later) and to launch satellites, as well as space probes with propulsion stages. All of these will support applications and technology as well as scientific experiments. It is anticipated that not only NASA personnel but also individuals from other government agencies, universities, and industry, as well as the international community will use the Shuttle (Figure 1) (1).

Program Objectives

The Space Shuttle Program Objectives are twofold: 1) to reduce the cost of space operations, and 2) to provide a capability designed to support a wide range of scientific, applications, defense, commercial and international uses as mentioned earlier. This is to help establish a national space transportation capability (Figure 2). The Space Shuttle System as it is called is composed of 4 major parts. The Shuttle Orbiter (an aircraft-like structure), the large external hydrogen/oxygen tank, and the 2 rocket engines. The Orbiter is 122 feet (37.2 m) in length, and it has a wing span of 78 feet (23.8 m). In comparison to other known air or spacecraft, the Shuttle is slightly larger than the DC9. It is approximately 22 feet longer than the Boeing 737, half the height of the Saturn V (the rockets that took the Apollo crews to the moon).

Space Shuttle Mission Profile

Initially, the Shuttle will be launched from the Kennedy Space Center. Approximately 2 years later it is planned that the Western Test Range, located at Vandenberg Air Force Base, will be added as a launch site. The Shuttle Orbiter will be taken into near Earth orbit by the solid rocket engines which will be jettisoned and returned to Earth by a parachute system for reuse. The large external hydrogen/oxygen tank's propellants will continue to carry the Shuttle Orbiter into orbit and then the tank will be jettisoned. It will not be returned by parachute system and will not be reused. Mainly because of its large size and configuration, it will not be able to reenter the Earth's atmosphere without damage, so the crash point for the tank will be programmed for a remote place in one of the oceans. The orbiter will then remain in Earth orbit, nominally 100 to 200 miles from the Earth's surface. It will remain there from one week initially up to a maximum of 30 days during which time the various payloads will be activated, tested, exposed or launched. The cargo bay doors may be opened to allow for launch or to place spacecraft into orbit or, if there is a Spacelab attached within the cargo bay, it will remain and work will be performed in the Spacelab. After the mission is completed, the Orbiter will enter the Earth's atmosphere with a high angle of attack and will coast with the minimal assistance of non-airbreathing engines. It will coast in a preprogramed flight plan and will land at Kennedy Space Center or Vandenberg Air Force Base. It is planned that there will be no more than a 2-week turnaround time between the time of landing and the next launch of a scheduled Shuttle mission (Figure 3) (1, 2).

Space Shuttle Program Activities

The Space Shuttle Program is made up of a number of planned activities. The development phase is now underway. Some of the key events are scheduled as follows: The first Space Shuttle Orbiter will be "brought out of the hangar" in the fall of 1976. The first captive flight test at Edwards Air Force Base is scheduled for the spring of 1977. Approach and landing tests also will be conducted at Edwards Air Force Base starting in the late summer of 1977. The first manned orbital test flights are scheduled for 1979. The operational flights then will begin early in the 1980s (Figure 4).

Space Shuttle Operations

The Space Shuttle is planned for a number of different operations. It will assist in orbital missions where we will have, for example, a telescope to permit astronomers to view heavenly bodies from above most of the Earth's atmosphere. The Shuttle Orbiter may also

serve as the propulsion stage for delivery and retrieval of orbiting spacecraft. It may
replace the first two launch stages for a spacecraft that will be launched into deep space.
It will also allow repair and servicing of satellites that are already in Earth orbit, thus
providing an extension of the life of spacecraft in orbit. It will also permit the carrying
of passengers and crewmembers to near Earth orbit for a space station when it is developed
and becomes operational. In addition, rescue operations may be conducted if a crewmember
were to become ill or when there is a problem in a spacecraft (Figure 5).

2) SPACELAB DESCRIPTION

The Spacelab actually may have 2 major parts - a pressurized volume and/or a pallet,
carried separately or together (Figure 6). These are contained and carried into near Earth
orbit in the cargo bay of the Shuttle Orbiter. The Spacelab remains attached in the cargo
bay for the entire mission since it relies on the Shuttle Orbiter for many of the life support
systems.

The Spacelab has been developed through international cooperation by the European Space
Agency (ESA). ESA is made up of representatives from a number of European countries, with
the major contributor, Germany, contributing over 53% of the financial backing. The
United Kingdom, Italy and France are other large contributors, although nowhere near the
percentage that Germany has contributed. ESA has its headquarters in Paris, and ESTEC
(the space center which might be compared to the Johnson Space Center) is located in the
Netherlands. This is where the Spacelab Program is managed. The major contractor working
and coordinating the work of other contractors for Spacelab is ERNO, located in Bremen,
Germany. It turns out that the nations contributing money into the program, to a large
extent, have contractors receiving funds approximately equal to the amount that the country
has contributed to ESA (Figure 7).

Experiment Accommodations of Spacelab
(Pressurized Module)

The Spacelab will be in orbit initially 7 days, as I mentioned before, and it is planned
that it may be extended to 30 days. The experiment weight that it will carry into orbit will
be from 4600-16700 lbs. (2100-7600 kg). The experiment volume is 790 ft^3 (22.3m^3). The
volume planned is for biological specimens. It cannot carry an elephant but could carry
primates, man or his surrogates. The atmosphere is to be an Earth-like environment, i.e.,
20% oxygen in nitrogen at 14.7 psi or 760 mmHg pressure. The temperature will be selectable
and will be a shirtsleeve environment, 18-27°C. The utilities available are virtually those
that can be expected in an Earth laboratory. There will be electrical power, thermal con-
trol, data processing as needed for tests currently considered. There will be support
equipment, and storage, i.e., places to store film so that it can be used effectively, and
there will be viewports to view the Earth or heavenly bodies from the pressurized volume of
the Spacelab.

This has been a brief review of what the Spacelab is and plans to be to support
scientific endeavors.

3) LIFE SCIENCES PROGRAM

Five main objectives are currently identified for the Life Sciences Program. The first is to explore and resolve the problems associated with future ventures of man in the exploration, exploitation, and eventual colonization of space. Next is to expand our knowledge of life sciences, i.e., life sciences as we perceive it to be related to Earth organisms. Another is to develop technology. This is something that we will be looking into. Coupled with this is an improvement in man's living conditions, both from an ecological and a biological standpoint. Lastly, prepare for space manufacturing and processing of biochemicals and biological materials. For instance, certain pharmaceuticals may be produced in space more effectively, purer, and less expensively than on Earth.

Life Sciences Program Overview

From the standpoint of presenting an overview of the Life Sciences Program, it can be divided into 3 segments. The first segment might be called the "Preparation Decade," i.e., from 1971 to 1981. The "Investigation Decade" would extend from 1981 to 1991, and beyond that, from 1991 to 2001, would be the "Exploitation Decade." During the preparation decade, i.e., from 1971 to 1981, you can further divide that into 2 parts. That part before 1974 had a number of programs that have led to the planning and developing of the Shuttle and the Spacelab. The Mercury, Gemini, Apollo and Skylab Programs all have certainly given us extensive information. There have been mission models and planning studies (which are actually payload definition studies) that have aided. The second part of this preparation decade can be identified as the "Development Period" and that is where we are right now. We are looking at the kinds of experiments which can benefit from the space environment and what support it will take to execute these experiments. Common Operational Research Equipment (CORE) is being investigated which will support the experiments and operational procedures. This will be described later. Some of the other activities that are taking place are the examination of carry-on laboratories. What can be done in the crew quarters of the Shuttle Orbiter in the way of experimentation in the very limited space that is available? What can be done in mini-labs when another discipline than life sciences is using the pressurized volume of the Spacelab? What can be done in mini-labs? What are those things that can be done life sciences-wise when the pressurized volume of the Spacelab is completely dedicated to life sciences? What are those experiments that can best fly on the proposed Biomedical Experiments Scientific Satellite (BESS)? These are the kinds of questions being considered now and will continue to be until the Shuttle/Spacelab becomes operational in the 1980s. Then the investigation decade will begin when operational flights will be available for life scientists to fly experiments. Probing type experiments and then in-depth experiments will naturally build on these for exploitation and building in breadth and depth of life sciences knowledge (Figure 8).

Space Transportation System Payload Program Elements

Not all individuals are aware of the various areas that can benefit from the space environment through the use of the Shuttle Spacelab. They range from Space Physics, Materials Sciences, Earth and Ocean Physics, Solar Physics, Astronomy, Earth Observations, Communications/Navigation, Technology, and High Energy Astrophysics, to Life Sciences. So many people do not appreciate that Life Sciences is not merely biomedical studies but ranges beyond biomedical studies to include areas such as the study of vertebrates, invertebrates,

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plants, cells, tissues, bacteria and viruses, as well as environmental control and man/
systems integration studies. These are areas of Life Sciences that we currently visualize
that can benefit from studies in the space environment. There may be others.

Major Space Life Sciences Research Areas

The major Life Sciences research areas that are predicted to benefit from the space
environment experimentation are Space Medicine, Clinical Medicine, Life Support Technology,
and Space Biology. These are the 4 major categories that include areas in Life Sciences
mentioned earlier (1, 2, 3, 4, 5).

Space Medicine.- In Space Medicine to date the general conclusion is that man can adapt
to weightlessness with good health for extended periods of time with appropriate exercise,
sleep, diet, working, and recreation. So far, no major physiological problems have been
encountered, but we do need to understand and we do need experiments which will permit inves-
tigation of the mechanisms of changes that are occurring. Some remedial or preventive
measures may be required for missions longer than the experience of 84 days that we had man
in a weightless condition up to now. For voyages of man to Mars or other regions in space
where he will be in the weightless condition as long as 9-12 months or perhaps several
years, we certainly must look at longer term remedial or preventive measures.

Shuttle research emphasis in space medicine will probably focus on the vestibular,
cardiovascular, mineral/fluid balance/electrolytes, and hematology areas, i.e., from the
perspective of our experience on Skylab and the other manned programs.

Clinical Medicine.- In Clinical Medicine, the effects on physical condition will be
studied. We must be prepared to treat fractures that might occur in a space environment and
learn how healing will progress. We will examine wound healing, burn therapy, cardiovascular
disease and therapy, and emergency and dental therapy. These are all conditions that are to
be expected in individuals who will be in space. They are to be expected in individuals on
Earth, and if man is going to be in space for extended periods, we must know how to handle
these kinds of problems. In addition, there will be opportunities for new research in areas
where we may have ailments of vision, muscle or cardiovascular function.

Life Support Technology.- In Life Support Technology, biological research involving both
the crew and passengers as well as biological specimens will be examined. Regenerative life
support systems involving atmospheric revitalization, reusing water that will be there in the
spacecraft as a by-product and looking at sources of food that should be grown will demand
attention.

Space Biology.- In Space Biology, vertebrates, invertebrates, cells and tissues and
plants will be studied in the weightless context and radiobiologically. Again, this is a
projection from past research efforts that have not been directed into an organized space
research program.

NASA-Shuttle/Spacelab/Life Sciences
Laboratory Traffic Model

The NASA-Shuttle Spacelab Traffic Model, or actually the projected plan of opportunities
to fly in space during the decade of the 1980s, from the perspective of today, is quite
encouraging. It is planned that approximately 10% of all the Shuttle flights will be flights

which will include Life Sciences research. To be specific, in the current plan, the Shuttle is to go into space 501 times during the years from 1979 through 1991. Of those 276 flights will contain Spacelabs, and Life Sciences can expect to have 22 dedicated laboratories and 25 missions where we will have a mini-lab aboard a flight that would be primarily dedicated to another discipline (Figure 9).

4) LIFE SCIENCES PAYLOAD CARRIER CHARACTERISTICS

Earlier in the paper, payloads carriers were briefly mentioned. There are 3 main carriers that will be contained within the Shuttle and remain there the entire mission (Figure 10). The other is the BESS or the Biomedical Experiments Scientific Satellite which will be launched and remain in Earth orbit for 6 months to perhaps 1 year.

Carry-on experiments.- Carry-on laboratory (COL) experiments will be contained in the crew compartment of the Shuttle Orbiter (Figure 11). They will be small experiment packages weighing less than 23 kg (50 lbs.), and will require minimum involvement of the crewmembers and an insignificant amount of power. They will be carried when weight and space are available in the crew compartment.

Mini-labs.- Mini-labs will be contained in racks installed in the pressurized volume of the Spacelab, perhaps in 1 rack, 19 inches wide, or 2 equalling approximately 1 meter (Figure 12). As indicated, this mini-lab would be flown on a shared discipline mission. It would generally weigh less than 500 kg and there could very well be a significant interface with the Spacelab or orbiter as far as power, heat balance, command, control, or electronic countermeasures are concerned. A payloads or mission specialist, who would oversee the mini-lab, would not necessarily be trained in a Life Sciences discipline.

Dedicated laboratories.- The next and perhaps most important is the dedicated laboratory where up to 7,600 kg of laboratory equipment would fly (Figure 13). There would be extensive interfaces with orbiter as far as power, thermal control, etc. are concerned, and up to 3 discipline specialists operating 12 hours/day on a 7-day mission might fly. It could be extended up to a 30-day mission as our experience and capability is extended (2).

Biomedical Experiments Scientific Satellite (BESS).- The Biomedical Experiments Scientific Satellite is a free-flying satellite which is under development now and which will become operational some time later than the Spacelab, perhaps around 1983. It will provide a 6-month to 12-month time period in orbit for specimens. It will be deployed from the cargo bay of the Shuttle Orbiter and will allow for longer duration, zero gravity exposures for both animals and plants with a built-in partial or one-g experiment control capability provided by a specimen centrifuge. It is planned that the specimens will be inserted into the BESS from the spacecraft after orbit is achieved. Checkout will occur to insure that all specimens are in good order. If some problem should exist, a correction could be made even after release of the BESS into orbit. Upon retrieval, specimen examination could be made in a Spacelab that might be carried aloft at the time of retrieval. It is a very exciting capability that is now being planned.

Earlier the Common Operational Research Equipment (CORE) was mentioned. CORE can be further subdivided into 3 areas (Figure 14). Regular equipment items such as a microscope, or a mass measuring device, which would be used in weightlessness to obtain measurements, will be used over and over again and may be required by many different biological experiments. CORE Intermittent would be items that would support several different experiments but would not be as universally useful as those mentioned under the regular equipment items. Examples would be lower body negative pressure device or certain racks for test tubes. Another piece of CORE equipment might be a rotating litter chair to exercise orientation and balance organs which very few experiments would require.

5) DEVELOPMENT OF PROGRAM PLANNING AND OPERATIONAL APPROACHES

An examination of the phases of a Life Sciences Payloads Mission would show it can be divided into 4 major segments or phases: Experiment Selection Phase, Preparation Phase, Flight Operations Phase, and Postflight Phase. Each of these can be further subdivided and then examined. In the Experiment Selection Phase, formulation of an overall plan takes place; solicitation, receiving of proposals, and selection takes place. In the Preparation Phase, mission scheduling, experiment development, experiment testing and training, and integrated simulations are some of the major areas. In the Flight Operations Phase, at prelaunch there is checkout, mating of the experiment carrier with the Orbiter, launch, flight operations, flight support, as well as landing operations. The Postflight Phase is composed of debriefing, examination of the data and distribution of the data and samples to the investigators for analysis and reporting by the experimenter. This is an example in a generalized way of what takes place in all flights. It is presented to give a concept of those things that must be considered for space flight.

Spacelab Mission Simulation

Some of the aspects of the mission have been investigated and reviewed through a Spacelab Mission Simulation. The current simulation plan at JSC projects a total of 5 Spacelab Mission Simulations or SMS. Two simulations have been conducted, 3 more are to occur, planned before the operational period of the Shuttle beginning in 1980 or 1981. Each one of the scheduled simulations will look at and emphasize specific aspects of operational planning. Totally, we will be considering science community involvement, management systems, experiment and payload processing, mission operation systems and approaches, equipment and facilities, supporting documentation, and Spacelab configurations as well as roles of organizations and key personnel.

## Involvement of Non-NASA Scientists

The simulation that we are preparing for now will involve visiting scientists, those from organizations other than at the Johnson Space Center and other elements of NASA. The Spacelab Mission Simulation Program is considered key in preparing for the operational period that will soon be upon us.

Through an Invitation to Participate in Life Sciences Space Program Planning, an announcement of planning opportunity was issued in the spring of 1975. The announcement was sent to over 27,000 scientists in this country. As of early this year, over 1,400 responses with over 2,500 ideas for experiments were received. The responses are being used by NASA to determine the range and types of biological specimens that will be required. The data will be used for determining the scientific disciplines (specialties) that we must accommodate in the Shuttle, and to determine and identify the laboratory equipment that will be required. An Announcement of Flight Opportunity will be issued later in 1976. The first call will be for the first US/European Space Agency Spacelab flight scheduled for 1981. Although experiments will be somewhat limited for this Spacelab flight, later a general announcement of flight opportunity will be made for Life Sciences (6,7).

6) SUMMARY

In summary, it can be said that the Space Transportation System Program is well under way and will provide a broad new capability by the 1980s; a capability which will permit flying many Life Sciences experiments. The development of the Spacelab/Common Operational Research Equipment and the Biological Specimen Holding Facility will allow for the involvement of many scientists with minimum hardware costs. There will be a broad Life Sciences Program which is currently in the conceptual phase (some few ideas are now represented by preliminary designs). Perhaps the most important point is that for the diverse program to be a success the participation of life scientists such as you is required.
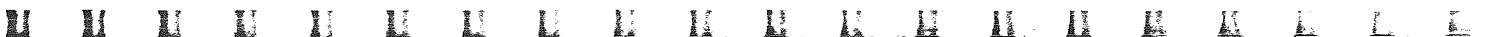
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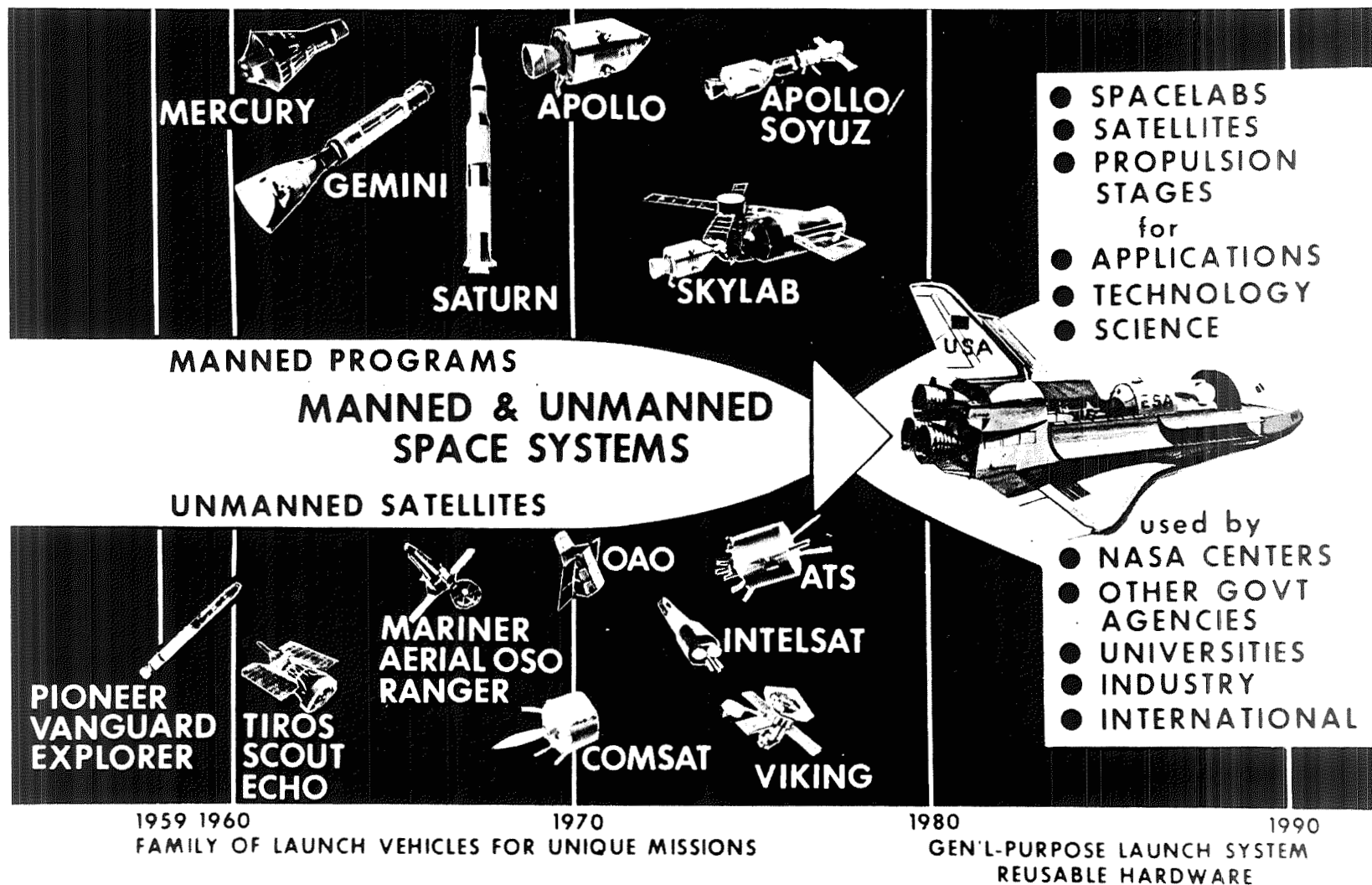
Figure 1.- Space Shuttle era (NASA-S-74-5358A).

TO ESTABLISH A NATIONAL SPACE TRANSPORTATION CAPABILITY THAT WILL
- **SUBSTANTIALLY REDUCE THE COST OF SPACE OPERATIONS AND**
- **PROVIDE A CAPABILITY DESIGNED TO SUPPORT A WIDE RANGE OF SCIENTIFIC, APPLICATIONS, DEFENSE, COMMERCIAL AND INTERNATIONAL USES**

Figure 2.- Space Shuttle Program objective (NASA-S-74-5359A).

ORBITER BURN CONTINUES

EXTERNAL TANK JETTISON - SUBORBITAL

ORBITAL INSERTION

SATELLITE PLACEMENT/RETRIEVAL AND/OR EXPERIMENTS

DEORBIT

SOLID ROCKET BOOSTER JETTISON

ATMOSPHERIC ENTRY

LAUNCH ORBITER AND SOLID ROCKET BOOSTER PARALLEL BURN

SOLID ROCKET BOOSTER RECOVERY

TURNAROUND MAINTENANCE AND REFURBISHMENT

CROSS-RANGE MANEUVER CAPABILITY

LAUNCH PAD

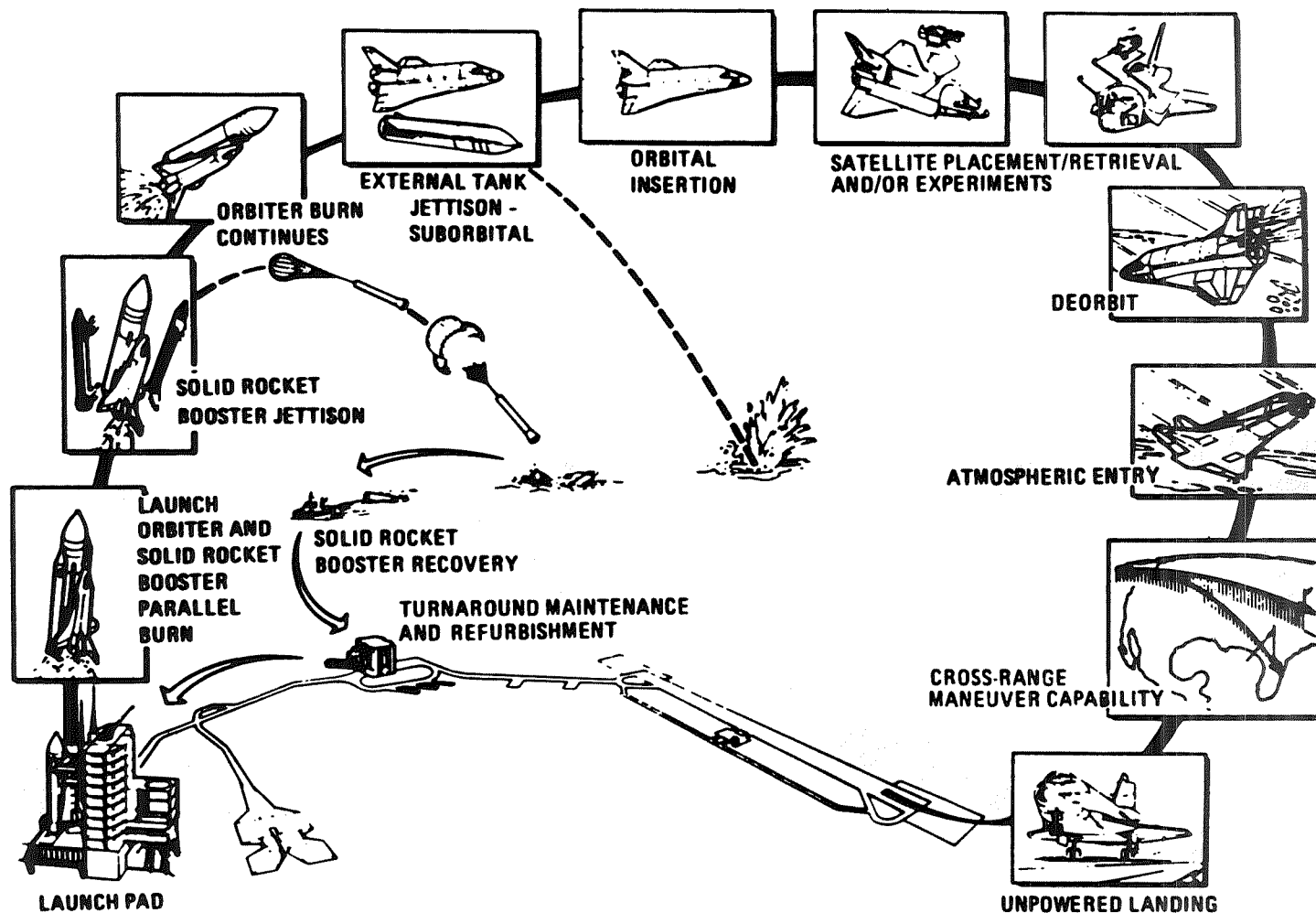UNPOWERED LANDING

Figure 3.- Space Shuttle mission profile (NASA-S-75-868).

Figure 4.- Space Shuttle Program activities (NASA-S-75-15027).

SHORT-DURATION ORBITAL MISSIONS

PROPULSION STAGES DELIVERY AND RETRIEVAL

PLACEMENT, REPAIR & SERVICE OF SATELLITES

PASSENGER AND CREW TRANSPORTATION
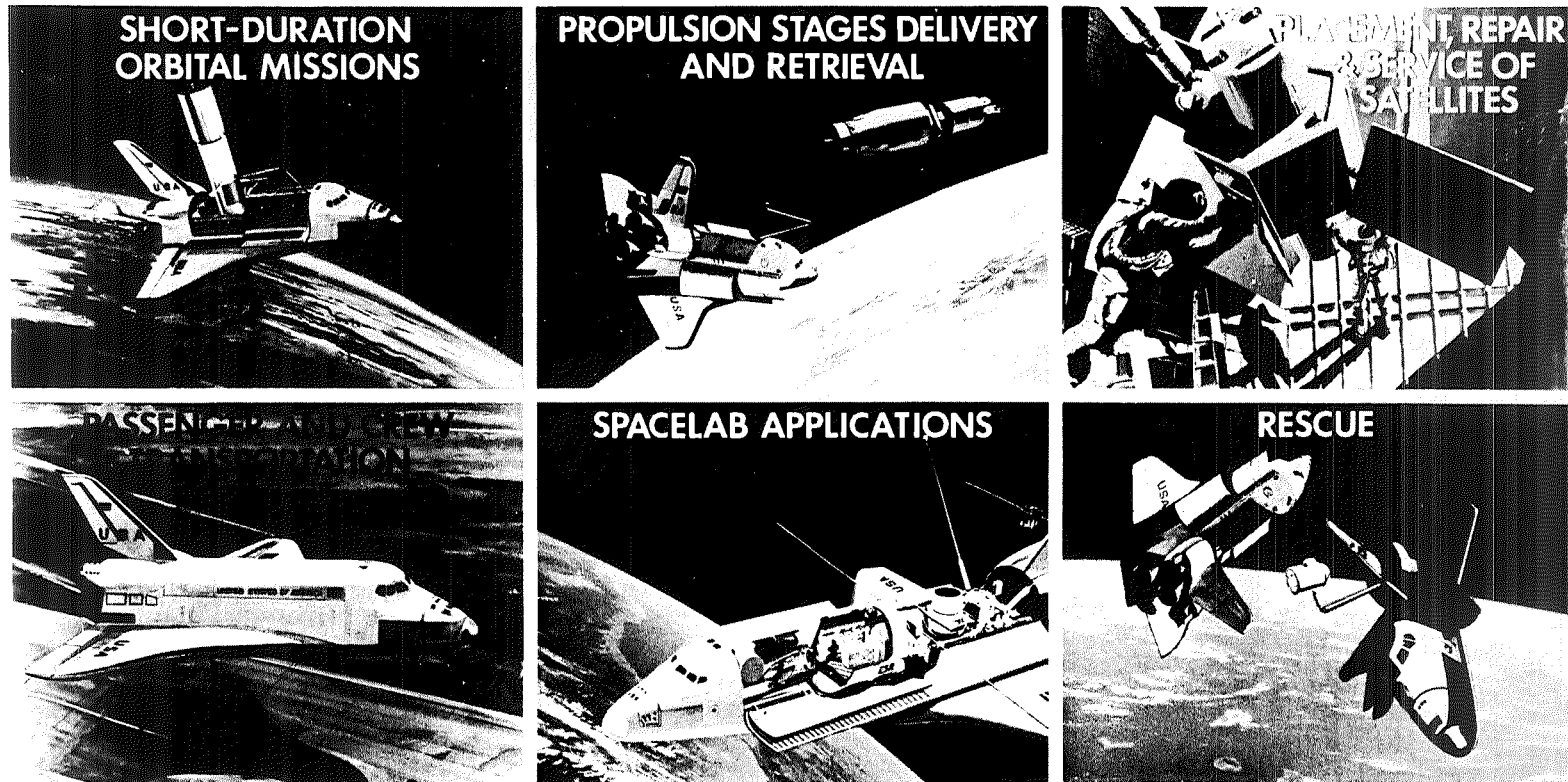
SPACELAB APPLICATIONS

RESCUE

Figure 5.- Space Shuttle operations (NASA-S-73-2380B).
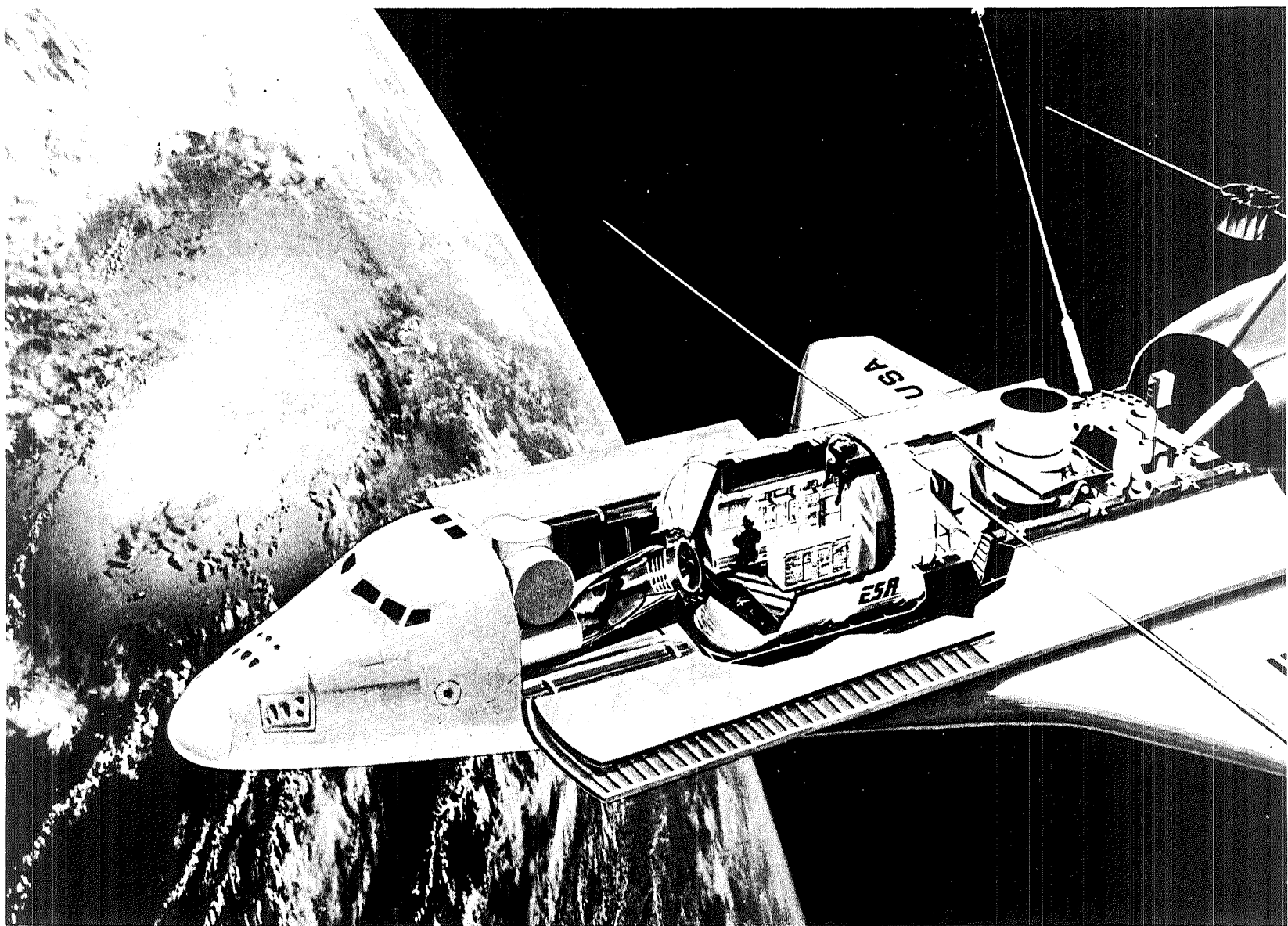
Figure 6.- Spacelab (NASA-S-75-15017B).

SPACE SHUTTLE

SPACELAB [   ]

EUROPEAN SPACE AGENCY (ESA)

* SWEDEN

1.5% DENMARK

53.1% W. GERMANY

2.1% NETHERLANDS

6.3% UNITED
KINGDOM

4.2% BELGIUM

1%** AUSTRIA

10% FRANCE

1% SWITZERLAND

USSR

2.8% SPAIN

18% ITALY

* ESA MEMBER BUT
  NOT INVOLVED IN SPACE LAB
** NON MEMBER OF ESA

20

Figure 7.— Extent of international cooperation in manned space flight (NASA-S-74-3431C).
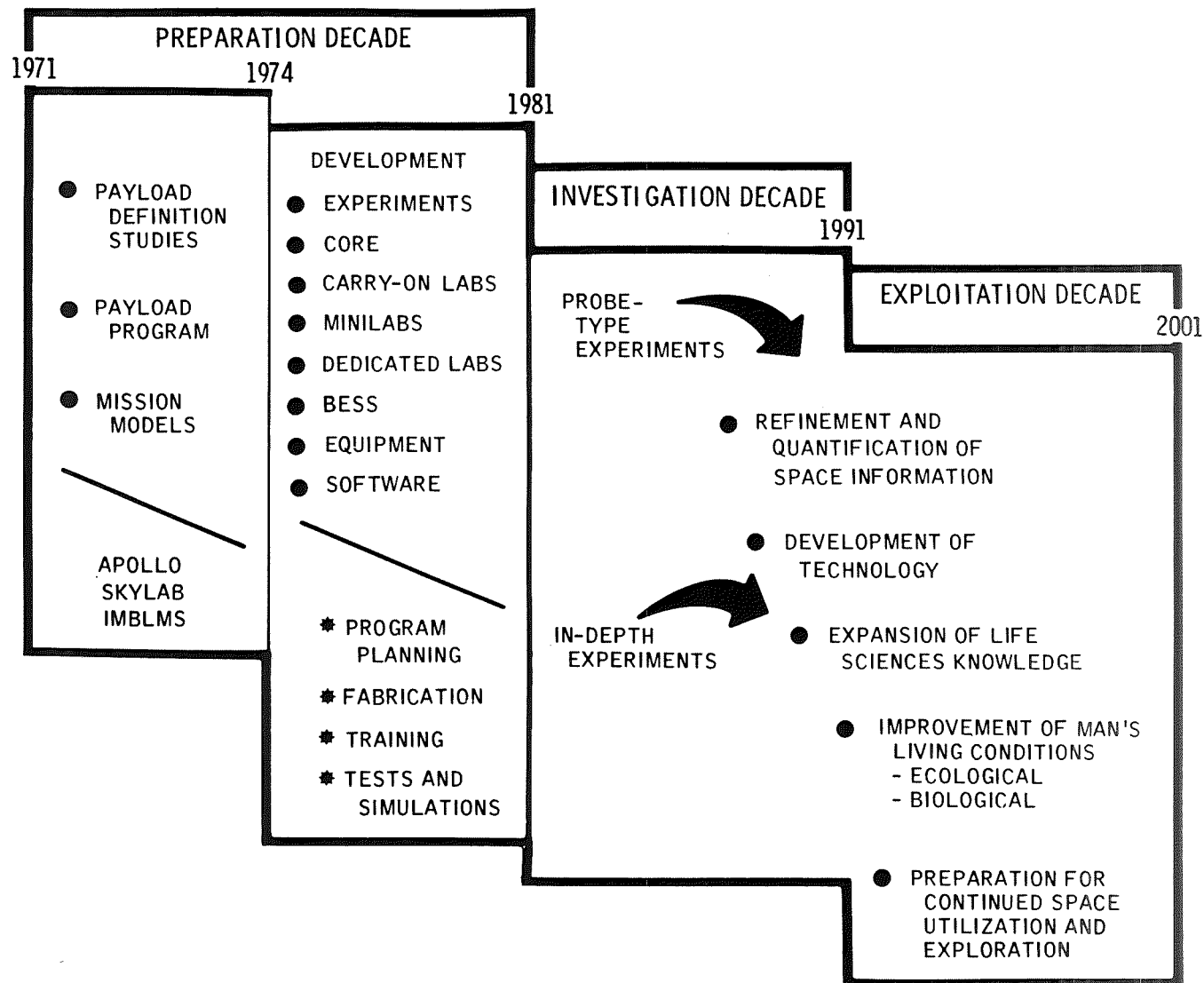
Figure 8.- Life science program overview (NASA-S-76-10159A).

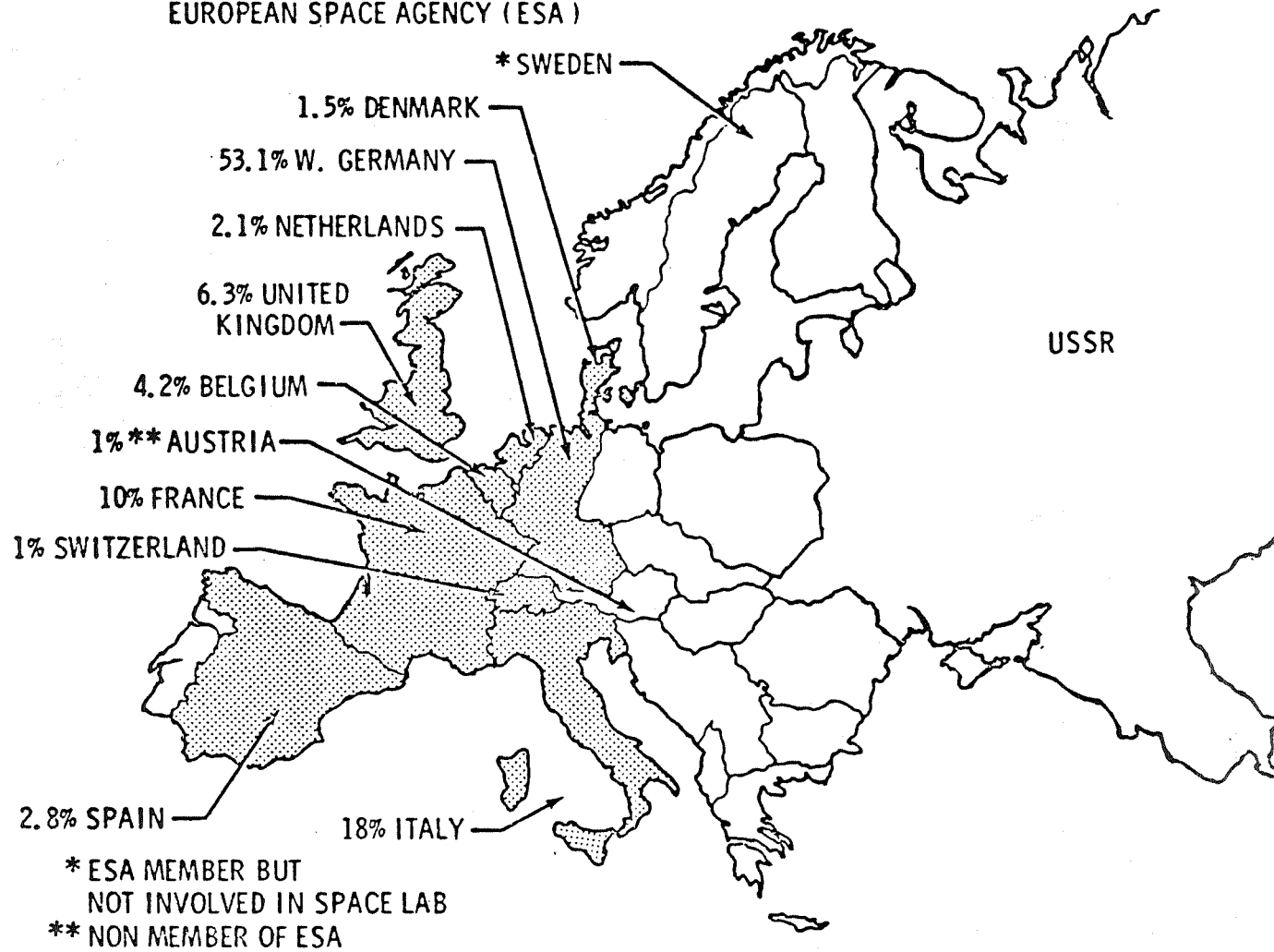| CALENDAR YEAR	1979	1980	1981	1982	1983	1984	1985	1991	

SHUTTLE FLIGHTS/CY ←3→ ←8→ ←35→ ←28→ ←41→ ←45→ ←51→ ←290→
CUM TOTAL FLIGHTS 1 2 4 5 7 9 10 13 11 14 46 74 115 160 211 501

3 6 8 12

SPACELAB FLIGHTS/CY ←11→ ←17→ ←19→ ←21→ ←23→ ←27→ 158→
CUM TOTAL FLIGHTS 11 28 47 68 91 118 276

LIFE SCIENCES STUDY

● CARRY-ON LABORATORIES   1 2

● MINI-LAB/MULTI-DISCIPLINE   1

MINI-LAB
FLIGHTS CONTINUE AT 2+/YEAR   25

● DEDICATED MODULES
    7 DAYS
    30 DAYS

MOD 1   MOD 2   MOD 2
1 2   3 4   1 2

(1)   4

22

(1) FLIGHTS CONTINUE AT 2/YEAR THRU 1987.
THEN 3/YEAR THRU 1991 FOR A TOTAL OF
26 LSL DEDICATED SPACELAB MISSIONS
(4;7 DAY AND 22;30 DAY)

Figure 9.- Space Shuttle/Spacelab/Life Sciences Laboratory (LSL) traffic model (NASA-S-76-10161).
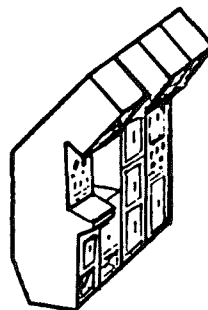
CARRY-ON

- ORBITER CREW COMPARTMENT
- LESS THAN 23 Kg (50 LB)
- MINIMAL INTERFACES - POWER
- FLIGHTS OF OPPORTUNITY
- 1 TO 7-DAY MISSION

MINI-LAB

- SHARED MISSION
- GENERALLY LESS THAN 500 Kg
- ONE TO SEVERAL RACKS OF EQUIPMENT
- SIGNIFICANT INTERFACES WITH SPACELAB - CDMS, POWER, THERMAL, ECS
- SHARED P/L SPECIALIST
- 7 TO 30-DAY MISSIONS

DEDICATED LABORATORIES

- UP TO 7,600 Kg
- FULLY DEDICATED SPACELAB MISSION
- EXTENSIVE INTERFACES WITH SPACELAB - CDMS, POWER, THERMAL, ECS
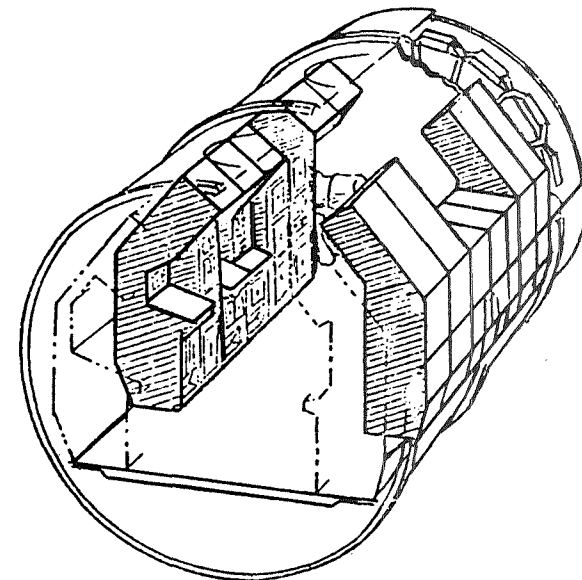- UP TO 3 DISCIPLINE SPECIALISTS, 12-HR/DAY ON 7-DAY MISSIONS
- 7 TO 30-DAY MISSIONS

Figure 10.- Life science payload characteristics (NASA-S-76-10157).

23

URINE ACQUISITION KIT
URINE STORAGE BAGS
200 cc SYRINGE (3)
LABSTIX
NEEDLES

TYPICAL CARRYING
HANDLE

BLOOD ACQUISITION KIT
SYRINGES
NEEDLES
COTTON SWABS
ALCOHOL
TOURNIQUET
BAND-AIDS
LANCETS
PRESTAINED SLIDES
SPENCER
HEMOGLOBINOMETER

WASTE STORAGE

TYPICAL ELASTIC
RESTRAINTS

SEPARABLE KIT
UNITS (4)

PHYSICAL
EXAMINATION KIT
BLOOD PRESSURE
CUFF
STETHESCOPE
DOPPLER FLOW METER
OTO-OPHTHALMASCOPE
TUNING FORK
THERMOMETER

AUXILIARY ATTACHMENT
POINTS FOR EQUIPMENT
RESTRAINT

WASTE
STORAGE

EQUIPMENT
RESTRAINT

Figure 11.- Conceptual design sketch of biomedical COL (NASA-S-76-10158).

KITS

LINEAR MEASUREMENT
DISSECTION
MICROBIOLOGY
OTHERS

MICROSCOPE
STORAGE

CREW WORK STATION

CHEMICALS

STAINING SYSTEM
AND MISCELLANEOUS

MMB PLANT CAGE

COLONY COUNTER

CRYOGENIC
FREEZER

LOW-TEMPERATURE
FREEZER

MASS MEASUREMENT
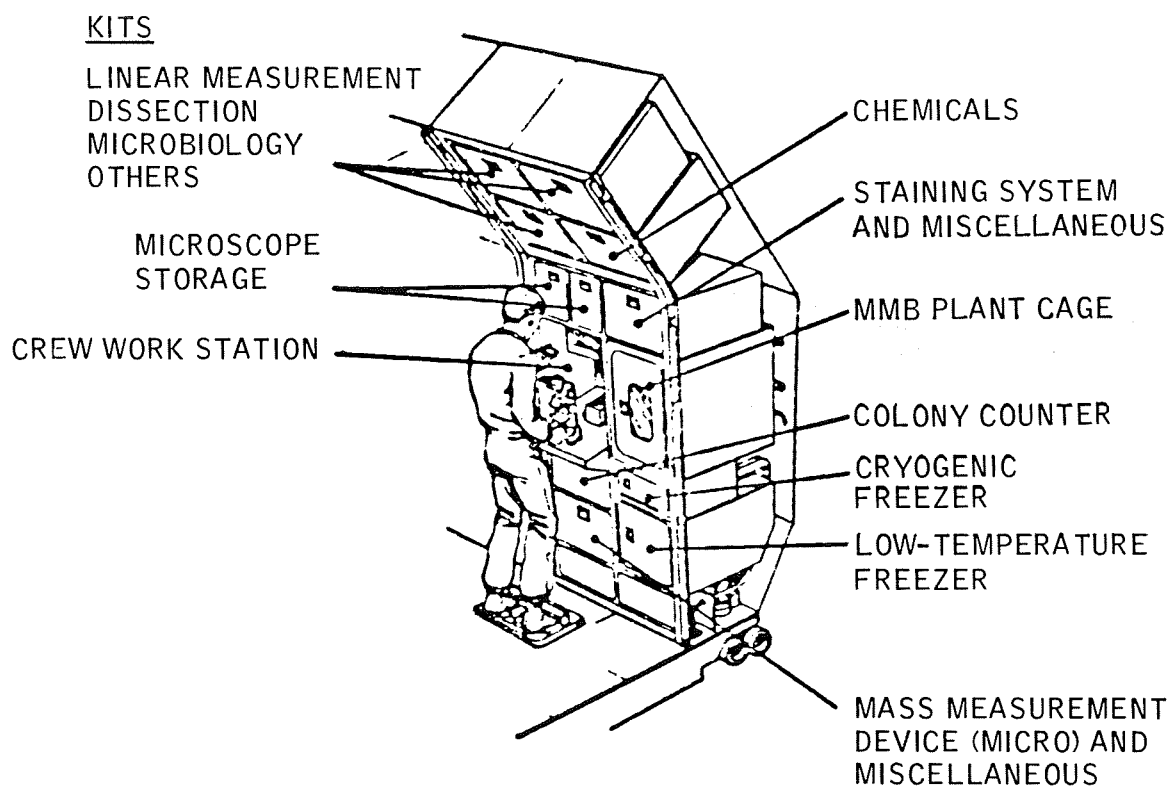DEVICE (MICRO) AND
MISCELLANEOUS

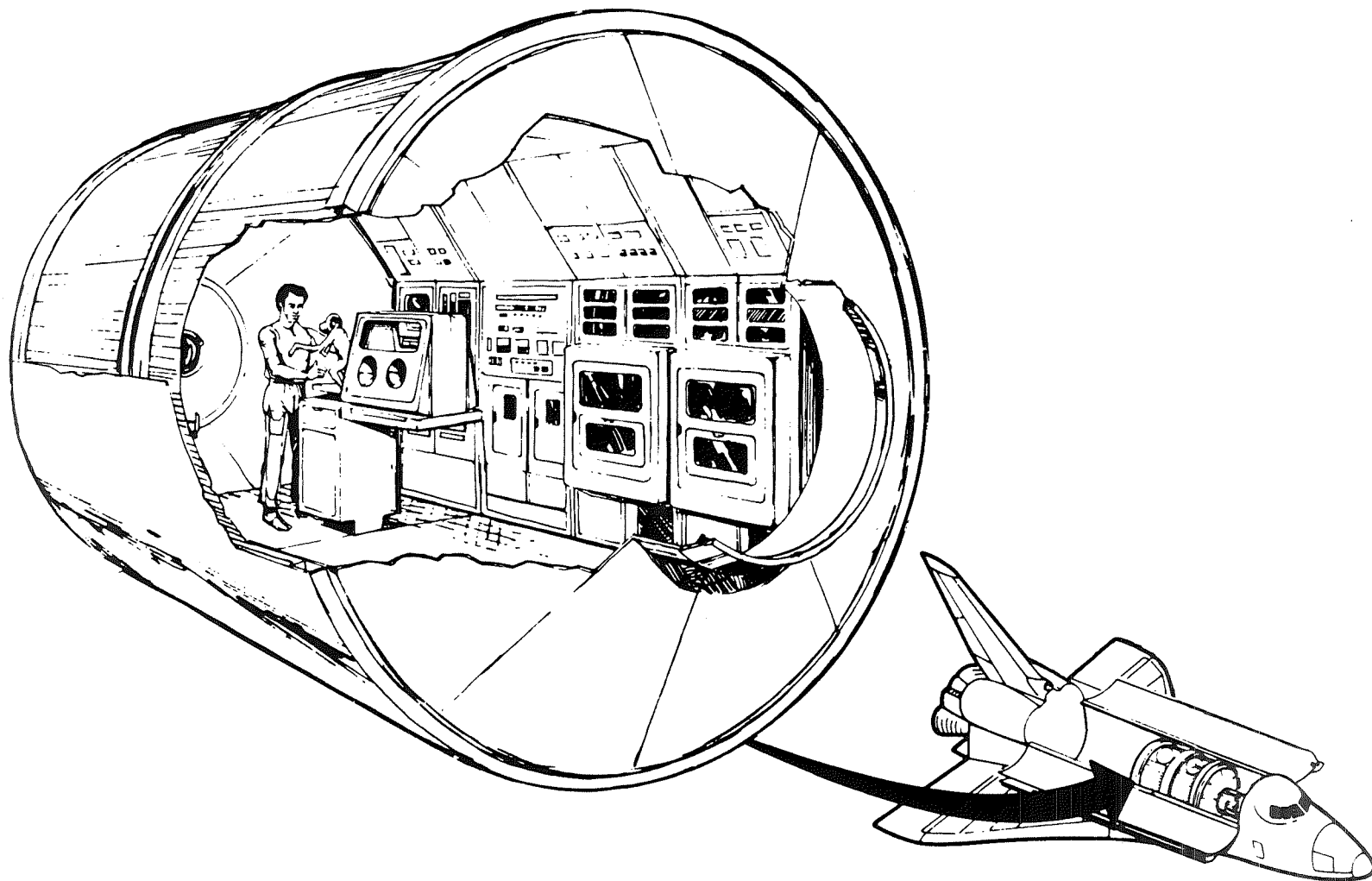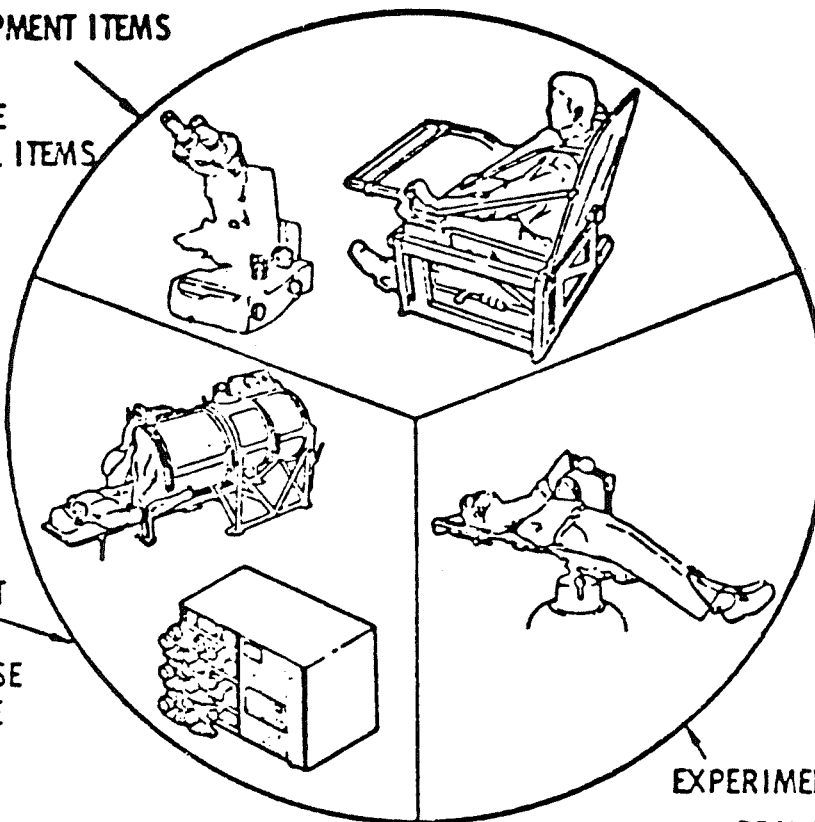Figure 12.- Typical minilab for biological specimen examination and experimentation.

Figure 13.- Life sciences in Spacelab (NASA-S-76-1604).

CORE REGULAR EQUIPMENT ITEMS
- MULTI-PURPOSE
- HIGH REUSE RATE
- MAJOR CRITICAL ITEMS

CORE INTERMITTENT ITEMS
- MULTI-PURPOSE
- LIMITED REUSE

EXPERIMENT SPECIFIC
- PRINCIPAL INVESTIGATORS SPECIAL USE ITEMS

Figure 14.- Common operational research equipment.

NASA's SPACE PROCESSING PROGRAM

By James H. Bredt, NASA Office of Applications, Washington, D.C.

ABSTRACT

The primary goal of NASA's Space Processing Program is to initiate utilization of space flight capabilities for economically beneficial activities in all branches of materials science and technology.

It is expected that the condition of virtual weightlessness obtained in space flight will permit unprecedentedly precise control over many known processes and development of many novel processes to manipulate and pre- pare biological materials intended for use on the ground. This expecta- tion has already been verified in the case of electrophoretic separation of living cells through a sequence of experiments performed on the Apollo, Skylab, and Apollo-Soyuz Test Project (ASTP) missions. It is believed that further applications will be found as well, and that future reductions in the costs of space operations will make a large increase in the scope of space experimentation possible.

Plans are now being made for payload equipment to implement materials processing experiments on the missions of the Space Transportation System (STS). This equipment is intended to support a diversified program of NASA-sponsored materials processing experiments by all classes of scientists, as well as pilot activities by non-NASA sponsors. It appears feasible to organize payload systems that can implement a wide variety of activities without undue constraints from spacecraft resources, and on this basis we expect that STS payloads can begin to provide supporting services for research and applications more or less routinely fairly early in the 1980's.

The NASA Space Processing Program is conducted by the Office of Applications to develop uses of space flight that will support research efforts and manufacturing operations on the ground by processing materials in space. It is expected that the unique conditions that are available in space will provide a basis for a wide variety of economically beneficial services to science and industry in fields such as metallurgy, electronic materials, glass technology, fluid physics and chemistry, and in biological material preparation as well.

The primary advantage that such applications will seek to exploit is the condition of virtual weightlessness obtained in a freely moving spacecraft during unpowered flight. In low Earth orbit, which has been the usual setting for materials processing experiments in space, the gravitational field intensity is only a little less than it is on the Earth's surface. However, in unpowered flight a spacecraft and everything in it are freely accelerated by the force of gravity, and these accelerations are all equal with an accuracy of the order of one part in ten million. Therefore, gravity effects cannot produce appreciable relative velocities between objects in the spacecraft. For example, a lump of lead that was released very carefully so as to avoid imparting any impulse to it would typically drift a meter or so from its original station and then return during the orbital period of approximately 90 minutes, because its orbit would differ slightly from that of the spacecraft unless the two bodies' centers of mass happened to coincide. The forces needed to hold the lump of lead in a constant position relative to the interior of the spacecraft would be very small, and in fact they could be provided easily by air currents, acoustic radiation pressure, or electromagnetic interactions.

In liquid media the relative velocities that can be produced by gravity effects are much smaller, because the very small forces that are involved have to act against viscous drag forces that are typically a hundred times as great as those found in gases. For all practical purposes, therefore, the driving forces for thermal convection, sedimentation and other buoyancy effects are completely removed from liquid systems in space. This is a factor of considerable significance for chemical and biological procedures, because it becomes possible to work with heterogeneous chemical systems under experimental conditions where the only heat and mass transport effects to be considered are heat conduction and chemical diffusion. Since both of these effects are precisely calculable according to a relatively simple mathematical theory, it should be possible to apply extremely precise techniques of control and measurement to experiments with such systems in space.

The effects of weightlessness have been exploited to study a variety of processes in solidification, crystal growth, fluid physics, and physical separation methods in space flight experiments conducted over the past five years. Flights that have carried space processing experiments are indicated in the top section of the schedule chart shown in Figure 1. They include the Apollo 14, 16 and 17 lunar missions, the four flights of the Skylab program, and last year's Apollo-Soyuz Test Project (ASTP) mission. These flights have carried a combined total of 41 experiments and demonstrations related to materials processing.

In order to continue experimentation through the rest of the 1970's, the Space Processing Program has undertaken a series of rocket missions that will carry payloads on ballistic flights that each afford between five and ten minutes of experiment time. This project is called the Space Processing Applications Rocket (SPAR) project, and it is planned to conduct three flights per year until the project is superseded by experiment operations using the Space Shuttle and Spacelab. The first SPAR flight was carried out on December 11, 1975. It carried nine experiments, thus bringing the total for the whole program to 50; the distribution of experiments and demonstrations among the missions flown to date is given in Table I.

Five of the program's experiments have been directed toward developing new methods for biological preparations. In these experiments, electrophoretic separations have been performed in aqueous media that were stabilized against convection by weightlessness rather than by porous supports or laminar flow, and protracted separation runs have been accomplished on particles that would have sedimented very rapidly on Earth. These early experiments have mainly served to establish the technology needed for more advanced work in the future, but their results indicate that further development can be expected to result in refined and powerful separation methods that should be capable of quantitatively predictable results.

As the second section of Figure 1 indicates, the development (Phase C/D) of the Space Shuttle was initiated at about the time of the Apollo 16 flight, and the Preliminary Design Review (PDR) was held shortly after the ASTP mission. The first Shuttle Orbiter is scheduled to be rolled out toward the end of this year and will be flight tested within the atmosphere during 1977. Thus the design phase of the Shuttle project has been going forward concurrently with the experiments that NASA has been performing to verify the promise of materials processing in space.

During the design work on the Shuttle, the Space Processing Program
has performed substantially continuous studies of potential modes
of Shuttle and Spacelab utilization. However, it has been necessary
to wait until the experiment results from the manned missions of the
1970's were available and the Shuttle design was substantially complete
before embarking on final definition of Space Processing Applications
(SPA) payloads for the Shuttle missions.

Phase I/II payload definition studies are now in progress and a com-
petitive procurement will be held in the latter part of 1976 for payload
design and development work which will begin in the first quarter of
1977. The development of specific equipment items will be phased to
provide for deliveries at the approximate points shown in the third
section of Figure 1, comprising two initial payloads compatible with
the test flights of the Shuttle and two payloads for operational flights
with the Spacelab in 1981.

In defining payload equipment for the Space Shuttle and Spacelab we
have followed approaches intended to give experimenters easy access
to space and maximize the scientific output of their experiment
while minimizing the costs of operating them in space. Typical payload
configurations that have been derived to implement this design philoso-
phy are illustrated in Figure 2. In general space processing payload
systems will be built up of modular, reusable equipment taken from a
standard inventory of apparatus and supporting equipment, so that each
item of equipment can serve many investigators and little apparatus will
need to be developed specifically for individual experiments. The usual
mode of operation will be to fly space processing payloads on missions
shared with other disciplines, and the modular nature of the equipment
will make it possible to take advantage of a wide variety of shared
mission opportunities because each payload will only need to include
the equipment necessary to its mission.

Payloads will also be organized for maximum productivity, so that the
unit costs of processing material samples will be minimized. The
system is being designed to that all of the equipment in a given payload
can be operated during all of the time it spends in space. Since this
concept requires many experiments to operate at once, the space pro-
cessing equipment inventory will include an Auxiliary Payload Power
System (APPS) that provides power from Shuttle type fuel cells and has
a radiator to dispose of the resulting heat. The system will be capable
of supplying 15 kw of electric power, and it is expected to free space

processing experiment operations substantially from constraints due to limitations on the resources of the Shuttle.

Investment and operating cost questions are critical for materials processing in space because one of NASA's goals is to introduce activity sponsored by other government and private sponsors into space. This will be possible only if the costs of performing space experiments are within the range that such sponsors are accustomed to pay for research. The results of the Space Processing Program's current payload definition studies indicate that this may be achieved if the number of investigators served on each mission is large enough, and therefore the program is planning for payloads that will be capable of processing literally hundreds of samples for a diversified community of users on every flight.

This type of operation is unprecedented in space experimentation, and it will obviously provide flight opportunities for larger than usual numbers of investigators. Initially these investigators will perform their experiments un er NASA's sponsorship to develop experimental methods and demonstrate that space flight provides capabilities for fresh approaches and new discoveries in their fields. The Space Processing Program is employing a variety of means to engage the interests of qualified experimenters and define worthwhile applications; among these means are meetings such as the present one, working group activities in support of the payload planning effort, and participation in the SPAR project. In the early years of space processing experiments on the Shuttle and Spacelab, we expect of the order of 100 investigators to conduct research projects that use space activities to achieve their objectives. Among these will be projects dealing with the feasibility of biological and biomedical applications of space flight, some of which may be based on ideas brought out by this Colloquium. Assuming that these projects are as fruitful and inexpensive as planned, we believe that the investigators will soon find other sponsors wishing to support their work as well. If this is the case, then by the late 1980's we can expect to find that space experiments will have become a more or less routine resource in the kinds of biological and other work for which they offer substantial advantages.
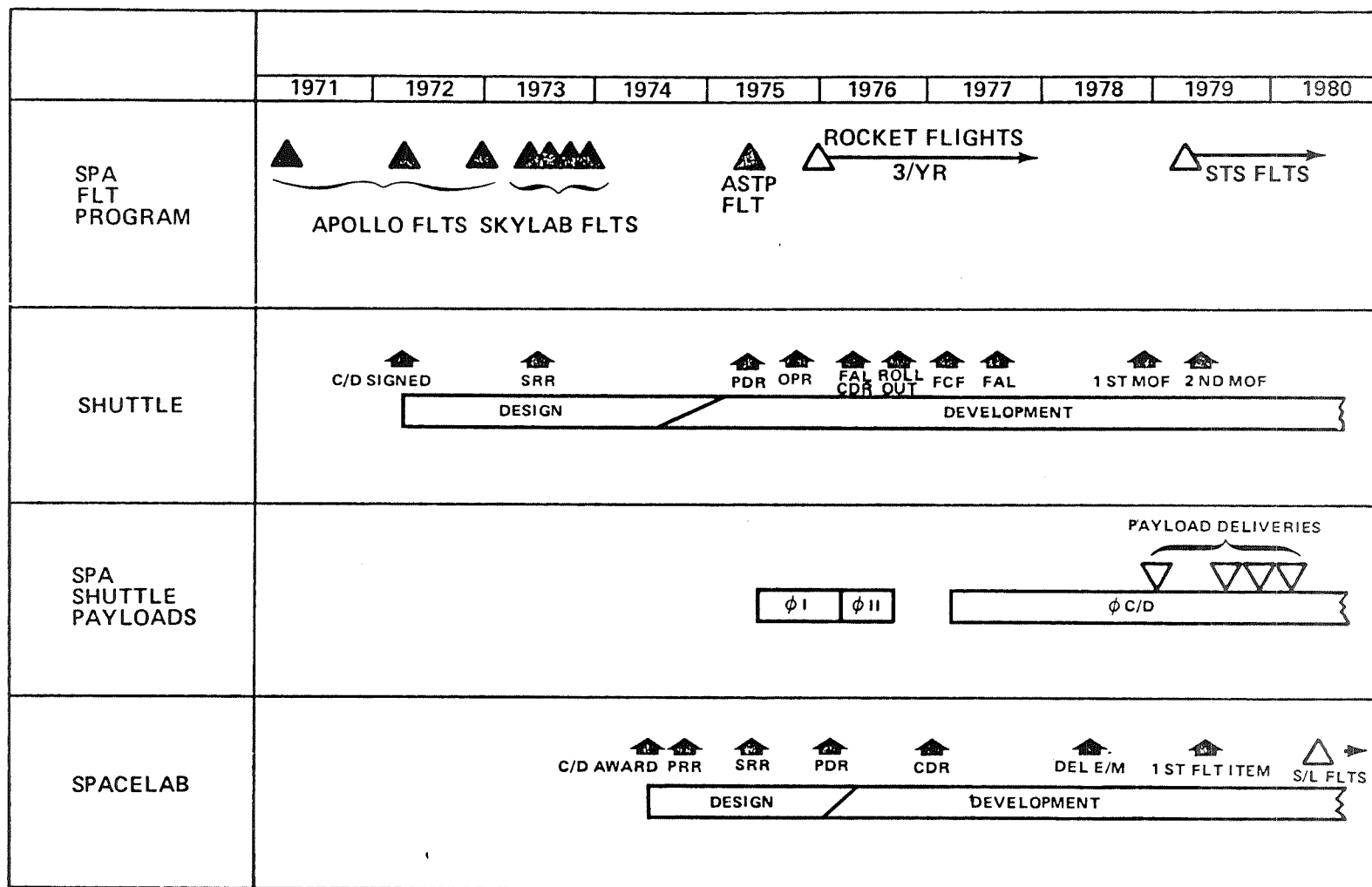
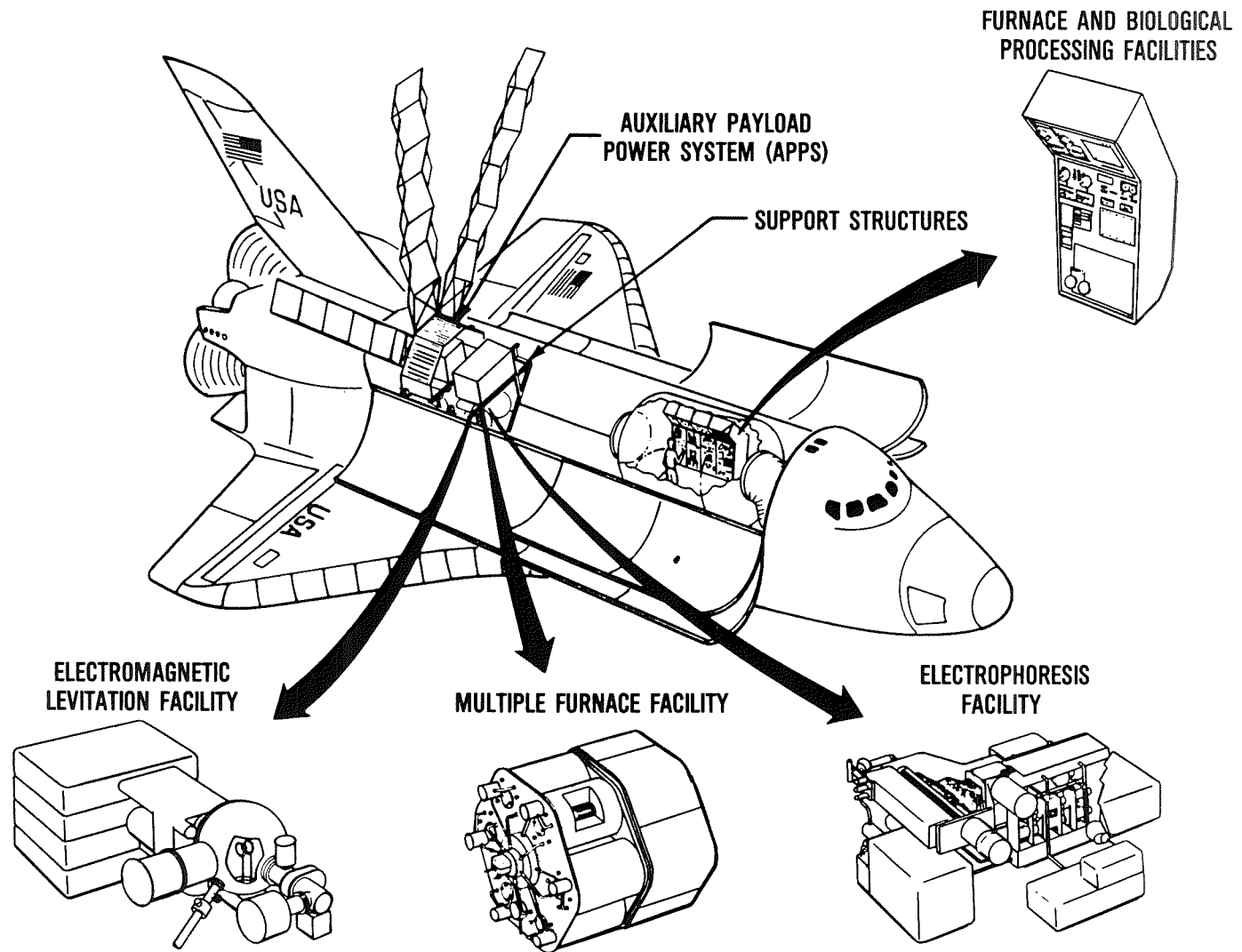Figure 1.- Overview schedules for Space Transportation System, Space Processing Applications, and Spacelab.

FURNACE AND BIOLOGICAL
PROCESSING FACILITIES

AUXILIARY PAYLOAD
POWER SYSTEM (APPS)

SUPPORT STRUCTURES

ELECTROMAGNETIC
LEVITATION FACILITY

MULTIPLE FURNACE FACILITY

ELECTROPHORESIS
FACILITY

Figure 2.- Space processing of Spacelab payloads.

D_3 1

BEHAVIOR OF FLUIDS IN A WEIGHTLESS ENVIRONMENT

By Dale A. Fester, Ralph N. Eberhardt and James R. Tegart
Martin Marietta Corporation, Denver, Colorado

ABSTRACT

Fluid behavior in space differs markedly from that in a gravity-dominated environment. This must be considered for all fluid usage, whether for vehicle operations or payload experiments. Numerous investigations have shown that the behavior of fluids in a low-g environment is controlled primarily by surface tension forces. Certain fluid and system characteristics determine the magnitude of these forces for both a free liquid surface and liquid in contact with a solid. These characteristics, including surface tension, wettability or contact angle, system geometry, and the relationships governing their interaction, are discussed. Various aspects of fluid behavior in a low-g environment are then presented. This includes the formation of static interface shapes, oscillation and rotation of drops, coalescence, the formation of foams, tendency for cavitation, and diffusion in liquids which were observed during the Skylab fluid mechanics science demonstrations. Liquid reorientation and capillary pumping to establish equilibrium configurations for various system geometries, observed during various free-fall (drop-tower) low-g tests, are also presented. Several passive low-g fluid storage and transfer systems are discussed. These systems use surface tension forces to control the liquid/vapor interface and provide gas-free liquid transfer and liquid-free vapor venting.

INTRODUCTION

Fluid behavior in space is controlled by surface tension forces which are generally insignificant in a gravity dominated environment. Basic phenomena such as buoyancy, settling, convection, mixing and diffusion are considerably different when gravity is not present. The reaction rates for many processes may be altered. These, and other differences in behavior, may offer advantages in conducting biological experiments. For example, longer residence times are available for oxygen bubbles in contact with immiscible substrates, and strong shear forces induced by agitation in liquid media can be eliminated. The lack of significant buoyancy forces, on the other hand, may introduce certain handling concerns such as the separation and control of liquid and vapor. An understanding of how surface tension controls fluid behavior in low-g is thus needed in planning any experiments where liquids will be interacting with gases and surfaces.

A discussion of fluid and system characteristics that determine the magnitude of the surface tension forces is presented in this paper. Examples of surface tension dominated behavior illustrating some of the basic phenomena are available from both drop tower and Skylab fluid mechanics demonstrations. The separation of liquid and vapor by surface tension forces to accomplish low-g storage and transfer is presented as a practical application of the basic principles to a real system.

FLUID CHARACTERISTICS

The shape of a gas-liquid interface in low-gravity (gravity forces are negligible) is determined solely by capillary forces. The Young-Laplace equation relates liquid surface tension and the curvature of the interface to the pressure differential between the gas and liquid,

$$P_L - P_G = \sigma \left(\frac{1}{R_1} + \frac{1}{R_2} \right) \tag{1}$$

where the subscripts L and G refer to the liquid and gas respectively, σ is the surface

37

tension, and R_1 and R_2 are the radii of curvature of the interface. The radii of curvature are considered positive when their center of curvature is within the liquid. The sum of the reciprocals of the radii, $(1/R_1 + 1/R_2)$, is referred to as the curvature of the interface[1].

Under static conditions, the pressure of the gas in contact with the liquid is uniform over the entire liquid surface. An equilibrium interface will be established when the pressure in the liquid is also uniform and the pressure differential defined by equation (1) is a constant. If the pressure difference is constant, then the curvature must also be uniform over the entire surface.

Considering a globule of liquid in low-gravity, not in contact with any surface, the requirement of uniform curvature can only be satisfied if both radii of curvature are equal. The globule then has the form of a sphere, with the radii of curvature equal to its radius. Equation (1) simplifies to:

$$P_L - P_G = \frac{2\sigma}{r} \qquad (2)$$

where r is the radius of the sphere. The pressure within the drop of liquid is greater than that of the surrounding gas by an amount that is directly proportional to the surface tension and inversely proportional to the radius of the drop. The same holds true for a bubble surrounded by liquid except that the pressure within the gas is greater than that in the liquid.

When the liquid is in contact with a surface, the determination of the interface becomes more complex. The curvature of the surface will still be a constant, but the two radii of curvature will vary over the surface. The liquid-to-solid contact angle θ is a boundary condition that must be satisfied. This is the angle formed between the liquid and the surface, measured within the liquid.

Variations in σ due to temperature and in σ and θ due to contamination, dissolved pressurant, and liquid purity will affect interface shape. The value of contact angle primarily depends on the liquid surface tension and the solid boundary surface energy. The latter can be expressed as a so-called "critical surface tension." If the liquid surface tension is less than the critical value, the contact angle is zero. If the surface tension is a greater than the critical value, the cosine of the contact angle is a linear proportion to the difference between the liquid and the critical surface tensions. Clean metal surfaces have high critical surface tensions and most liquids will completely wet them. Maintaining a contaminant-free surface is difficult to achieve in practice, however. Most monolayer contaminant films (except fluorocarbons) have critical surface tensions between 20 and 45 dynes/cm[2, 3]. This is also true of plastics. Water, with its high surface tension of 72 dynes/cm, will have high contact angles on these surfaces. A contact angle of 33.5° is shown in Figure 1.

Very few liquids (other than liquid metals) have a higher surface tension than water. Since water has such a high surface energy, it is readily contaminated and a considerable lowering of the surface tension takes place[4]. Contaminants that lower the surface tension of a liquid are referred to as surface active agents. A small amount of a surface active agent will impose its low surface tension on a liquid of much higher surface tension. As the concentration of the impurity is increased the surface tension of the solution decreases until it becomes saturated. Further addition of the impurity does not cause any change in surface tension[5]. For example, adding soap of the type used on Skylab to water, in approximately the concentrations used in the demonstrations, reduced the surface tension to 20 dynes/cm[6].

While the Young-Laplace equation can define more than one interface for any given set of conditions, the equation for surface energy defines the preferred configuration. A stable interface shape is achieved when the surface energy is a minimum. In simplified form, surface energy is defined by

$$S.E. = \sigma_{LV} (A_{LV} - A_{SL} \cos\theta) \tag{3}$$

where A is area and LV denotes liquid-vapor and SL denotes solid liquid. A capillary area can be defined as

$$A_C = A_{LV} - A_{SL} \cos\theta \tag{4}$$

for any given interface. Then surface energy can be further simplified to

$$S.E. = \sigma_{LV} A_C \tag{5}$$

Again consider a free-floating globule of liquid. The surface energy will be a minimum when the capillary area, which is the area of the liquid-vapor interface in this case, is a minimum. This occurs when the globule assumes a spherical shape, again confirming the static shape for a liquid drop.

The capillary area is reduced when a liquid drop contacts a solid surface. Therefore, contact with a surface is a preferred equilibrium configuration for a liquid drop. This decrease in surface energy accounts for the adhesiveness of a liquid on a surface. Energy must be added to remove a drop from a surface.

The equilibrium interface configuration is established by the mechanism of surface-tension-driven flow, termed capillary pumping. Liquid will preferentially orient within a container by capillary pumping if the system is in a low-g environment[7]. The geometry can be modified by compartmentation in one area of the tank such that a lower characteristic dimension results. This reduced length can be provided by a vane structure (surface tension device). The device can reduce the pressure of liquid adjacent to and within the device to a value lower than the pressure of liquid located away from the device. The low-pressure region will be created when the device causes the curvature of the interface about the device to be large (small radius of curvature) in comparison to the curvature of the liquid elsewhere in the tank. The pressure difference will have an effect only if the two liquid volumes are in communication. Under near-zero-g conditions, spreading of the liquid as it wets the tank walls will usually bring the liquid into communication with the surface tension device. If this is not possible, some sort of communication channel must be provided.

With a communication path provided, liquid will be transferred, as shown in Figure 2, until the curvature of the interface throughout the tank is the same, i.e., pressure is uniform. The surface tension device is designed so the curvature of the interface remains high until the device has filled with liquid. In comparison, liquid in contact with only the tank wall has a relatively low curvature.

For a spherical gas-liquid interface with liquid in contact with a surface, equation (2) becomes

$$P_G - P_L = \frac{2\sigma}{r} \tag{6}$$

This pressure differential can be related to a dimension (other than the radius of curvature) such as the pore radius R and a second parameter, the liquid-to-solid contact angle θ. This is done by introducing the relationship between R, θ, and r as shown in Figure 3. Then

$$P_G - P_L = \frac{2\sigma}{r} \cos\theta \qquad (7)$$

A final criterion for determining interface stability is the Bond number (Bo), a dimensionless ratio of acceleration forces to capillary forces[8]

$$Bo = \frac{\rho_a L^2}{\sigma} \qquad (8)$$

where L is the characteristic system dimension. The liquid/gas interface is stable in a cylindrical tank or circular pore when Bo ≤ 0.84. The critical Bo for square weave screen is 0.45[8]. Surface tension forces become significant, producing highly curved interfaces, for Bo in the range of five or below. The interface is essentially flat for Bo ≥ 50[9]. Other scaling parameters, such as the Weber, Froude, and Reynolds numbers, are applied when liquid flow is involved[8].

LOW-G FLUID MECHANICS

The above described characteristics of fluid behavior in a low-g environment have been demonstrated in space and in simulated low-g environments on earth. These experiments illustrate the basic phenomena of low-g fluid mechanics. A number of fluid mechanics science demonstrations were performed on Skylab. While these demonstrations did not follow rigorous experimental protocols, they did provide interesting demonstrations of basic phenomena, some of which had not been previously observed[6].

Skylab Fluid Mechanics Demonstrations

The following are examples of fluid mechanics demonstrations performed aboard Skylab to illustrate low-g fluid behavior.

Static Interface Shape.- Surface tension and contact angle work together, as discussed previously, to yield the static shape of a gas/liquid interface in low-g. It was demonstrated that a free floating drop of water assumes a spherical shape, as shown in Figure 4. Due to disturbances induced in forming the drop and the relative acceleration of the Skylab and the drop, a completely static interface was difficult to form. Before the lightly damped oscillations ceased, the drop impacted a surface.

The astronauts found that these difficulties could be overcome by placing the drop on a thread. For the drop sizes used (30 to 100 cc), the thread retained and centered the drop. An evaluation of the data showed that the thread had a significant effect on the damping of the drop oscillations and caused a distortion of the drop shape (∼ 5% elongation along the thread axis)[6].

When the drop was in contact with a larger surface like a straw, the surface influence was stronger and the equilibrium interface shape positioned the drop tangent to surface. On a large flat surface, the contact angle became a significant factor in establishing the interface shape, as illustrated in Figure 5. Water was the liquid for all these demonstrations, giving contact angles ranging from 30 to 90 degrees, depending on the surface material. A liquid with a lower surface tension would wet these surfaces and give somewhat different interface shapes[6].

Oscillating Drop.- Drops were oscillated in their basic first and second modes. Surface tension is the restoring force that sustains the oscillation. Opposite sides of the drop were pulled by rods to induce oscillation. A thread was again used to initially stabilize the drop. Measured oscillation frequencies were found to correlate well with theory. Damping of the oscillation was found to be at least an order of magnitude greater than predicted by theory due to the presence of higher modes of oscillation (a result of the method of inducing oscillation) and the damping effect of the thread[6].

Coalescence.- Coalescence depends upon the interaction of two liquid interfaces when they meet. Depending upon the angle of incidence and relative velocities, the drops can bounce off one another or combine. Momentum effects can cause them to separate again.

Drops were impacted by holding one drop stationary on a thread and maneuvering the second drop onto a collision course. Drops ranging from 1.8- to 5.2-cm diameter could be observed as they coalesced. The drops were colored differently so the rate of mixing (found to be fairly slow) could be observed. The coalescence observed in Skylab was consistent with available theory[6].

Rotating Drop.- When a liquid drop is rotated, centrifugal and surface tension forces balance to produce the resulting interface shape. This was the most unique demonstration performed on Skylab. Such a test was not previously feasible because of the long low-g period required and the need to manipulate the drop. Available theory predicts a completely different result, but does hint that other results may be possible.

When rotated at low rates, the drop has a watermelon shape. At higher rates, it pinches off, assuming a peanut shape, as shown in Figure 6. If the rate is increased further, an equilibrium shape cannot be achieved and the drop divides into two drops[6].

Immiscible Liquids.- A dispersion of two immiscible liquids can be formed if they are strongly mixed. If the densities of the two liquids are different, the dispersion will quickly separate in one-g. When gravity forces are small, the mechanism for separation of a dispersion is very different. One liquid can separate from the other only by coalescence of the finely divided drops. If drops of one liquid do come into contact and do coalesce, separation can proceed at some slow rate.

An experiment using various proportions of oil and water was performed to examine this phenomena. The two liquids were separated centrifugally and then mixed by shaking. They were observed for a period of 10 hours to see if any separation could be observed. Only a "cellular structure that grew coarse" could be observed[6]. On Earth, the two liquids completely separate in less than 10 seconds[10].

Ice Melting.- This demonstration provided an indication of the influence of a low-g environment on the mechanisms of heat transfer[6]. Instead of draining away as it does in one-g, the liquid surrounded the ice, acting to insulate it from the surrounding air. It took 190 minutes for the ice to completely melt to a liquid drop in low-g. The ice melted in 130 minutes in the same experiment on earth[11].

Since convection is thought of as being driven by buoyant forces, conduction and radiation heat transfer are usually presumed to be the means of heat transfer in low-g. While not established from this experiment, convection could still be a mechanism of heat transfer in low-g. Convection can be driven by surface tension forces (Marangoni flow) since gradients in temperature along an interface also produce gradients in surface tension[12]. Thermoacoustic effects, mechanical vibrations, electric and magnetic fields, concentration gradients and chemical potentials are also possible mechanisms for convective heat transfer in low-g[13].

Cavitation.- A bubble within a liquid can oscillate in much the same manner as a liquid drop oscillates. Both hydrodynamic and surface tension forces act at the surface of the bubble. If the magnitude of the hydrodynamic forces exceeds the surface tension forces at some point on the surface, the bubble can become unstable and collapse upon itself. Each time an unstable drop collapses a jet of liquid forms that shoots across the bubble.

This phenomena was demonstrated on Skylab in the following manner. A bubble was formed inside a drop such that only a thin film separated them at one area on the surface. When the surface was touched with a plunger, the bubble ruptured. While the source of the instability of the bubble was not a hydrodynamic force, the resulting collapse was the same as cavitation. A jet of liquid shot out of the drop at the point the bubble had been ruptured, as shown in Figure 7. As the velocity of the jet decreased, surface tension forces acted to retract part of the jet back into the drop, while some of the jet pinched off into additional drops[6].

<center>Drop Tower Testing</center>

Drop tower test facilities have been extensively employed to investigate some of the basic low-g fluid behavior. Although limited by relatively short test times, on the order of 2 to 5 sec, they do provide a quite accurately controlled acceleration environment (acceleration range between 10^{-5} and 10^{-1} g)[14]. Examples of interface shapes within containers and liquid motion resulting in reorientation within a container are presented below.

Capillary Pumping and Interface Shapes.- Liquids are usually stored in a container and the shape assumed by the liquid/vapor interface is important to the draining or filling of the container in low-g. The tank shape and any internal structures influence the interface shape. Differences in capillary pressure will cause liquid to be transferred from a region of low curvature to a region of higher curvature, as discussed previously. This capillary pumping establishes the equilibrium interface shapes. Interface shapes and pumping rates have been established for numerous geometries[7]. As an example, orientation of small liquid volumes by various vane configurations is shown in Figure 8. Orientation of the liquid in a bare tank is shown in the upper left hand corner. Liquid positioning with each of the five different vane devices, however, is such that gas-free liquid could be supplied to an outlet located at the 6 o'clock position.

Liquid Motion.- Disturbing forces acting on a container can cause the liquid within to reorient. A small lateral acceleration component will make the liquid flow along one side of the tank as it reorients. In a tank with a smooth interior wall the flow adheres to the tank wall continuing past its final equilibrium position. A typical example is shown in Figure 9[15]. As the liquid began to move, the liquid interface remained relatively flat so the motion appeared as a rotation of the interface about its center. Very little splashing of the liquid occurred during the reorientation. Once the leading edge of the flow reached the tank dome, the liquid interface began to acquire some curvature. The liquid overshot its final equilibrium position, continuing around the tank and recirculating some of the liquid. Baffles or surface tension device structure will breakup this recirculation and speed the achievement of its new static position. Baffles do induce turbulence and produce gas bubbles within the bulk liquid however.

<center>STORAGE AND TRANSFER</center>

Operation of passive devices for the storage and transfer of liquid in low-g illustrates a practical application of the previously described, surface tension dominated, behavior. Devices are available to handle a wide range of fluids such as alcohols, freons, propellants and cryogens[16,17]. Configurations differ because of varied functional requirements; however, the operational principle for each system relies on the relatively small

<center>42</center>

pressure differential that exists across any curved gas/liquid interface due to intermolecular forces. Liquid surface tension and ullage pressure support are used to passively provide near-instantaneous, gas-free liquid expulsion on demand.

In general the surface tension devices are divided into two categories: devices that use fine-mesh screen for liquid orientation and control and those that use sheet or vane-type structures. The characteristic dimension, pore size, is the significant parameter that differentiates between the two categories. The vane devices with larger characteristic pore dimensions operate only in low acceleration environments on the order of 10^{-3} g or less, depending on the size of the tank or container. Fine-mesh screen devices, by virtue of very small pore sizes and small radii of curvature, can provide retention and control of large liquid masses over a wide range of spacecraft accelerations. The small capillary pressure differences that exists at the screen pores must balance or exceed the sum of other pressure differences tending to breakdown the passively-controlled liquid/gas interface. Premature interface breakdown reduces the quantity of gas-free liquid that can be expelled from the tank. During storage with no liquid outflow the capillary pressure difference must exceed the hydrostatic head supported by the screen. Additional losses which must also be balanced by the capillary pressure difference are introduced with a flowing system.

For applications requiring a screen system, there are two categories of devices: total communication systems and partial retention or trap systems[18]. Trap devices are screen reservoirs which position only a small percentage of the total liquid load over the outlet. Total communication devices are composed of screen liners or individual liquid supply channels which are in continuous communication with the bulk liquid. An example of a total communication channel device is shown in Figure 10. If the tank is large, on the order of 3 feet and greater, or the acceleration is relatively large, the tank can be compartmented so that the hydrostatic head to which any segment of the device is exposed is reduced. A schematic of such a device is shown in Figure 11.

An example of a device which uses vanes to orient propellants for gas-free liquid expulsion in a very low-g environment is provided by Martin Marietta Viking 1975 Orbiter propellant tankage[19]. The surface tension propellant management device (PMD) orients the liquid over the tank outlet so that a bubble-free supply of propellant is available to the rocket engines. Ullage control is provided by the PMD for liquid-free gas venting of the tank during all maneuvers of the vehicle following separation from the Centaur upper stage. During spacecraft cruise and immediately before engine burns, the PMD orients the propellant symmetrically about the tank centerline to enhance spacecraft pointing accuracy during engine firings.

A schematic of the propellant management device is shown in Figure 12. The primary elements of the PMD were the vane assembly, the communication channel assembly, and the mounting cap assembly. The vane assembly, shown in Figure 13, consisted of a hollow central tube or standpipe to which twelve 6Al-4V titanium sheet vanes were attached. A communication channel was positioned along the wall to provide for the capillary pumping of the propellants from the top to the bottom of the tank during low-g. Cleaning of the PMD and the inside of the tank was important to assure near-zero contact angles between the propellants and metallic surfaces.

The efficient transfer of liquid from one tank to another in a weightless environment is primarily dependent on the initial liquid/vapor interface shapes and their locations in both the supply and receiver containers, and the flowrate of the transfer. Liquid/vapor separation and control is required in the supply container to accomplish gas-free liquid flow from the container. Several kinds of devices are available for accomplishing expulsion from the supply container including the previously discussed passive surface tension devices, positive expulsion devices such as bladders and metal diaphragms, and bellows.

The particular device selected depends upon factors such as fluid compatibility, cycle life, expulsion efficiency, cost. etc.

Pressure control in the receiver tank during filling is a key design consideration. When small quantities of liquid and small containers are being used, evacuating the container to achieve transfer is a rather simple approach. On a larger scale, structural considerations and/or vaporization of the liquid usually rule out this method of transfer. For a high vapor pressure fluid or a cryogen the receiver container must be vented during the transfer, and the location of the gas must be carefully controlled.

An example of evacuating one container to achieve transfer in a low-g environment was demonstrated during the Skylab missions[6]. A sample of blood was drawn in a large syringe and transferred to an evacuated bottle. The pressure within the bottle was selected so that the addition of a given volume of blood reduced the pressure differential between the syringe and bottle to zero.

The needle of the syringe was inserted into the bottle, piercing a diaphragm. It extended part way into the bottle. Blood immediately began to transfer from the syringe to the bottle, since the bottle was at a pressure somewhat below ambient. As the flow of liquid began, a drop could be observed forming at the tip of the syringe. No turbulence or geysering of the blood was observed. The drop continued to expand until it contacted the wall of the bottle. At this point there was a volume of liquid, located near one end of the bottle, dividing the gas into two separate volumes. The interface on each side of the liquid volume moved toward the ends of the bottle compressing the two gas volumes as the transfer continued. The volume of gas near the diaphragm of the bottle was the smaller of the two. Each volume was at the same pressure with the liquid acting as a piston between them. Some pressure was applied to the plunger of the syringe to achieve complete transfer of the liquid.

The transfer of liquid from one tank to a second vented tank in a weightless environment was demonstrated by the crew of Apollo 14[20]. Two surface-tension baffle designs were incorporated in separate tanks of a scale-model liquid-transfer system with each tank being used alternatively as the supply and reciever tank. A sketch of the tanks is presented in Figure 14. One tank contained a standpipe-liner baffle structure consisting of a perforated standpipe located over the drain/fill port and a wall-liner spaced a fixed distance away from the tank wall. The second tank contained a curved-web baffle structure consisting of three circular perforated plates nested around a small feeder capillary section. The curved web baffles are arranged off-center such that the cross-sectional area between baffles increases gradually from the feeder section towards the opposite end of the tank. This arrangement tends to retain the bulk liquid adjacent to the feeder.

Testing was performed to determine the ability to achieve gas-free outflow from the supply tank and orderly inflow into the receiver tank with gas located at the tank vent and liquid at the fill port. Gas-free liquid was transferred to and from either baffled tank to within 2 percent of the liquid available for transfer and the receiver tank vent remained in contact with gas. Transfer between unbaffled tanks was included for comparison and gas ingestion occurred when less than 12 percent of the supply tank volume had been delivered. At the termination of transfer liquid had ingested into the receiver tank vent.

CONCLUDING REMARKS

The parameters that govern fluid behavior in a weightless environment have been partially characterized and verified by ground and orbital testing. Because the environment, geometry, and fluids of interest influence this behavior and also differ from system to system, each new application must be evaluated to assure that the desired performance is achieved. Early assessment of fluid behavior and control for each experiment is required. One-g bench and/or drop tower testing can provide verification prior to orbital operation.

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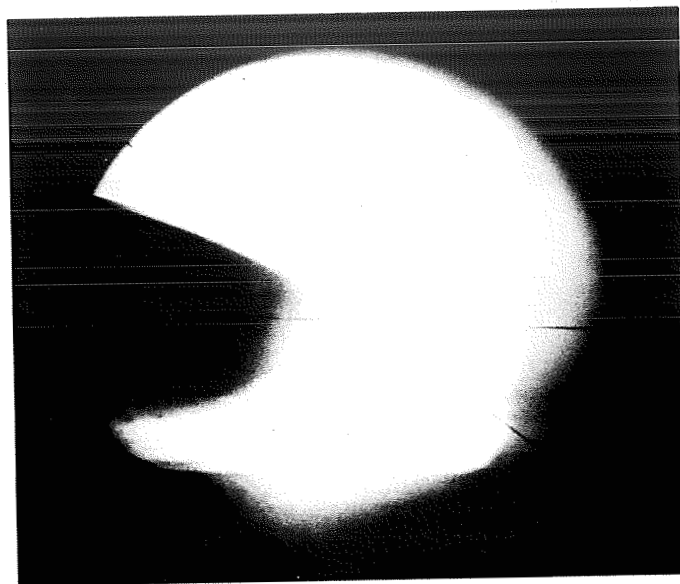
Figure 1.- 33.5° contact angle of a drop on
a contaminated surface.
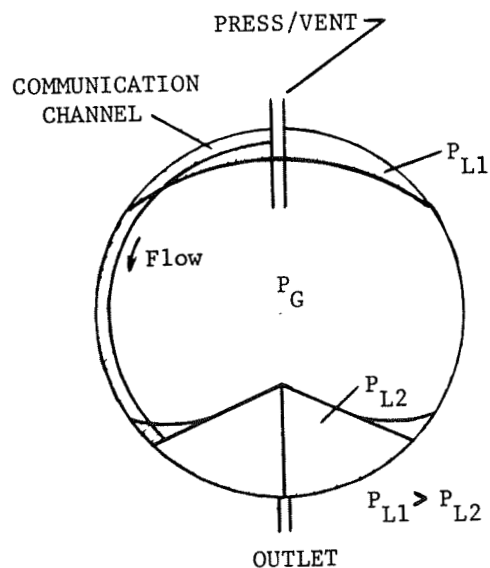
Figure 2.- Capillary pumping with a vane
surface-tension device.
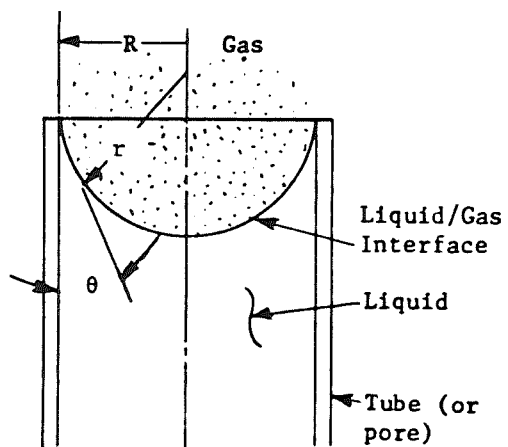
Figure 3.- Liquid/gas-interface shape in a pore.
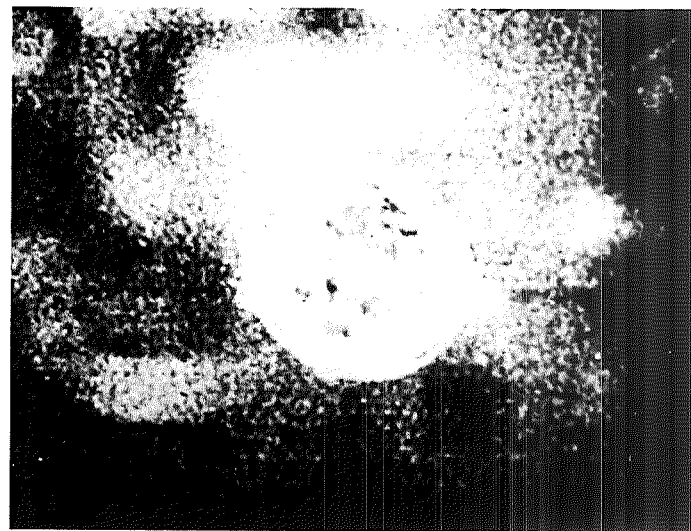
Figure 4.- Free-floating water drop.

Figure 5.- Water drop on a flat surface (tube freely resting on drop surface).

Figure 6.- Peanut shape assumed by a rotating drop.

48

49
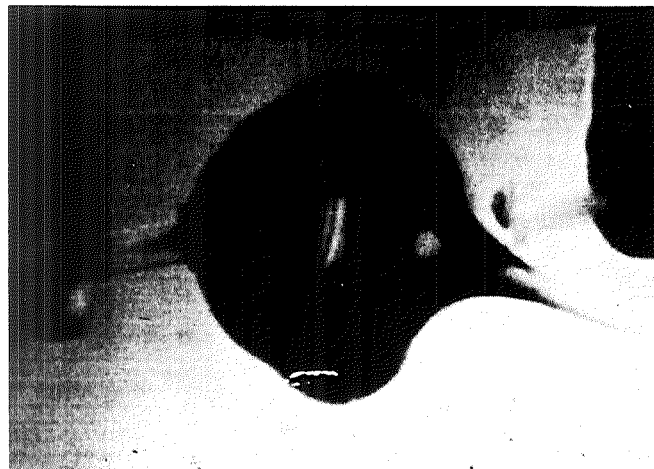
Figure 7.- Bubble collapse showing liquid jet
leaving the drop.

(a)

(b)

(c)

(d)

Figure 9.- Reorientation in cylindrical tank with
off-axis acceleration.

Figure 8.- Liquid orientation in low-g for
various vane configurations.
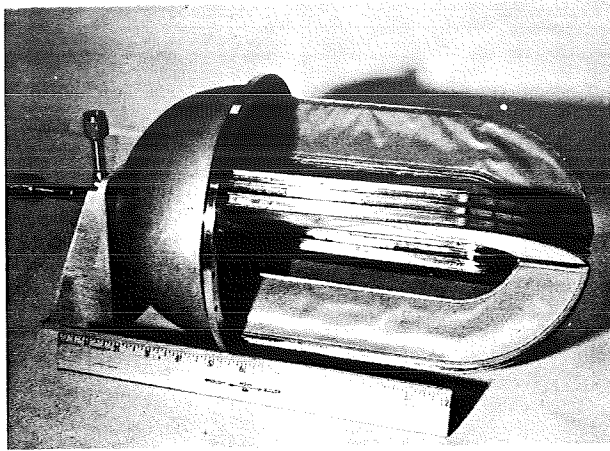
Figure 10.- Total communication screen device.
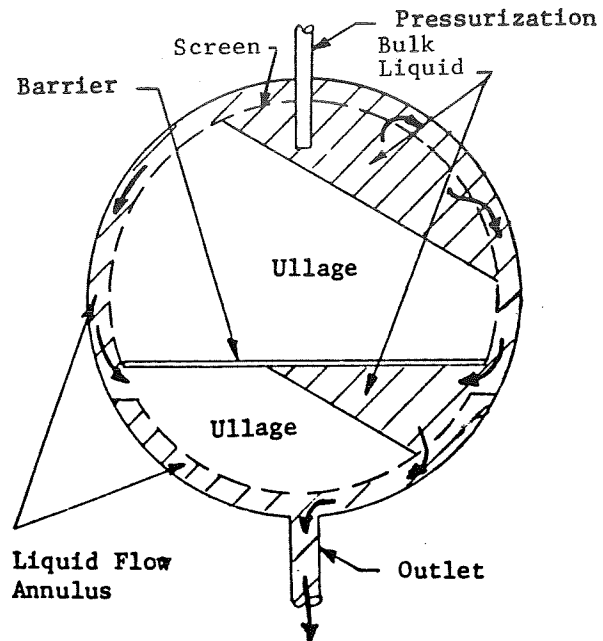
Figure 11.- Schematic of a compartmentalized screen device.
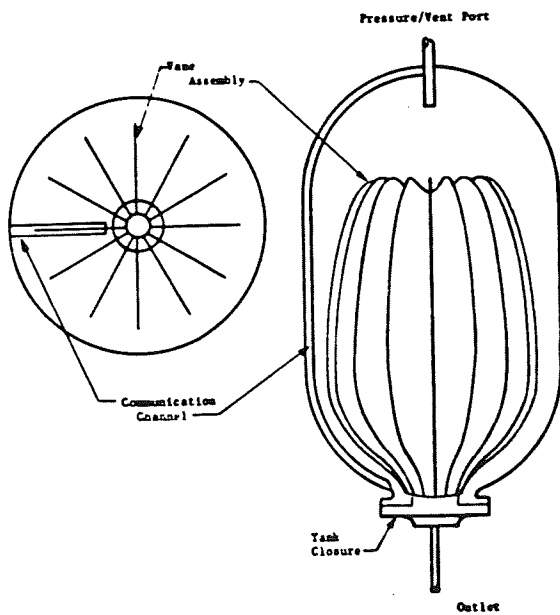
Figure 12.- Schematic of the Viking orbiter 1975 propellant management device.
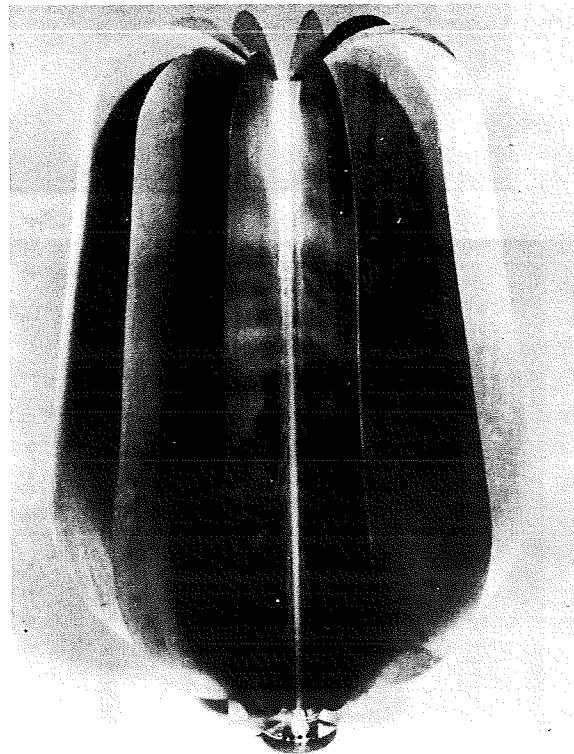
Figure 13.- Photograph of the Viking orbiter 1975 vane structure.

Liner

Vent

Curved-Web Baffles

Standpipe

Direction of Flow

Capillary Feeder
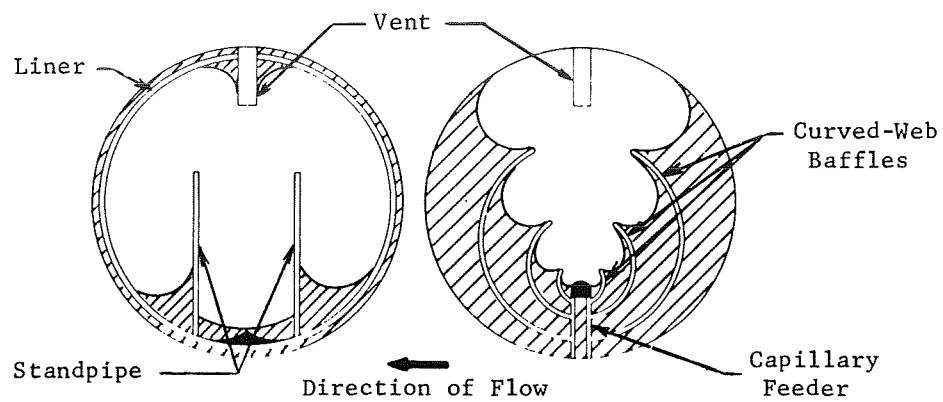
(a)   Filling of standpipe.

Direction of Flow

(b)   Standpipe section nearly empty.

Figure 14.- Apollo 14 fluid transfer with baffled tanks (ref. 20).

SPACE PROCESSING ON SKYLAB AND ASTP

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ABSTRACT

The Skylab and Apollo-Soyuz Test Project (ASTP) missions provided the opportunity to examine the influence of micro-gravity on the processing of various materials. The majority of the experiments dealt with the solidification of alloys, semiconductors, and composite materials or basic liquid-liquid and liquid-solid interactions necessary to understand complex processing. The space results have yielded basic data and increased knowledge of fundamental materials behavior. Potential advantages of space processing to several materials disciplines have been identified.

INTRODUCTION

In the following paragraphs the results of physical and engineering experiments in metallurgy, fluids handling and crystal growth on two space missions, Skylab and the Apollo-Soyuz Test Project (ASTP), will be discussed. The presentation is incorporated into a colloquium concerned with bioprocessing in space because there are similarities in process objectives in the two fields and the results may trigger some ideas in biological applications even though the metallurgists and solid state physicists were concerned with metals and semiconductors. Some of the properti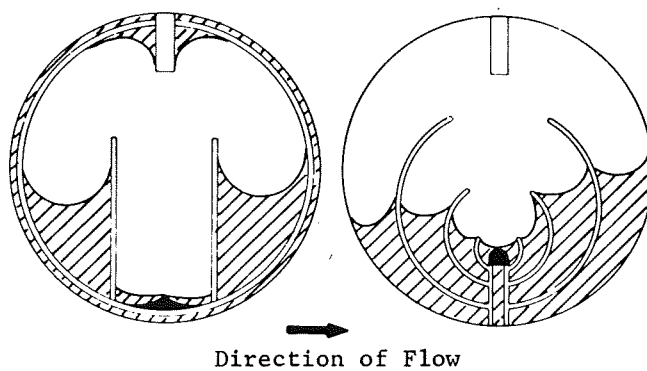es explored in space should have utility in all scientific fields. For example, many disciplines are now expressing an interest in freely suspending their material and then performing manipulations or measurments with no contamination or uncontrolled disturbance. Homogeneous isotropic materials as well as ordered highly anisotropic material have been proposed for space investigation.

The unique advantage of the space environment for modifying or improving the properties of materials is the long term weightlessness. The extended weightlessness in space is created by the motion of the spacecraft under the influence of its high orbital velocity and the gravitational pull of earth. Objects located at the center of mass within the spacecraft are not accelerated by any additional outside force and they will "float". Objects displaced from this point will follow trajectories prescribed by well known equations of motion. These motions have been frequently photographed in space. Space is also characterized by a vast vacuum capacity and the possibility of attaining a vacuum significantly below the capability of any vacuum pump system on earth. The Skylab and ASTP experiments, however, were designed to utilize primarily the micro-gravity advantage of space.

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These two missions encompassed a wide variety of experiments intended to explore the capabilities of the space environment and the details of each of them cannot be given in this brief overview paper. Several review articles of the experiments are already available[1] in addition to the individual reports of the principal investigators.

SKYLAB

A group of experiments that could be done in a common facility on Skylab, shown in Figure 1, were selected in 1969. An electron beam was incorporated to melt metals typically used in welding and to form freely floating metal spheres. The brazing of two tubes with an exothermic chemical reaction was proposed to investigate the capillary flow of the melt around the tube ends. The growth of a gallium arsenide single crystal in a solution of liquid gallium was designed to investigate an improved method to grow this important semiconductor material. All control functions, battery power and storage containers were included in this facility.

By 1971, interest in space processing had developed considerably. The Apollo 14 mission had flown three simple demonstrations of high interest to the program: electrophoresis; melting and solidification of composite materials; and fluid behavior under various thermal conditions. These experiments, done during the return of the Apollo from the moon, showed both the advantages and some of the difficulties of processing materials in space. In 1972, an additional set of eleven experiments were proposed to use the furnace concept of the gallium arsenide experiment but modified for multiple purpose use. (Figure 2) These are listed in Table 1 to show the variety of different experiments that were planned for this versatile facility. As is shown, several experiments were done on Skylab III and repeated with additional samples on Skylab IV. Since the furnace processed three sample cartridges each time, a significant amount of material was returned to Earth for analysis.

The "Vapor Growth of IV-VI Compounds" experiment investigated a technique for growing crystals of electronic materials by condensing vapor evolved from a heated source of the same material. Turbulence in the vapor due to the imposed temperature gradient limits the results on earth but swirling convection currents should not occur in weightlessness. The results of this experiment were unanticipated since up to ten times more crystalline material was produced in space. Dr. Wiedemeier proposes that this is primarily due to an inadequate theory used to determine growth on the ground as well as in space and the Skylab results will modify the fundamental understanding of this technology on Earth. Additionally, the structural perfection of the space grown crystals was clearly superior and the size of one crystal, shown in Figure 3, was six times larger than ever achieved.

Two experiments with different semiconductor materials, germanium and indium antimonide, were done to give a direct comparison of ground and space characteristics. A single crystal, contained in a cylindrical ampoule, was partially remelted and then solidified in space. The principal objective was to achieve improved homogeneity of a specific impurity by removing thermal convection at the solidification interface. The availability of precise electrical measurements systems and high resolution techniques to study segregation inhomogeneities on the order of fractions of a micrometer make semiconductor materials excellent candidates for space processing. Figure 4 shows a comparison of Earth and space-grown crystal of indium antimonide from Dr. Gato's experiment.

Dr. Hans Walter made crystals in a way that is impossible on Earth. A containerless melt was formed on the end of a seed crystal and then directionally solidified to form single crystals typified by Figure 5. The initial samples were cylinders of single crystal indium antimonide, one end fastened in a heat sink and the other in a cavity that was heated above the sample melting point. The cylinder was partially melted in space forming a spherical melt in contact with the solid seed material. The temperature of the cavity was then slowly lowered. The melt solidified first near the seed and finally at the tip in contact with the inner wall of the cavity. Although the melt was surely spherical, the final oblong crystal shape was determined by the forces intrinsic to the solidification process itself. These crystals were all characterized by flat surface facets and decreased internal imperfections.

The measurement of diffusion of one particle species through another in liquids is difficult on Earth because gravity will act on any small difference in particle density to produce convection currents. Dr. Ukanwa designed an experiment to detect and measure any disturbances to pure diffusion caused by any source. In this experiment, cylindrical rods of zinc containing radioactive zinc-65 atoms confined to a zone at the end of the cylinder were melted and solidified in space. By slicing the cylinder on Earth, the diffusion of radioactive zinc atoms was measured, the results show agreement with theory, again confirming the absence of convection currents driven by a temperature gradient.

APOLLO SOYUZ TEST PROJECT (ASTP)

The success of the Skylab experiments led to the proposal of over 30 experiments that could be accommodated in the Skylab furnace with minimum modification. The ASTP timeline allowed only seven experiments to be done by utilizing almost the entire mission in the heat-up, soak at high temperature and controlled cool-down required to fulfill the experiment requirements. The experiments selected for ASTP are listed in Table II. Three proposals were follow-on to Skylab experiments in which the same scientists wanted to obtain additional data. The USSR proposed an experiment to melt several different material combinations during the joint Russian American part of the mission but their analysis has not been completed.

The ASTP experiment on "Surface Tension Induced Convection" further investigated the elimination of convection currents in liquids metals. The Skylab experiment on diffusion in zinc indicated that gravity-induced thermal convection is effectively eliminated in space processing. However, as a result of this condition, it was hypothesized that convection effects caused by surface tension gradients may become significant in space processing. Since surface tension gradients occur due to thermal or concentration differences which are not gravity dependent factors, the objective of this experiment was to determine if similar surface tension induced convection effects would result from concentration gradients. The experiment concept consisted of melting three samples of bi-metallic materials (lead and lead-gold alloy) in wetting and non-wetting capsules in the multipurpose furnace, allowing inter-diffusion of the two components, and then re-solidification. If no convective stirring effects due to the surface tension difference between the materials exist, then a normal concentration - distance profile for the gold would result. The preliminary examination of the flight samples indicates that a normal concentration-distance profile of gold in the sample was obtained. Autoradiographs compare favorably with distance predictions based on the zinc self-diffusion data obtained on Skylab indicating that the diffusion of gold in lead was in the typical liquid diffusion range.

"Monotectic and Syntectic Alloys", investigated the effects of weightlessness on the melting and solidification of two material systems, lead-zinc and aluminum antimonide. The objectives were to investigate phase segregation effects in low gravity for the

immiscible binary lead-zinc and to determine the influences of low-gravity solidification on the microstructural homogeneity and Stoichiometry of semiconducting compound aluminum antimonide. The large density difference between the two metals makes it difficult to avoid severe gravity separation of the phases during solidification of earth-prepared systems. The comparison of the microstructure of the ground and space pro-cessed material, Figure 6, clearly shows an improvement of homogeneity obtained in space.

The experiment, "Interface Marking in Crystals", was developed to study quanti-tatively the basic solidification behavior of high temperature melts under near zero-gravity conditions and answer questions raised by the earlier experiment by Professor Gatos of MIT on Skylab. The experimental hardware on ASTP differed from the related Skylab experiment in that an electrical pulsing unit was utilized to provide interface demarcation during solidification from the melt. Evaluation of the demarcation lines which were clearly established throughout the samples show that the microscopic growth rate was subject to an initial transient which did not stabilize immediately. The observed rate behavior in space was comparable to the ground-based samples and at variance with the behavior predicted by theory. Segregation analysis again showed striking differences between one-gravity and zero-gravity conditions.

ASTP experiment "Processing of Magnets" was conducted to determine the potential advantage of space processing to modify critical magnetic properties by improving chemical homogeneity, morphological and crystalline perfection of the magnetic sub-structure. Preliminary results now indicate that a magnetic alloy of manganese and bismuth resolidified directionally in space has attained a significantly higher coercive strength.

The objectives of "Crystal Growth from the Vapor Phase" were to continue the Skylab investigation into the effects of micro-gravity on the structure of single crystals of mixed systems and to determine the mass transport rates of these systems using the chemical transport technique. The materials, germanium selenide and germanium telluride were chosen to be similar to Dr. Wiedemeier's Skylab experiment. The ASTP experiment gave close agreement of data which confirms predictions that the vapor trans-port technique is suitable for the growth of crystals of significantly better quality in a micro-gravity environment. Crystals of high structural uniformity were produced and the vapor transport rate in space has consistently been greater than predicted by earth models resulting in much larger single crystals than can be produced on earth by identi-cal techniques.

Experiment MA-131, "Halide Eutectic Growth", was developed to investigate pro-duction in space of a eutectic mixture consisting of continuous fibers of LiF embedded in a NaCl matrix. When grown on earth, this material has not realized its full potential for optical transmission due to discontinuous fibers resulting from convection currents in the melt. Dr. Yue's related Skylab experiment demonstrated that continuous fibers of NaF embedded in a NaCl matrix could be grown in space by the directional solidification technique resulting in a material that exhibited improved optical properties in compari-son to earth-grown samples. This technique was again successfully utilized in the ASTP experiment and improved optical transmission was obtained. Figure 7 shows a compari-son of the optical transparency for the best Earth-grown material and the sample from space.

Demonstrations of fluid banding in space and two electrophoresis experiments done on ASTP are described elsewhere in this Colloquium. There are other bioprocessing

experiments that could be done advantageously in space and they could be related to the solidification experiments just discussed. The ease with which liquids can be immobilized and manipulated should have relevance to bioprocessing. Ideas for new experiments should be developed and proposed to NASA for the Spacelab missions of the 1980's.

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SKYLAB EXPERIMENTS	SKYLAB MISSION		
	II	III	IV

MATERIALS PROCESSING FACILITY

	II	III	IV
M551: METALS-MELTING EXPERIMENT, R. R. POORMAN, MSFC ASTRONAUTICS LAB.....................X			
M552: EXOTHERMIC BRAZING EXPERIMENT, J. R. WILLIAMS, MSFC PRODUCT ENG. LAB..............X			
M553: SPHERE-FORMING EXPERIMENT, E. A. HASEMEYER, MSFC ENG. LAB.........................X			
M555: *GALLIUM ARSENIDE CRYSTAL-GROWTH EXPERIMENT, R. S. SEIDENSTICKER, WESTINGHOUSE RESEARCH LAB..			

MUTTIPURPOSE FURNACE SYSTEM

	II	III	IV
M556: VAPOR GROWTH OF IV-VI COMPOUNDS, H. WIEDEMEIER, RENSSELAER POLY- TECHNIC INSTITUTE...		X	X
M557: IMMISCIBLE ALLOY COMPOSITIONS, J. L. REGER, TRW SYSTEMS.............................		X	X
M558: RADIOACTIVE-TRACER DIFFUSION, A. O. UKANWA, MSFC SPACE SCIENCES LAB...............		X	
M559: MICROSECRATION IN GERMANIUM, F. A. PADOVANI, TEXAS INSTRUMENTS....................		X	
M560: GROWTH OF SPHERICAL CRYSTALS, H. U. WALTER, UNIV. OF ALABAMA......................		X	X
M561: WHISKER-REINFORCED COMPOSITES, T. KAWADA, NATIONAL INSTITUTE FOR METALS RESEARCH..		X	X
M562: INDIUM ANTIMONIDE CRYSTALS, H. C. GATOS, MIT.....................................		X	X
M563: MIXED III-V CRYSTAL GROWTH, W. R. WILCOX, USC....................................		X	X
M564: ALKALI HALIDE EUTECTICS, A.S. YUE, UCLA...		X	
M565: SILVER GRIDS MELTED IN SPACE, A. DERUYTHERRE, KATHOLIEKE UNIV., LEUVEN, BELGIUM...		X	
M566: COPPER-ALUMINUM EUTECTIC, E. A. HASEMEYER, MSFC PRODUCT ENG. LAB..................		X	X

EXPERIMENTS PERFORMED ON EACH MISSION:	3	11	7

* NOT FLOWN BECAUSE STORAGE AREA PREEMPTED BY SKYLAB REPAIR KIT.

TABLE I

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ASTP MULTIPURPOSE FURNACE EXPERIMENTS

MA-010: MULTIPURPOSE FURNACE - J. McHUGH, WESTINGHOUSE................ ..
MA-041: SURFACE TENSION INDUCED CONVECTION - R. E. REED, OAK RIDGE....
MA-044: MONOTECTIC AND SYNTECTIC ALLOYS - C. Y. ANG, USRA/MSFC........
MA-060: INTERFACE MARKING IN CRYSTALS - H. C. GATOS, MIT.............. ..
MA-070: PROCESSING OF MAGNETS - D. J. LARSON, GRUMMAN.................. ....
MA-085: CRYSTAL GROWTH FROM THE VAPOR PHASE - H. WIEDEMEIER, RPI.....
MA-131: HALIDE EUTECTIC GROWTH - A. S. YUE, UCLA......................... ....
MA-150: MULTIPLE MATERIALS MELTING - I. IVANOV, USSR................... ....

59

TABLE II
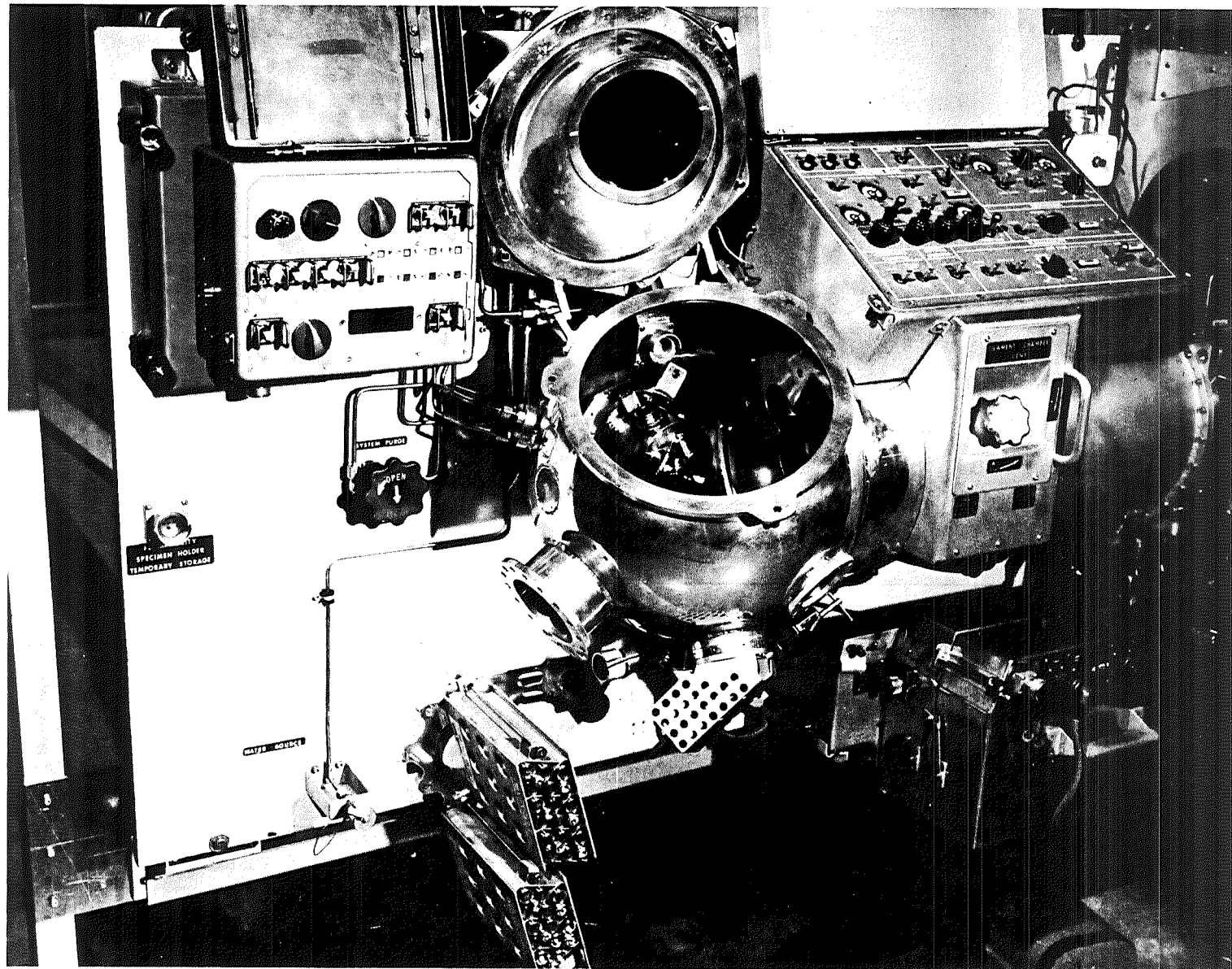
Figure 1.- M512 space processing experiment facility on Skylab.

S/S CASE

MOLY HEAT SHIELDS

MOUNTING FLANGE

MEASUREMENT THERMOCOUPLE

HEATER AND HEAT LEVELER

EXPERIMENT CARTRIDGE

M512 FACILITY

HEAT EXTRACTION PLATE

CONTROL THERMOCOUPLE

ACCESS PLUGS

VENT VALVE

ELECTRICAL RECEPTACLE
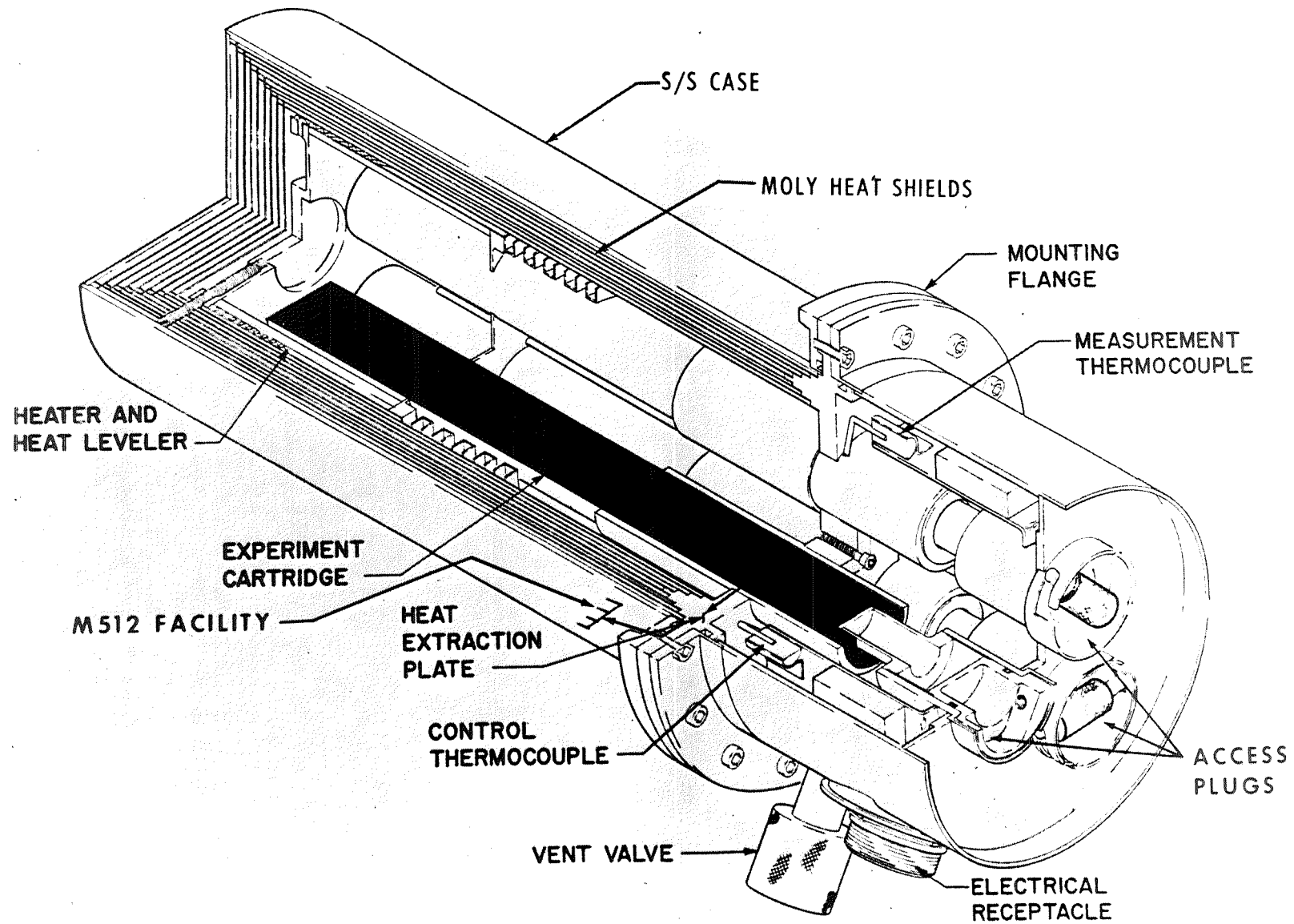
S&E-PE

Figure 2.- M518 multipurpose electric furnace.
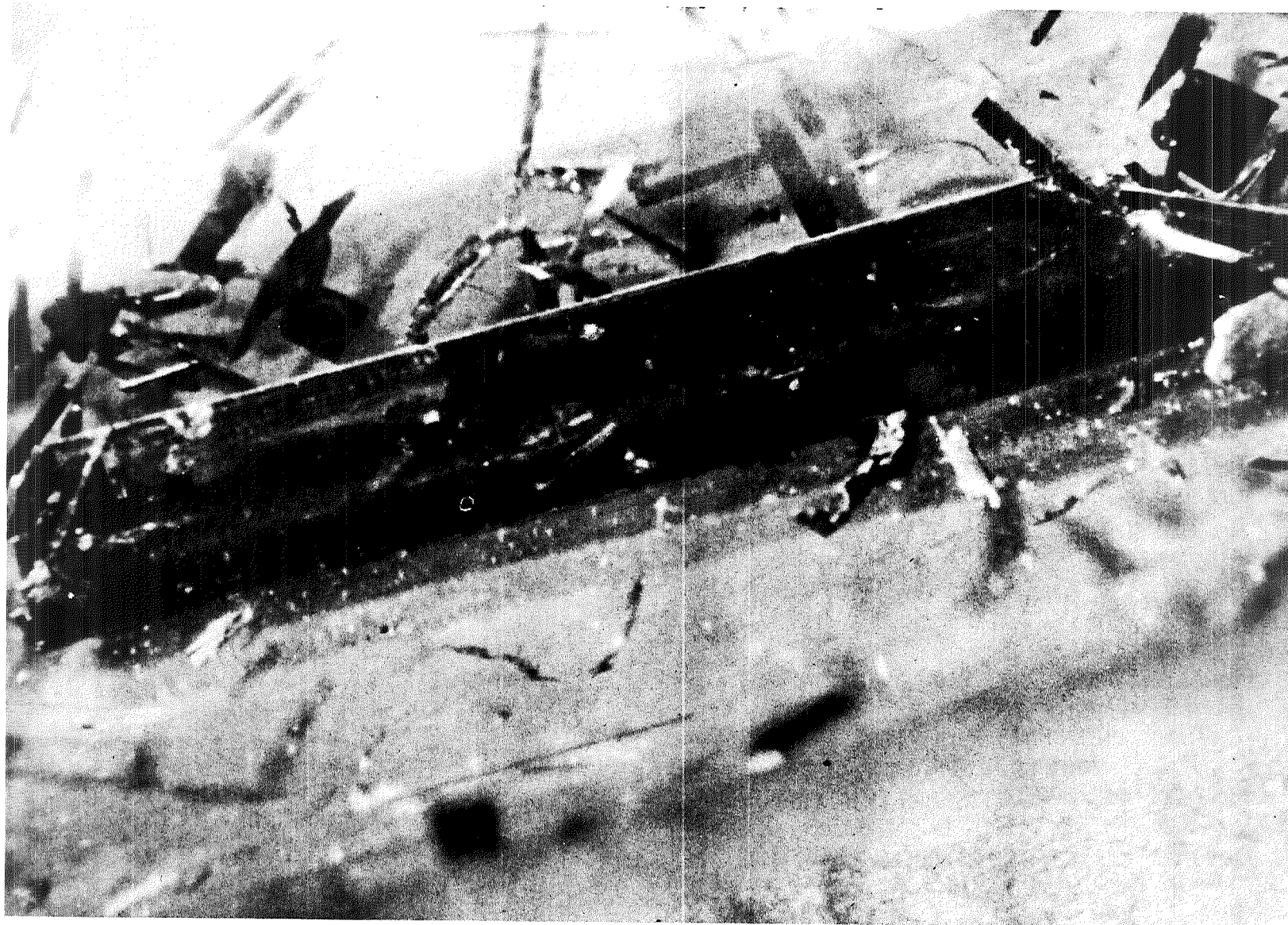
Figure 3.- Space-grown (zero g) semiconductor crystal (germanium selenide); Skylab experiment M556, single crystal, 20-mm length).
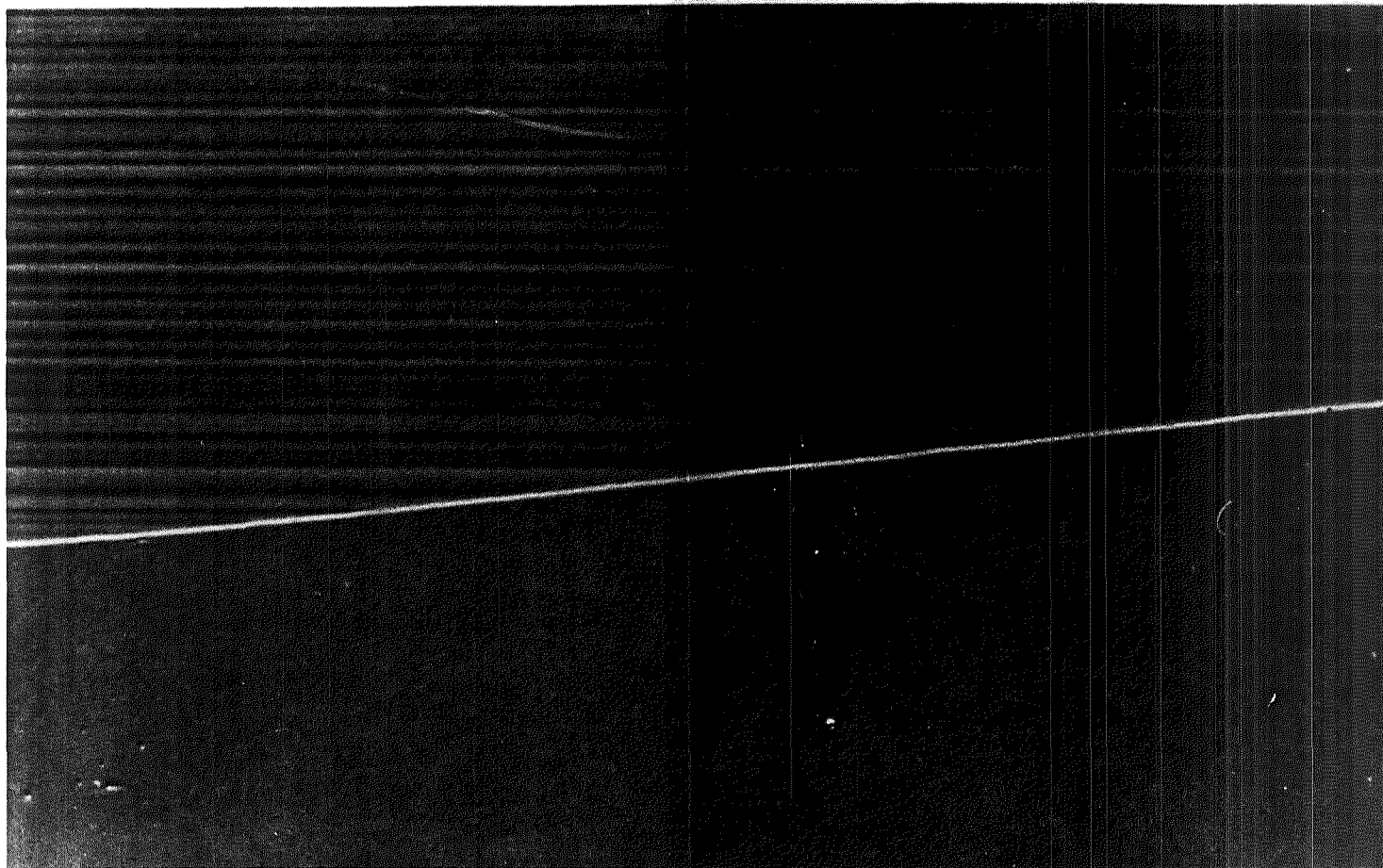
Figure 4.- Segment of the initial regrowth interface of tellurium-doped InSb crystal (B-1); dopant inhomogeneities are seen in the Earth-grown (upper) section; no dopant inhomogeneities are present in the space-grown (lower) section; 125X magnification.
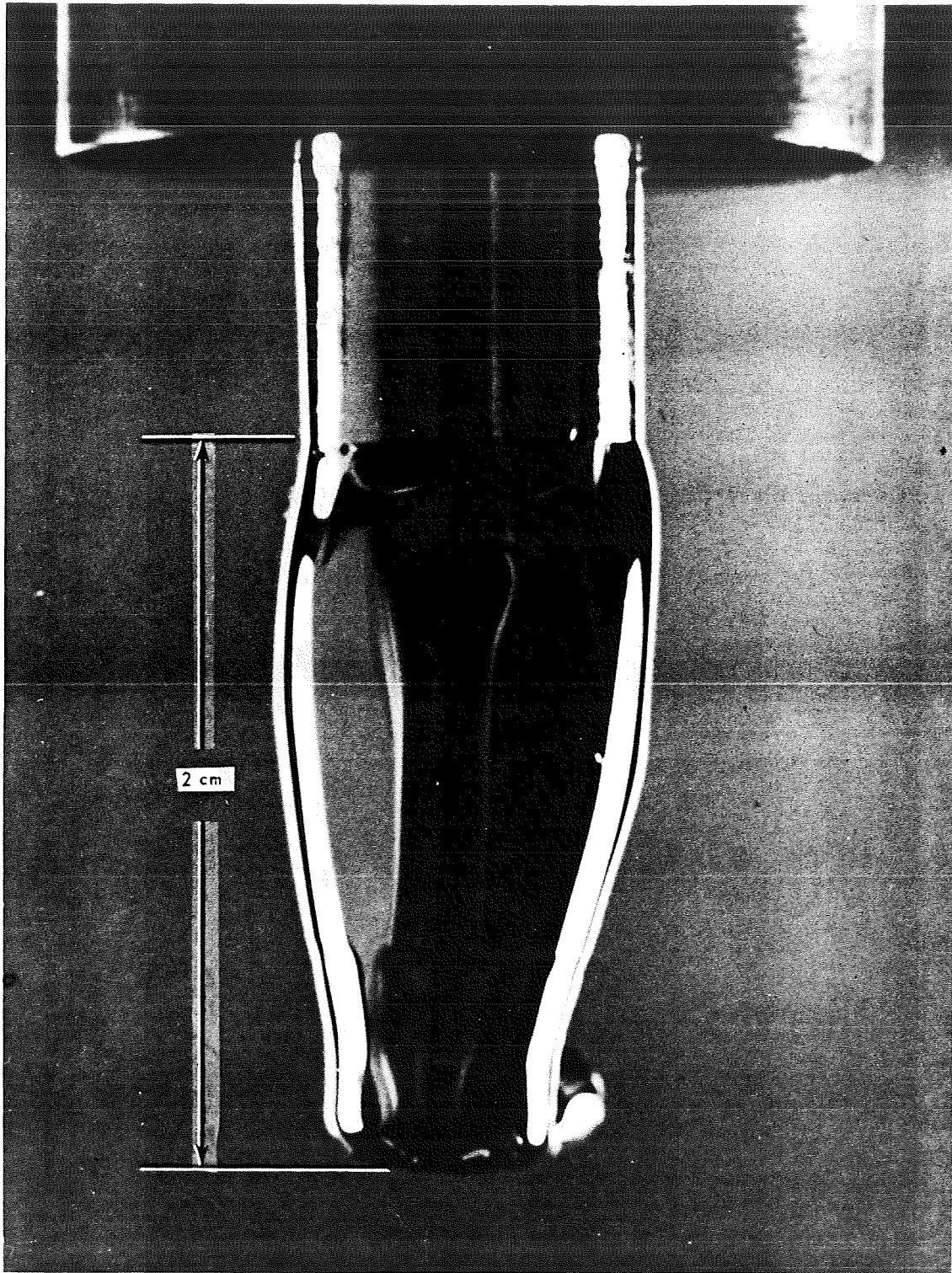
Figure 5.- Space-grown (containerless) single crystal of semiconductor indium
antimonide; Skylab experiment M560 (special illumination).

(a) Low g (A185).
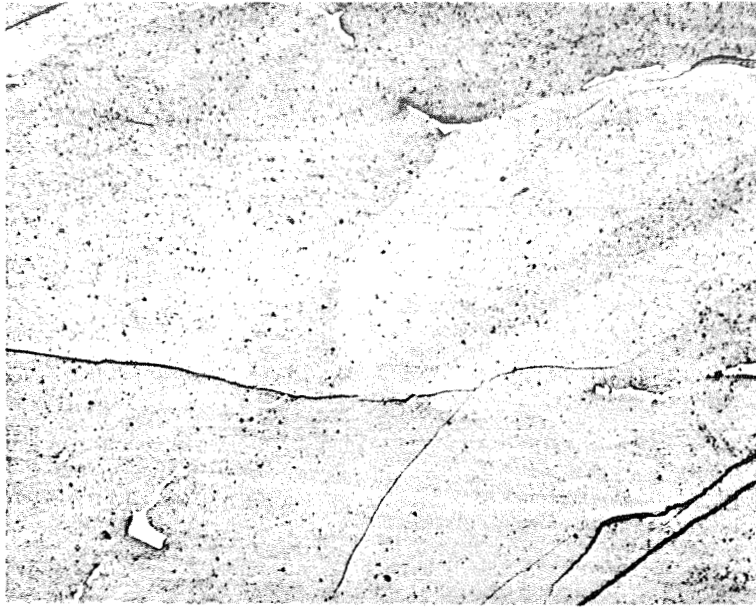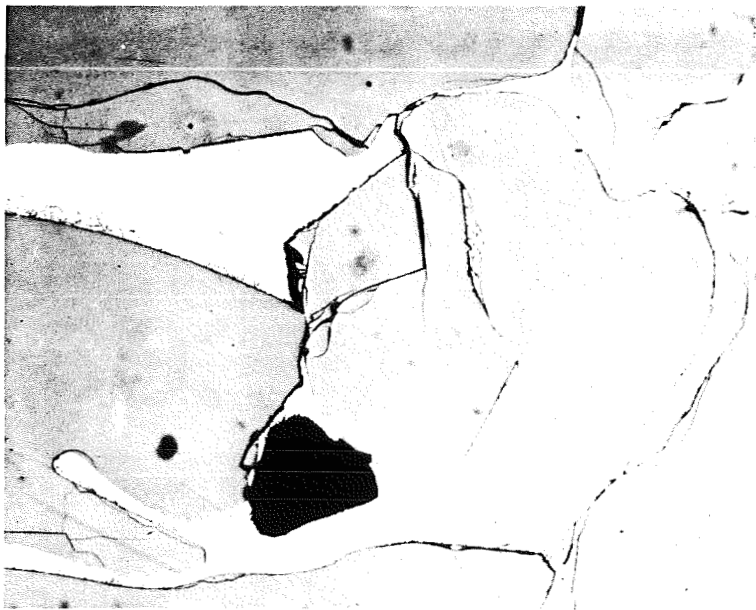
(b) One g (A141).

Figure 6.- Comparison of ASTP and GBT-2
AlSb microstructure.

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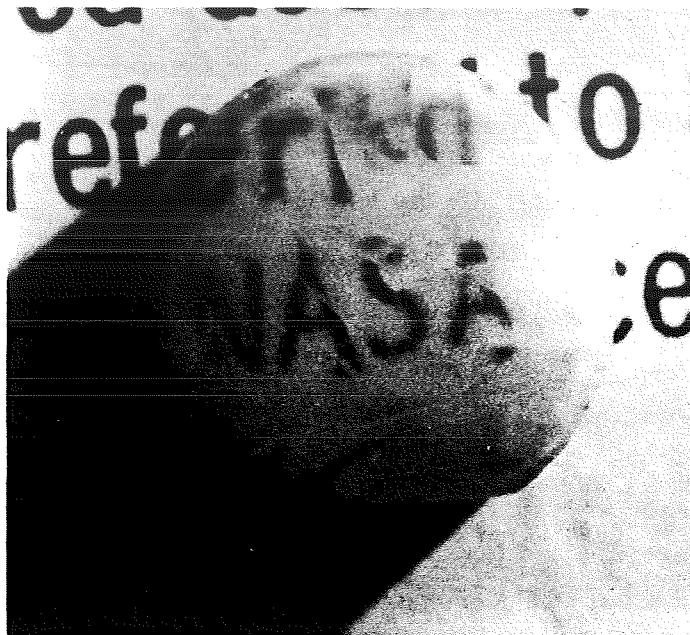
(a) Space-grown.
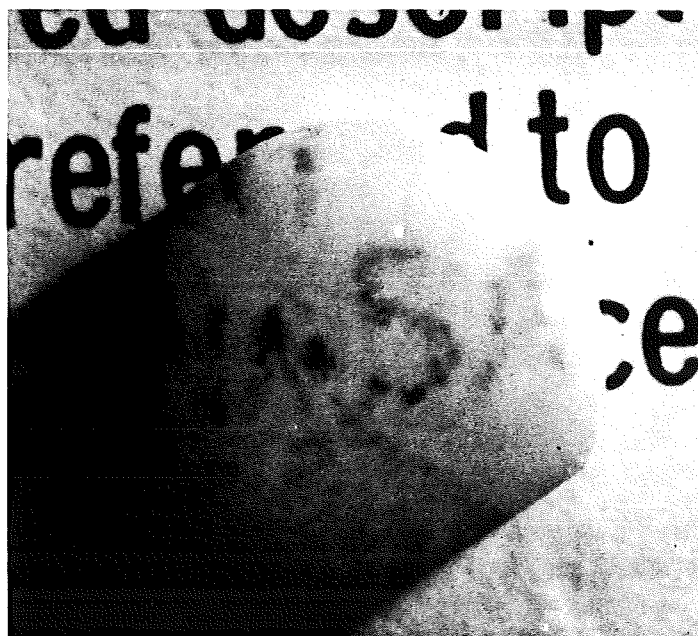
(b) Earth-grown.

Figure 7.- Optical transparency of samples
from halide eutectics experiment M564.

THREE MODEL SPACE EXPERIMENTS ON CHEMICAL REACTIONS

By Philomena Grodzka, Lockheed Missiles and Space Co., P. O. Box 1103,
Huntsville, Alabama 35807, and Barbara Facemire, NASA-Marshall Space
Flight Center, Alabama 35812

ABSTRACT

Three simple science demonstrations conducted aboard Skylab IV and Apollo-Soyuz
involved phenomena that are of interest to the biochemistry community. The three experiments are identified here as the Formaldehyde Clock Reaction, the Equilibrium Shift
Reaction, and the Electrodeposition Reaction. The Formaldehyde Clock Reaction and the
Equilibrium Shift Reaction experiments conducted aboard Apollo-Soyuz demonstrated the
effect of low-g foams or air/liquid dispersions on reaction rate and chemical equilibrium.
The Electrodeposition Reaction experiment conducted aboard Skylab IV demonstrated the
effect of a low-g environment on an electrochemical displacement reaction.

In a formaldehyde clock reaction, a number of chemical reactions occur simultaneously
and at such rates that the end of the reaction, signaled by a change of color from colorless
to red, does not occur until about 20 seconds after the reactant solutions have been mixed.
A clock reaction embodies some of the features of periodic chemical reactions which are of
great interest at present because they suggest a relevancy to mechanisms controlling
biological rhythms. In exploratory ground experiments, the purpose of which was to
identify a good space demonstration experiment, it was discovered that a formaldehyde
clock reaction displays effects that can be attributed to Gibbs (or van der Waal) adsorption
of polymeric formaldehyde solution species. Also discovered were internal effects that
are caused either by internal shear as the result of residual fluid flow or by formation of
three-dimensional formaldehyde species networks. The various noted behaviors of the
Formaldehyde Clock Reaction in ground tests and in the low-g tests are described. It is
concluded that the unique behaviors observed in low-g are the result of the presence of
many more small air bubbles than were present in the one-g cases.

In the Chemical Shift Reaction a reversible chemical equilibrium is caused to shift
by means of foam formation. Evidence of the chemical shift is given by the color of the
foam (pink) which is different from the color of the bulk solution (amber brown). In low-g
the pink foam was many times more stable than under one-g conditions.

In the Skylab Electrodeposition Experiment, a chemical displacement reaction caused
silver crystals to be deposited. The silver crystals obtained in low-g were quite different
than those obtained in one-g because of the differing convection currents generated in the
two situations. This experiment is not discussed in detail. Only the implications of the
conclusions for biochemical type reactions are considered.

The implications of the three space experiments for various applications are considered.

THE FORMALDEHYDE CLOCK REACTION

It is well known that the rates of many, if not most, chemical reactions are heavily
dependent on the concentrations of the reacting species. Thus, if reacting chemical
species are not uniformly distributed throughout the solution, a reaction can occur faster
in one part of the solution than in another. At constant temperature and pressure, a non-
uniform distribution of solute species in a well mixed solution can occur as the result
either of adsorption at a liquid/gas or a liquid/solid interface or of a hydrostatic pressure

effect on chemical potential. The first of these effects is the one of interest here. For the sake of clarity, it may be well to note that the type of adsorption we are considering here is Gibbs adsorption, i.e., a solution becomes more or less concentrated (positive and negative adsorption) in solute species in the liquid-gas or liquid/solid interface zones but no change in phase occurs nor is there any chemical reaction between solute species and species in the gas or solid phases.

It is generally well known that Gibbs adsorption can cause the surface tension of a solvent to be either increased or decreased when solute is added. The effect of Gibbs adsorption on rates of chemical reaction, however, has not, to the best of our knowledge, been directly demonstrated. A number of previous investigators have conducted chemical reaction experiments, however, in which either insoluble monomolecular films were involved or chemisorption had occurred. In some cases where Gibbs adsorption was undoubtedly involved, the evidence was indirect. These various cases as well as some prior, reported speculations are briefly reviewed in the following paragraphs.

E. K. Rideal was a very active investigator in this area and published a number of works on chemical reactions involving monolayers of one reactant (Refs. 1-4). Among some of the systems investigated by Rideal and others are (Refs. 1-9):

Type Reaction	Monolayer Film	Bulk Reactant
oxidation	oleic acid	permanganate
hydrolysis	ethyl butyrate	pancreatin enzyme
hydrolysis	ethyl palmitate	aqueous alkali
hydrolysis	lecithin	snake venom enzymes
photolysis	stearanilide	
	benzylstearylamine	
	β-phenylethylstearyl-amine	
	proteins	
	barium stearate-stearic acid	
polymerization	maleic anhydride compound of eleostearin	
chemical reaction	amines and aldehydes	
chemical reaction	egg albumin	trypsin enzyme
	caseinogen	
chemical reaction	sterol	sodium cetyl sulfate
	carcinogenic hydrocarbons and sterols	
lactonization	γ-hydroxystearic acid	
complex formation	stearic acid	heavy metal ions

The rates of chemical reaction were shown to be strongly affected by species adsorption at charcoal interfaces in the hydrolysis of bromoethylamine and in the conversion of dimethyleneimine hydrobromide into bromoethylamine in hydrobromic acid solution (Ref. 10). Foaming was found to promote the lipolysis of milk (Ref. 11). The cited study concluded that a foam promotes lipolysis by providing optimum conditions as follows (i) greatly increased liquid surface, (ii) selective concentration of enzyme at the air-liquid interface, (iii) activation of the substrate by surface denaturation of the membrane materials surrounding fat globules, and (iv) intimate contact of enzyme and activated substrate.

In ground studies conducted to identify a good space demonstration experiment, the discovery was made that a formaldehyde clock reaction can display both a Gibbs adsorption and an internal structure or a shear effect on the rate of the reaction. A report of the observed evidences of Gibbs adsorption and internal shear or three-dimensional structure

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effects in a formaldehyde clock reaction has not yet appeared in the open literature although a submission is currently being reviewed for publication (Ref. 12). A brief summary, therefore, is given here.

The surface and internal effects to be described were observed with solutions and procedures which are as follows:

Stock solutions: (a) 3.3% formaldehyde (9.0 ml 37.7% reagent grade formaldehyde diluted to 100 ml with distilled water), (b) 1 gm phenolphthalein dissolved and diluted to 500 ml with 50% ethanol-water, and (c) 10.0 gms sodium metabisulfite ($Na_2S_2O_5$) and 1.5 gms of sodium sulfite (Na_2SO_3), reagent or certified grades, diluted to 100 ml with distilled water. Procedures: One ml of formaldehyde stock and 0.5 ml of phenolphthalein stock are added to 8 ml of distilled water in a test tube. A 0.5 ml portion of the bisulfite/sulfite stock solution is then added rapidly, the test tube capped, and shaken vigorously for about 5 sec. The mixed solution remains colorless for about 20 sec at the end of which time a sudden appearance of a red color occurs. The time interval between the time of bisulfite/sulfite addition to the time of red color appearance can be varied by adjustment of solution concentrations (Ref. 13). Plastic syringes bought in drugstores for a few cents make handy devices for measuring and adding the small amounts of reagents involved. The stock solutions of formaldehyde and phenolphthalein are stable indefinitely. The sulfite/bisulfite solution, however, deteriorates. The deterioration rate, however, is fairly slow if oxygen and light exposure are kept to a minimum.

The chemical reactions involved are as follows (Ref. 13):

Rate Constants

2.8 l/mol sec

$$HCHO + HSO_3^- \longrightarrow CH_2OHSO_3^-$$

0.14 l/mol sec

$$H_2O + HCHO + SO_3^= \longrightarrow CH_2OHSO_3^- + OH^-$$

instantaneous

$$OH^- + HSO_3^- \longrightarrow SO_3^= + H_2O$$
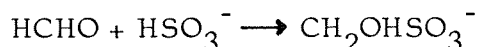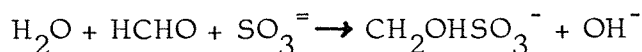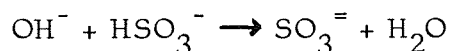
Thus, excess hydroxide ion becomes available to react with phenolphthalein indicator only when all of the bisulfite ion is used up.

The surface and internal structure effects observed are briefly as follows: In plastic (Lexan) test tubes the color change is most frequently seen to occur first in the small drops that cling to the sides of the tube. Or the color change will start at a point in the liquid/vapor/solid interface or in the bulk of the solution and then spread out as a wave into the remainder of the solution. If the reaction is allowed to occur in contact with a polystyrene surface, color spots appear at the solid/liquid interface sites, the color change then proceeding into the bulk of the solution. In chilled solutions the surface effects are greatly enhanced. In addition to the described surface effects, internal structure effects, as evidenced by complex colored shapes in the bulk of the solution, are also seen. The internal structure effects may be caused either by shear as the result of residual fluid flow or by a three-dimensional formaldehyde species network. In stirred solutions the color changes outline vividly fluid flow phenomena such as vortices. Various size drops of reacting solution placed on various surfaces change color most frequently in the order of large drops first, medium-sized drops next, and small drops last. Further evidences of the Gibbs and internal structure effects are given in the not-yet-published report (Ref. 12) and in film strips of the various ground tests. In the Apollo-Soyuz experiment Astronaut D. K. Slayton performed in earth orbit the formaldehyde clock reaction in capped Lexan centrifuge tubes. The objective of the space experiment was to determine the effect of a

low-g gas/liquid dispersion or "foam" structure on the reaction. The low-g experiment showed that in low-g "foams" the red color first appears at the gas/liquid/solid interface and then spreads out rather evenly from this interface. A homogeneous light pink color appearing about the same time as the first red color is also noted. Further results such as reaction times and velocity of color wave advance await a complete analysis of the flight data.

THE EQUILIBRIUM SHIFT REACTION

Only a few workers have delved into the area of chemical equilibrium shift induced by foam or emulsion formation. The following indicator equilibria are reported by Freundlich to be shifted by emulsion formation (Ref. 10):

$$A + H^+ \rightleftharpoons AH^+$$
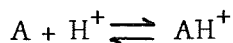
| Violet (2.0) | Green (1.0) | Methyl-violet |

| Green (1.5) | Yellow (0.5) | Malachite-green |

$$A^- + H^+ \rightleftharpoons AH$$
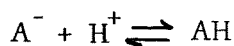
| Blue (7.4) | Yellow (6.2) | Brom-thymol blue |

The numbers in the brackets are pH values. Thus, if an aqueous solution of bromothymol blue of pH of 7.4 is shaken with benzene, the color changes from blue to yellow. The yellow color remains as long as fine drops of benzene remain. Similarly a solution of thymol blue of pH of about 2.8 will show a change of color from brown-amber to pink when foamed by shaking. The foam under one-g conditions disappears in a few seconds, however. The preceding data would indicate that the interface regions favor the undissociated forms of the indicators while the bulk phases favor the dissociated forms, although to the best of our knowledge, the matter is still not conclusively settled. Other reactions along these same lines are:

Rhodarnine-O (colorless base of)

benzene solution + water ——————— emulsion
   colorless   red

benzene solution ——————— on filter paper or quartz powder
   colorless   red

colorless form probably lactoid form
colored form probably betaine form

Rhodaime 6G extra and Rhodamine 3G extra

organic liquids ——————— organic liquid + water emulsion
   one color   another color

Bases of the preceding dyes

benzene solution ——————— benzene + water emulsion
   yellow   red

water solution
  <u>red</u>

benzene solution   ————————————   on quartz powder
  <u>yellow</u>   <u>red</u>

<u>Silver Eosinate</u>

aqueous solution containing silver nitrate ——— adsorbed on surface of silver halide
  <u>pinkish-yellow with green fluorescence</u>   <u>red</u>

         "   ——— solution + benzene emulsion
                            <u>red precipitate at interface</u>
         "   ——— shaken in air
                            <u>red precipitate at interface</u>

Probably not all of the preceding reactions are strictly reversible. The illustrative point, however, is not affected.

In the Apollo-Soyuz experiment an aqueous solution of thymol blue was shaken. The pink foam lasted a great deal longer than one generated on the ground. Times for foam collapse, however, were not measured.

<center>THE ELECTRODEPOSITION REACTION</center>

The Electrodeposition Reaction experiment conducted aboard Skylab IV was designed primarily to study metal crystal growth in a low-g environment. However, because the reaction involved an electrochemical displacement reaction, certain features of it should be of interest to the biochemical community. The experiment consisted of the astronaut inserting a copper wire into a 5 wt % silver nitrate solution. The following electrochemical displacement reaction occurred:

$$Cu + 2Ag^+ \longrightarrow Cu^{++} + 2Ag$$
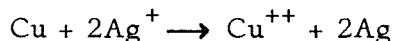
The silver crystals grown in space are quite different from those grown on the ground because of the differing convection currents generated in each case. Full details will be found in a report which will appear shortly (Ref. 14) and in a paper which is currently being reviewed for publication (Ref. 15). The experiment, while not immediately relevant to biochemistry, does raise some common questions. For example, what are the gravity effects on e.m.f. and what is the nature of low-g convection in cases where electric fields are involved. These common aspects are covered in more detail in the following discussions.

<u>Gravity effects on e.m.f.</u>: The fact that gravity can affect e.m.f. values has been long known (Ref. 16). The gravity dependency of e.m.f. in ordinary cells is the result of the change of chemical potential of the ions with change in gravity level. This dependency is given by:

$$d\mu_i = (M_i - \bar{V}_i \rho) \, gdh$$

where μ_i is the chemical potential of the ion in question, M_i its molecular weight, $\bar{V}_i$ the partial molal volume, ρ the density of the solution, g the gravitational acceleration, and h the height measured from some reference height. One early worker found that e.m.f. changed about 0.510×10^{-8} volt per cm of height for a cell employing a 2.71 molal potassium chloride solution. Thus, on earth an e.m.f. may be generated just by having one half cell higher than another identical half cell. In living systems gravity has been shown to electrically polarize an organ placed horizontally. For example, after shoots and leaf

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stalks had been placed horizontally, their upper sides assumed a potential several milli-volts more negative than the lower (Ref. 17). The explanation given for this effect is that the diffusion potentials already existing across membranes becomes modified as the result of gravity. The diffusion potentials can be modified by a change of ion mobilities due to gravity and by displacement of growth hormone which raises the ionic selectivity of the membranes concerned in the region of its enrichment (Ref. 17).

Low-g electro- and other types of convection: Various types of relatively ill-under-stood convections undoubtedly play important roles in biochemical processes. For example, Dr. W. Dorst, Amsterdam, believes that convective processes within a millipore membrane are the major factors in his experiments dealing with the effect of gravity on the permeabil-ity of a synthetic membrane (Ref. 18). Adding substance to this belief is the demonstration of free convection in electrolysis cells whose total volume was 0.25 ml and which contained solution of depths of less than 2 mm (Ref. 19). Convective processes have undoubtedly also played roles in experiments on anomalous and thermo-osmosis which gave puzzling results (Ref. 20). Recently Dr. J. R. Melcher of MIT conducted some preliminary tests on elec-trically-driven convections in aqueous solutions (Ref. 21). It might be added that the electro-convection Dr. Melcher was concerned with had to do with electrical forces on zones of concentration inhomogeneities within the bulk of the solution and not with electro-osmosis type convection. It is interesting to speculate what role electro-convection might play in various electrolysis and electrophoretic processes, but speculations would be fruitless at present when so little is known about the basic phenomenon. The literature is so scanty on convective phenomena in biological processes and processing that we expect that a whole new exciting era will be initiated once serious attention is turned in this direction. It may be well to add that gravity and electric fields are not the only forces that can drive fluid flow. Surface and interfacial tensions, phase changes, thermally induced volume changes, vibrations or g-jitter, magnetic fields are also possible driving forces. In addition, care must be taken when analyzing data from low-g environments not to tacitly assume that gravity-driven convection was absent. Even in low-g, gravity can be a major driving force. It all depends on the particulars of a given situation. The general nature of convection in low-g environments was recently reviewed (Ref. 21).

POSSIBLE APPLICATIONS

The implications of the space experiments with regard to possible applications fall into two areas. One area is concerned with directions for basic research on biophysical processes and the other with directions for processing applications in low-g. In the area of basic research, the Formaldehyde Clock Reaction would appear to have relevancy to phe-nomena such as the clotting of blood and the formation of cataracts. For example, the following description of how a lobster's blood clots on a glass slide sounds much like a des-cription of how a formaldehyde clock reaction occurs in a plastic tube:

"A wave of changes must start at the interface between the glass and blood, and pro-gress through the latter, involving these sensitive corpuscles in its path. . . The two impressive features of this phenomenon are (1) that a chemical change of catastrophic character can be started at an interface, and (2) that the change can be propagated appa-rently indefinitely through one of the phases." (Ref. 22)

A description of the processes involved in the formation of cataracts (Ref. 23) also sounds as if processes similar to those found in the Formaldehyde Clock Reaction are in-volved. For example, the formation of regions of cortical opacities within a cataractous lens can be compared to the formation of red spots within the solution in the Formaldehyde Clock Reaction because an opacity is caused by an abrupt or irregular change in protein concentration. Also interesting is the fact that shear or internal structure effects appear to play a role both in the Formaldehyde Clock Reaction and in the formation of cataracts.

Also solution size or volume effects are notable in both phenomena. The hints that the two phenomena may be related are tantalizing and a number of experiments immediately suggest themselves. With regard to electrical phenomena in low-g, it would appear that space experiments could help a great deal in explaining the role of convection in a number of membrane transport phenomena.

In the area of processing applications, the demonstration of a formaldehyde clock reaction and an equilibrium shift reaction in low-g indicates that low-g forms can be unique environments for conducting biochemical reactions. Also the demonstration of a longer lasting foam in low-g indicates foam separation processes that cannot be done on earth because of the long times required for adsorption, i.e., the foam on earth does not last long enough for adsorption to occur. The demonstration of an electrodeposition in space points towards organic syntheses and separations utilizing electrolysis.

ADDENDUM

In the discussion that occurred after this paper was given at the Bioprocessing Colloquium, a couple of questions were raised on which we should like to elaborate. The first concerns the role of oxygen in the surface effects noted in the Formaldehyde Clock Reaction. Oxygen may affect significantly only the sulfite or bisulfite species through the reaction

$$H_2SO_3 + \tfrac{1}{2}O_2 \rightleftharpoons HSO_4^- + H^+$$
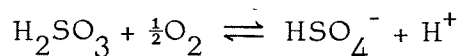
The reaction is slow in the absence of catalysts. Even if it did occur to any appreciable extent at the liquid/air interface, however, the effect would be to make the interface solution more acid. Thus the onset of red color would be expected to be considerably delayed at air/liquid interfaces, not accelerated as is actually observed. It may, therefore, be concluded that oxygen is not a significant variable in the reaction. To verify this conclusion, a test was run which included a nitrogen purge in a closed test tube prior to performance of the reaction. The reaction was observed to proceed in all respects the same as it does when no purging is employed.

It was also mentioned after the talk that the red color is seen to form first at a negatively charged platinum electrode. The question was raised whether the red color was due to the clock reaction or to electrolysis of hydrogen ions. A check of our notes verified that no red color develops at the negative electrode if formaldehyde is left out of the reaction mixture, i.e., no or insufficient electrolysis occurs with the $1\tfrac{1}{2}$ volt system used to change the color of the phenolphthalein indicator. The accelerating effect of the negative electrode on the Formaldehyde Clock Reaction, therefore, is real.
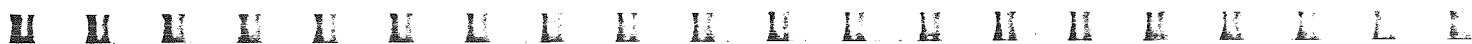
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SURVEY OF CELL BIOLOGY EXPERIMENTS

IN REDUCED GRAVITY

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ABSTRACT

Although single cells are generally considered to be less vulnerable than higher organisms to variations in gravitational forces, many cell experiments have been conducted in the reduced gravity of space. Studies involving isolated viruses, bacteria, yeasts, filamentous fungi, protozoans, and cells in small groups (such as tissue cultures and early embryos) are reviewed to illustrate the variety of species examined. Early studies, conducted with high altitude balloons, sounding rockets, and primitive orbital satellites, demonstrated the capability of cells to survive the space flight environment. These results revived interest in Panspermia and demonstrated the possible requirement for sterilization of all space-craft landing on foreign heavenly bodies.

Because space vehicles, equipment, and passengers are not sterilized before flight, it has been important to study the effects, if any, of spaceflight on terrestrial cell systems. With some important exceptions, which are discussed, static cell systems carried aboard USA and USSR space flights have failed to reveal space-related anomalies. Some sophisticated devices which have been developed for viewing directly, or continuously recording, the growth of cells, tissue cultures and eggs in flight, are described and the results summarized. The unique presence of high energy, multicharged (HZE) particles and full-range ultraviolet irradiation in space has prompted several investigators to evaluate the response of single cells to these factors.

Summary results and general conclusions are presented. Potential areas of research in future space flights are identified.

INTRODUCTION

Experimental evidence that organisms are affected by gravitational forces was first obtained in 1806 by Knight who demonstrated, with the

aid of a water-driver centrifuge, that orientation in plants was determined
by the gravitational vector (1). The dependence of animals upon gravity
was first observed in 1883 by Pfluger with demonstrations that the develop-
ment of frog eggs in an inverted position resulted in a high rate of abnor-
malities (2). Many studies, designed to evaluate the effects of resultant
forces in excess of one gravitational unit, issued from these beginnings.
In addition, some investigators have observed organisms in devices which
compensate for, or oppose, the Earth's gravitational attraction. The neu-
tral bouyancy tank which provides a flexible lift equal to the mass of the
test object, and the rotating clinostat which continually alters the di-
rection of the gravitational vector, are the two most widely used devices
(3).

With the advent of the space age it became possible to reduce the total
force upon test systems to less than one gravitational unit by removing
them from the Earth. This opportunity was recognized by many investigators
who conducted experiments on a large variety of different types of living
test systems. Those systems which involve single cells or small groups
of cells (such as blastulas or tissue culture) are reviewed and summarized
in Tables I through VI, in an effort to demonstrate the variety of tests
that have been conducted in space. In addition, general conclusions are
presented and areas potentially worthy of future space research are identi-
fied.

REVIEW

Survival Of Cells In Space

Prepatory to studies on orbital spaceflights, several microbial species
were exposed to altitudes up to 1900 Km in balloon and sounding rocket
flights (Tables I, II, III, and IV). These exposures, which were initi-
ated in 1935 (4), were conducted to determine if microorganisms could sur-
vive high altitude flight and have been thoroughly reviewed (5, 6, 7, 8).
Although rudimentary, these studies permitted the investigators to observe
that a large percentage of fungal spores and dormant vegetative cells could
survive short-duration direct exposure to the space environment at these
altitudes (9,10).

Beginning with the USSR recoverable Sputnik 5 flight in 1960 (11) and
the USA Gemini/Agena missions in 1963, the requirement to sterilize space
vehicles destined to land on other heavenly bodies has been studied (8).
In a typical example, a variety of microbial species (Penicillium
roqueforti, Bacillus subtilis spores, Tobacco Mosiac Virus, and T_1
coliphage) were carried aboard the Gemini 9A and Gemini 12 spacecraft (9).
Viable representatives of all species were recovered following nearly 17
hours of "direct exposure" to space conditions. These same species, when
protected from direct solar irradiation, survived 4 months of exposure on

the Agena 8 orbiter (10). Similar tests on the Soviet Cosmos 368 Earth-orbital satellite, and the Zond 8 automatic lunar station, revealed that Hydrogenomonas eutropha, Saccharomyces ellipsoides, Zygosaccharomyces baili, and Escherichia coli, cells were all able to survive spaceflight (12,13).

In the ensuing years, viability measurements have generally been included in all space cell biology studies. As a result it has been established that microorganisms in and on interplanetary spacecraft may be capable of surviving to contaminate extraterrestrial bodies (8, 9, 10, 12, 13, 14, 15, 16, 17). The record for viability in space was reported for Streptococcus mitis which was recovered from internal components of a Surveyor III television camera that had resided on the surface of the Moon for 2.5 years (18). Even though the possibility of survival in space has been repeatedly proved, it was considered operationally non-feasible to sterilize space vehicles, equipment, and passengers before flight. Accordingly it became important to evaluate the effects, if any, of spaceflight on terrestrial cell systems.

Although interested in the same objective, the American and Soviet space programs proceeded differently to evaluate these effects. This difference is outlined by Jenkins (6) who demonstrated that in the first decade of orbital flight, Soviet scientists evaluated 56 different species (or preparations) including viruses, bacteria, yeasts, fungi, plants, animals, and tissue cultures. During the same period the USA evaluated only 35 different species and cellular preparations. More importantly, several of the Soviet satellites were flown primarily to obtain biological data to qualify man for spaceflight. In contrast, the early American biology studies were operated on a non-interference basis and no success-ful, dedicated biology satellite was flown until the launch of Biosatellite II in September 1967 (6).

Effect Of Spaceflight On Growing Cultures

In addition to the previously mentioned viability tests which involved static or dormant cells, spores, or cysts, some important studies, outlined in Table VII, have been conducted on growing cells. Inflight microbial growth was first monitored during the flights of Sputnik 5 (19) and other, non-recovered Soviet satellites (20), with the aid of an automated device known as "Bioelements". This device was designed to measure the rate of gas production in actively growing Clostridium butyricum cultures and to relay these data to earth. Data from this test, and from Vostok 1 and 2 where a modified "Bioelements" was used, showed gas production rates in-distinguishable from ground controls.

Growing and reproducing protozoans have been variously studied. Planel et al. (21) have reported an increase in cellular growth rate for Paramecium aurelia exposed to high-altitude balloon flight for 6 hours. Additionally, amoebae were observed following the 45 hour flight of Bio-satellite II. There were no significant differences between flight cells

and ground controls, but Ekberg et al. (22) reported a "trend" towards a higher division rate during flight. It is well known that amoebae require gravity (or some force vector) to attach to substrates. Although this attachment is generally considered to be required for locomotion and feeding, these organisms survived the flight and fed, assimilated food, grew, and performed all other measured functions in a manner indistinguishable from the ground controls (23). These results generally confirm data obtained from earlier simulation studies aboard C-131 aircraft in Keplerian trajectory (24).

Another test system, which was unusually refined for automated satellite studies, was designed to study the developing frog egg under reduced gravity conditions. This series, flown aboard the Gemini 8, Gemini 12, and Biosatellite II spacecraft, provided for inflight growth and differentiation of fertile eggs from the 2 cell stage. Developing frog eggs on Earth exhibit a marked sensitivity to disorientation with respect to the normal gravity vector, with the early embryo (up to the eight-cell stage) being the most sensitive (25). In spite of this known sensitivity no differences could be determined between flight and ground controls. The authors point out that, to complete this line of research, frog eggs should be fertilized after launch and maintained for a longer time in the reduced gravity state (25, 26).

In a similar manner, young Killifish eggs (Fundulus heteroclitus), were allowed to develop and hatch during the 56-day Skylab, the 20-day Cosmos 782, and the 10-day Apollo Soyuz Test Project (ASTP) flights. In all cases space-hatched fry exhibited no observable tendency toward disoriented swimming activity (27, 28) although dependence on visual orientation cues aboard the Skylab and following return to Earth suggested the possible absence of vestibular input (28).

One of the most elegant and complex growth studies to date was conducted with Wistar-38 human embryonic lung cells in tissue culture aboard the middle Skylab flight (Skylab 3). Continuous cultures were maintained at 36° C and photographed with time-lapse motion picture cameras, through phase-contrast microscopes at 20X and 40X magnification, for 28 days (29). Many parameters were evaluated, including growth curves, mitotic indices, cell migration rates, vacuole formation, cell size, nuclear size and location, nucleolar size and number, and G- and C-band patterns in chromosomes. Although the experiment operated according to plan, no differences were detected between flight cells and suitable ground controls (30).

More recently, growing colonies of Streptomyces lavoris were flown aboard the Soyuz 16 and the Apollo Soyuz Test Project flights (31). The formation of alternating rings of spore-bearing and sterile mycelium allowed continuous analysis of changes in cyclic growth and provided a method for keeping track of certain inflight mutations. A correlation between the cyclic spore formation and spaceflight was not demonstrated. Although analytical data are not yet available it should also be noted that Soviet investigators have reported active observation of cultures of

coliform bacili, fertilized frog eggs and Serian Hamster cell tissue
cultures in the "flying oasis" of Soyuz 17 - Salyut 4 (32).

Genetic Studies

Bacteriophage induction has been extensively employed, by Soviet in-
vestigators, as a model system for visualizing the effects of spaceflight
on the genetic apparatus of microorganisms (Table VIII). Escherichia
coli K-12 (λ) bacteriophage have been carried aboard most of the flights
of the Sputnik series, all six of the manned Vostok flights, Voskhod 1
and 2, the unmanned biosatellite Cosmos 110, and Zond 5 and 7, both of
which circled the Moon (19, 33, 34). This system was used as a radiation
dosimeter because increases in phage production could be stimulated by as
little as 0.3 rad of gamma radiation or by small doses of protons or rapid
neutrons (33, 35). Because phage induction involves injury to the genetic
apparatus, the lysogenic bacteria system was used to provide information
about the potential mutagenic activity of cosmic radiation. It was re-
ported that the spaceflight effect (measured in terms of increased phage
production in space as compared to the magnitude of spontaneous phage
production in the ground controls) increased with mission duration through-
out the Vostok series (7, 35). This relationship is summarized in figure
1. Laboratory studies demonstrated that simulated launch vibration
followed by exposure to ^{60}Co gamma radiation resulted in an increased
mutation rate which was higher than that obtained by gamma radiation or
simulated launch vibration alone (33, 35). This was interpreted as indi-
cating that the Vostok launch vibrations "sensitized" the cells so that
they were not susceptible to inflight irradiation.

Two different bacteriophage systems were tested as part of the 45-hour
Earth-orbital flight of the American Biosatellite II (36, 37). Salmonella
typhimurium BS-5 (P-22)/P-22, and E. coli C-60 (λ)/λ were tested for
alterations in bacterial cell growth and bacterial prophage induction
following spaceflight (Table VIII). During the flight, different aliquots
of cells were exposed to a total dose of from 265 to 1648 rad of ^{85}Sr
gamma radiation with the resulting radiation response curves being compared
with appropriate ground control curves. Neither ultrastructural nor
viability differences were noted between flight and ground-control E. coli
systems. However, with the S. typhimurium system the authors reported an
increased cell density in the space-flown culture fluid indicating in-
creased growth activity. This same result was later duplicated in clino-
stat studies which supplied a continually shifting gravitation vector,
did not allow settling of cells, and kept the growth medium continually
agitated. Even though the resultant increase in growth could be simulated
in the clinostat the authors speculated that the mechanism was probably
different (36, 37).

Testable numbers of phage were not produced with the E. coli system because the flight was shorter than had been planned. In the S. typhimurium system there was no differences in the free P-22 density of the flight and ground cultures, although the space-flown cells were more resistant to gamma radiation, as indicated by a decrease in phage production. Efforts to reproduce these results with acceleration, vibration, and clinostat tests were unsuccessful. This decrease in phage induction supports the results reported for the E. coli system flown on Cosmos 110 but is counter to the results reported for all of the other Russian coliphage studies (34).

Additional spaceflight irradiation studies have been conducted which did not involve phage induction systems (Table IX). A variety of microorganisms, carried aboard the Cosmos 368 earth-orbital satellite, were irradiated with ^{60}Co gamma irradiation before flight and/or after return to earth. There was no evidence that the spaceflight had sensitized these species in a way that altered their viability or mutability (15).

During the flight of Gemini XI, conidia of Neurospora crassa were exposed to a ^{32}P beta source, and cells of the same species were exposed to a ^{85}Sr gamma source during the 45-hour Biosatellite II flight (38, 39). For both experiments the assayed system was a genetically marked two-component heterokaryon which was heterozygous for two different genes that control sequential steps in purine biosynthesis. The exposure of ground control and inflight cells to a range of radiation in both tests allowed for comparative analyses of dose-response curves.

Analyses of the Gemini XI samples indicated that neither the survival rate nor the mutation frequency of conidia deposited on membrane filters was altered by 71 hours of orbital flight. However, the flight cells suspended in agar demonstrated higher levels of survival and lower frequencies of induction, indicating that the spaceflight affected a protective influence (39). The authors point out that these data must be considered equivocal since they could have been the result of anoxia caused by high temperatures in the spacecraft. However, when the experiment was repeated 12 months later in the Biosatellite II unmanned orbitor agar suspensions were not used and this portion of the test was never repeated. As in the Gemini XI test, there were no differences between the flight and ground control radiation survival curves or overall induction.

In addition to the studies with ionizing radiation, possible synergistic relationships between spaceflight and solar ultraviolet light have also been tested. The data presented in Table X illustrate that the T1 coliphage, P. roqueforti, and tobacco mosiac virus (TMV) particles have been flown on various space vehicles. From these studies, Lorenz et al. (40) concluded that solar ultraviolet irradiation with wavelengths between 200 and 300 nm was the main cause of inflight inactivation of these microorganisms. These data do not differ from the results of the many laboratory UV-response experiments, suggesting that ground-based studies may be used as model systems for preparation of inflight experiments.

In another study, prepared by a group of American and European investigators, eight microbial species were exposed to solar UV and space vacuum outside of the Apollo 16 command module during its return from the Moon (41, 42). The use of various combinations of optical filters to provide exposure of different test aliquots to varying amounts of solar irradiation at peak wavelengths of 254, 280, and 300 nm, allowed for a different dose-response curve at each of these three wavelengths (43). The T-7 bacteriophage preparations of E. coli which were exposed to inflight irradiation were found to be more sensitive to UV light than were irradiated ground controls (44). There were no significant differences reported between postflight survival rates of non-irradiated fungal cells when compared with appropriate ground controls (45) although the survival rate of space-flown Chaetomium globosum, Rhodotorula rubra, and Saccharomyces cerevisiae was slightly depressed and samples of Trichophyton terrestre, and S. cerevisiae demonstrated some sensitivity to inflight solar UV when measured in terms of a loss of cell viability (corresponding ground control data were not reported). No changes in survival rate, mutation rate, or toxin production could be detected with postflight analyses of Bacillus thuringiensis and Aeromonas proteolytica (46). However, it was reported that the combination of solar UV and space vacuum resulted in a greater loss of viability in dried Bacillus subtilis cultures than with UV alone, indicating that the spores were sensitized to UV by the vacuum (17).

Cell Studies With Multicharged, High Energy (HZE), Cosmic Particles

Experiments designed to study the biological effects of individual heavy nuclei of cosmic radiation during space flight outside the magnetosphere of the Earth have been repeatedly conducted by a consortium of European investigators (47, 48). These experiments were housed in the BIOSTACK, a complex package consisting of alternating layers of nuclear track detectors, and biological objects imbedded in polyvinyl alcohol (PVA). Among other species, spores of Bacillus subtilis and cysts of Artemia salina were exposed to HZE particles during the flights of Apollo 16, 17, and the Apollo-Soyuz Test Project (Table XI). Individual cells or cysts in the path of HZE particles were evaluated for germination, outgrowth, and production of abnormals. The first vegetative cells issuing from bacterial spores lying in the path of high energy, multicharged particles were frequently found to be abnormally swollen. Artemia salina cysts, lying along nuclear tracks, showed reduced hatching and larval emergence and an increase in the incidence of developmental anomalies.

In a further attempt to understand the effect of galactic HZE particles upon biological objects, Soviet investigators included the yeast Saccharomyces cerevisiae in the "Bioblock" which was aboard the 2 month Cosmos 613 earth orbital flight. Although many of the colonies did not survive the long storage, a ten-fold increase in the incidence of "radiation damaged cells" was reported (49).

CONCLUSIONS

The above review has illustrated that, whereas a large variety of cell biology studies have been conducted in space, consistent space-mediated alterations have not been identified. Although individual studies often produced equivocal data, evaluation of the aggregate results indicates that cell systems are generally no less stable in space than they are in the Earth-based laboratory. Of course the conditions to which cell systems are exposed in space are usually less well controled (and less controlable), often leading to more variable and erratic results.

It has not yet been demonstrated that the spaceflight environment could be used to affect unique or hitherto unknown cell changes. On the contrary, cell systems appear to remain sufficiently stable to permit experimentation with models which require a fixed cell line. Therefore, taken as a unit, the cell biology studies conducted during the preceding two decades should definitely be considered a success. It is now possible to prepare cell biology experiments for the Space Shuttle era with a reasonable probability that the cells will not react engimatically to the unique environment encountered within the spacecraft.

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TABLE I. – SPACE-FLOWN ISOLATED VIRUSES
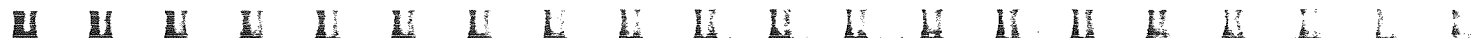
Microorganism	Flight	Condition	Reference	Number
Tobacco mosaic virus	U.S.S.R.	Unknown	Parfenov 1973	7
	Gemini IXA			
	Gemini X/ Agena VIII	Dry	Lorenz 1968	10
			Hotchin 1969	9
	Gemini XII			
Poliomyelitis virus	U.S. Balloon	34 to 155 km, altitude	Parfenov 1973	7
Vaccinia virus	Gemini XII	Dry	Hotchin 1969	9
Influenza virus	U.S.S.R.	Unknown	Jenkins 1968	6
Influenza (PR-8 strain) Canine hepatitis Infectious bovine Rhinotracheitis	Gemini XII	Dry	Hotchin 1969	9

TABLE II. – SPACE-FLOWN BACTERIAPHAGE AND HOST

Microorganism	Flight	Condition	Reference	Number
Escherichia coli K-12/K-12	Sputnik 4 and 5	Unknown	Antipov 1967	50
	Vostok 1, 2, 3, 4, 5, 6	Nutrient suspension $^{60}Co-\delta$		
	Cosmos 110	Nutrient suspension $^{60}Co-\delta$	Jenkins 1968	6
Escherichia coli T_1	U.S. Balloon Aerobee Gemini IXA Gemini X/ Agena VIII Gemini XII	Dry	Hotchin 1969	9
	Sputnik 5 and 6 Voskhod 1 and 2	Dry	Jenkins 1968	6
Escherichia coli T_4	U.S. Balloon	34 to 155 km, altitude	Parfenov 1973	7
Escherichia coli B/T_2	Vostok 2	Unknown	Zukov-Verezhnikov 1966	35
Escherichia coli $T_{4b}r^+$	ASTP	Dry	Rogers 1976	31
Escherichia coli T_7	Apollo 16	UV Exposure	Spizizen 1975	44
Escherichia coli C-600	Biosatellite II (p-1135)	Growing in liquid $^{85}Sr.-\delta$	Mattoni 1968	36
Salmonella typhimurium BS-5(P-22)/P-22	Biosatellite II (p-1135)	Growing in in liquid $^{85}Sr.-\delta$	DeSerres 1969	39
Aerobacter aerogenes 1321	Vostok 2	Unknown	Parfenov 1973	7

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TABLE III. — SPACE-FLOWN BACTERIA

Microorganism	Flight	Condition	Reference	Number
Escherichia coli	Soyuz 17/ Salyut 4	Growing in nutrient	Apenchenko 1975	32
Escherichia coli K-12 ( ) Escherichia coli B Aerobacter aerogenes 1321 Staphylococcus aureus 0.15	Vostok 1	Agar cultures	Parfenov 1973 Zhukov- Verezhnikov 1966	7 35
Clostridium butyricum	Vostok 1	Spore suspension		
Clostridium sporogenes	Discoverer XVII Discoverer XVIII	Unknown	Parefenov 1973	7
Bacillus brevis	Voskhod 1	Spores		
Bacillus subtilis ATCC 6052	Gemini IXA Gemini X/ Agena VIII Gemini XII	Dry	Lorenz 1968 Hotchin 1969	10 9
Bacillus subtilis 168	Apollo 16 Apollo 17 ASTP	Dry Dry Dry	Bücker 1974 Bücker 1976	47 48
Bacillus subtilis HA101 and HA101 (59) F	Apollo 16	UV Exposure	Spizizen 1975	44
Bacillus thuringiensis	Apollo 16	UV Exposure	Simmonds 1974	46
Aeromonas proteolytica	Apollo 16	UV Exposure	Foster 1973	51
Streptomyces erythraeus 2577 Streptomyces erythraeus 8594	Vostok 2	Aqueous spore suspension and liquid mycellium suspension	Glembotskiy 1962	52
Streptomyces streptomycini Kras LS-3	Vostok 2	Unknown	Khvostova 1962	53
Streptomyces aureofaciens LSB 2201	Vostok 4 Vostok 5	Aqueous spore	Glembotskiy 1962	52
Streptomyces levoris	ASTP	Growing colonies	Rogers 1976	31
	Soyuz 16	Growing colonies	Izuvestiya 5 Dec. 1974 p. 5	
Hydrogenomonas eutropha Z-1	Cosmos 368 Zond 8	Cells in aqueous suspension	Grigoryev 1972 Romanova 1971	15 13

TABLE IV. – SPACE-FLOWN YEASTS AND FILAMENTOUS FUNGI

Microorganism	Flight	Condition	Reference	Number
Zygosaccharomyces	Cosmos 368	Cells on Agar	Grigoryev 1972	15
Saccharomyces (Zygosaccharomyces) 40-2587 (haploid)	Vostok 2	Suspensions both unsensitized and sensitized with olic acid	Kovyazin 1962	54
Saccharomyces (diploid) 139-B	Cosmos 368	On agar and in aqueous suspension	Grigoryev 1972	15
	Cosmos 613	Colonies on agar (0.5 – 1.0 mm d.)	Benevolensky 1976	49
	Voskhod 1	Suspensions both unsensitized and sensitized with olic acid	Kovyazin 1962	54
Saccharomyces cerevisiae	Apollo 16	UV Exposure	Volz 1973	45
Penicillium roqueforti	U.S. Balloon	Dry spores (34 km altitude for 6 hrs.)	Parfenov 1973 / Hotchin 1969	7 / 9
	Gemini XII	Dry spores	Hotchin 1969	9
	Gemini IXA / Gemini X/ Agena VIII	Dry spores	Hotchin 1969	9
Neurospora crassa	Biosatellite II (p.-1037)	Dry spores ^{85}Sr-	DeSerres 1971	38
Neurospora species	U.S. Balloon	Unknown	Hotchin 1969	9
	Gemini XI	Dry spores phosphorus-32 $(32_p) - \delta$ – and metabolizing spore suspension $32_p - \delta$	DeSerres 1969	39
	Nerv I	1900 Km altitude for 28 min	Jenkins 1968	6
	Discoverer XVIII	Dry Spores		
Chaetomium globosum / Trichophyton terrestre / Rhodotorula rubra	Apollo 16	UV Exposure	Volz 1973	45
Candida tropicalis SK-4	Zond 8	On Agar	Romanova 1971	13

TABLE V. - SPACE-FLOWN PROTOZOANS

Species	Flight	Condition	Reference	Number
Colpoda cucullus	Apollo 17 (Biostack II)	Cysts in mono-layers of polyvinyl alcohol	Bücker 1974	47
Pelomyxa carolinensis (giant multinucleate Amoeba)	Biosatellite II	Dividing, Free-feeding cells	Abel 1971 Ekberg 1971	23 22
Amoeba	C-131 Aircraft in Keplerian trajectory	Growing cells	McKinney 1963	24
Paramecium aurelia	USSR Balloon	Growing cultures	Planel 1975	21

TABLE VI. – SPACE-FLOWN CELLS IN SMALL GROUPS

Species	Flight	Condition	Reference	Number
Rana pipiens (Leopard frog)	Biosatellite II	Developing eggs from 2-cell stage	Young 1971	25
Frog Eggs	Gemini 8 Gemini 12	Developing eggs from first cleavage	Young 1968	26
Frog Eggs	Soyuz 10 Soyuz 17/ Salyut 4	Fertile Frog Eggs	Apenchenko 1975	32
Artemia salina (Brine shrimp)	Biosatellite II	Dry Blastocysts	von Borstel 1971	55
	Apollo 16 (Biostack I) Apollo 17 Biostack II) ASTP (Biostack III)	Encysted blastula in monolayers of polyvinyl alcohol	Bücker 1974 Planel 1974 Bücker 1976	47 56 48
Carausius morosus (grasshopper)	Apollo 17 (Biostack II)	Eggs in monolayers of polyvinyl alcohol	Bücker 1974	47
Fundulus heteroclitus (killifish)	ASTP	32-336 hr embryos in sea water	Scheld 1976	28
	Skylab 3	5-day old fertile eggs in sea water		
	Cosmos 782	32-128 hr embryos in sea water		
Danio rerio (fish)	Soyuz 16	Fertilized eggs	Izvestiya 8 Dec. 1974 p. 3	
WI-38 diploid human embryonic lung cells	Skylab 3	Growing cultures from single cells	Montgomery 1974	30
Serian Hamster cells	Soyuz 17/ Salyut 4	Tissue culture	Apenchenko 1975	32
Carrot Tissue culture	Cosmos 782	Crown gall and proembryonic cells	Scheld 1976	28

TABLE VII. - MAJOR SPACEFLIGHT STUDIES WITH GROWING CELLS

FLIGHT	DEVICE	TEST SYSTEM	RESULTS
Sputnik 5 Vostok 1 & 2	"Bioelements"	Clostridium butyricum	Gas production rate same in flight as for ground controls.
Biosatellite II	Experiment P-1035	Pelomyxa carolinensis (Amoeba)	"Trend" towards higher division rate during flight. No change in survival, food assimilation, growth, etc.
Gemini 8 & 12 Biosatellite II	Experiment P-1047	Rana pipiens Frog eggs in 2-cell stage	No difference between flight and ground control specimens. Authors recommend repeat with inflight fertilization.
Skylab 3 ASTP COSMOS 782	Experiment MA 161	Fundulus heteroclitus (Killifish)	Dependence of hatched fry on visual cues suggestive of absence of vestibular input. No other differences resulting from flight.
SKYLAB 3	Experiment SQ 15	Wistar-38 human embryonic lung tissue culture	No differences in growth curves, mitotic indices, cell migration rates, cell size, nuclear size and location, nucleolus size, etc.
Soyuz 16 ASTP	"Biorhythm I"	Streptomyces levoris	No differneces in cyclic spore formation inflight. No biological indications of HZE damage.

TABLE VIII. - BACTERIOPHAGE INDUCTION SYSTEMS TESTED IN SPACE

SYSTEM	FLIGHT	RESULTS
Escherichia coli K-12 λ	Most Sputniks All 6 Vostoks Voskhod 1 & 2 COSMOS 110 ZOND 5 and 7	Number of phages inflight exceeded ground controls. Excess proportional to length of mission. Simulated launch vibration plus ^{60}Co γ irradiation gave increases higher than irradiation alone. No increases from launch vibration alone or after ^{60}Co γ irradiation.
Salmonella typhimurium BS-5 (P-22/ P-22)	Biosatellite II	Increased cell density following 45 hr flight. Space-flown cells more resistant to ^{85}Sr γ irradiation (inflight 265-1648 rads) as indicated by decreased phage production.
Escherichia coli C-60 (λ)/λ	Biosatellite II	No postflight differences in growth when exposed to ^{85}Sr γ inflight. Flight terminated early, no opportunity for phage production.

TABLE IX. - ADDITIONAL SPACEFLIGHT STUDIES WITH RADIATION SOURCES

SOURCE	SYSTEM	FLIGHT	RESULTS
^{60}Co gamma (preflight and postflight)	Hydrogenomonas eutropha Z-1 Saccharomyces ellipsoides (diploid) Zygosaccharomyces baili (haploid)	COSMOS 368	No measurable loss of viability or change in radiosensitivity
32p beta (inflight)	Neurospora crassa conidia	Gemini XI	Neither survival rate or mutation frequency altered for dry cells. Better survival and lower mutation frequency for agar-suspended cells
^{85}Sr gamma (inflight)	Neurospora crassa conidia	Biosatellite II	No inflight effect on dry cells

TABLE X. - INFLIGHT CELL STUDIES WITH ULTRAVIOLET IRRADIATION

FLIGHT	EVENT	TEST SYSTEM	RESULTS
6 sounding rockets 6 balloon flights 3 orbital satellites	Exposed To Direct UV Irradiation	T 1 Coliphage Penicillium roqueforti Tobacco Mosiac Virus	Confirms that UV between 200 and 300 nm is major cause of inflight inactivation.
Apollo 16	Exposed To Direct UV plus Components at 254, 280, and 300 nm	Escherichia coli T-7 bacteriophage	Flight specimens more sensitive to UV than ground controls although shape of dose response curves similar.
		Rhodotorula rubra Saccharomyces cerevisiae Chaetomium globosum Trichophyton terrestre	No evidence of synergism between inflight UV irradiation and reduced gravity
		Bacillus subtilis	No change in survival rate at 1 atm. Combined UV and vacuum resulted in greater loss of viability than UV alone. (Spores sensitized to UV by vacuum)
		Bacillus thuringiensis Aeromonas proteolytica	No change in survival rates. No change in ability to produce toxins

TABLE XI. - CELL STUDIES WITH COSMIC HZE* PARTICLES

EXPERIMENT	FLIGHT	SPECIES	RESULTS
BIOSTACK (Bücker)	Apollo 16 and 17 ASTP	Bacillus subtilis spores Artemia salina cysts	Swelling during growth of first vegetative cells from "hit" spores. Those "hit" by HZE showed reduction in larval emergence and hatching. Incidence of developmental anomalies increased.
BIOBLOCK (Benevolensky)	COSMOS 613	Saccharomyces cerevisiae 139-B	Of 1045 colonies, 169 hits with $Z \gtrsim 8$ and 12 hits with $Z \gtrsim 5$ over 2 months. 1.3% of cells demonstrated "radiation damage" compared with 0.15% normally. 2×10^4 cells damaged per particle.

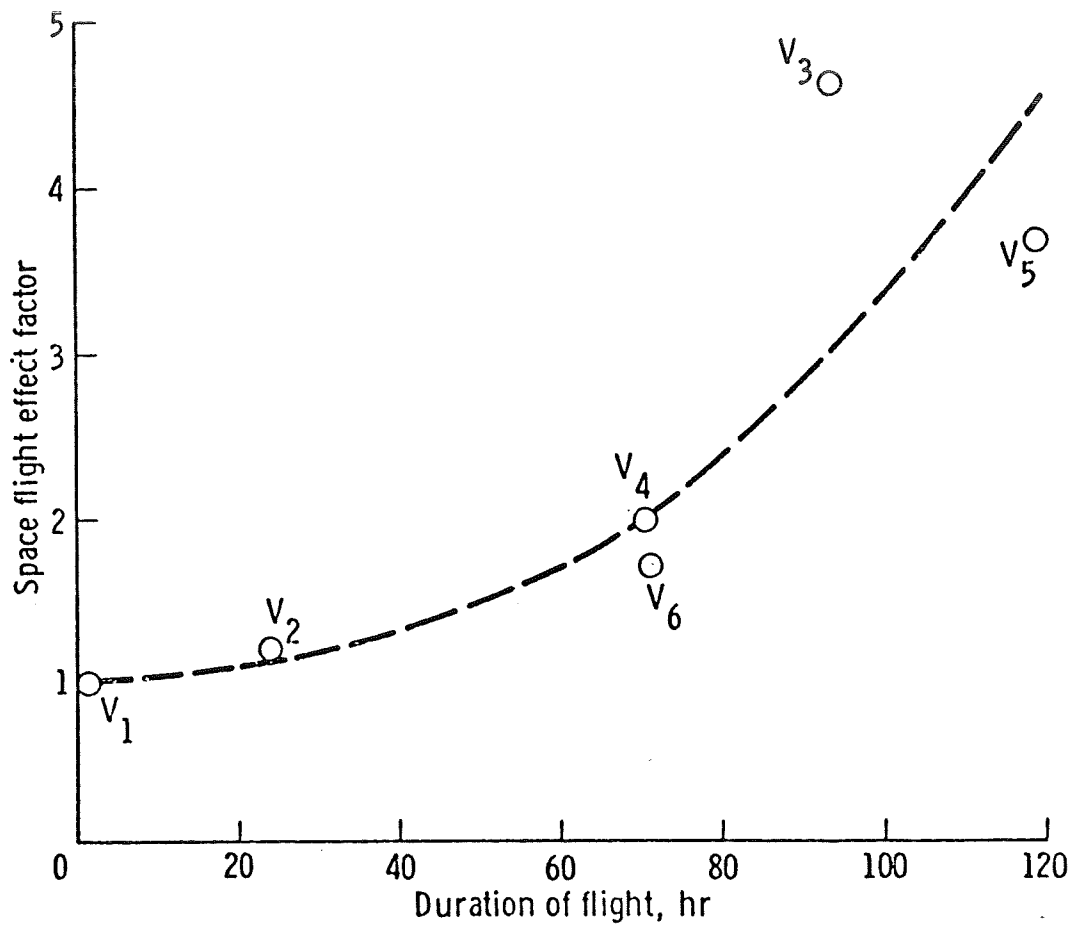* HZE = Heavy (high atomic number) high-energy particles

Figure 1.- Effect of duration of Vostok space missions on K-12 (λ) bacteriophage induction in <u>Escherichia</u> <u>coli</u> from data compiled in reference 6. V_1 to V_6 denote Vostok flight number. Space-flight-effect factor = number of bacteriophage particles per ground control cell.

GRAVITY AND THE CELL: INTRACELLULAR STRUCTURES AND STOKES SEDIMENTATION

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ABSTRACT

Plant and certain animal embryos appear to be responsive to the gravity vector during early stages of development. The sensing of gravity of individual cells could be based upon convection of particle sedimentation. Various intracellular particles have been proposed as gravity sensors in the cells of developing plants, and the participation of amyloplasts and dictyosomes has been suggested but not proven. An exploration of the mammalian cell for sedimenting particles reveals that their existence is unlikely, especially in the presence of a network of microtubules and microfilaments considered to be responsible for intracellular organization. Destruction of these structures renders the cell susceptible to accelerations several times g. Large dense particles, such as chromosomes, nucleoli, and cytoplasmic organelles are acted upon by forces much larger than that due to gravity, and their positions in the cell appear to be insensitive to gravity.

INTRODUCTION

Space Biology Research was originally designed to answer the question, Is Space Safe?, and the next phase of research is designed around the use of the conditions of space flight as a biological research tool. The latter phase is designed to answer such questions as, Can We Learn Something of Fundamental Significance by Performing Experiments Under Space Flight Conditions and Obtain Biological Insights that Cannot be Acquired on the Ground? At the inception of space research some 20 years ago, there was concern in both the U.S. and the Soviet Union about the effects of weightlessness on living things. It needed to be known in particular whether the absence of gravity had no effect or a catastrophic effect on biological systems under space flight conditions. It was easy to solve problems introduced by the space environment by the use of engineering to protect against the lack of an atmosphere and the presence of radiation, but engineering against weightlessness and its possible biological effects proved to be extremely difficult. Fortunately, early experiments indicated that the biological effects of zero G was certainly not catastrophic and the 84-day Skylab mission suffered no catastrophes as a consequence of the absence of a gravitational field.

In view of the conclusion that the absence of gravity has no catastrophic effect on man in space, future research is directed at the basic study of what we presume to be gravity dependent environmental responses. In other words, space flight conditions are to be made available for basic science experiments. Due to volume limitations and other limitations on spacecraft, it is logical to begin with research at the cellular level.

Although we know of many biological phenomena affected by gravity, their connection to molecular and physical concepts are extremely poorly understood. In this sense, the effect of gravity is paradoxical because the cell is the basic structure of living things, and the organisms' properties depend upon cells. Yet it is much easier to think of gravity as acting on larger systems as cells are at the limit of size and mass which is influenced by the gravitational field.

DEVELOPING SYSTEMS

The effect of abnormal gravitational exposure upon embryonic development was noted during the previous century (1). The most remarkable gravity-dependent phenomena in
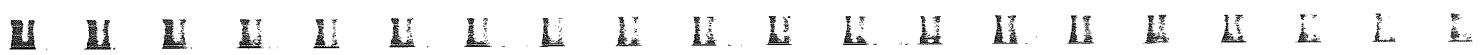
developmental biology include the obvious polarization of amphibian egg cell division at early stages and the reliable upward growth of coleoptiles and downward growth of roots in germinating plant seedlings. It should be no surprise that these phenomena have been the favorite subjects of investigations of the effects of gravity compensation and weightlessness (2,3).

Amphibian Embryos

The inversion of embryos of Rana sp. before they reach the 4-cell stage can lead to the formation of double embryos (1,4). Gravity compensation and centrifugation can lead, under appropriate conditions, to similar effects (4,5). Evidently, very soon after fertilization, events occur which orient the egg and establish the planes of further cell divisions and the ultimate symmetry of the organism. The gravity sensing mechanism in this system is thought to be associated with a density gradient in the materials of the yolk.

Attempts to induce developmental abnormalities in weightlessness during orbital flight of Rana eggs yielded negative results (6,7,8), presumably because exposure to weightlessness did not adequately coincide with the gravity-sensitive period of orientation or possibly because Rana pipiens, used in orbital experiments, is not as sensitive to orientation as Rana fusca, which was used in classical experiments (1). There was also no microscopic evidence for the redistribution of morphological structures during orbital weightlessness (8).

Plant Geotropism

Cytological studies on the distribution of amyloplasts in wheat seedlings flown on Biosatellite II led to the conclusion that these granules were distributed at random under weightlessness, as in seedlings grown on a clinostat, rather than being clumped on the lower cell wall as in erect control seedlings (9,10). Fixation experiments indicated that these plastids return to their normal position in the cell in less than 4 hr. These organelles were observed because they are thought by some (11,12), but not others (13) to play a role as "statoliths"--intracellular indicators of the gravity vector. The identification of "statoliths", however, depends on the ability of the plant physiologist to distinguish between cause and effect. It remains to be determined whether the elongation plant cell responds to sedimenting amyloplasts or positions its amyloplasts in response to a metabolic gradient formed by activities other than sedimentation.

Other plant cell organelles have been considered with respect to possible roles in geotropism. These include mitochondria (14) and the Golgi apparatus (15-18). The dictyosomes of the Golgi apparatus, despite their generally accepted relationship to internal membranes, appear to be positioned in a manner strongly related to the gravity vector (16,17). Whether they are serving as statolith or responding to metabolic gradients is unknown, but one might consider the following metabolic interrelationships as a testable alternative to the statolith theory: 1) Cell wall compression produces a membrane response. 2) This response consumes auxin. 3) Auxin is transported down its gradient. 4) Cell wall synthesis is stimulated. 5) New wall synthesis depletes Golgi products. 6) The cell produces more active dictyosomes. 7) Golgi forms in direction of the secretion (as in animal systems).

ORGANELLES IN MAMMALIAN CELLS

Animal cells differ explicitly from plant cells in their lack of a need to synthesize a cell wall in a particular direction. If plant cells need to respond to gravity for this purpose only, then one would not expect the intracellular activities of animal cells to be very responsive to gravity. An analysis of the constituents of the mammalian cell should indicate whether or not there exist any organelles that can sediment under the influence of gravity. Biophysical research in the past decade has added considerably

to our knowledge of the structural and hydrodynamic properties of chromosomes, plasma membranes, nuclear membranes, cytoplasm, nucleoplasm, chromatin, nucleolus and membranous organelles. Using recent measurements, an attempt is made here to estimate the effects of the gravitational field upon the position and motion of the cells' densest structures.

The Nucleolus

Earlier theoretical work indicated that the nucleolus might be a sufficiently large dense structure to be influenced by gravity (19). This would certainly be the case if the nucleolus could be considered as a solid object suspended in a viscous liquid medium. However, this is not the case. Our current understanding of the nucleolus (see Fig. 1) is that its role is the synthesis of ribosomal RNA and the assembly of ribosomes (20,21). Although it is truly a densely packed structure, it is not isolated from the surrounding nucleoplasm as a solitary hydrodynamic unit. Instead it has threads of chromatin running through it--presumably the chromatin which contains ribosomal DNA genes (21). The nucleolus is therefore suspended in the nucleus by a number of threads, and its motion is therefore constrained by the motion of the chromatin with which it is associated. Hence, as shown in the electron micrographs of Fig. 1, there is little or no evidence for the sedimentation of nucleoli to the bottoms of nuclei in cultured human cells. On the average, the nucleolus is just about as close to the top of the nuclear membrane as it is to the bottom. It is to be learned from this discussion that fibrous materials in the cell can greatly influence the response of its organelles to gravity.

The Cell Nucleus

Now let us consider the nucleus as a whole. Recent studies have indicated that the cell cytoplasm can be considered as a network of microfilaments and microtubules (23). The increasing rate at which contractile proteins are being discovered in so-called non-contractile cells is so alarming that we wonder why they were not previously found. Two classes of structure are of interest to our discussion. The main protein of microtubules is tubulin (24). The tubulin exists in sub-units of microtubules. The sub-units are assembled into tubules for such purposes as the guiding of chromosomes at mitosis, the strength and movement of cilia, and for axoplasmic flow in nerve axons. The assembly of these sub-units into tubules is inhibited by colchicine and similar vinca alkaloids. Microfilaments, on the other hand, appear to consist of a mixture of actin, myosin, and other contractile muscle proteins (25). Microfilaments have been considered essential for the normal migratory behavior of cultured fibroblasts (26). Cytochalasin B interferes with the normal action of microfilaments (27). Figure 2 indicates the presence of both actin and myosin in the microfilaments of cultured fibroblasts and shows that these microfilaments envelope the cell nucleus.

It appears that the nucleus is positioned in the cytoplasm under constraints imposed by microfilaments and/or microtubules. If cultured cells attached to coverslips are centrifuged at moderate speed, one finds that cells remain intact without significant displacement of their nuclei. If, on the other hand, one treats cultured cells attached to coverslips with cytochalasin B and then subjects the attached cells to a centrifugal field, it is found that the centrifugal acceleration is then adequate to enucleate the cells (28). If one were to assume that the nucleus is a hydrodynamic unit approximated as a sphere 12 microns in diameter with density 1.14 suspended in a fluid with viscosity 17 centipoise and density 1.03, then one would anticipate a sedimentation velocity of the cell nucleus equal to about 20 micrometers per hour. Clearly, all nuclei would sediment to the bottoms of their cells in a few minutes. That this is not the case is observable in mammalian tissue sections in which the nuclei are always central and in vertical sections of cultured cells (Fig. 1), where the nuclei are also rather centrally positioned. Evidently, microfilaments or other cellular structures deny the cell nucleus any motion induced by gravity.

The effect of gravity on nuclear shape is now considered. It has been noted that isolated cell nuclei are more susceptible to deforming forces than are nuclei within

cells. Evidently the deformibility of cell nuclei is also influenced by cytoplasmic materials. If the nucleus were to be pictured as a colloidal sol inside a deformable bag, one would expect nuclei to be broader at the bottom than at the top where up and down are defined by the gravitational vector. If cells from sectioned tissue ever indicated such an anisotropic feature it was never reported. Upon examining human cells in culture such as in Fig. 1, in which the gravity vector is clearly defined, one might seek a gravitational affect in the form of nuclei which are broader in their lower halves than in their upper halves and in which the top radius of curvature is much less than the lower radius of curvature of the nucleus. Indeed, one finds evidence for this occurring in a significant proportion of cells examined. It should be cautioned, however, that such anisotropic nuclear shape might have nothing to do with the gravitational field because the nucleus may assume this shape simply because the cell which surrounds it is broader at the bottom as a consequence of being attached and spread at its bottom surface and not at its top surface. There is, therefore, no concrete evidence that gravity influences nuclear position or nuclear shape.

Chromosomes

Finally, let us consider the possibility of a gravitational effect on chromosomes at mitosis. Ever since the discovery of chromosomes, scientists have been fascinated by their movements during cell division. Their kinematics and mechanics have been considered in detailed physical theories of the mitotic process. As we have done with the other organelles, let us consider the sedimentation velocity of a free chromosome suspended in the fluid matrix of the mitotic cell. The general equation of motion for a sedimenting particle is

$$m \frac{d^2 x}{dt^2} = F_{grav} - F_{buoy} - F_{drag}$$

or

$$0 = mg - V_{\rho o}g - fv \tag{1}$$

where the friction factor, f, for a long prolate ellipsoid is estimated as

$$f = 9\pi \sqrt[3]{3V/4\pi} . \tag{2}$$

Substituting for f and solving equation (1) for the terminal velocity we find that

$$v = \frac{Vg\,(\rho - \rho o)}{9\,\pi\,\sqrt[3]{3V/4\pi}} \tag{3}$$

in which all of the values in equation (3) are known. These values and their sources are tabulated in Table I.

We may also use the equation of motion (equation 1) as a force balance equation. By using the boundary conditions that velocity and acceleration equal 0, we may determine the difference between the gravitational and buoyant force and thereby estimate the force required to prevent the chromosome from sedimentating in the cytoplasm. The constants needed for this calculation are given in Table I and Figure 3. The calculations applied to a "typical" mammalian chromosome and indicate that if the chromosome were suspended in a free solution with cytoplasmic density and viscosity it would sediment with $v \simeq 2 \times 10^{-7}$ cm/sec. Assuming $v = 0$ in equation (1) leads to a balancing force of about 10^{-8} dyne, or less than that of the covalent bonds which exist within the cross section of a spindle fibre.

One may now question whether or not the mitotic spindle can exert the force required to prevent chromosome sedimentation in the cytoplasm. A set of experiments was done in the following way: cultured Chinese hamster M3-1 cells (34) and cultured human kidney T-1 cells (35) were allowed to attach and grow on the surface of plastic tissue culture bottles (Falcon #3012) for 24 hours in the horizontal position, after which half of the sample bottles were filled with medium and oriented vertically. After 18-20 more hours of incubation at 37°C, the cultures were rinsed with Hanks' balanced salts solution and fixed without changing their orientation. They were stained in the horizontal position with Harris' hematoxylin and mordanted tap water. The angle Θ subtended by the direction of the spindle and a vertical line (Fig. 4) was estimated within 30° intervals microscopically, and the number of dividing cells lying in each 30- degree interval was determined (Table II). The following results are to be expected: 1) If mitosis is oriented by the growth surface only, there will be an equal proportion of cells in each 30-degree interval in both vertical and horizontal cultures, and a preference for chromosome motion parallel to the growth surface. 2) If mitosis is oriented by gravity alone, there will be a preferred orientation around $\Theta=90°$ (interval 3) in the vertical culture. 3) If both gravity and the growth surface act to orient mitosis, there will be a preferred orientation around $\Theta=90°$ and a preference for chromosome motion parallel to the growth surface in the vertical cultures.

Cells were assigned to groups 1 through 6 according to the value of Θ. An isotropic culture should have roughly equal numbers of dividing cells in each group. The existence of anisotropy should be indicated by an excess of dividing cells in one or two of the angular intervals. In horizontal flasks isotropic distributions were generally found. Nevertheless, the proportion of mitoses oriented at each angle in horizontal cultures was used as a baseline against which to compare the proportion at the same angle in vertical cultures, and to determine the effect of growth on the vertical surface, the following vertical-to-horizontal ratio was defined:

$$\frac{V}{H} = \frac{\text{proportion of cells in } \Theta \text{ interval, vertical}}{\text{proportion of cells in } \Theta \text{ interval, horizontal}} , \tag{4}$$

then histograms were prepared of V/H vs. Θ interval.

An example of such a histogram is given in Figure 5, which suggests that the proportion of mitoses oriented at each angle did not differ significantly between horizontal and vertical cultures in this experiment.

Chinese hamster M3-1 cells grow into colonies with a large axial ratio. If the plane of division occurs with greater frequency at a particular position for cells grown on a vertical surface, then the long axis of the resultant colonies should be preferentially oriented. The angle subtended by the long axis of the colonies and the long axis of the bottle was estimated for vertically- and horizontally- grown cultures and the corresponding V/H ratio determined for each angle in Figure 6.

In order to avert the ambiguities associated with counting small numbers of dividing cells (about 300 cells per dish were measured), experiments were designed so that the direction of division could be determined for a large number of cells plated at relatively low density. Human kidney T1 cells were plated and the vertical bottles were oriented as soon as the cells were firmly attached; thus, the first division occurred after the bottles had been oriented. The oriented bottles were then incubated for exactly one generation time (about 24 hours) and stained. The plane of division was determined for 1,000 cells in two experiments. The V/H ratio presumably has the same meaning as in experiments in which only dividing cells were measured, as the angles were determined only for colonies containing two cells. The distribution of the V/H ratio is given in Figure 7.

If there is any effect of vertical incubation upon orientation of cell division, it is probably small and difficult to reproduce.

Cultured Human Cells in Weightlessness

The above conclusions concerning a lack of obvious effect of the gravity vector on the orientation of mammalian cell division is borne out in the studies of Montgomery (36), in which cultured human WI-38 fibroblasts were grown during the 59-day mission of Skylab. The population doubling time in flight, 22.3 ± 3.1 hr did not differ significantly from that at 1g, 20.4 ± 4.8. The speed of cell migration on the culture vessel surface was the same, and no ultrastructural or karyotypic differences could be observed. Cells that had rounded for mitosis did not even require the gravitational force to reattach to the surface upon which they were growing.

Experiments in the laboratory and in space indicate that the cell division process in cultured mammalian cells is rather sensitive to the influence of gravity.

DISCUSSION

Some of these concepts lead to interesting questions concerning the role of gravity in organic or chemical evolution. For example, one might ask would the ideal shape of an organism in the absence of gravity always be a sphere? In other words, would an organism evolving in space be spherical rather than shapely as organisms evolved on earth in the presence of gravity? At the subcellular or organelle level even more serious questions persist: Do particles that sediment in plant cytoplasm really behave as geotropic sensors? If they do, how do they inform the cell what to do? Does gravitational stress lead to an intracellular contractile response? Many of these considerations overlook the existence of internal cellular membranes which, in eukaryotic cells, exist in great abundance.

Perhaps the sedimentation of particles in cells has been considered too simplicistically and one needs to include considerations of such phenomena as the Dorn effect in which an electric field results when a particle sediments. Such fields can be as great as 20 millivolts.

Also, droplet sedimentation should probably be given more serious consideration as it is a phenomenon related to larger hydrodynamic units whose density depends on particle concentration.

Other questions of biological interest include, Why are plant tumors not geotropic? Do plant tumor cells disregard gravity? Is something missing in their differentiated structure? Also, simple plants such as the mold, Phycomyces, respond to gravity without possessing any apparent sedimenting cytoplasmic particles.

Research on earth and in space has not yet led to concrete evidence that sedimenting intracellular particles play a role in determining the relationship between cellular activities and the gravity vector.

ACKNOWLEDGMENTS

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TABLE I. HYDRODYNAMIC VALUES FOR A METAPHASE CHROMOSOME (SEE FIGURE 3) USED FOR APPLI-CATION TO EQUATION (3). CHROMOSOMES HAVE BEEN EXAMINED HYDRODYNAMICALLY IN ISOLATION (31,32), AND CYTOPLASMIC VISCOSITY HAS BEEN STUDIED BY PARAMAGNETIC RESONANCE (33).

$$V = 2\pi r^2 \ell = 25 \times 10^{-12} \text{ cm}^3$$

$$g = 980 \text{ cm/sec}^2$$

$$\rho - \rho_o = 1.35 - 1.04 = 0.31 \text{ g/cm}^3$$

$$\sqrt[3]{3V/4\pi} = 2.1 \times 10^{-4} \text{ cm}$$

$$\eta = 5 \pm 2 \text{ dyn-sec/cm}^2$$

$$v \simeq 2 \times 10^{-7} \text{ cm/sec}$$

TABLE II. ANGULAR INTERVALS (SEE FIGURE 4) USED TO CLASSIFY ORIENTATION OF MITOTIC
          CELLS AND COLONIES ON HORIZONTAL AND VERTICAL CULTURE FLASKS.

CLASS	RANGE OF Θ (DEGREES)
1	135 - 165
2	105 - 135
3	75 - 105
4	45 - 75
5	15 - 45
6	-15 - 15

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Figure 1.- Electron micrographs of vertical sections of cultured human liver cells grown on horizontal Millipore filters. The location of nucleoli is variable, and the nuclei tend to be broader at the base. (Micrographs courtesy of Helge Dalen (ref. 29).)

Figure 2.- Fluorescence micrographs of cultured human embryonic lung cells fixed in acetone, extracted with glycerol, and "stained" with fluorescent antibody against heavy meromyosin to show presence of myosin (left) and "stained" with heavy meromyosin in addition to the same fluorescent antibody to show presence of actin in filaments (right). (Micrographs courtesy of Alex L. Miller (ref. 30).)

Figure 3.- Assumed properties of a metaphase chromosome suspended in cytoplasm. (See table I.)
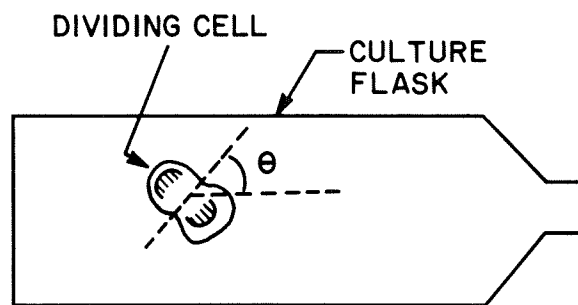
Figure 4.- Illustration of analysis of orientation of mitosis in horizontal and vertical cell culture flasks. The diatram defines the mitosis orientation angle θ.
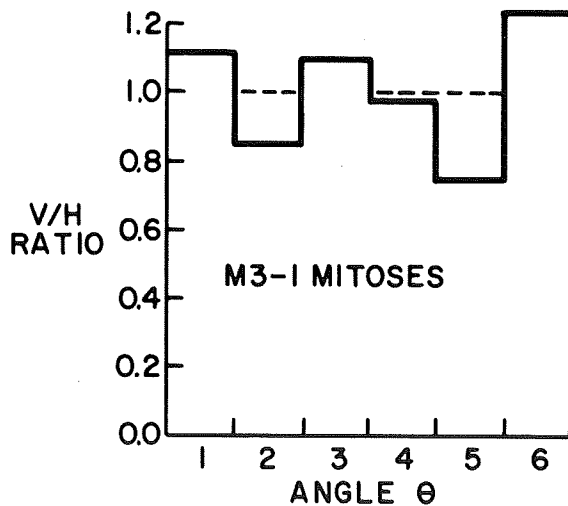
Figure 5.- Histogram showing the ratios of mitoses in vertical to those in horizontal culture flasks at each interval of the mitosis orientation angle θ, defined in figure 4 and in table II.
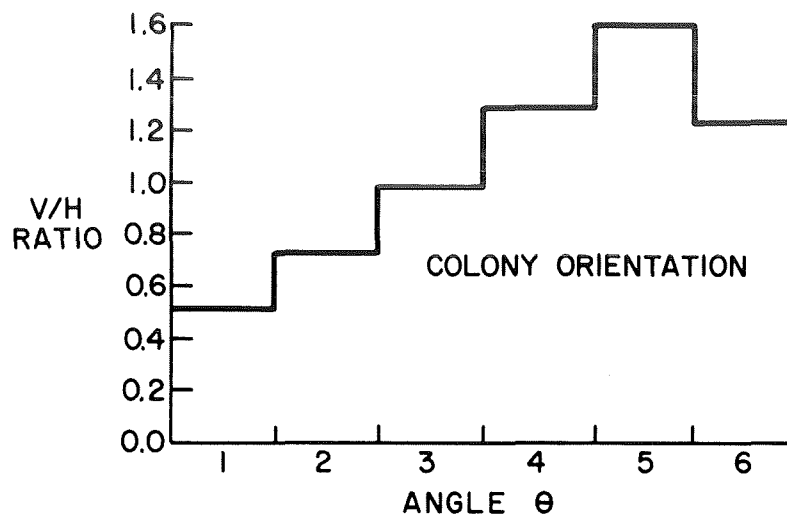
Figure 6.- Histogram showing the ratios of M3-1 cell colonies in vertical to those in horizontal culture flasks oriented with their long axes in each interval of the colony orientation angle θ, defined in figure 4 and in table II.
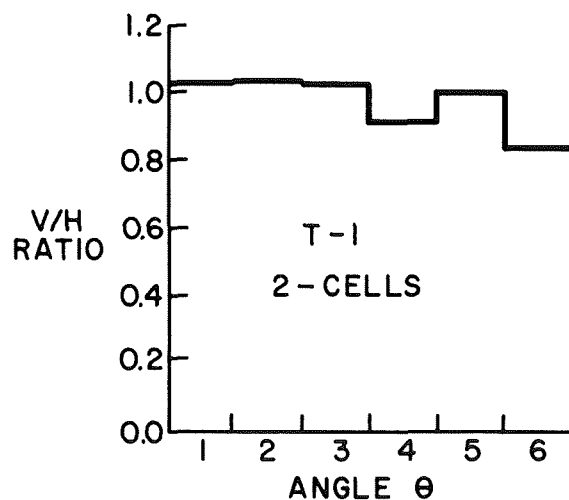
Figure 7.- Histogram showing the ratios of T-1 (two-cell) colonies in vertical to those in horizontal culture flasks having their plane of division in each interval of the division plane angle θ, defined in figure 4 and in table II.

BIOPROCESSING: PROSPECTS FOR SPACE ELECTROPHORESIS

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ABSTRACT

The basic principles of electrophoresis will be reviewed in light of its past contributions to biology and medicine. Preliminary experiments aboard of Apollo 14 and 16, Skylab and the recent Apollo-Soyuz Mission have confirmed the feasibility and advantages of a possible space electrophoresis facility. This has to be viewed primarily as a unique national research resource, which may eventually yield significant benefits for the advancement of biomedical knowledge and its technological utilization. Primary objectives of the facility should be the increase of resolution and throughput for critical electrophoretic fractionation of living cells and biologically active macromolecules.

INTRODUCTION

The technology of bioprocessing comprises two distinct categories of activity; on the one hand, there is need to grow and propagate various organisms through bacterial, mold or tissue culture and much work has been dedicated to optimize techniques and isolate strains possessing maximal activity. On the other hand, specific products have to be isolated from the biomass, often a difficult problem in view of the complexity of composition of living matter, and the similarity of the species to be separated. As a result, separation processes have acquired a unique importance in biotechnology, reflecting their importance in basic biochemistry.

Both of these aspects of bioprocessing may benefit from space research, and the last two speakers have discussed specialized aspects of space effects on growth and reproduction. In a later presentation, Dr. Barlow will show an example of where cell activity may have been enriched by separation in space. My present paper will focus on possible contribution of space electrophoresis, a separation process which has been identified as being most likely to benefit from the near-zero gravity environment prevailing in orbiting spacecraft.

The possible contribution of NASA's space capabilities to bioprocessing should be evaluated not only in terms of the economic importance of this industry, but primarily in terms of its social impact, through advances in medicine and its contributions to the quality of our lives.

ELECTROPHORESIS

Electrophoresis (1) is defined as the transport of electrically charged species under the influence of a direct current electrical field. Most materials in aqueous solution or suspension acquire an electrical charge due to ionization of their functional groups, ion absorption, or other more complex phenomena, and are therefore attracted by electrodes of opposite polarity. The charged species may be simple ions, complex macromolecules or even particles, such as living cells, emulsion droplets, clay, etc. Their migration velocity in unit electrical field is referred to as their electrophoretic mobility, and is a complex function not only of their electrical charge, but also of their molecular size, shape and hydration, as well as the dielectric characteristics of the solvent. As a result, electrophoresis is capable of providing a high degree of characterization of individual ionized species, which is most important for macromolecular systems and living cells, where structural parameters are difficult to determine.

Based on this uniqueness of information provided by electrophoresis, a number of applications have been developed. To categorize them in their broadest outlines, these are as follows:

(a) Identification and characterization of an ionized species.

(b) Determination of the quantitative composition of a complex mixture.

(c) Actual isolation of components of a mixture, separation being achieved on the basis of differences in transport rates.

Originally, electrophoresis was carried out in free solutions but it was soon recognized that problems arise due to convective disturbances in the bulk of fluid. We can categorize several major causes of these disturbances:

(a) The solute to be separated, if present in significant concentration, adds to the density of the supporting electrolyte. This difference in density between solution and pure solvent causes gravity-caused convective flow, unless means are found to prevent it.

(b) In some instances the particles may be sufficiently large to sediment noticeably. While there are techniques which utilize differential sedimentation to accomplish meaningful separations, within the context of electrophoresis such sedimentation is usually undesirable, especially as it is superimposed on the convective flow of the suspension as a whole, described above.

(c) The passage of electric current causes heating of the solution. As the vessels are externally cooled, a radial temperature gradient arises, again causing gravity-conditioned convection.

(d) The electric charge exhibited by the vessel walls within which electrophoresis is carried out causes an electroosmotic streaming of the fluid. This disturbance is independent of gravity and is a consequence of the electrical properties of the system as a whole.

In order to eliminate some or all of the above problems, a variety of techniques has been evolved, and a systematic classification is next to impossible. Thus, the techniques are differentiated according to their primary purpose (preparative or analytical), the mode of operation

(batchwise or continuous flow), shape of vessel (cylindrical, flat, annular, etc.), and other operational parameters. In the context of this paper, the most important classification is based on the anticonvective means employed to circumvent the effects of gravity. Three basic approaches were taken:

(a) migration can be carried out in gels, where all convective flow is prevented,

(b) fine porous structures of packed granules, or the interstitial spaces of filters and various specially developed membranes are also effective in preventing gross fluid motion without interfering in molecular transport, and

(c) a density gradient can be artificially created within the liquid by using a non-migrating solute such as sucrose, of sufficient steepness to overcome the density gradients caused by the electrophoretic process.

To these three approaches, we now must add the radically new concept to avoid gravity altogether, by using orbiting spacecraft. The soundness of this approach has been confirmed in pilot experiments conducted aboard Apollo 14 and 16 (2), Skylab (3), and the recent Apollo-Soyuz Mission (4).

As defined above, electrophoresis is a separation process occurring within the bulk of the liquid phase (and not at the electrodes) and is based on the differences in electrical transport rates. Electrophoresis alone, however, does not provide for the ultimate separation of various molecular species of proteins present, as their mobilities may be overlapping. Highest resolution is obtained if a second separation parameter is employed by introducing an element of discontinuity into the liquid phase. Two methods are most often used. In high density gel electrophoresis an element of molecular sieving is superimposed on the electrical separation process by progressively increasing the density of the supporting gel matrix. In isoelectric focusing a continuous pH gradient is established and the proteins become immobilized at the pH corresponding to their characteristic isoelectric point (mobility of proteins is pH dependent - the narrow pH zone of zero mobility is the isoelectric point). The separation obtainable by isoelectric focusing is comparable to that in high density gels, and both are much superior in resolution to plain electrophoresis.

ELECTROPHORESIS OF LIVING CELLS

Because of its nondestructive nature, electrophoresis is one of the few separative methods applicable to living cells. Nevertheless, in comparison to proteins, cell electrophoresis is only in its infancy. Most of the techniques and instruments developed for protein electrophoresis are not applicable, and cell electrophoresis has remained the province of a few highly specialized laboratories. One of the great merits of the NASA program is that it has focused the attention of a number of scientists here and abroad on this long neglected field. At present, cell separation is the main objective of NASA's space electrophoresis facility.

Basic knowledge in this field is sorely needed. While there is a multitude of analytical electrophoretic methods applicable to proteins, until quite recently there was only one method suitable for cell electrophoresis. This technique involves direct visual microscopic measurement of electrophoretic migration velocity of individual cells, and has remained essentially unchanged for over 50 years (5,6). It is an inherently slow and unreliable method, burdensome and tedious

for the observer. As a result, while there is adequate information on some normal cell populations, such as red blood cells and lymphocytes, there are almost no reliable data on changes of cell properties in most clinical or pathologic conditions. This situation is intolerable, since the present state of the art would readily permit computer assisted automation of the microscopic method, resulting in rapid accumulation of important basic data on cell mobilities in health and disease. It is hoped that through NASA sponsorship, such an instrument will soon become available. Other alternatives to more rapid accumulation of data involve the measurement of the Doppler Effect caused by migrating particles under laser illumination (7). Both of these two types of instruments are operable in presence of gravity, but at zero gravity their scope of application would be extended to larger cells, characterized by rapid sedimentation in a normal gravity field. Moreover, such instruments will be essential for the space facility, to provide real time information on the quality of separation achieved in space in the preparative instruments.

Similar considerations prevail in preparative electrophoresis. Several techniques have been developed, including thin film free-flow electrophoresis (8), stable-flow electrophoresis (9), electromagnetophoresis (10), and rotationally stabilized instruments (11, 12), but most have remained almost exclusively in the hands of their original developers. This is largely due to their complexity and the paucity of basic analytical data, which are indispensable in pinpointing the most important areas of preparative application. Moreover, the throughput of the instruments is limited, and their resolution less than optimal.

It was previously emphasized that highest resolution of proteins is obtained only when a second discriminating parameter is superimposed on electrophoresis, as in high density gel electrophoresis. The same situation may prevail with cells, and we are presently developing a system where electrophoretic separation is followed by an in-line discrimination according to cell size. Other secondary discrimination factors may be usable, such as presence of fluorescent markers. Such systems bear the promise of much higher resolution than that obtainable by electrophoresis only.

SPACE ELECTROPHORESIS

Gravity is not an unmitigated enemy of electrophoresis, and, to the contrary, in numerous techniques it is utilized to great advantage. The determining factor is the objective one seeks and one has to consider in different light separation of proteins and that of living cells. With proteins, there is a profusion of excellent methods for analytical or micropreparative work, i.e. fractionation and separation of products on a small, laboratory scale operation, and no foreseeable advantage is to be gained from a zero gravity facility.

The situation is different when scaling up of these techniques to larger volumes is attempted. In this realm, ground-based electrophoresis has failed completely and all attempts to scale up high resolution micropreparative procedures have been unsuccessful. The zero gravity facility may provide the hoped-for breakthrough by allowing the use of novel instruments specifically designed for the weightless environment. As an example, in our laboratory we are currently engaged in collaborative efforts to purify two trace components of human serum, Somatomedin, a growth promoting polypeptide, and Phagocytosis Recognition Factor, a potential antitumor agent. The technique utilized is isotachophoresis, a relatively new variant among the many electrophoretic techniques (13), and we have designed a novel type of instrument (14) particularly suited for space use. A diagram of this instrument is shown in Fig. 1. It is constructed from a parallel array of feeder spacers and knife edge separators, assuring laminarity of liquid flow. Time does not per-
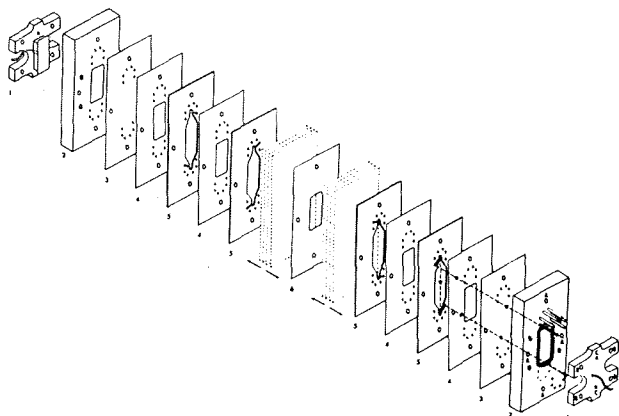
Fig. 1. Schematic presentation of a miniaturized flow electrophoresis apparatus designed for space electrophoresis. The parallel array of spacers and knife edge separators provide for laminar flow within the cell. The apparatus is envisioned for rapid flow-through with minimal migration distance, and may be particularly suited for isotachophoresis.

mit to go into the rationale why this apparatus promises a higher throughput in space operation than that of other similar continuous flow instruments available on ground. There are obviously a great number of other proteins which may benefit from space processing, including clotting factors, enzymes, and other protein hormones.

If the prospects of space electrophoresis were to be realized for proteins, there would be an immediate widespread usage for the products. Nevertheless, the primary emphasis of the current NASA program is centered on separations of living cells. The reason for it is that most techniques to circumvent gravity effects developed for protein electrophoresis are not applicable to cells. Moreover, cell electrophoresis is only in its infancy, and the development of better techniques may result in significant advancement of our knowledge of cell biology. This is the most opportune time for such a development, as it is only in recent years that cell biology has come into its own, with the recognition that apparently similar cells may have a variety of distinct functions. This is particularly true of lymphocytes, the mediators of immune reactions, which are of direct and immediate importance in such diverse areas of medicine as allergies, autoimmune diseases, leukemia and other forms of lymphocyte neoplasias, resistance to cancer, etc. Thus, there is a widespread current interest in cell separations by any and all means. Reliance on electrophoresis is based on the as yet fragmentary but significant evidence that functional, pathologic, genetic and environmental factors affect the electrophoretic behavior of lymphocytes (15).

The recent Apollo-Soyuz Mission provided an opportunity to test two prototypes of instruments suitable for zero gravity operation. The NASA prepared flight module was similar to those previously flown in Apollo 14 and 16 (2), though more ambitious in its aims. The essential part of the instrument was the electrophoresis column, reproduced schematically in Fig. 2. The two electrode compartments were detachable from the main body of the column, permitting a total of eight different columns to be tested. The columns were preloaded with sterile buffer, and the samples to be electrophoresed were frozen in liquid nitrogen till immediately before use by the astronauts. The samples contained kidney cells, lymphocytes and fresh and fixed lymphocytes. Photographs could be taken during the run to record the migration of the cells, while at the end of the run, the

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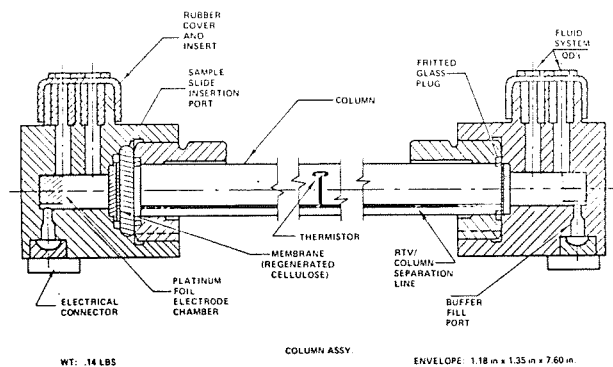
Fig. 2. Schematic diagram of the column
assembly for the static electrophoresis ex-
periment carried out as part of the Apollo-
Soyuz Mission. The center part of the
column is detachable from the electrode
compartments on both ends.
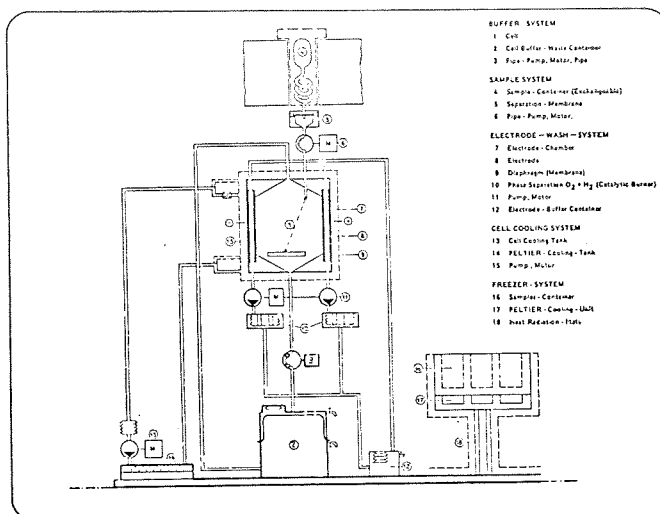
Fig. 3. Schematic drawing of the continuous
flow electrophoresis apparatus used in the
Apollo-Soyuz Mission. The cell itself is in
the center. Accessories provide for frozen
sample storage, sample insertion, and buffer
circulation.

columns were frozen in situ, and returned to earth in liquid nitrogen. The kidney cells were sub-
sequently grown in tissue culture, demonstrating the viability of the cells so recovered and we
will hear more about it later on. Time does not permit to discuss in greater details the other
experiments, or the apparatus.

A second apparatus was also included in this Mission. It was designed by Hannig of the
Max Planck Institute for Biochemistry in Munich, Germany, and was constructed by the Messer-
schmitt-Bolkow-Blohm consortium, and financed by the German Government. The diagram of this
apparatus is presented in Fig. 3, and it can be readily seen that it is far more complex than the
first apparatus. It is an automated and miniaturized version of the well known continuous flow in-
strument of Hannig (8), and contained three samples: a mixture of human and rabbit erythrocytes,
a mixture of B and T lymphocytes, and a suspension of bone marrow cells. Their migration was
followed photometrically, and no recovery of fractions was intended.

Both of above instruments were space adaptations of ground based equipment, and rep-
resent prototypes of two basic concepts in electrophoresis: stationary fluid versus continuous
flow operations. Their designs were kept within the narrow constraints inherent in experiments
aboard manned rockets. The availability of the Shuttle will probably eliminate most of these con-
straints, and there have been already several proposals within the NASA program of instruments
more specifically designed for the space application.

CONCLUSIONS

The near-zero gravity environment of orbiting spacecraft may present some unique advantages for a variety of processes, by abolishing the major source of convection in fluids. As the ground-based development of electrophoresis was heavily influenced by the need to circumvent the effects of gravity, this process should be a prime candidate for space operation. Nevertheless, while a space facility for electrophoresis may overcome the limitations imposed by gravity, it will not necessarily overcome all problems inherent in electrophoresis. These are, mainly, electroosmosis and the dissipation of the heat generated by the electric field. The NASA program has already led to excellent coatings to prevent electroosmosis, while the need for heat dissipation will continue to impose limits on the actual size of equipment. It is also not excluded that, once the dominant force of gravity is eliminated, disturbances in fluid stability may originate from weaker forces, such as surface tension.

There is as yet no consensus on the best apparatus for space electrophoresis. Reflecting the diversity of ground-based electrophoresis instruments, it is likely that more than one instrument will be needed for the space facility as well. It is important to consider the nature of the possible advantages to be derived from space electrophoresis: these are only a matter of degree. In all separation processes, one has to consider the factors of resolution and throughput, there being usually a trade-off between the two. For cell separation in space, both may be of importance, while for proteins, only a quantum increase of throughput would constitute a definitive advantage. To achieve either or both of these advantages, optimization of the design of the space instruments is essential. Less than the best designed instruments may well jeopardize the whole program, particularly when one considers that its operators in space may not have the skills of principal investigators or highly skilled technicians. The interdisciplinary expertise available to NASA offers a unique opportunity not only to optimize the space but also ground-based equipment.

The space facility for electrophoresis has to be considered primarily as a unique research tool for the advancement of our knowledge of cell biology, though its potential technological applications should not be overlooked. In view of the cost of space experimentation, greatest care should be given to the selection of candidate materials for space processing. To accomplish this, it would be desirable if the NASA program were to be integrated or correlated with the work of other agencies or organizations having a more primary interest in the health field. The process of selection should encompass a thorough evaluation of the material by ground-based electrophoresis and the consideration of alternatives. As we are dealing with a rapidly advancing area of science, maximum flexibility in planning is essential for both, instrument design and their application.

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Electrophoretic Separation of Human Kidney Cells at Zero Gravity

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Acute thromboembolic vascular disease remains the greatest single cause of mortality in the middle and elder age population of the U.S. In recent years the use of fibrinolytic therapy has shown the feasibility of clot lysis in vivo in such diseases as pulmonary emboli and deep vein thrombosis. The UPET (1) and USPET (2) multicentered clinical trials have demonstrated the safety of such therapy.

The presence in urine of a substance capable of effecting the transformation of plasminogen to plasmin, the agent necessary to bring about fibrinolysis was first described by Williams (3) in 1951 and in the following year by Astrup and Sterndorff (4). Sobel (5) et al assigned the name urokinase (UK) to this activator in 1952.

It was apparent from the start that the logistics of urine supply and the cost of production made the urine derivative of the enzyme impracticable. For the 4 million unit dose used in the clinical trials at least 1500 liters of urine were required and the cost was prohibitive. These facts motivated us to search for another source of this fibrinolytic agent; and since this agent is protein in nature, it is not desirable for immunogenic reasons, to use other animal sources aside from man.

In 1959, Barnett and Baron (6) demonstrated the production of a plasminogen activator from KB cells, derived from a human epidermoid carcinoma and primary monkey kidney cells. In a later paper (7) the same authors showed that many continuous primary cell cultures would produce either or both plasminogen and protease activators. Painter and Charles (8) demonstrated the accumulation of a fibrinolytic agent in cultures of primary monkey kidney cells and in an established line of canine renal cells. This agent was shown to be an activator of plasminogen with properties similar to those of urokinase. Finally, Bernik and Kwaan

(9, 10) demonstrated (a) fibrinolytic activity in cultures of human kidneys, (b) that this activity was immunologically indistinguishable from urinary urokinase and (c) that this fibrinolytic agent was produced to the greatest degree in cultures of human renal cells from a 26 - 32 week old fetus. These findings and the development by Weiss and Schleicher (11, 12) of cell equipment known as the Mass Tissue Culture Propagator (MTCP) which allows for the culturing of cells on a large scale prompted us to initiate a program to produce urokinase from human embryo kidney cells. The basic methodology has been described (13) and the research has continued for improvement of the process.

The observation by Bernik and Kwaan (10) using a fibrin slide technique that only about 5% of the cells produced activator led to the design of the experiments described here. A method was sought to isolate the producing cells that could be used eventually on a large scale. One possible way is electrophoretically. However, a drawback in the electrophoresis of cells is the loss of resolving power due to the sedimentation of the cells in the media. An electrophoretic separation at zero gravity should obviously negate this drawback. Thus, the experiments described here were performed on the Apolly-Soyuz space mission.

Methods

Electrophoresis including instrumentation, electrophoretic conditions ang gel slicing techniques will be described elsewhere by NASA (14).

Cell Viability was performed using a 0.4% stock solution of erythrosine B made in phosphate buffer, pH 7 (15).

Urokinase Activity determined using a modification of the fibrin plate technique as described by Brakman (16).

HGCF Activity determined using human bone marrow cells in a modification of the method described by Stanley and Metcalf (17).

Electrophoretic Mobility determined by the method described by Seaman (19).

Results

The frozen fractions were thawed rapidly at 37^0C, centrifuged and resuspended in growth media. The fractions were weighed and tared to determine the weight of each fraction. The pH on each fraction was also determined. These results are shown in Table I. An aliquot was taken for viable cell count and based on this information the cells were cultured. The distribution of viable cells is shown in Figure 1. As can be seen about 4 subpopulations of cells can be identified.

After 28 days only fractions between 11 and 19 had reached confluency. The other fractions were removed from the culture plates and tested for urokinase activity by fibrin plate method and showed no fibrinolytic activity. The sequence of events with the confluent plates are shown in Table 2 and of the subculture in Tables 3 and 4. Those plates that went to production were put on production media and tested for urokinase activity at various times. The cells that were subcultured were removed from the dishes with EDTA and then recultured.

Table 5 shows the results of the urokinase production obtained with the primary and subculture 1 cells after 35 days on production. There is an obvious enrichment of urokinase activity in Fraction 15 when it is recorded as units of activity per 100 cells. An optimization is also seen in several other fractions. Control experiment with the same cells at ground base conditions gave the value 0.28/100 cells. The subculture 2 cells did not produce urokinase when placed on production media.

Part of the cells from subculture 2 were analyzed for mobility distribution and the results on three such fractions is shown in Figures 2, 3 and 4.

The material from SC-1 was also tested for the presence of Human Granulocyte Conditioning Factor and the results are shown in Table 6.

Discussion

The results show that cells can be separated under sterile conditions and returned from orbit in such a manner that they retained their ability to grow in culture.

The electrophoresis in space showed good separation of the kidney cells into subpopulations. The results indicated that there were at least 3 and maybe 4 subpopulations. This result is in agreement with some results obtained using the endless belt electrophoresis.

Even though each fraction showed viable cells by the stain technique and they all attached to the glass surface only the few fractions between 11 and 20 grew. The reason for this is not known. The only possible explanation is that the non growers were more sensitive to unfavorable conditions and therefore could not recover from the shock to grow in culture.

The data indicates an enrichment of producing cells in the area centering around Fraction 15. The results can be interpreted to show an incomplete resolution between a producing cell population and a non producing population. Considering the fact that the conditions of the experiment were not optimized for kidney cells but generalized for three different separations, this result is not surprising. This is probably why the cells failed to produce at subculture 2 when normally under optimized growth conditions they produce to subculture 7.

It would appear that the area for maximum production of Human Granulocyte Conditioning Factor does not coincide with that for urokinase. This is a very interesting finding and indicates that the two products are most likely not produced by the same cell.

The analytical mobility data at the subculture 2 data shows each fraction at this stage to have a rather broad distribution. This is rather disappointing in that one would anticipate a rather sharp distribution based on the narrow fraction one starts with. This is probably explained by the fact that the starting fraction is heterogeneous and the cell attachment and growth pattern is a random event which can broaden at each reculture level. More analyses should help prove this point.

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TABLE 1

Tube Number	Wt in grams Tube, ring, sample	Wt in grams tube	Wt in grams teflon ring	Wt in grams frozen sample	buffer pH
R	1.4292	1.1061	None	0.3257	7.85
L	1.3623	1.08425	None	0.27805	7.05
1	1.3483	1.11375	0.0855	0.15406	7.8
2	1.3381	1.0926	0.0944	0.15110	7.9
3	1.3150	1.0861	0.08695	0.14195	7.8
4	1.3612	1.1047	0.08955	0.16695	7.8
5	1.3521	1.09775	0.09115	0.16320	7.85
6	1.3350	1.0905	0.08625	0.15825	7.6
7	1.3290	1.09205	0.08850	0.14845	7.85
8	1.3568	1.104	0.09030	0.16250	7.75
9	1.3573	1.0943	0.09105	0.17195	7.75
10	1.3350	1.0869	0.09005	0.15805	7.85
11	1.3581	1.11405	0.08970	0.15435	----
12	1.3749	1.10465	0.09260	0.17765	7.65
13	1.3301	1.08585	0.08805	0.15620	7.55
14	1.3156	1.10695	0.01765	0.10100	7.45
15	1.2970	1.08865	0.07495	0.13340	7.5
16	1.3154	1.08785	0.08830	0.13525	7.65
17	1.4090	1.10985	0.09510	0.20405	7.25
18	1.3296	1.0915	0.08505	0.14975	----
19	1.2915	1.0854	0.08595	0.12015	7.45
20	1.3240	1.08645	0.08640	0.14915	----
21	1.3443	1.0908	0.09235	0.16175	----
22	1.3714	1.10855	0.09690	0.16595	7.45
23	1.3494	1.09885	0.09025	0.16230	----
24	1.3408	1.09785	0.09210	0.15605	----
25	1.3829	1.09895	0.09850	0.18545	----
26	1.2727	1.1061	0.04525	0.11575	7.4

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TABLE 2

SEQUENCE OF EVENTS

PRIMARY CULTURE
(35 mm) Petri Dishes

FRACTION	CONDITION	DISPOSITION
11 1 dish	Confluent	To production
13 1 dish	Confluent	To production
14 1 dish	Confluent	To subculture (2)
15 1 dish	Confluent	To production
16 1 dish	Confluent	To subculture
17 2 dishes	Confluent	To production (1), to subculture (1)
19 1 dish	Confluent	To subculture
B Control 1 dish	Confluent	To production
D Control 1 dish	Confluent	To production

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TABLE 3

SEQUENCE OF EVENTS

SUBCULTURE 1

FRACTION	CONDITION	DISPOSITION
14-1	Confluent	To subculture-25 cm^2 flask
14-1	Confluent	To production
16-1	50% Confluent	To production
16-1	50% Confluent	Reculture-no growth
17-1	Confluent	To subculture 2-25 cm^2 flask
17-1	Confluent	To production
19-1	Confluent	To subculture 2-25 cm^2 flask
19-1	Confluent	To production

TABLE 4

SEQUENCE OF EVENTS

SUBCULTURE 2

Fraction	Condition	Disposition
14-2	Confluent	To Production
14-2	Confluent	To Mobility Detn.
17-2	Confluent	To Production
17-2	Confluent	To Mobility Detn.
19-2	Confluent	To Production
19-2	Confluent	To Mobility Detn.

TABLE 5

RESULTS
PRIMARY CULTURE

FRACTION	UK ASSAY units/dish	VIABLE CELLS $\times 10^5$	UK UNITS 100 cells
11	45	.07	.64
13	535	.696	.77
15	240	.12	2.0
17	225	.744	.3
B CONTROL	61	.068	.9
D CONTROL	81	.288	.3
SUBCULTURE 1			
14-1	85	.60	.14
16-1	124	.132	.94
17-1	205	.9	.23
19-1	359	.222	1.62

TABLE 6

RESULTS

Human Granulocyte Conditioning Factor

Subculture 1

Fraction	HGCF (Colonies formed)*
14-1	40
16-1	( )
17-1	123
19-1	0

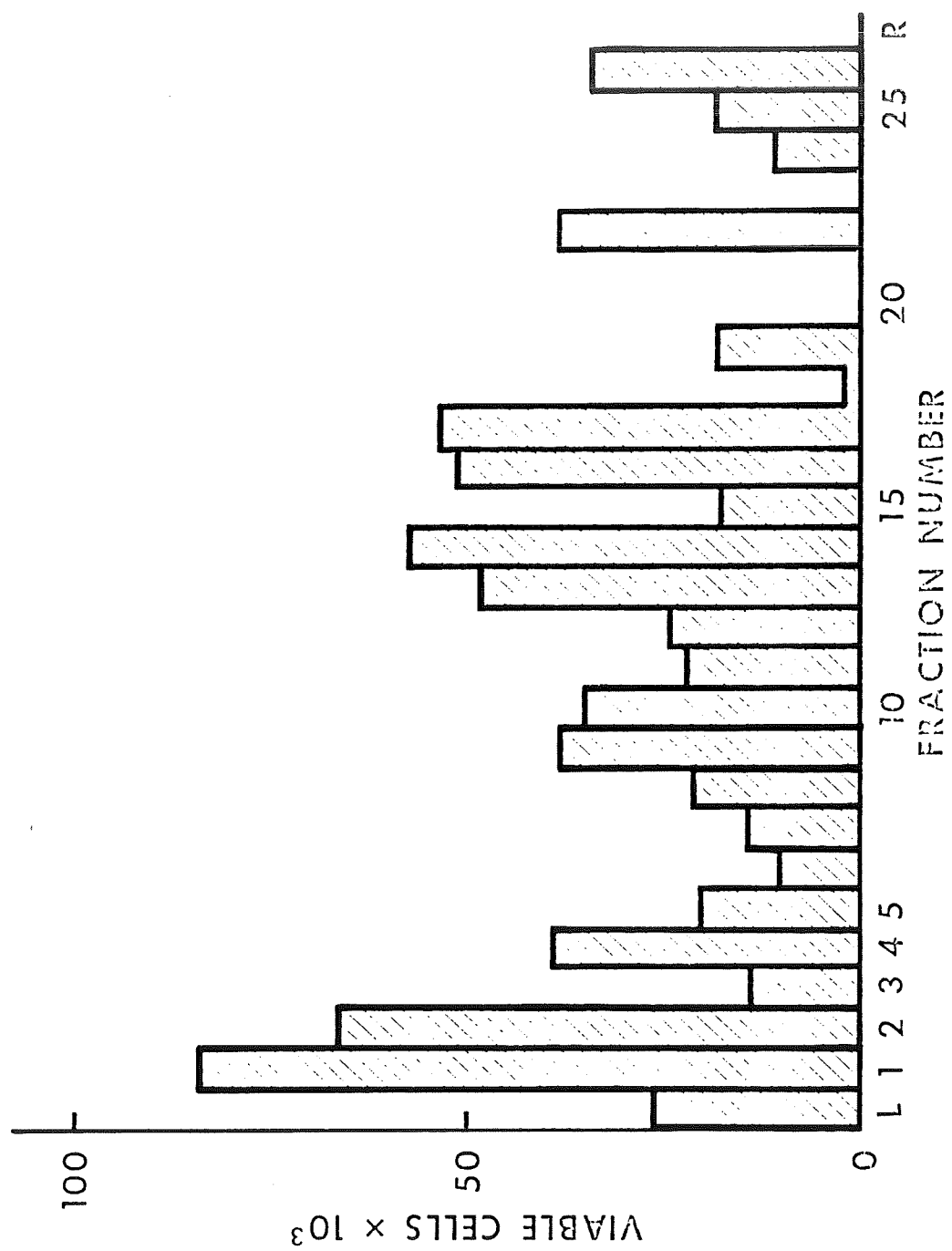*Colonies formed corrected for control plate

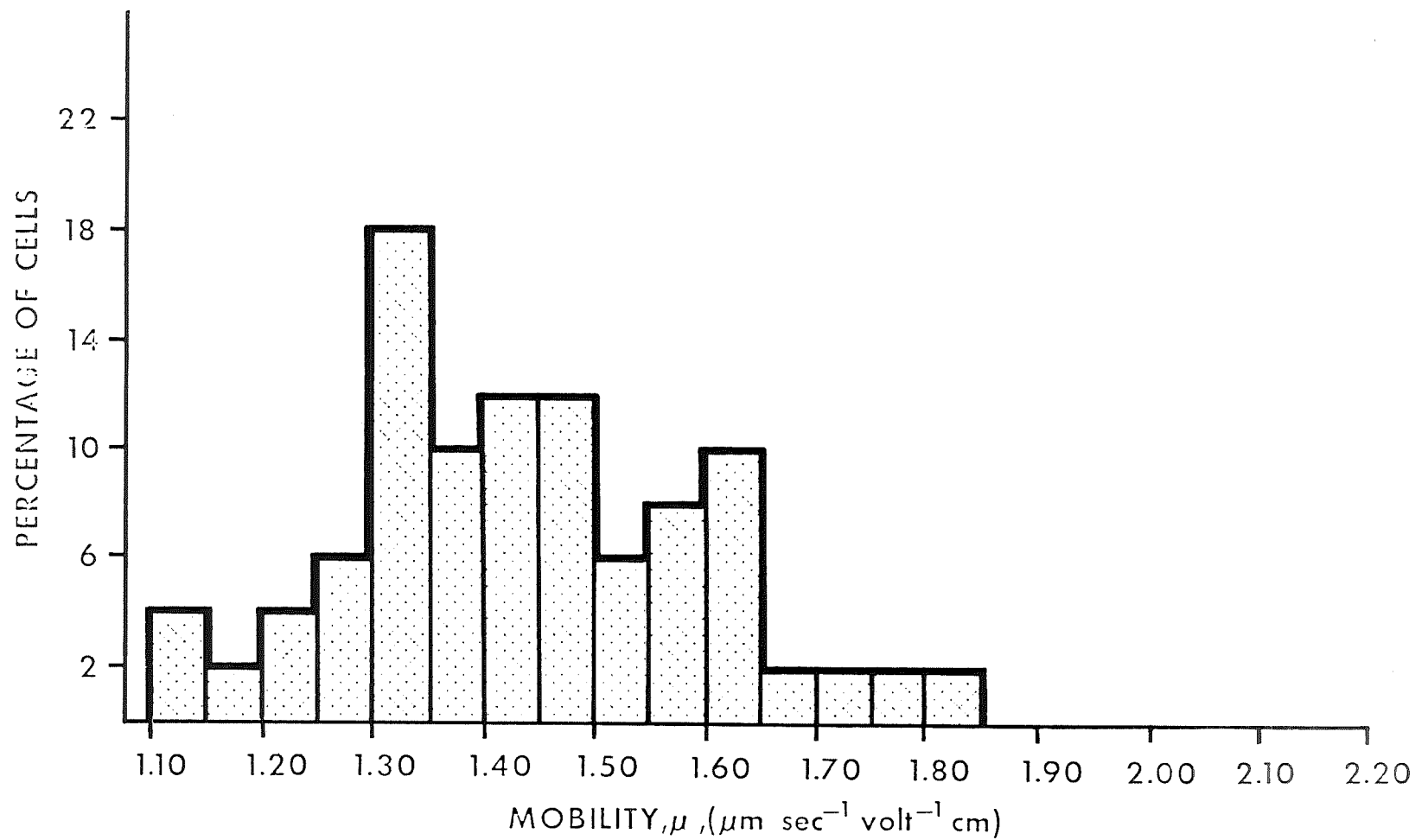Figure 1.— Viable cell distribution in fractions from sliced gel.

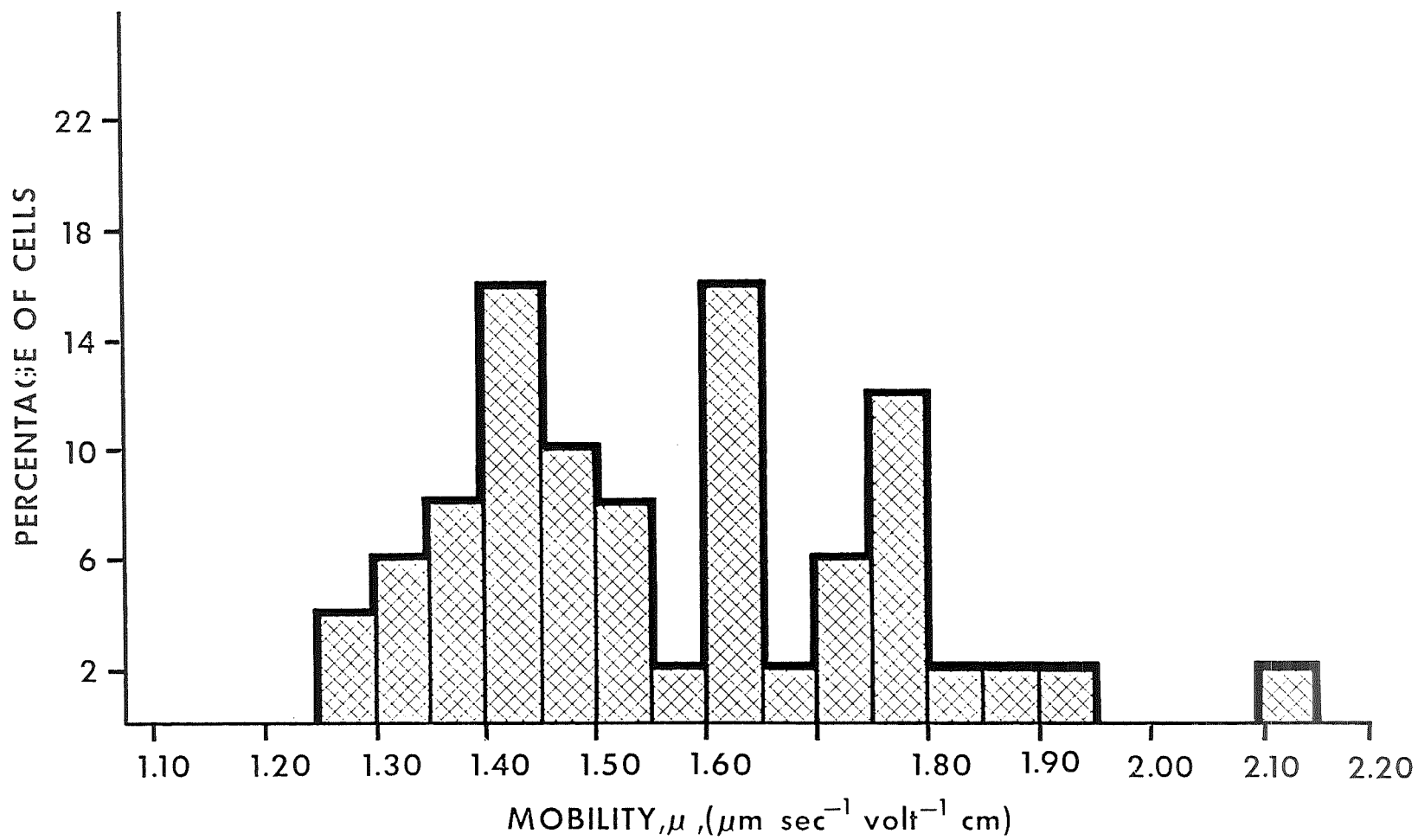Figure 2.- Analytical electrophoretic mobility distribution on cell subculture 2 of fraction 14.

Figure 3.— Analytic electrophoretic mobility distribution on cell subculture 2 of fraction 17.
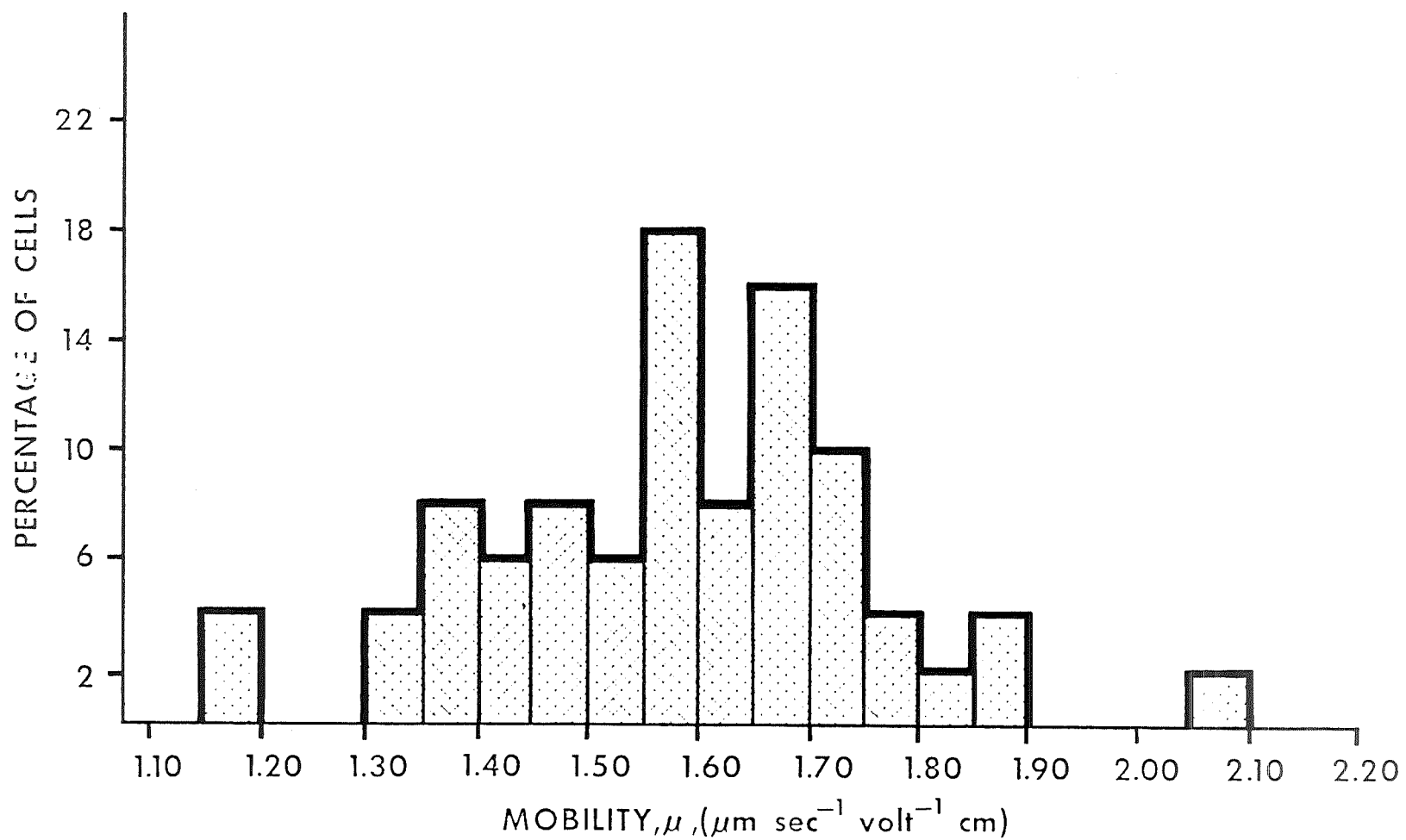
Figure 4.– Analytic electrophoretic mobility distribution on cell subculture 2 of fraction 19.

ELECTROPHORESIS FOR BIOLOGICAL PRODUCTION

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Abstract

Preparative electrophoresis may provide a unique method for meeting ever more stringent purity requirements. Prolonged near zero gravity in space may permit the operation of preparative electrophoresis equipment with 100 times greater throughput than is currently available. Some experiments with Influenza Virus Antigen, Erythropoietin and Antihemophaliac Factor, along with process and economic projections, will be briefly reviewed.

Introduction

The idea of preparing biologicals of improved purity and specificity in space has both great technical and economic basis. It could become a multi-billion dollar business and be an important use for the STS. There is however a great deal of research and development to perform first.

This paper reviews some of the early work; from the initiation of the idea through some flight demonstrations and on to the current development of a sounding rocket experimental unit and some ground based separations work. A brief concluding section then outlines some of the projections for possible future preparative electrophoresis in space.

Early History of Electrophoresis in Space

Electrophoresis has been widely used for several decades, primarily based on the work of Tiselius, for analysis of biological materials. There are now an estimated 30,000 research and analysis personnel who utilize the technique in the U.S. alone and several hundred technical papers are based on this work annually.

Unlike other analysis and process techniques it has not been possible however to scale up the electrophoretic analytical technique to provide a truly preparative scale of operation. This is primarily due to gravity induced convection and sedimentation which can be sufficiently counteracted by such approaches as the use of gels, orientation, cooling, and small dimensions in the case of analytical devices, but are too restrictive to permit scaling up to an economical preparative level.

Thus in a "brain storming" type of discussion on space processing ideas among staff members of the Wyeth Laboratories and General Electric Space Sciences Laboratory in the spring of 1969, preparative scale electrophoresis was suggested among the several ideas at that meeting. Several possible product examples such as vaccines, hormones, enzymes, and cells were suggested by Wyeth as well as other organizations over the next year or so.

About a year later, specific R&D work was initiated on the idea and almost immediately an opportunity arose to have a flight demonstration on Apollo 14. In about four months, we then designed and developed a small flight demonstration shown in Figures 1 and 2.

Samples of salmon sperm DNA, hemoglobin, and a mixture of red and blue dye were chosen to represent a broad range of molecular weights and to demonstrate electrophoretic mobility under microgravity conditions. Only the red and blue dye were expected to be, and were, electrophoretically separated.

Although the results were not up to our high expectations, the red and blue dyes did separate but the photography did not provide clear pictures. The biologicals were destroyed, apparently by bacteria, during the four month flight and quarantine period, but the engineering aspects of the unit were excellent and were reused on later flights.

A second flight demonstration was then scheduled on Apollo 16 with again about four months to develop it. Steps were taken to overcome the problems of the Apollo 14 flight, namely: a tripod and lens extension tube system was provided to improve the photography (Figure 3) and PSL (polystyrene latex) was suggested by the USRA (University Space Research Association) as a more stable non-biological sample.

Along with the choice of PSL as the sample, ground based work using sucrose solution density gradients was suggested and used to indicate (Figure 4) and define the separation of the 0.2 and 0.8 micron PSL which was flown as a mixture in the upper tube and individually in the bottom and middle tubes, respectively, of the apparatus.

An example of the results of the Apollo 16 demonstration is shown in Figure 5 with a ground based view for comparison. A clear indication of the possible improvements in electrophoretic separation performed in space is indicated even though electroosmosis and some bubbles are also indicated. The latter two problems warrant some brief mention.

The bubbles were, we now believe, caused by the permeability of the silicone tubing - especially when it is subjected to a rapid external depressurization as was the custom on Apollo flights. During the development of the Apollo 16 unit there had been some indication of stress corrosion problems with the Lexan used to fabricate the electrophoresis cells. This was primarily due to the use of thin wall sections for greater transparency as compared to the more massive monolithic machined block used for Apollo 14. While the design was indeed demanding of the full capabilities of the Lexan, it was the best choice of transparent plastic and probably not the source of fluid leaks that permitted bubbles to form.

The electroosmosis is the result of the high zeta potential on the walls of the electrophoresis chambers. At that time there was no low zeta potential coating available which would adhere adequately to the chamber walls, and ground based tests in surcrose density gradients indicated no benefit from the use of a collodion coating as compared to leaving the Lexan uncoated. Since applying a coating may have been detrimental to the Lexan from the stress-corrosion standpoint, it was decided to leave it uncoated. The problem then is one of either not being able to translate the density gradient work to the flight demonstration unit or in not being able to obtain ground based results from the flight demonstration unit that would show the electroosmosis problem.

Happily, the remaining problems with these two flight demonstrations have been overcome in the more major experiment MA-011 (which on the other hand had some other difficulties which are being assessed and reported separately). The MA-011 also again made use of the phase separators and small peristaltic pump plus other technologies from Apollo 14 and 16. Thus it appears that while each flight has corrected the deficiencies of the previous flight, new problems have arisen by virtue of the changes made in

the demonstration materials, or design, or equipment, as each unit was being hurriedly developed for a singular flight opportunity.

It is therefore highly satisfying to see the sounding rocket and space shuttle flight schedules and the possibility for repeated flights of an experiment until it is satisfactory for as long as warranted.

Preparative Electrophoresis

Equipment - With the completion of the sufficiently satisfactory Apollo 14 and 16 flights, our attention was turned toward meeting the original and still desirable goal of developing a truly preparative unit for space experiments. We chose the continuous flow type of unit as offering the greatest ease of inserting and removing samples and sample fractions. While the engineering of such a unit requires ingenuity, it is not extremely difficult and the basic idea for the electrophoresis cell is to simply make it thicker than the 0.5 to 1.5 mm commonly used for such units on earth. It was estimated that the cell in such a unit for space could be as much as 8-10 mm thick and provide an improvement by a factor of about 5-10 in resolution or an improvement of about 80-100 in throughput. This much greater performance is simply due to being able to scale up the thickness without gravity induced convection and to increase the sample concentration without sedimentation problems.

This has now been demonstrated with a 4 mm thick cell, at least partially, on the ASTP-MA-014 experiment by Hannig. A similar unit has also been developed in our laboratory for sounding rocket usage. It is shown in Figure 6 as currently equipped with a camera for data acquisition. Other work is now underway on modifications to permit collecting up to 50 fractions of sample and to detect them by a U.V. scanner system. These are being done on a schedule to permit a flight test in late 1976. The cell is 5 mm thick by 5 cm wide and has a 10 cm long electrode section. It is supplied with approximately $4^{\circ}C$ buffer, coolant, and samples by the use of a passive refrigerant system. The possible operating conditions such as flow rates, volts/cm across the cell, etc. are very broad and can be adjusted over several decades by a choice of gear ratios on the pumps and by plug-in power supplies, as well as by "fine tuning" electrically up to a short time before flight.

Math Modeling and Computer Simulation - Prior to designing the current sounding rocket unit, a math model was prepared both to aid the design and to predict the performance of thick cell electrophoretic separators in space. A separate publication is in preparation on this work so it will only be briefly reviewed here. Figure 7 shows the factors considered in the math model along with indications of which are controllable. As compared to previous efforts to describe the operation of a continuous electrophoresis cell, the parameters in our model are allowed to interact and are calculated primarily as to their effect on resolution and secondarily, throughput. Some typical results for a realistic although hypothetical separation of four samples (as defined by zeta potential and other conditions in each of three different thickness flow cells) are shown in Figures 8, 9, and 10.

Extensive ground based testing in prototype electrophoresis cells built for the previously described sounding rocket, as well as with other electrophoresis units, has generally corroborated the calculations for at least low levels of power. A plot of the power versus resident time (as a measure of flow rate) and thickness for stable and unstable conditions (hydraulically) is presented in Figure 11. Unfortunately, gravitational convection induced by the joule heating in these thick cells occurs at levels of power (10-15 watts) which are an order of magnitude below the power levels useful for separation.

Therefore, while the calculations and ground based tests at low levels are in good agreement, the more realistic level tests will have to be accomplished in space under microgravity conditions.

Biological Tests - Ground based tests for both the development of equipment and for establishing operating conditions for biologicals are being accomplished in a commercially available unit shown in Figure 12. It is a Beckman CPE II which is well designed for model studies and to which we are making additions and changes for more easily handling biologicals. These include fused silica windows and a U.V. scanner, for example. Studies ranging from single experiments, so far, to about 20 experiments, plus numerous calibration runs with PSL, have been undertaken with each of various biologicals including:

Hepatitis Vaccine
Sperm
Lymphocytes
AHF
Erythropoietin
Influenza Virus Antigen

The results are generally encouraging but not necessarily easily achieved nor sufficiently complete. Considerably more effort has to be expended in this area before flight tests since it seems unlikely that a space flight test will accomplish a separation that has not at least been shown to be feasible on earth. Examples of some of the separations studied and results obtained are shown in Figures 13-15.

Projections for the Future - Contacts in numerous pharmaceutical houses indicate that, while indeed cells of various types and sources are an intriguing problem for separation science, numerous hormones, enzymes, blood and urinary source materials, and vaccines need or would be benefited by a great deal of improvement in purity. The practical limitation on the use of electrophoresis to prepare these products in sufficient purity to be of value is throughput efficiency. While absolute purity is required in certain cases, many products are only needed in more concentrated form and therefore resolution is often a subjective parameter which can perhaps be traded off against throughput. An estimate of the throughput in grams/hour versus cell thickness for two cases is shown in Figure 16. The upper right hand area of the figure depicts a high throughput case, i.e. for a case where sufficient resolution is easily obtainable and the extra capacity of the equipment can be utilized for throughput. The lower portion of the figure depicts a situation where resolution is to be stressed. In each case a range of 1-10% sample concentration is shown which should be compared with typical ground based practice of using about 0.1 to 0.5%. In any case, some 2 to 3 orders of magnitude improvement in throughput are predictable. This is far beyond the degree of improvement for which one might consider simply duplicating the ground based facilities when greater throughput is needed even if resolution were satisfactory.

Economic Predictions - Three general areas of potential payoff for this work are foreseen. First is the possibility of the research and development being beneficial to ground based electrophoresis equipment and techniques. Secondly is the possibility of preparing more specific strains or products in space which can then be used to culture and produce greater quantities of particular products on earth. Thirdly, when the first two or other approaches are insufficient, products may actually be produced in space.

Examples of the first two approaches already being productive are available. Improved electrophoresis equipment and coatings with nearly zero zeta potential are now available

and are examples of the first area of benefits. Increased yield of Urokinase through the improved separation of fetal kidney cells on the Apollo Soyus Test Project flight in the summer of 1976 is an early indication of a potential benefit in the second area. Preliminary examples of the third area must await further work but may well come from current projects for the sounding rocket and early shuttle flights.

Several products could potentially benefit from these capabilities and further work is recommended to establish the necessary protocols and reference data on which to base flight tests.

The human value of more effective biologicals is of course impossible to measure. The preparation of purer erythropoietin could free some 15,000 U.S. renal failure patients from repeated blood transfusions. Thus humanitarian and societal motivation in this area is unusually high, and even greater than the basic economic value. Some simple projections for space processing of biologicals can be made based on certain assumptions.

It is presumed first that for efficiency and economy, as much of the processing as possible will be done here on Earth. Then, only a reasonably pure concentrate will be taken to space for one more, or perhaps a few, processing steps. In addition, the large quantities of water normally used in biological processing are presumed to be recoverable and reusable in space so that this commodity will not need to be completely resupplied from Earth for each product. Finally, however, the general rule that each biological product should be prepared in isolation from other products in order to avoid cross contamination is likely to be necessary. This may necessitate some special scheduling, but should not create any insurmountable problems.

Vaccines are the best defined available product on which to base projections for the future. In the U.S., some 60 million doses of vaccine are used annually. If we utilize the World Health Organization's estimates of World population in 1990-2000 as 5 billion and assume the same rate of vaccine applications world-wide as is now current in the U.S., we project the need for about 1.5 billion doses of vaccine per year. Using a conservative average number of 100,000 doses per gram of active ingredient, we calculate the need for 15,000 grams of active ingredients per year. Many currently used and very fine biological products are at best however quite dilute or impure (but not necessarily with harmful impurities). The purity may range from less than 1% to about 50%. This is assumed to be the starting material for a space purification operation. Therefore, the weight of starting material could range from 2 to 100 times the 15,000 gram final product weight derived above. Assuming a conservative average of 50, it is expected that some 750 Kg of partially purified vaccines might be used as the starting materials. In addition, some several hundred kilograms of water would be required. While vaccines generally cost about 20¢ per unit to produce, some examples of higher costs for greater specificity indicate that $1.00 per unit may be an acceptable value. This then indicates a $1.5 billion dollar activity in vaccines alone, a fraction of which may require space operations.

The processing of some other biological products such as cells and the blood derivatives in space while less specifically calcuable could easily exceed the estimates for vaccines by up to an order of magnitude in volume and value.

Acknowledgement
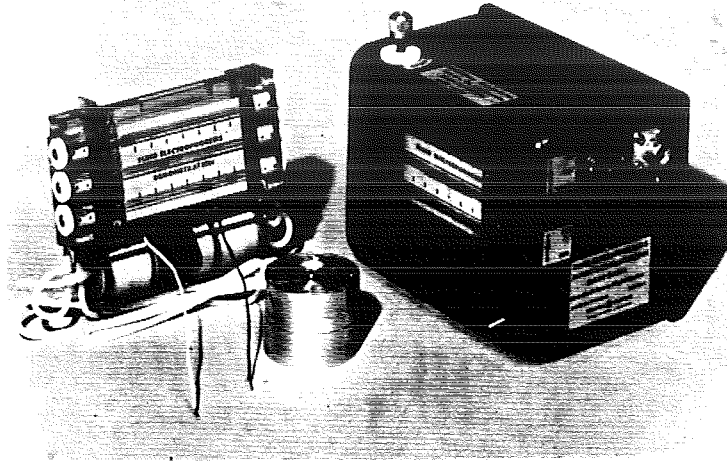
Figure 1.- Apollo 14 fluid electrophoresis demonstration unit on right with some of the major components at left. These include the three electrophoresis cells machined in a monolithic block of Lexan, with the phase separators below and the peristaltic pump at center. The overall dimensions of the experiment are approximately 4 by 5 by 7 in. plus appurtenances.
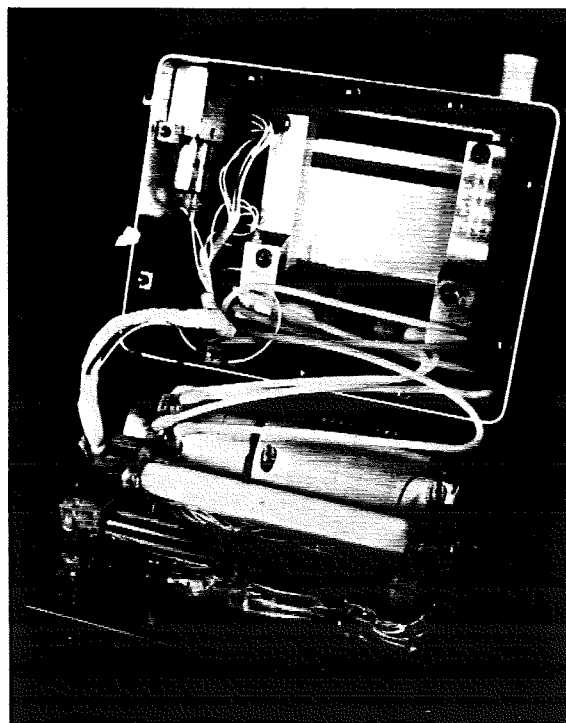
Figure 2.- Apollo 14 fluid electrophoresis demonstration unit in opened configuration showing back view of electrophoresis cell in upper portion of box with phase separators and peristaltic pump in middle and fluorescent lamps and potted electronics in lower area.

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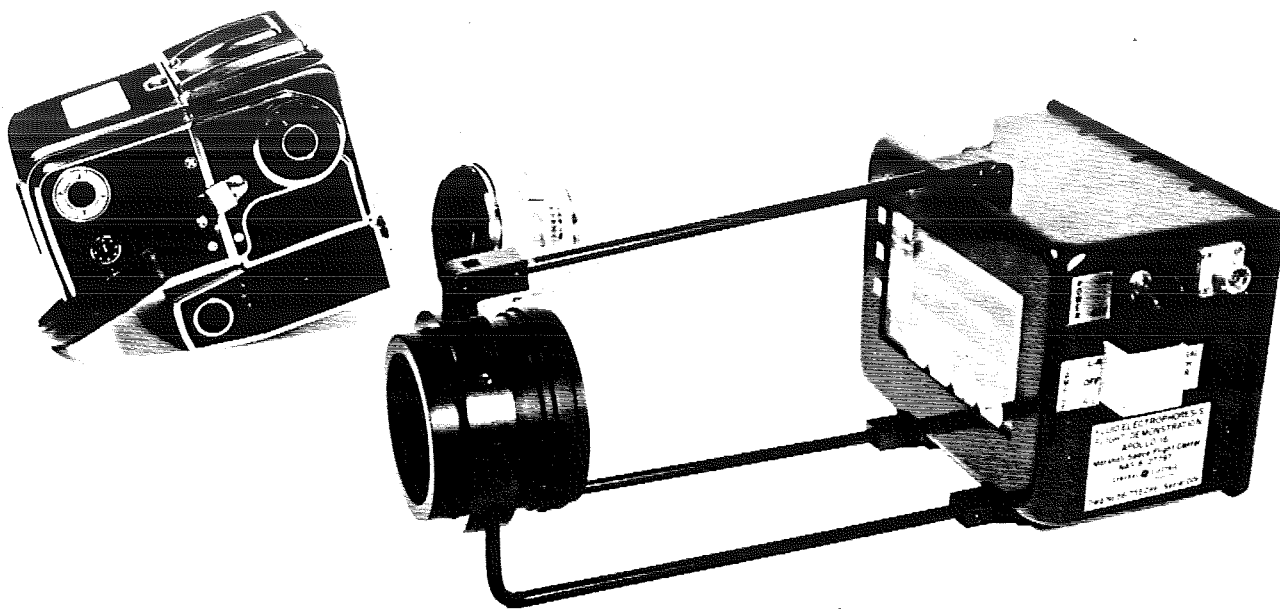
Figure 3.- Apollo 16 fluid electrophoresis demonstration mockup. Larger window, instruments, and camera-tripod arrangement improve data acquisition.
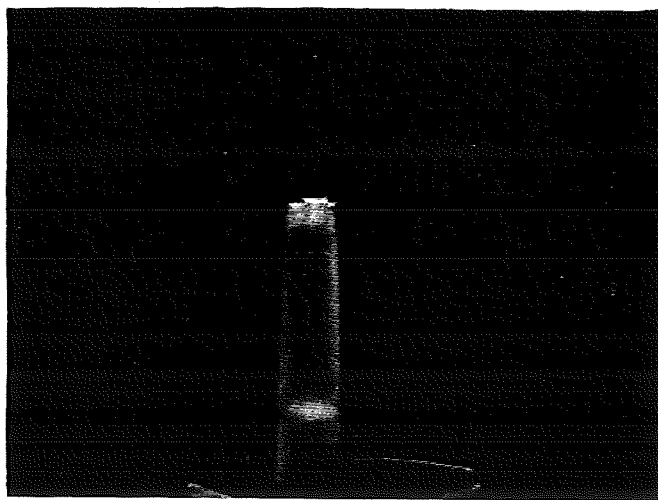
Figure 4.- Electrophoretic separation of 0.2- and 0.8-micrometer polystyrene latex in a sucrose density gradient after 40 minutes during which time the leading band (0.8 micrometer) traveled 8 centimeters and the trailing band 5.6 centimeters using a 0.085 M borate buffer of pH 8.5.

(a) Flight results.
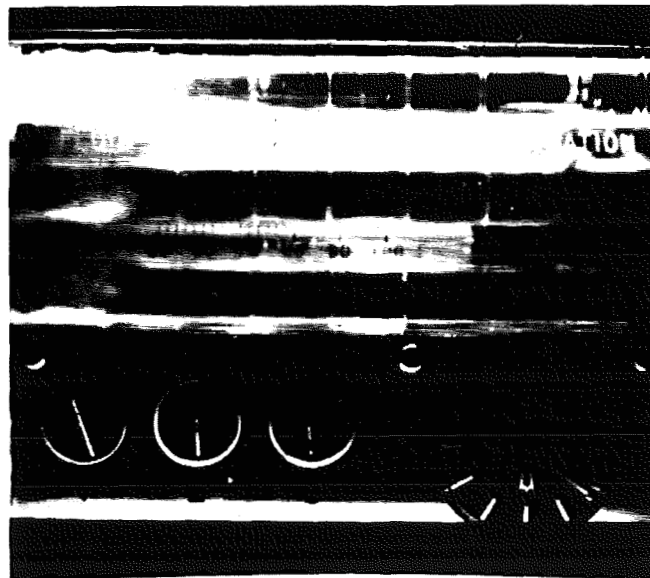
(b) Ground results.

Figure 5.- Results of Apollo 16 fluid electrophoresis demonstration. The flight results
clearly show the benefit of reduced gravity on the electrophoretic mobility at
30 V/cm in borate buffer of a mixture of 0.2- and 0.8-micrometer polystyrene latex
(PSL) in the upper tube, 0.8-micrometer PSL in the middle tube, and 0.2-micrometer
PSL in the lower tube. Equivalent samples in the ground-based results show the
detrimental effects of gravity-induced convection and sedimentation.

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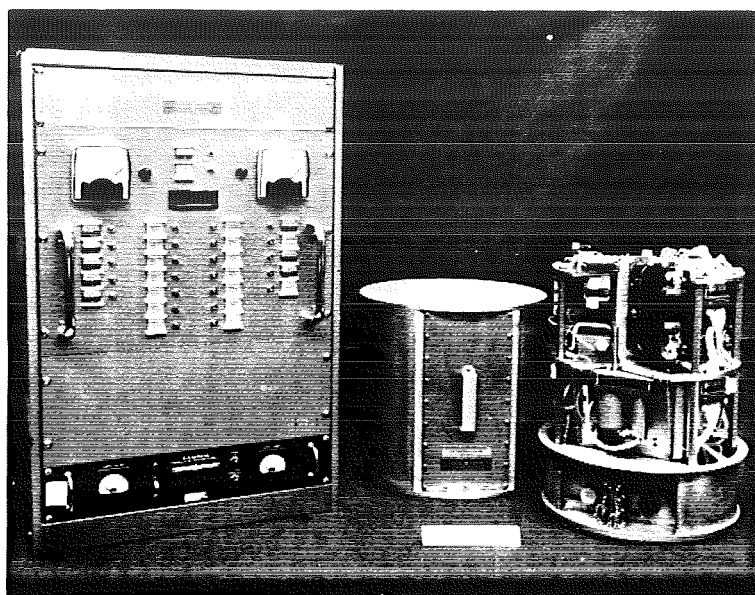
Figure 6.- Advanced applications flight experiment (AAFE) continuous-flow
electrophoretic separator (right) under development for use on a sounding rocket.
The upper enclosure (middle) with an access door for installing the sample, servicing
the camera, and setting experiment conditions before flight; and the test and control
panel including power supply (left) are also shown.

INTERDEPENDENCE OF CELL VARIABLES

*QUANTITIES EASILY CONTROLLED
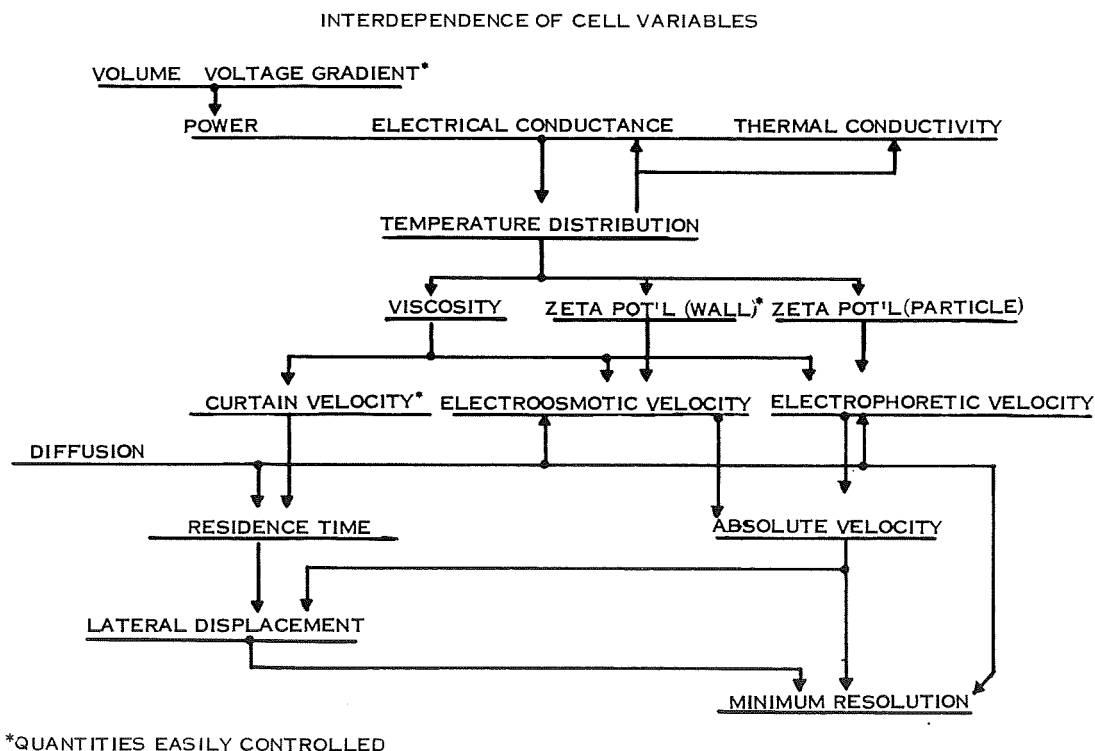
Figure 7.- Major features of a computerized mathematical model of a continuous-flow
electrophoretic separator. (Controllable variables are indicated by asterisks.)

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CELL: 10CM X 5CM
THICKNESS: 0.07CM
SAMPLE DIAM: 0.06CM
FIELD: 40V/CM
WALL POT'L: 5MV
PART. POT'L: 25, 29, 30, 34MV
CENTERLINE VELOCITY: 0.032CM/SEC
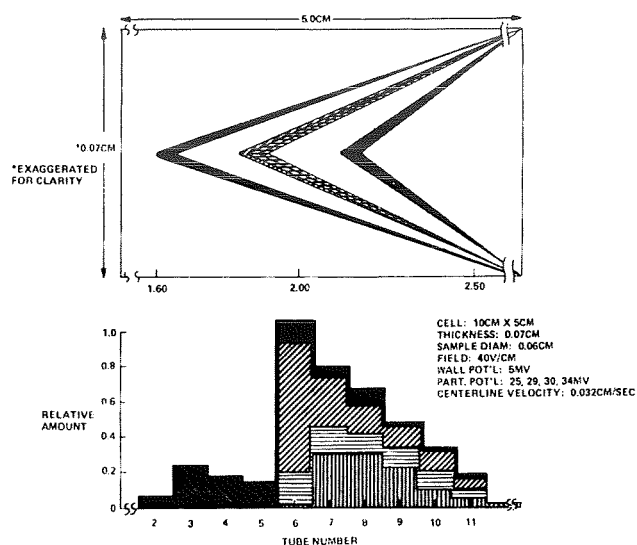
Figure 8.- Illustration of calculations for a four-component separation in a 0.5-mm-thick continuous-flow electrophoresis cell. (Note expanded scale; other assumed conditions as indicated.)

CELL: 10CM X 5CM
THICKNESS: 0.16CM
SAMPLE DIAM: 0.06CM
FIELD: 40V/CM
WALL POT'L: 5MV
PART. POT'L: 26, 29, 30, 34MV
CENTERLINE VELOCITY: 0.035CM/SEC
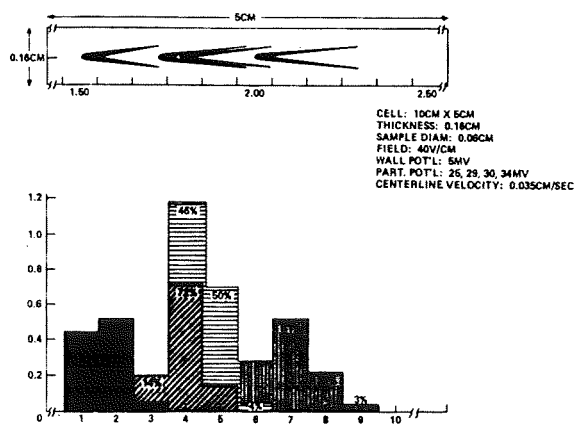
Figure 9.- Illustration of a calculated resolution for the same four-component separation as figure 8 in a 1.5-mm-thick cell.

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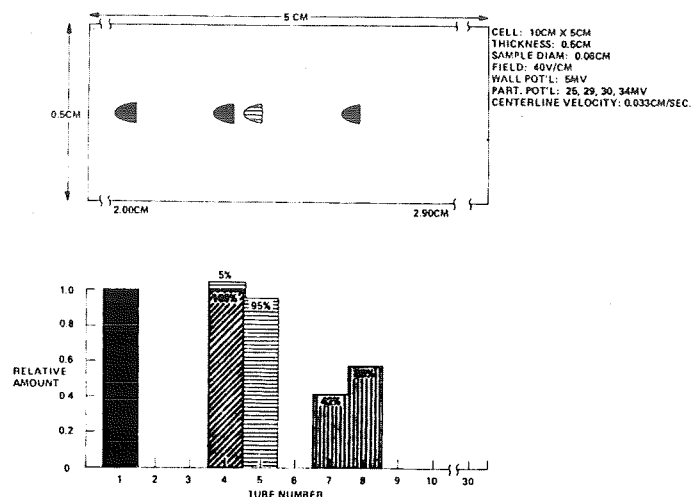
Figure 10.- Illustration of calculated resolution for the same four-component separation as figures 8 and 9 in a 5-mm-thick cell.

ACTIVE AREA 5CM X 10CM
SAMPLE: NEUTRAL DENSITY POLYSTYRENE LATEX
ORIENTATION 20° FROM VERTICAL
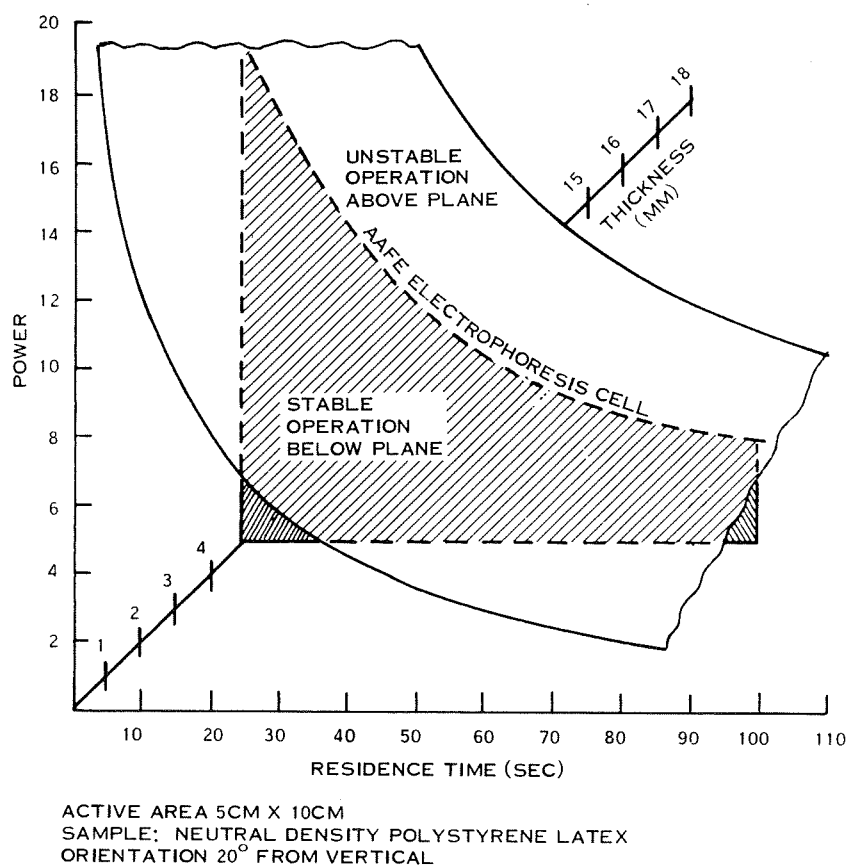
Figure 11.- Experimentally determined regions of stable and unstable operation of the AAFE (fig. 6) electrophoresis cell as a function of power and residence time for a 5-mm-thick cell with other conditions estimated.

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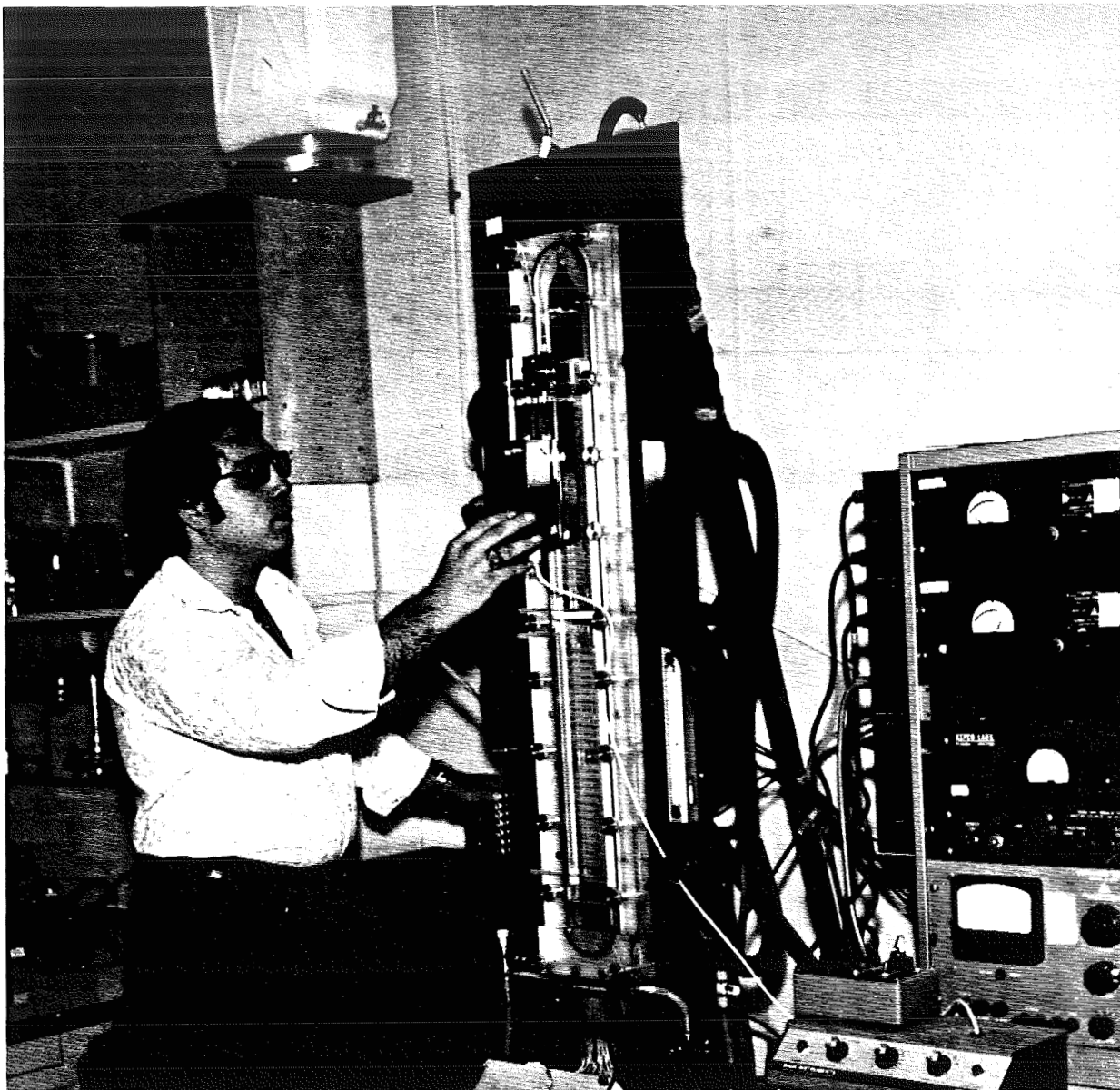
Figure 12.- Modern laboratory continuous-flow electrophoretic separator.
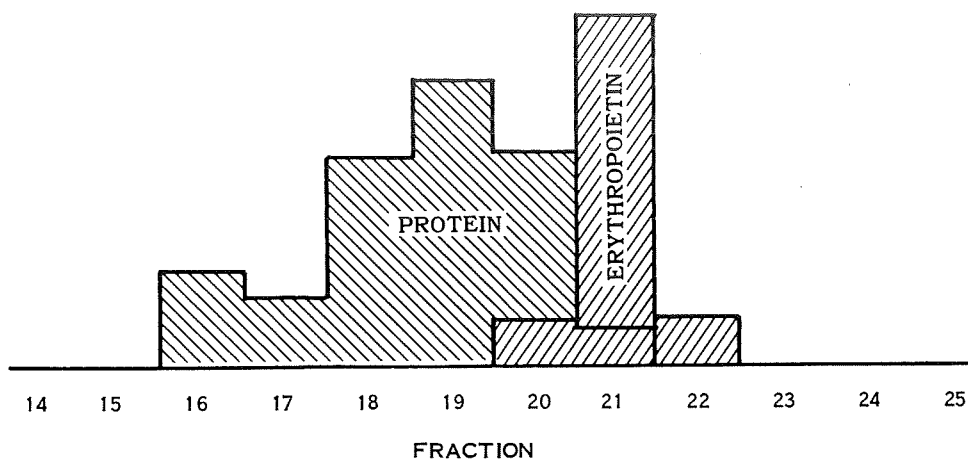
Figure 13.- Partial concentration of Erythropoietin from protein by electrophoresis.
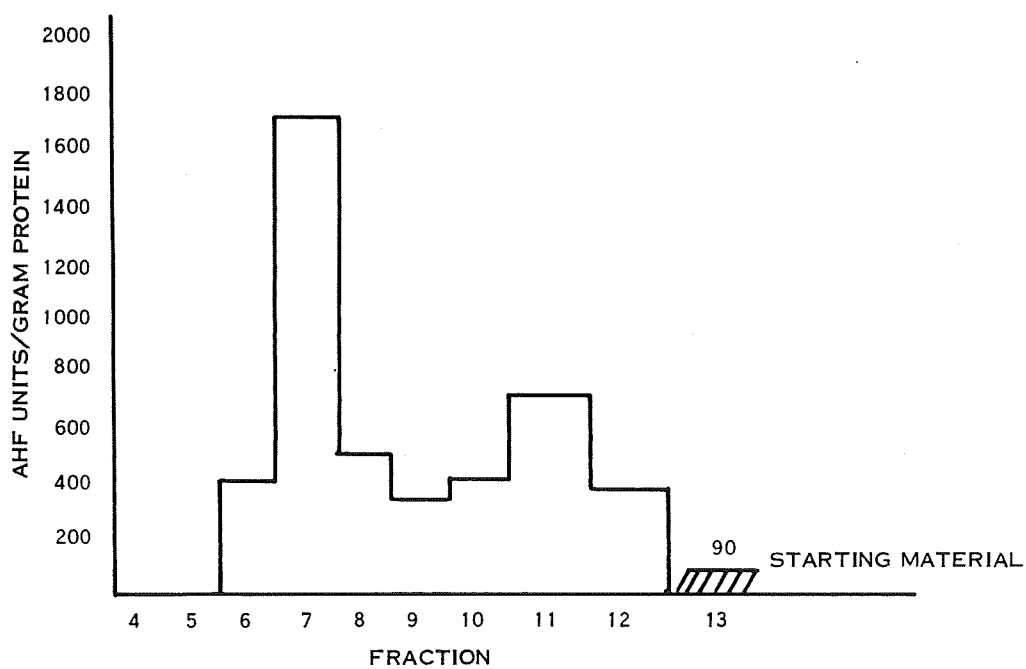
Figure 14.- Concentration of antihemophiliac factor VIII by continuous-flow electrophoresis.
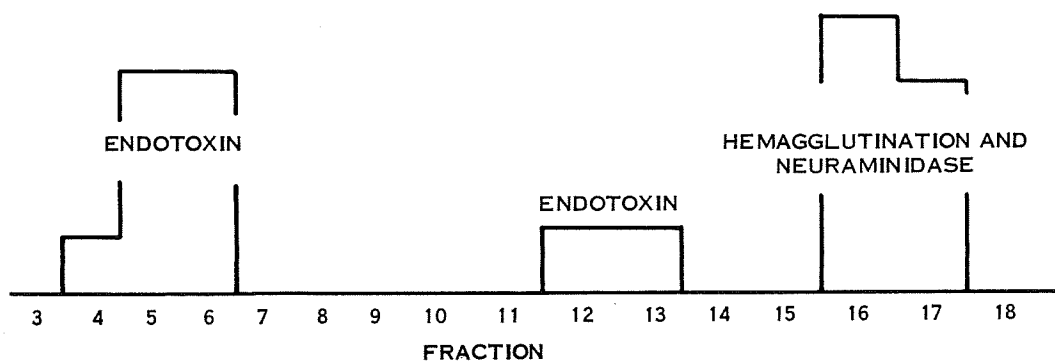
Figure 15.- Separation of influenza virus antigen from endotoxins by continuous-flow electrophoresis.
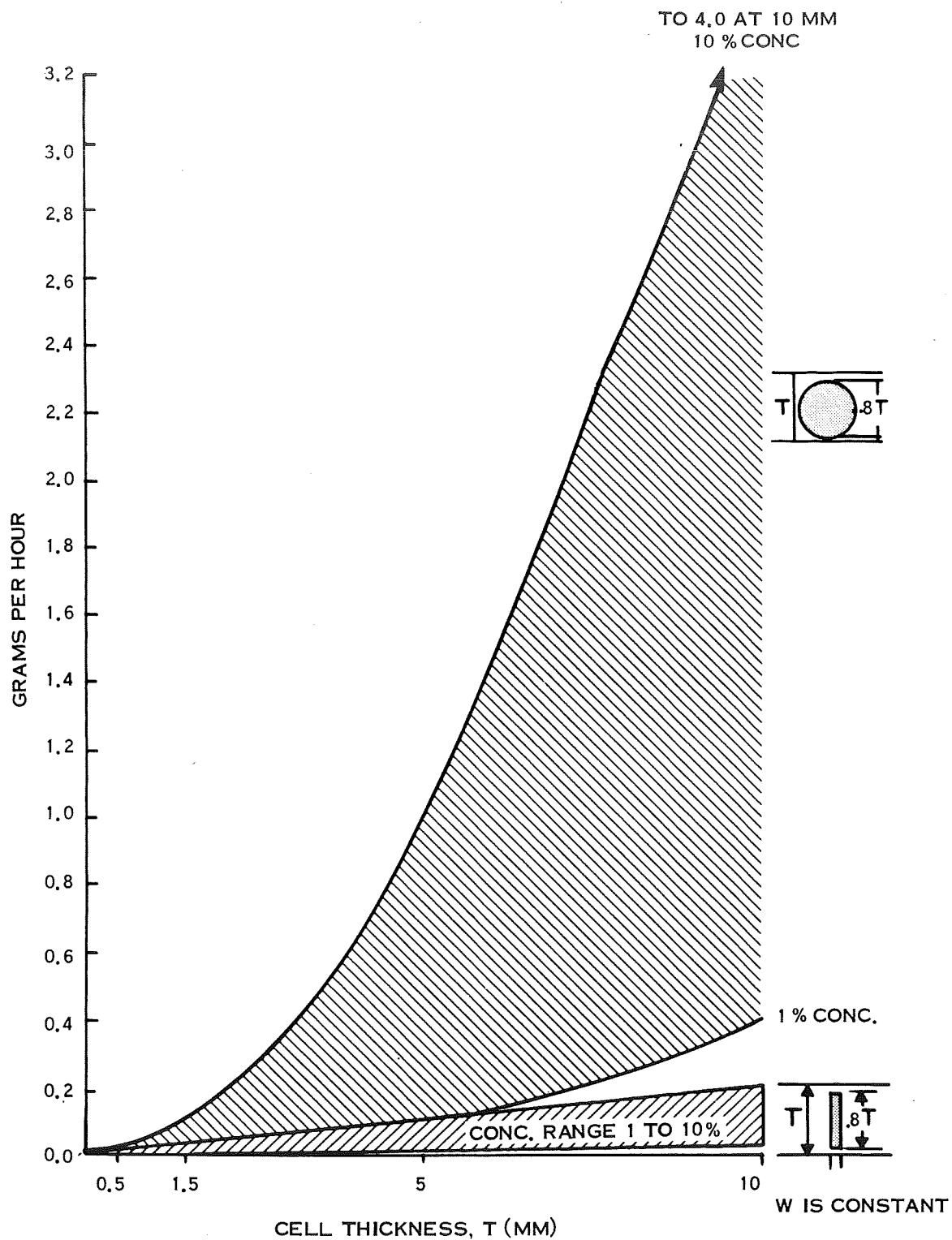
Figure 16.- Calculated throughput for continuous electrophoretic separators as a function of cell thickness and sample concentration for high-resolution (lower curves) and high-throughput examples.

SOME QUESTIONS OF SPACE BIOENGINEERING

BY

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COLLOQUIUM ON BIOPROCESSING IN SPACE

JOHNSON SPACE CENTER, HOUSTON, TEXAS
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SOME QUESTIONS OF SPACE BIOENGINEERING

BY

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...for the chief malady of man is a restless curiosity about things which he cannot understand; and it is not so bad for him to be in error as to be curious to no purpose.

PASCAL (Pensees, Section 1, #18)

ABSTRACT

Zero-gravity offers selective effect on growth and metabolic activity of unicellular organisms as well as unique opportunities in purification of organic compounds. These make it possible to consider the biosynthesis and recovery of certain metabolites economically feasible in space.

Design, construction and operation of systems for the above mentioned purposes requires interdisciplinary actions within the scope of a new discipline: space bioengineering. Paper discusses the problems and perspectives of this discipline particularly in the application of bio-reactor-recovery systems in space to manufacture metabolites of high economic and scientific value. Special attention is paid to pivotal factors such as various mass transport phenomena, contamination control, automatic control of optimum environment and synchronization of the operation of the biological (biosynthesis) and the physicochemical (recovery-purification) systems. Although the space bioengineering is in its early stage of development, its role will gradually increase with the full implementation of the Space Bioprocessing Program.

INTRODUCTION

In the course of technical developments, we always witness that the human knowledge on the physical behavior of inorganic materials precedes the information on biological systems. This tradition was observed again, most recently, during the Apollo and Skylab experiments which demonstrated significant changes in the physical behavior of fluids and gasses exposed to zero-gravity (1).

On the other hand, certain experiments related to the search of effects of gravitational acceleration, cosmic radiation and weightlessness on living systems revealed, among others, the possibility of culturing unicellular organisms in space. Increased specific growth rate and higher cell density obtained with S. typhimurium (2) as well as higher frequency of induction of bacteriophage in E. coli K-12 (3) indicated improved metabolic activities probably due to altered physicochemical properties of the liquids and gases under near weightless conditions.

These results initiated a study by JORDAN (4) concluding that there is a possibility to enhance the productivity of certain organic compounds if the biosynthesis takes place in space.

Most recent experiments with zero-gravity electrophoresis revealed the possibility of fine separation and (ultra) purification of biological materials (1).

These basic experimental data forecast a potential research activity and industrial application in space environment for processing biological materials. A new discipline seems to shape up which may be called as "space bioengineering".

Objective of this paper is to analyze some of the major questions related to this technology. It is emphasized, however, that the potentials and limits of this discipline have not been identified yet. All of our discussion is based on a few experimental data in space and the knowledge accumulated, mostly during the last 30 years, in biochemical engineering. It is felt, however, that this study gives some basis which can be utilized by space processing scientists during the implementation of Space Bioprocessing Program.

SPACE BIOENGINEERING

Unique properties of the space environment (particularly gravitational effects, cosmic rays) interactively influence the bioprocesses which, henceforward, requires hardware and technology substantially different from the ones employed on the Earth. Space bioengineering is concerned with the hardware design and technologies related to processing of organic compounds by means of biosynthesis and recovery-purification, at least one of which takes place in space.

As a discipline (Figure 1) space bioengineering is closely related to biochemical engineering (5) which generally deals with the theory and practice of bioconversion of materials and their recovery from a culture liquid. It has also a relationship with space microbiology (6) which deals with extra terrestrial detection of microorganisms, evaluation of behavior of terrestrial microorganisms in space and monitoring of spacecraft and astronaut microbial flora. The discipline has a close relationship with the material processing sciences specially utilizing the knowledge on unique behavior of liquids, gases and solids in zero-gravity.

Table I lists the main areas of concern related to activities in space bioengineering. At this time, we shall address ourselves only to few key questions closely related to implementation of NASA Life Sciences Program in Space, particularly the early stage of Space Shuttle and Spacelab experiments. It is anticipated, however, that gaining further practical information, the scope of discussions will broaden incorporating such questions as experimental trial of bioprocessed material in space for quality control purposes.

PROCESS DESIGN FOR SPACE EXPERIMENTS

A major objective for the initial stage of Bioprocessing Program is the demonstration of usefullness of space biosynthesis and biochemical separation techniques. Implementation of biosyntheses and recovery-purification processes in space, however, faces constraints from the viewpoint of payload, in particular regarding the requirement of relatively large quantity of water during each step of the operation. Another important constraint is the maintenance of aseptic condition during the culture and product recovery.

Usefullness of bioprocessing can be demonstrated in production of one or more organic compounds of high scientific or medical value in a quantity applicable for at least experimental purposes.

In an attempt to define the most promising materials, Table II lists various organic compounds currently produced by means of biosynthesis and biochemical recovery techniques on laboratory or industrial scale. Each process represents a type of metabolic pattern and has attractive features from experimental point of view. Accordingly,

1. Production of cell mass (SCP) or ETOH on carbohydrates can be the subject of experiments of shifting metabolic pathway in favor of one product accumulation (7),

2. Biosynthesis of gluconic acid from glucose is a classical example of combined and staged activity of various cell-bound, cell-free enzyme activities as well as nonenzymatic conversion of an intermediate into final product.

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Besides, the process is well known, therefore, comparative studies can be easily made.

3. Production of oxytetracycline (OTC) has the combined characteristics of the former two processes (with the exception of nonenzymatic catalysis step). In addition, the problem of contamination is greatly reduced because of the wide spectrum of the antibiotic activity.

4. Biosynthesis of vitamin B_{12} is an example of mixed culture operation incorporating complex growth and product formation kinetics.

As it is noted on Table II with the exception of the first process, product recovery can be implemented either by chromatography or by electrophoresis.

On the other hand, the absolute (scientific, commerical) value of products #1 – #4 is low, whereas the desired quantity for application is relatively large. Even in the case of substantial improvement in biosynthesis (assuming fourfold increase in space) the needs can be fulfilled only with moving of large quantities of water.

Because of these considerations, any of the processes has short range of applicability and scientific value only in the testing stage of space biosynthesis and recovery equipment.

Product #5 has the advantage of experimental trial of eucaryote cell growth exposed to space environment as well as production of compounds of scientific and medical significance. In particular, production of growth hormones (GH), adrenocortical steroid hormones, thyrocalcitonin and parathyroid hormone may be listed here as prime candidates. In addition, electrophoresis is considered as the best means in separation of the protein and polypeptide compounds from the culture medium components (e.g. from serum). POSNER, in a short discussion (8), describes the most recent achievements (notably, direct relationship between cell mass and GH production, suspension culture of pituitary tumor cells, enhancing effect of hydrocortisone on GH production, release of hormones into the extracellular liquid). With a potential increase of cell density to 10^{12} cells per liter from 10^9 cells per liter, gonadotropin hormone production can be substantially augmented (a cautious estimation is a fourfold increase in GH production). HIMMELFARB and his coworkers already reported 10^8 cells per ml in a perfusion-suspension apparatus (9).

On the basis of the first experiments in space relative to fluid and gas mixing conditions, it is anticipated that the enhanced oxygen transfer will improve the cell metabolic activity leading to increased cell number, hence larger hormone production. In case of achieving

10^{12} cells per liter, a semicontinuous culture can produce about 2 G GH/L/24 hours for further recovery and purification. Also changes in normal human cells "anchorage dependency" can be anticipated in zero-G, making production of hormones by non-malignant cells possible.

EQUIPMENT DESIGN

GENERAL CONSIDERATIONS

According to experiences accumulated in biochemical engineering the following main rules must be observed in the hardware design:

1. Systems integrity. The entire system consists of three major elements: 1) Biosynthesis equipment, 2) Recovery equipment and 3) Process support subsystem. From operation point of view all those elements are considered as one unit. This principle defines the type and number of monitoring and control elements as well as the mode of operation of the system.

2. Systems flexibility. At the beginning of the experiments, particularly in the test stages at least three types of reactions can be visualized: 1) Fast, enzymatic conversion of compound A into B (where compound B is the subject of recovery), 2) Relatively fast microbiological process (doubling time = 20-60 minutes, product formation rate (dP/dt) = > 5G/L/HR) and 3) Relatively slow process (doubling time = 10-20 hrs, product formation rate < 5G/L/HR).

   Accordingly, the internal design and instrumentation of the biosynthesis subsystem must allow sufficient interchangeability which makes the implementation of enzymatic reactions (CSTR), microbial fermentations and animal cell suspension cultures possible. It is necessary to note, that in all cases, the recovery sub-system (EFO) is unchanged. This makes the application of a "buffer subsystem" necessary. This system couples or uncouples the biosynthesis and recovery subsystems depending on the differences between the kinetics of biosynthesis and the recovery.

3. Automatic operation. Because of the anticipated workload aboard the Spacelab, the system must contain extensive electronic monitoring, process analysis and control equip-ment which alleviates the necessity for scientists to manually operate the equipment. This question, considered to be im-

portant, both from design and process implementation points of view, will be discussed in a subsequent chapter.
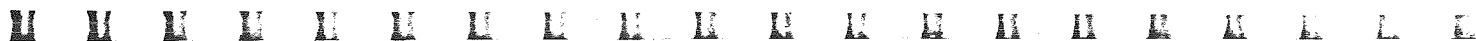
SPACE BIOPROCESSING SYSTEM

Figure 2 contains a basic concept of "convertible" equipment useable as CSTR for enzymatic reactions, as well as for culturing cells in suspension. In a typical process, culture medium in Culture Vessel, 1 is seeded from a Seed Chamber. Environmental conditions are maintained according to the physiological status of the culture. Upon a condition (maximum product concentration, nutrient depletion) the culture liquid (or reaction mixture) is transferred to a dialysis system, 2 (10) where the product (among other organic compounds of same cut-off molecular weight) is removed and stored in a reservoir, 3. The non-dialyzed part of the culture is either recycled or discarded depending on the type and physiological stage of the process. Also, fresh nutrients and/or inducers can be added assuring (semi) continuous operation. The reservoir serves as a "buffer tank" if capacity problems take place in the recovery system, which is an electrophoresis apparatus.

Specific emphasize is given to the on-line measurement of process variables. With the exception of wet chemical analysis all sensed variables have computer compatible output for further data analysis.

Wet chemical analysis is performed from time to time, however, with the accumulated knowledge on the process correlations can be found between wet chemical analytical data and direct sensorial analysis. In a more advanced form, pivotal process variable(s) (11) will govern the process. This requires computer analysis and control of the entire operation.

In particular, the system is sterilized by ethyleneoxide/CO_2 gas-mixture prior to use. This is a definite deviation from the "classical" fermentation practice where steam is the primary sterilization agent. The contamination control is twofold, namely:

a) excluding foreign microflora penetration into the system,

b) controlling the spread of the culture content in the working area.

In view of the most recent findings on fluid mechanics and fluid gas interface phenomena in zero-gravity conditions, the most problematic area is the assurance of proper liquid flow and mixing conditions. This question is yet to be further analyzed.

PROCESS CONTROL

Depending on the systems configuration and the type of the bio-processing, the control of the entire process is implemented on two levels:

1) Process kinetics control,

2) Systems operation control.

The question here is the proper definition of the pivotal process variables around which the process control can be built. This requires extensive on-line, real-time analysis of the process resulting in definition of the physiological stages and the overall process kinetics.

PROCESS KINETICS ANALYSIS AND CONTROL

Analysis of the process condition can be performed introducing the signals of the on-line operating sensors into a computer which further processes the data by multivariation of the individual process variables. Figure 3 presents the logic of such an operation. According to our experiences with a highly instrumented, computer coupled pilot-plant fermentor, information related to the gas exchange conditions was found useful in detection of the physiological conditions of the culture (12).

As an example, Figures 4 and 5 show an on-line, real-time follow-up of a C. utilis culture's gas exchange condition. In this case, among other process indicators, RQ was computed which has direct correlation with the cells' physiological conditions. During the operation, samples were taken and analyzed by wet chemical analytical techniques.

Chemical analysis of the culture revealed significant correlations between RQ and some biochemical events including: 1) nucleic acid, 2) protein, and 3) ethylalcohol synthesis (Figure 5).

The drop in RQ value during the elapsed time period of 1-3.0 hours coincided with the increase in specific nucleic acid content of the culture, while the minimum RQ (EFT = 3-4 hrs.) coincided with the start of increased specific protein concentration. The increase in RQ value in the fourth hour coincided with the start of ethylalcohol formation (shaded area). Correlation obtained between the culture's metabolic activity detected by wet chemical methods and the respiratory quotient obtained via computer operation demonstrate that the latter, after the definition of correlations, can be used to determine certain transition conditions in eucaryotic cell cultures.

As a consequence, process status indicator such as RQ can be used to control the optimum environmental condtions during the culture. Figure 6 shows a double control loop concept to implement interactive control of individual process variables (T, N, Q, P, etc.: inner control loop) the composite of which creates the environmental conditions. Alteration of setpoints on the individual process controllers is based on the status of the process obtained through an analysis of the available information on culture rheology, physiology and metabolic activity (outer control loop).

In the above mentioned particular case, the culture's RQ served as process status indicator and QO_2, QCO_2 were used to define the process kinetics. On this basis carbon and nitrogen compounds were fed in an optimum proportion resulting in suppression of ethyl-alcohol and increase in protein biosynthesis. By this means, environmental conditions were optimized and a fourfold increase in growth rate was obtained (7).

SYSTEMS OPERATION CONTROL

According to the principle of systems integrity the operation of bio-synthesis, recovery and support subsystems will be coordinated. Figure 7 shows a concept of the systems operation control. This assumes analysis of status both for the biosynthesis and for the recovery part of the process. As it was shown, the biosynthesis stages and the kinetics can be analyzed and controlled on-line, real-time. This part of the process is considered, however, to be the most complex one exposed to unexpected disturbances. In the case of product recovery (dialysis and EFO) liquid flow rates and material concentration are considered to be the pivotal variables.

Information on status of both processes is fed into the controllers of process support system. This assures the proper rates of gas and liquid removal and purification as well as defines the availability and supply of utilities. It is well known that the microbiological processes are water extensive. Therefore, particular care must be exercised to assure the proper water recycling schedules.

The task of construction of a system with such a complexity and the payload constraints require application of microprocessors for process control purposes. These are programmed according to findings while using minicomputers in process analysis and controls of biosynthesis and recovery systems during the test stages.

CONCLUSIONS

The above mentioned examples and design considerations indicate that implementation of a Space Bioprocessing Program is technically feasible.

In addition, there are three general comments worth emphasizing with regard to this project.

First, the Program has to demonstrate the economic and/or scientific value of bioprocessing under zero-gravity conditions. This is the question of well defined process(es) and well designed equipment. Presently, there are guesses in this area and only the experimentation will give us proper answers. We have to recognize, however, that because of the unique properties of fluid-gas mixing, new process and equipment designs are an absolute necessity.

Second, we all know that the majority of the presently existing fermentation plants are constructed with more respect toward material selection, motors, pipes than regarding the importance of cells' physiology in the biosynthesis. Whatever the outcome of the Spacelab experiments will be, information obtained on the cellular metabolism can be used as source for construction of more bioprocess oriented fermentation plants on the Earth.

Third, in case of success, the bioprocessing equipment constructed for Space application, will serve as a prototype of an ultramodern fermentation system which can open new areas for the industrial microbiology.

I believe that with proper interdisciplinary efforts, we shall achieve those goals which will be beneficial both for science and the industry.

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LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate,
CSTR	Continuously stirred reactor,
D_i	Diameter of impeller,
DEDT	Rate of ethylalcohol (C_2H_5OH) biosynthesis,
DO	Dissolved oxygen concentration,
DPRDT	Protein biosynthesis rate in the culture liquid,
EGA	Exit gas analysis,
k_La (KLA)	Oxygen mass transfer coefficient,
N	Agitation speed,
NA	Nucleic acid content of the culture liquid,
NADH, NADH + H^+	Pyridine nucleotide content of cells (oxidized, reduced forms),
N_{Re} (NRE)	Reynolds number,
ORP	Oxidation-reduction potential (eH),
OXUP	Oxygen uptake rate of the culture,
P	Vessel head pressure,
P·	Product formation rate (dP/dt),
Q_n	Air (gas) flow rates,
QCO_2	Specific CO_2 release rate,
QO_2	Specific O_2 uptake rate,
RQ	Respiratory Quotient,
S, S_1	Substrate, C_6 sugar,

LIST OF ABBREVIATIONS (CONTINUED)

S_2, S_n Nutrients,

T Culture liquid temperature,

T_d Doubling time,

V Culture liquid volume,

W Energy uptake for fluid mixing,

WCA   Wet chemical analysis,

X Cell mass,

$\dot{X}$ Growth rate,

GREEK LETTERS

μ Specific growth rate

ρ Liquid density

TABLE 1

MAIN AREAS OF CONCERN IN SPACE BIOTECHNOLOGY

PROCESS DESIGN

    UNIT PROCESSES
    UNIT OPERATION
    CONCERTED OPERATION OF THE SYSTEM ELEMENTS

EQUIPMENT DESIGN

    CULTURE VESSELS
    PRODUCT RECOVERY AND PURIFICATION SYSTEMS
    INSTRUMENTATION
    SUPPORT SYSTEMS

ASEPTIC OPERATION

    MEDIA AND GAS SUPPLY STERILIZATION
    BIOHAZARD CONDITIONS

PROCESS CONTROL

    KINETICS
    CONTROL OF ENVIRONMENTAL CONDITIONS
    SYSTEMS OPERATION CONTROL

PROCESS MANAGEMENT

    ECONOMICS OF OPERATION
    EARTH-SPACE COOPERATION
    SCALE-UP IN SPACE

TABLE 2

ANALYSIS OF PROCESSES FOR SPACE BIOTECHNOLOGY

	PRODUCT	PROCESS	MAIN PRODUCTS PRIMARY (CELLS)	SECONDARY	TYPE OF RECOVERY #1	#2	VALUE[A] #1 $	#2	ANNUAL REQUIREMENT[B] (ESTD)
1.	SCP/ETOH	C.UTILIS SUBMERGED	25 G/L	ETOH:80 G/L	FILTR.	DIST.	1.9×10^{-3}	2×10^{-2}	10^6 T
2.	GLUCONATE	P.OVALIS SUBMERGED	3 G/L	GA:40 G/L	FILTR. DIAL.	CHROM. EFO	NIL	1.9×10^{-2}	10^4 T
3.	OXYTETRA-CYCLINE	S.RIMOSUS SUBMERGED	8 G/L	OTC:30 G/L	FILTR. DIAL.	CHROM. EFO	NIL	2.5	10^3 T
4.	VITAMINE B_{12}	MIXED CULTURE SUBMERGED	6 G/L	B_{12}:0.3 G/L	FILTR. DIAL.	EFO	FEED	4.7	10^2 T
5.	GROWTH HORMONES	THYROTROPH MAMMOTROPH CELLS	10^9/L	GH:0.5 G/L	DIAL.	EFO	?	375	0.1 T

(A) PRODUCT VALUE ESTIMATED FOR PROCESSING ONE LITER CULTURE LIQUID WITH A RECOVERY LOSS OF 25%.

(B) ESTIMATED POTENTIAL NEED IN USA.
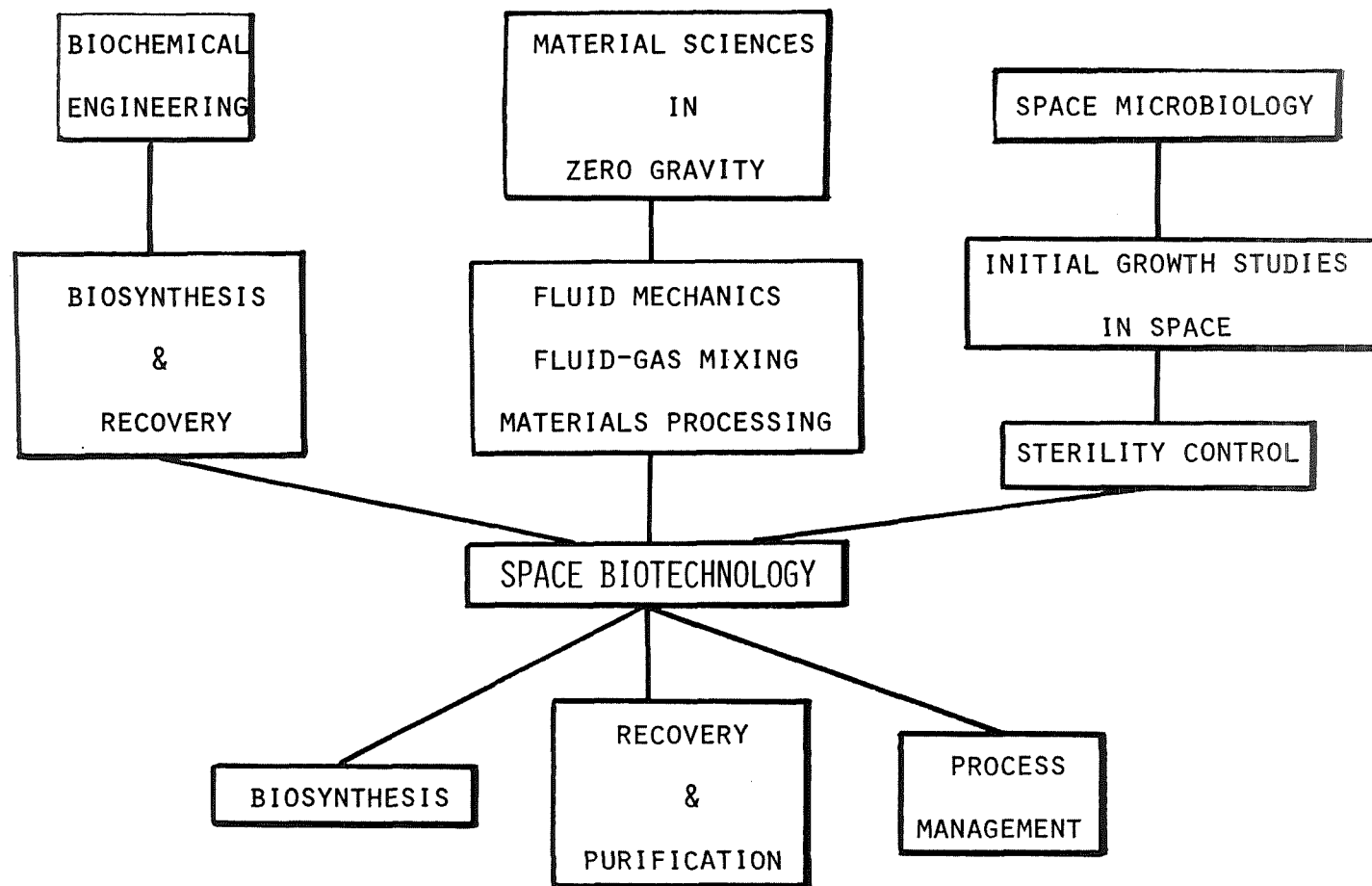
Figure 1.- Relationship between space biotechnology and other disciplines.

INLET GASES N_2, O_2, CO_2

SEED

RECYCLE

INDUCER

ACID   BASE

EGA

GAS PURIFICATION

O RESERVOIR   FRESH NUTRIENT

FEED

1

(SEMI)CONTINUOUS OPERATION

WCA

WATER IN

LEVEL SENSOR

INSTRUMENTATION

TEMPERATURE
PRESSURES
AGITATION SPEED
GASFLOW RATES
LIQUID FLOW RATES
ORP
DO
pH
CELL DENSITY
SPECIFIC ION CONCENTRATION
   (pCO_2, pNH_3, $PO_4{}^{3-}$)
C_6 ANALYZER
EXIT GAS ANALYSIS (EGA)
WET CHEMICAL ANALYSIS (WCA)

2 DIALYSIS

M

RECYCLE

WCA

RESERVOIR FOR MAIN PRODUCT *3*

WATER/SOLVENTS

RECOVERY *4*

WCA

WATER PURIFICATION

DISCARD OF NONDESIRED PRODUCTS

RECYCLE

FINAL PRODUCT COLLECTION *5*

Figure 2.- Design concept of bioprocessing equipment (suspension culture).
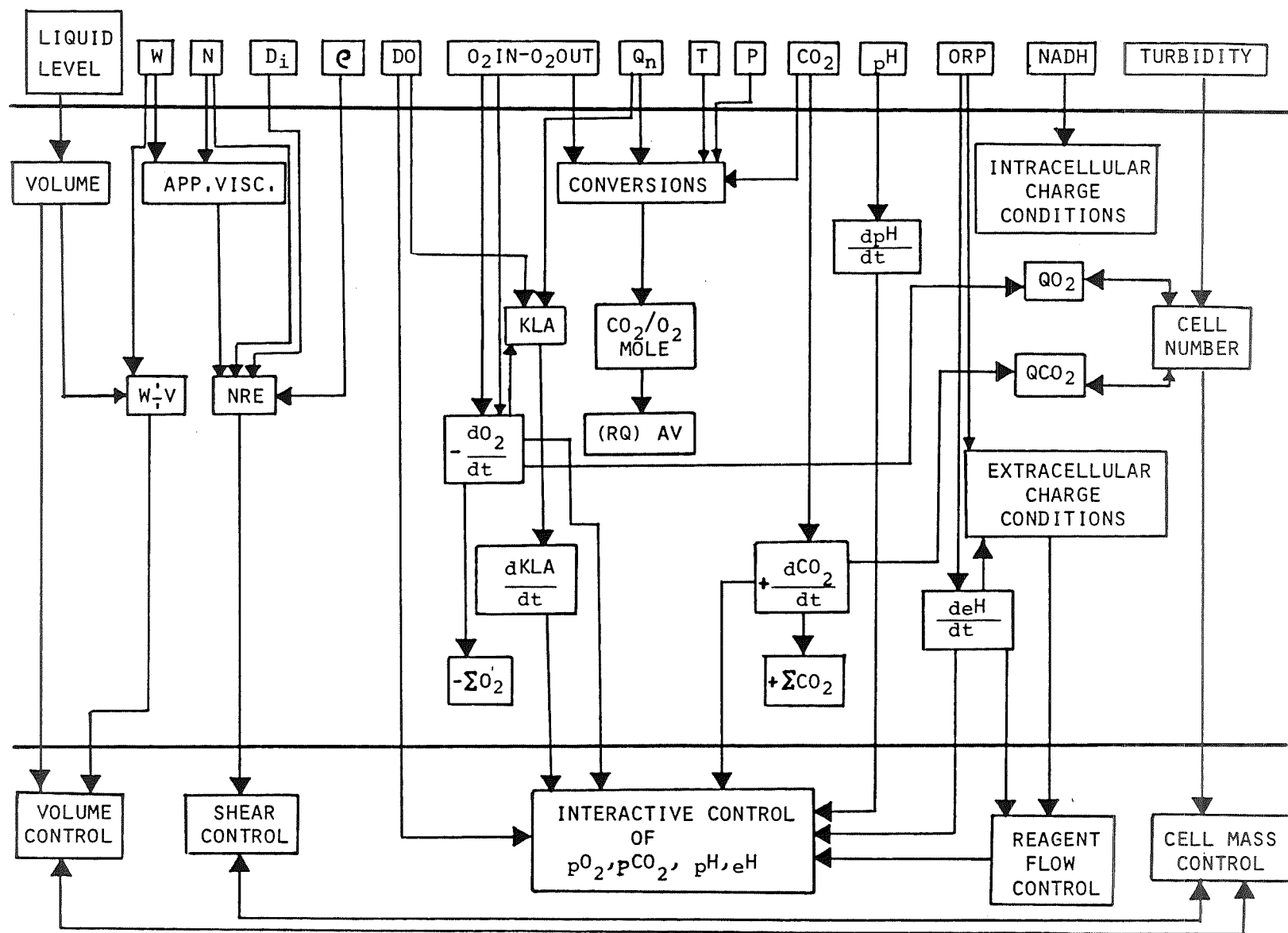
Figure 3.- Flow chart of electronic process analysis of biosynthesis processes (symbols explained in list of abbreviations).
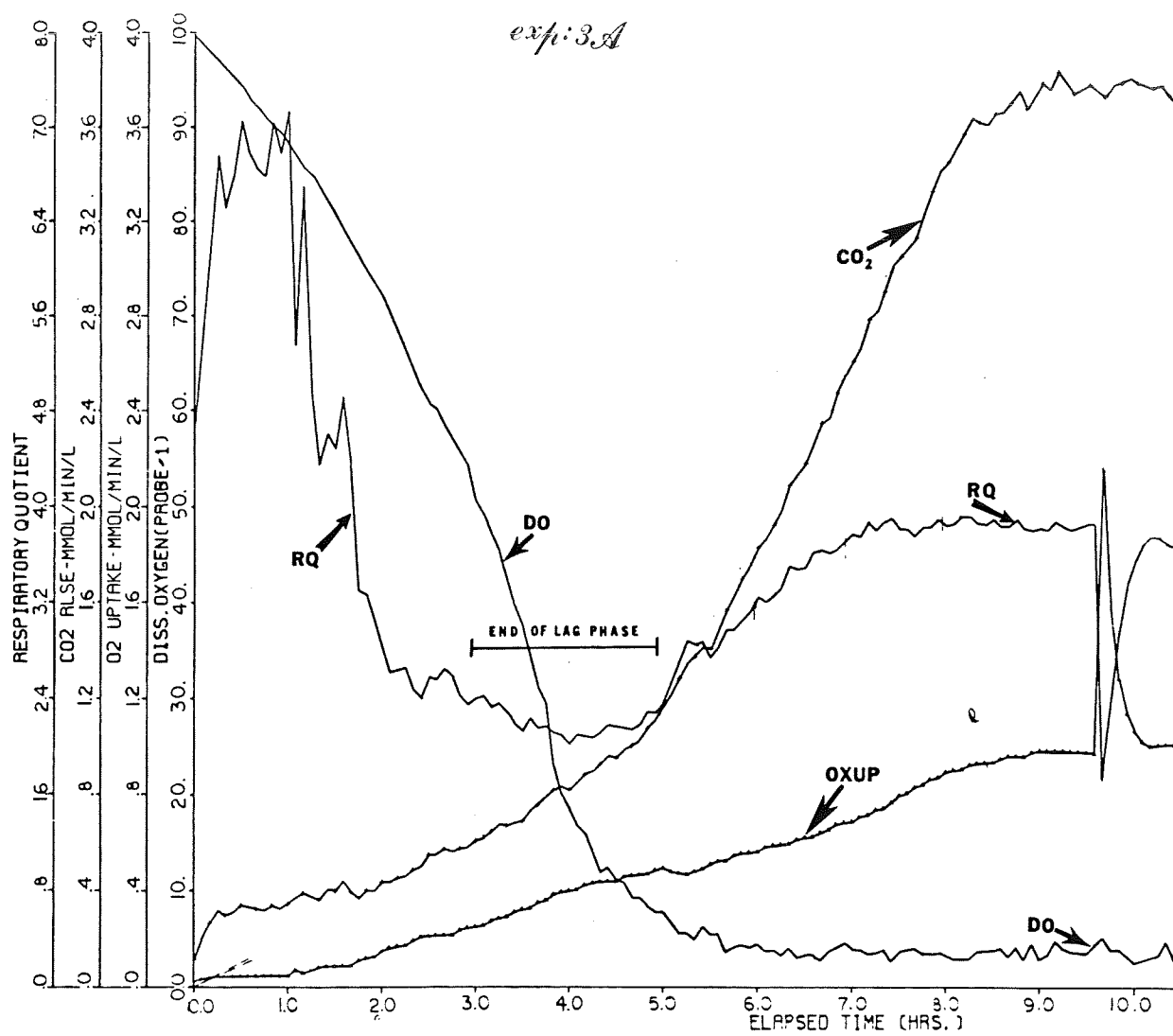
Figure 4.- On-line real-time determination of the physiological condition and process kinetics of an eucaryote cell culture.

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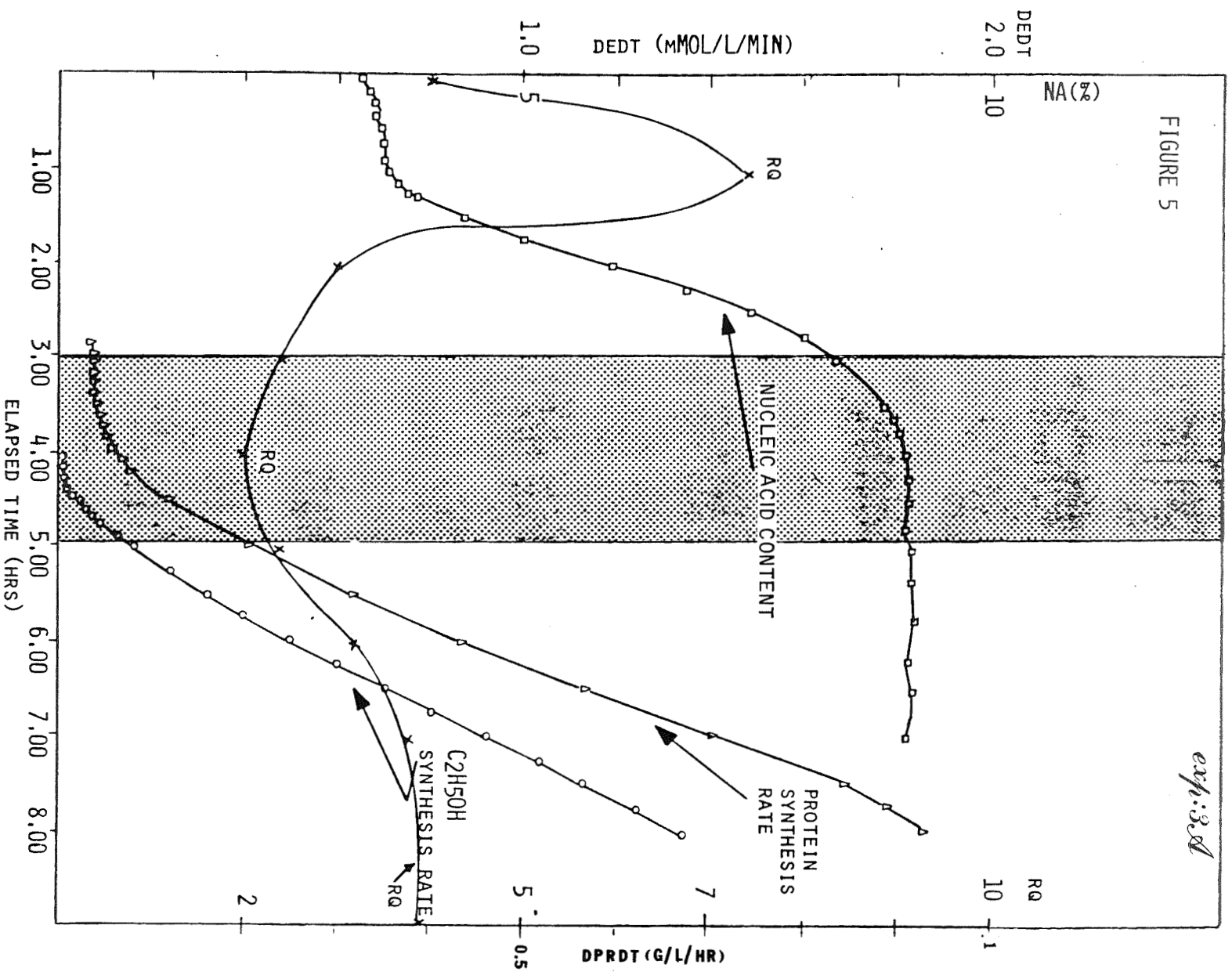
Figure 5.— Metabolic activity of _C. utilis_ in lag-log transition.
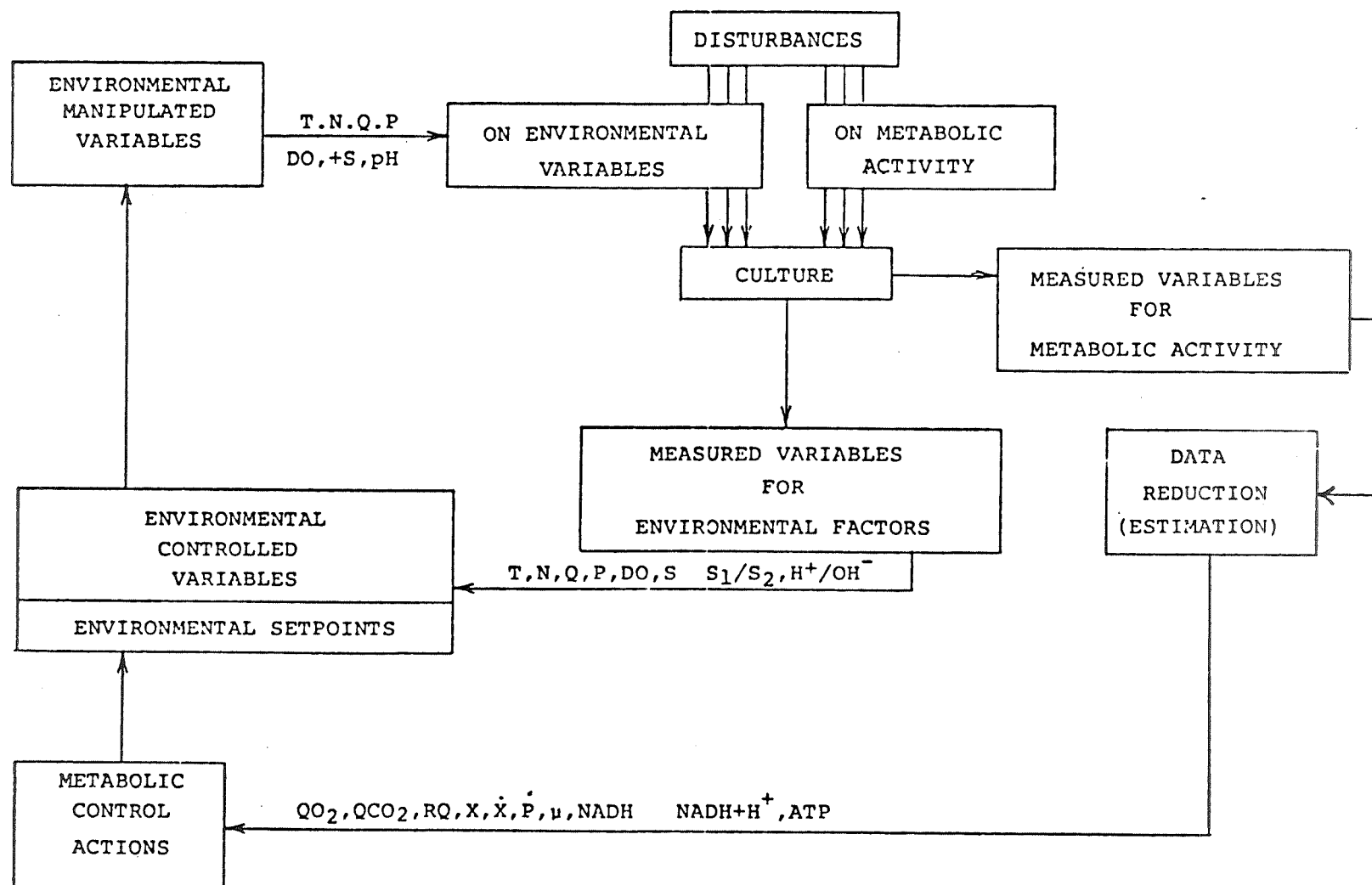
Figure 6.- Basic concept of biological interactive control.
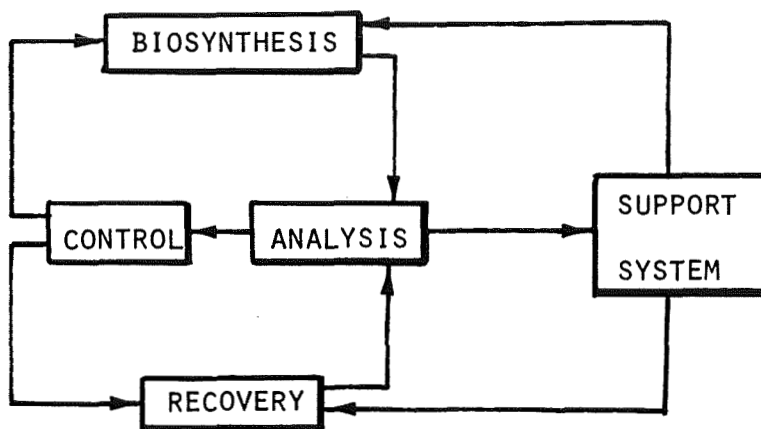
Figure 7.- Concept of systems operations control.

INFLUENCE OF ZERO-G ON SINGLE-CELL SYSTEMS AND ZERO-G FERMENTER DESIGN
CONCEPTS

By Jerry V. Mayeux, Bio Innovar, Inc., Storm Lake, Iowa

INTRODUCTION

Exploratory biological studies on the effect of the space environment on biological systems, including microorganisms, have been carried out on the United States' Discoverer, Biosatellite, Gemini, Apollo, Skylab, and ASTP programs and on several Russian satellites. In many cases, the results were incomplete or inconclusive; however, significant observations with respect to microbial interactions with the space-flight environment have been made. The data obtained have evoked considerable interest to continue a space-flight experimental program (ref. 1).

The advent of the shuttle era presents the first multiple-flight opportunity for performance of comprehensive and well-controlled space-flight experiments. Early shuttle payloads such as the Long-Duration-Exposure Facility (LDEF) and Biomedical Experiment Scientific Satellite (BESS) may provide opportunities for bioscientists to initiate experimental programs that can be expanded and continued when the shuttle dedicated labs become operational. These first-generation experiments are a necessary step in the process of developing an orderly space microbiology application. Early investigations can be accomplished by passive growth experiments involving a selection of viable organisms over an extended period of time. The results of these experiments could lead to developments that would eventually provide beneficial exploitation of the space environment for unique application in a space microbiology program.

An analysis has been made to identify potential gravity-sensitive mechanisms that may be present in the single-cell growth system (ref. 2). Natural convection (density gradients, induced sedimentation, and buoyancy) is important in microbial systems. The absence of natural convection in the space-flight environment could provide an opportunity for new approaches for developments in industrial fermentation and agriculture.

Some of the potential influences of gravity (i.e., convection, sedimentation, etc.) on the cell will be discussed to provide insight into what experimental areas may be pursued in future space-flight research programs.

INFLUENCE OF A CONVECTION-FREE ENVIRONMENT ON SINGLE CELLS

Mass and energy transport depends primarily on diffusion and convection in single-cell systems (ref. 3). Migration or relocation of products and substrates from one point of production to a point of utilization can be critical to the cell, especially in synthetic processes (refs. 4, 5, and 6). Some of these requirements and their interdependence on diffusion and convection are shown in table I.

Further evaluation of the cell functions identifies other potential convection-dependent functions at the subcellular level. Examples of these functions at the enzyme reaction site, for example, are as follows: heat dissipation, substrate transport, product removal, boundary layer formation by concentration gradients, enzyme induction or inhibition, and active or passive transport across membranes.

INFLUENCE OF A CONVECTION-FREE MEDIUM ON THE CELL ENVIRONMENT

Upon examination of the growth environment of the single cell, other convection-sensitive parameters can be identified. These parameters are in part determined by the nature of the growth system; i.e., air-water, air-water-particulate, air-water-hydrocarbon, or water-hydrocarbon. The major difficulty with these systems in unit gravity is maintaining homogeneity of the growth medium, because these nonmiscible materials are buoyant. The gravity-induced buoyancy affects nutrient transfer to the aqueous phase, sedimentation of cells, pH control, dissolved oxygen supply, and heat dissipation (refs. 4, 5, and 6).

The theoretical advantages of low gravity in the space-flight environment may provide the opportunity to work with stable multiphasic fermentation systems that are not currently available to the fermentation scientists. These multiphasic fermentation systems could provide the cell with a continuous maximum interfacial contact of oxygen, aqueous medium, and insoluble substrates, with a minimum of agitation (ref. 7). The lack of buoyancy should allow the residence time of oxygen bubbles and oil droplets to be increased, with a concomitant decrease in shear forces (due to reaction in the need for agitation)(ref. 8). This result may allow fragile cells or shear-sensitive enzyme systems to be utilized in production processes requiring high levels of oxygen.

The analysis of convection-dependent cellular functions and environmental growth factors has led to speculations about what would happen in the absence of gravity-induced convection (ref. 2). Intracellular biochemical reactions could exhibit an abnormal periodicity due to the time delay for translocation in regulatory substrates or metabolites. Multiphasic microbial fermentation systems that are stable, though not emulsified, could be prepared and maintained as a uniform mixture without the interference of natural convection (ref. 2). Single cells, suspended in a suitable liquid medium, could be made to grow and reproduce to form discrete colonies, originating from a single parent cell or clumps of cells (ref. 2).

ZERO-G FERMENTER DESIGN CONCEPTS

The unique opportunity that zero gravity provides for fermentation developments also provides some unique problems in equipment design for zero g.

Design Considerations

The oxygen bubbler (or aerator) generally depends upon bubble buoyancy and agitation for dispersion. Because there is no buoyancy, the bubbles will not automatically shear when a critical size is achieved. A shear device must be developed that will provide generation of fine bubbles without vigorous agitation of the growth liquids (refs. 2 and 9).

The mixer used in the fermenter must be capable of uniform dispersion of bubbles as well as other nonmiscible substrates. But most important, it must avoid promoting an increase in bubble coalescence, and a means must be provided to prevent bubble attachment to solid surfaces.

The oxygen, or other gas bubbles provided to the growth system, will serve as a reservoir for waste gases produced by the cells. Although carbon dioxide (CO_2) has a high solubility in water, it will establish an equilibrium with the gas bubble and thus prevent the bubble from decreasing in volume as the oxygen is utilized. A

mechanism for collecting and removing CO_2 from either the aqueous phase or the gas bubbles must be developed.

A necessary assumption is that, during the early stages of zero-g fermenter development, most of the preparation will be made on the ground and the events sequence will be programed and automated. Some of the factors that must be considered in overall fermenter design for zero-g use are discussed briefly below.

Mixer control and agitator size will depend on the nature of the growth environment. If a true fermentation system is used, substrate mixing and CO_2 removal are the main problems to be considered (ref. 9). The addition of oxygen or a gaseous substrate provides other unique problems already mentioned.

The logistics for handling the inoculant, the substrate, and other nutrients must be considered. If the cells should be in a freeze-dried state, they must be uniformly resuspended, added to the fermentation medium, and uniformly dispersed. The same holds for the substrate and other nutrients. Some of the questions that arise are as follows: How and when should the nutrients premixed in the reaction tank; should all the substrate be added initially, or should substrate be added gradually over the growth phase; and if the nutrients and substrates are added incrementally, how is it accomplished?

At the other end of the fermentation program, the opposite sequence of events must occur. The fermentation products, metabolites or cells, must be accumulated or stored. Can these be selectively recovered while the fermentation is in progress? Or, must they be allowed to accumulate for later recovery? If so, how are they handled - as a batch or can a continuous system be developed?

The question of sterilization and contamination controls is one that has received much attention from NASA as well as the fermentation industry. It is probable that the technology for these problem areas is already developed for use in zero g. The operational controls, startup and handling procedures, and monitoring of the oxygen supply, pH, pressure, and other environmental parameters have been developed to a highly sophisticated level by the fermentation industry and for other space-flight programs.

It is conceivable that the technology developed for handling liquid fuels in zero g will be directly applicable to the handling and storage of nutrients, fermentation liquids, and metabolites.

Zero-G Fermenter Design: Cylindrical Chamber Concepts

A considerable amount of design work on zero-g fermentor concepts has been conducted by the Martin Marietta Aerospace Corporation in Denver. The information on these designs has been provided by courtesy of Martin Marietta's Denver Division (ref. 9).

A cylindrical chamber concept of a zero-g fermentor is shown in figures 1 and 2 (ref. 9). As conceived, the fermentor chamber will initially contain water and possibly the required initial concentration of dissolved nutrients. The cell inoculant would be presuspended and pressure fed into the tank, together with the nutrients and substrate to start the process.

Slow-speed paddles would provide uniform mixing of the cells and substrate. It is probable that the paddles need only operate at intervals. The oxygen would be fed with a special bubbler head and dispersed by the paddles. Bubble coalescence could be a problem in this design.

The nutrients could be in the fermentation tank initially, and the nutrient level could be maintained by nutrient diffusion through the dialysis tube, with a partial shroud formed around the paddles. The metabolites could be removed by a separate set of dialysis membrane tubes.

Carbon dioxide and other gaseous wastes could be removed by diffusion through Teflon, silicone, or other selectively permeable membrane walls. This gas could be collected and vented as appropriate.

A microbial cell harvester is provided to collect cells for further processing in flight or for return to the ground-based laboratory. Preservation of the cells or extracellular metabolites could be accomplished by use of space vacuum for freeze drying.

Most of the handling and movement of fluids would be accomplished by the use of bladder tanks that can be subjected to a pressure gradient to force fluid flow in or out of the reservoir or collector.

This concept, although designed for use with oxygen in aerobic systems, may be more applicable to anaerobic systems. It does not appear that the concept will provide sufficiently small bubbles without vigorous initial agitation. The closed-loop concept discussed next appears to be more suitable for use with oxygen.

Zero-G Fermenter: Flow Circulation Loop

The flow circulation loop concept of a zero-g fermenter design is shown in figure 3 (ref. 9). This unit is designed with a continuous flow fermentation chamber that would be initially filled with water and that could contain a basal nutrient medium. The water is gently circulated by two pumps operating in synchrony.

The cell inoculum, substrate, and nutrients are introduced into the chamber vessel through a pressure-feed system. The oxygen required for growth of aerobic organisms is provided through the two sparger screens. These sparger screens are grids containing multiple bubbling sites. As the liquid flows across the bubbling sites, the bubbles are sheared off. The size of the bubbles can be regulated by the flow rate through the screen.

The nutrient supply in the fermenter is maintained by pumping the concentrates to the counterflow mass exchanger, which consists of a series of membrane tubes, to increase cross diffusion.

The carbon dioxide produced will be collected by a separator screen into a single large pocket at the acute angle between the screen and the closed-loop fermenter wall. Accumulation of the carbon dioxide will automatically activate an optical sensing device that will vent the CO_2 to the outside.

The metabolites will be removed by a second counterflow mass exchanger and accumulated for later use. The cells can be harvested continuously when growth reaches a steady state. These cells can then be stored in the collection reservoir or processed further in flight or preserved for return to a ground-based lab.

These concepts are preliminary and have not been proven to work, or fail to work, in flight. However, they do serve as a point of departure for discussion and planning of space-flight mission objectives in the field of applied biology. There are many steps that need to be taken before embarking on a space-flight fermentation program. The most critical question still remains to be answered. "To what degree does space flight influence single-cell systems?" Many projections have been made, but the experimentation to verify these predicted responses still remains to be conducted.

When the microbial behavior is determined and the facts bear out the predictions, then it is time to determine the advantages that may be gained from the space environment. Yet, it is not expedient to wait until the experimental results are collected and tabulated before proceeding with the conceptual design of the necessary hardware and the exploration of these new, theoretical possibilities for solving some very difficult engineering problems in the biosynthesis industry.

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TABLE I.- MECHANISM FOR MASS AND ENERGY TRANSPORT IN CELLULAR PROCESSES

	Conduction	Diffusion	Radiation	Convection[a]
Nutrient procurement				
Passive diffusion		X		X
Active transport		X		X
Nutrient utilization				
Heat dissipation	X	X	X	X
Energy conversion		X		X
Biosynthesis		X		X
Waste-product removal				
Passive diffusion		X		X
Active transport		X		X
Respiration				
Gas exchange		X		X

[a]Gravity-dependent natural convection.
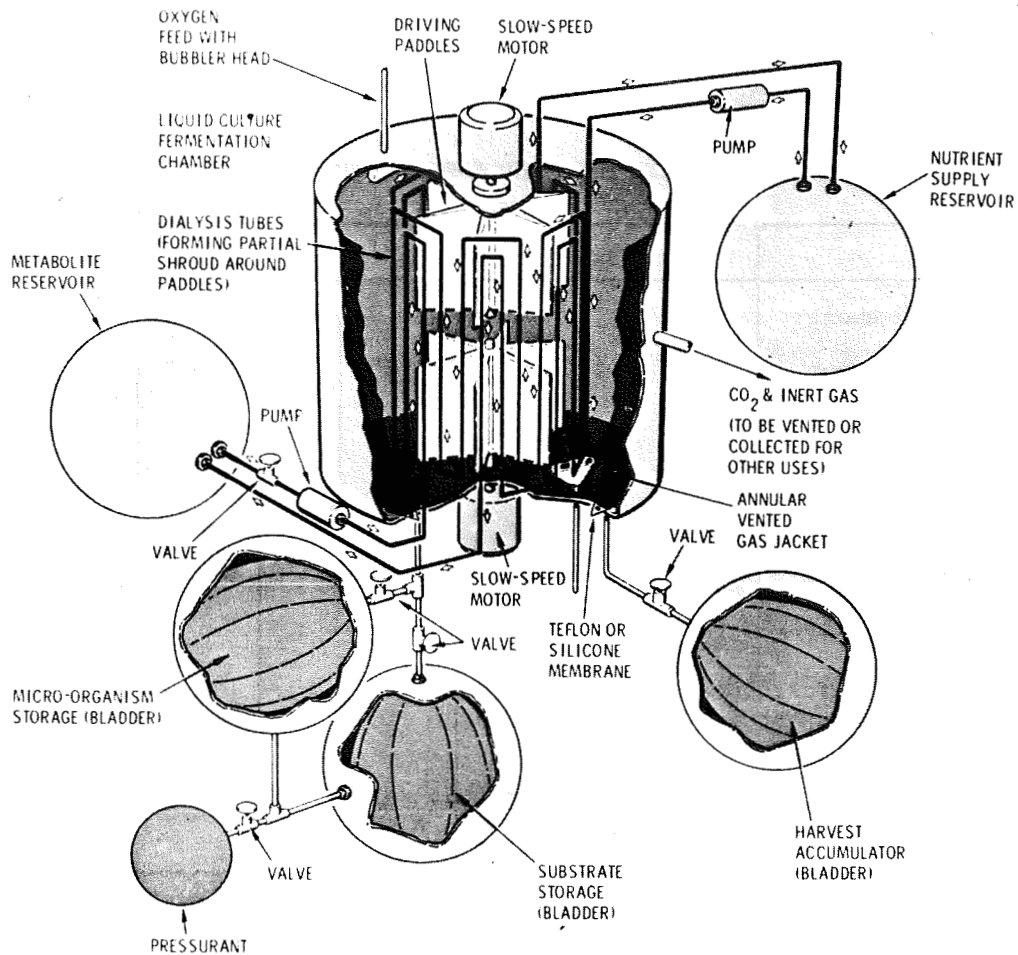
Figure 1.- Zero-g fermenter design, cylindrical chamber concept. (Courtesy Martin Marietta Corp.)

LIQUID CULTURE FERMENTATION CHAMBER

OXYGEN FEED WITH BUBBLER HEAD

SLOW-SPEED MOTOR

PUMP

DIALYSIS TUBES (FORMING PARTIAL SHROUD AROUND PADDLES)

DRIVING PADDLES

CO₂ & INERT GASES (TO BE VENTED OR COLLECTED FOR OTHER USES)

NUTRIENT SUPPLY RESERVOIR

PUMP

ANNULAR VENTED GAS JACKET

METABOLITE RESERVOIR

VALVE

TEFLON OR SILICONE MEMBRANE

SLOW-SPEED MOTOR

VALVES

MICRO-ORGANISM STORAGE (BLADDER)

HARVEST ACCUMULATOR (BLADDER)

VALVE

SUBSTRATE STORAGE (BLADDER)

PRESSURANT

Figure 2.- Concept for carbon dioxide removal by dialysis in the cylindrical fermenter design. (Courtesy Martin Marietta Corp.)

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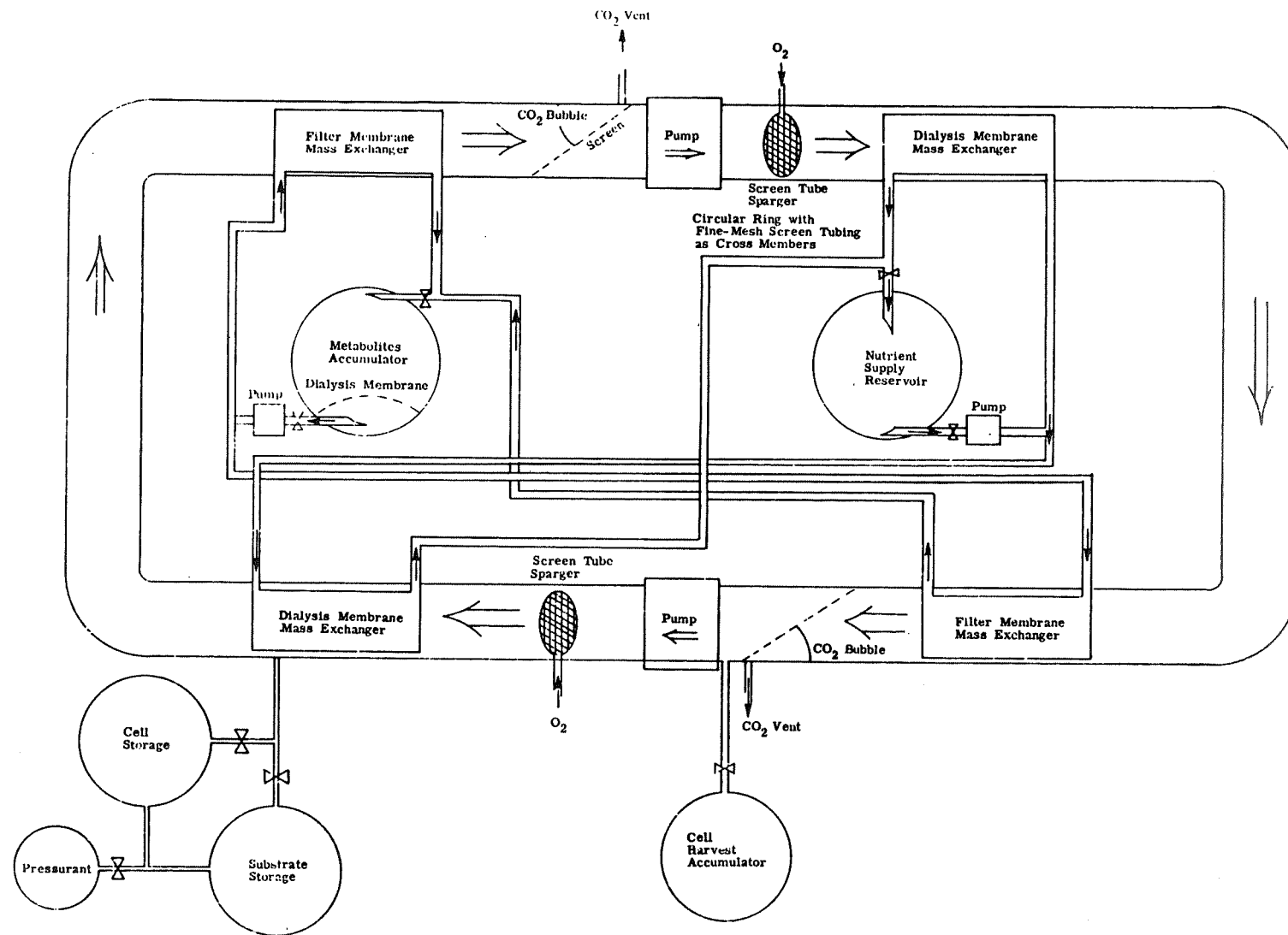
Figure 3.- A zero-g fermenter design, flow circulation loop concept (courtesy of the Martin Marietta Corp.).

SPACE SOLAR POWER SYSTEMS

By Curt Toliver, The Boeing Company, Houston, Texas

INTRODUCTION

Power from sunlight — a long-time dream of philosophers and inventors — is be-
coming an engineering reality. Solar heating and cooling is beginning to undergo com-
mercial development. A pilot plant phase has begun for ground-based solar electric
plants. The ultimate solar power plant — a power station in space called powersat —
is being studied by Boeing. But why a power station in space? Isn't that prohibitively
expensive and impractical? The Boeing Company does not think so, and its findings have
been summarized in this report.

GENERAL DESCRIPTION

Two primary candidates for a means of converting solar power to electrical power
in space exist: solar cells (photovoltaic) and thermal engines. Although Boeing is
investigating both candidates with equal vigor, this report primarily deals with a
thermal-engine concept called powersat.

The powersat will be essentially continuously illuminated by sunlight (no night,
no weather) and will collect over six times the solar energy falling on any equivalent
size area on Earth. Power beamed from the powersat can be coupled to a converter sta-
tion sited in any part of the nation — or the world, for that matter — to provide
continuous baseload electric power (see fig. 1). In contrast, early ground-based solar
plants will produce intermediate load (i.e., only daytime) power and only in sunny re-
gions. Continuous illumination at higher intensity offers a potential economic advan-
tage to spacebased solar power if the transportation to space can be accomplished at a
sufficiently low cost. Boeing's studies of the system economics indicate that this ac-
complishment is possible and that the outlook for commercially competitive electric
power from satellites is promising.

The powersat envisioned by Boeing would use lightweight mirrors to concentrate sun-
light into a cavity and thereby heat the cavity so that it serves as a "boiler." The
heat would then be supplied to turbine generators similar to those in use at convention-
al powerplants. These machines convert about a third of the input heat energy to elec-
tricity; the other two-thirds (the thermal pollution of conventional powerplants) is
returned to the environment. The powersat would use space radiators to reradiate this
unusable heat to space far from the Earth. The electricity would be converted to a
microwave beam for transmission to a receiving antenna on Earth for commercial distri-
bution as electric power.

The powersat would be large — many square kilometers in size — but would produce
great amounts of power. A typical design requires 48.6 square kilometers (12 000 acres)
of mirrors for 10 000 000 kilowatts of electric output from the ground station. Most of
the satellite area consists of thin, reflective plastic film, which minimizes the weight
to be transported to space.

The powersat illustrated here (fig. 2) is the result of conceptual design studies
performed at Boeing over the past year. The four power generation modules shown pro-
vide a reasonable compromise between the simplicity of a single large module and the
practical considerations of transportation and operation.

Each module consists of a mainframe structure formed from fold-out trusses, a spiderweblike fill-in structure to support the plastic film mirrors, 10 000 to 12 000 1011.71-square-meter (0.25 acre) mirrors and their spreader frames, a cavity heat absorber surrounded by twelve 300-megawatt helium turbogenerators, and a heat radiator. Attached to one of the modules on a rotating joint are the microwave generator and antenna. The electric power produced by the turbogenerators is routed to the microwave generator for conversion and transmission.

The parts of the satellite are designed as subassemblies for transportation by the space freighter. For example, one turbogenerator with its heat exchangers and accessories can be packaged on a pallet for a single-launch delivery; the pallet forms a portion of the wall of the cavity heat absorber. Hexagonal plastic film mirrors can be folded and rolled so that many reflectors can be launched together.

Located in a stationary orbit 35 405.6 kilometers (22 000 miles) above the Earth, the powersats will be illuminated by sunlight more than 99 percent of the time. They will appear to hang motionless in the sky, and a simple fixed-position array of antenna elements (dipoles) will serve as the ground-based converter for the power beam. The converter array will be approximately 8.0 kilometers (5 miles) in diameter. Its construction and appearance will resemble cyclone fencing.

ECONOMIC CONSIDERATIONS

Cost and economic analyses have indicated promise of commercially competitive electric power costs even if the development of the powersat is amortized by operational revenues. A busbar cost of 6.9 mills ($0.0069) per megajoule (25 mills ($0.025) per kilowatt hour) has been used in the Boeing studies. This rate is about equal to projected busbar costs for nuclear power in the 1990's; it is slightly more than that for most current coal-fired electric power generation but is cheaper than that for current oil-fired power. The 25-mill busbar cost enables an allotment of $60 billion for development and $13 billion per satellite, with use of an 8-percent discount rate, an investment horizon of 30 years beyond beginning of service of the first satellite, and addition of one satellite (10 000-megawatt ground output) per year to the system. Cost studies indicate that the $13 billion per satellite should be divided roughly as $8 billion for satellite hardware (including orbital assembly costs), $4 billion for space transportation, and $1 billion for the ground station. Preliminary estimates of powersat system costs fall within these targets.

Technical feasibility of the powersat concept is not in question. It could undoubtedly work. The issue is implementation at an acceptable cost, such that an economically feasible project is the result. Boeing's studies have outlined practical approaches to an economically promising system (see fig. 3).

Closed-cycle turbogenerators offer efficiency, compactness, light weight, and low cost for conversion of heat energy to electricity. Helium turbines are under development for Earth-based applications — the largest yet operated has a 50-megawatt rating. Scale-up to the projected powersat size of 300 megawatts would be a straightforward development; this size has been studied for nuclear reactor applications. The closed-cycle helium gas turbine provides compatibility with desirable cycle-limit temperatures and enables use of high temperatures without corrosion or oxidation.

A simple geometric principle was used by Boeing to construct and test intrinsically flat mirrors of metallized plastic film during a heliostat research project for ground-based solar power. These mirrors are very light in weight and do not require precision parts. The test mirrors had 2.5 square meters (27 square feet) of reflector and weighed

approximately 453.6 grams (1 pound) each. The powersat would use thousands of mirrors and similar design, approximately 1114.8 square meters in size (12 000 square feet or approximately 0.25 acre). Each would be individually steered by a small servo system to direct its energy to the central cavity. The use of steered, flat mirrors provides the requisite high concentration of sunlight at light weight and low cost and eliminates the need for a large, massive high-precision structure. In space, the protective plastic bubble is not needed.

The 54 431.1-megagram (60 000 ton) satellite cannot be transported to orbit in a single flight. It will be assembled in space from subassemblies of manageable size. The National Aeronautics and Space Administration (NASA) Skylab program demonstrated the effectiveness of men working in space. The space assembly job is analogous to the construction of powerplants on Earth, with transportable elements at a field location.

Transmission of the converted power to Earth was early recognized as a key problem. Microwave transmission studies and experiments by the NASA, Jet Propulsion Laboratory (JPL), and Raytheon have demonstrated the principle of efficient transmission. Efficiencies of about 90 percent for each of the three transmission steps (electric-to-microwave conversion, antenna-to-antenna transmission, and microwave-to-electric conversion) are indicated, and these values lead to a predicted transmission link efficiency of 70 percent or better.

System economics are dependent on low-cost space transportation. Flight to orbit is not intrinsically expensive; that is, the basic energy cost is not high. The cost of propellants for a large rocket (Saturn or the projected space freighter) is less than $11.02/kg ($5/lb) of payload. The history of rocket development from Vanguard to Saturn shows reduced cost through increased efficiency and size. By partial reuse, the shuttle will eliminate a large part of the highest cost element of Saturn. Through complete reuse, efficiency that results from large size, and efficiency that results from using launch crews at high launch rates, the space freighter can keep costs below $44.09/kg ($20/lb), according to Boeing studies.

<center>SETUP AND OPERATION METHODOLOGY</center>

The size of the powersat is awesome, primarily because normally space payloads are throught of as being fully assembled on Earth and then launched into space. Skyscrapers, dams, or nuclear powerplants would be equally awesome if they were thought of as being transported, fully assembled, from one place to another on Earth.

Powersat parts and subassemblies would be transported to a low Earth orbit, similar to the Skylab orbit, in hundreds of flights of a low-cost space freighter, shown in comparison with the Apollo-Saturn moon rocket in fig. 4. The freighter would burn non-polluting hydrogen fuel as did the Saturn upper stages. Its "fat Albert" shape provides a large-volume payload bay and enables controlled aerodynamic return to Earth and a rocket-arrested soft landing. The freighter shown has been sized for 226 796.2 kilograms (250 tons) of payload and would use space shuttle engine technology and a water-cooled heat shield to achieve total reusability and a ground handling time between flights of less than 1 day.

The destination of the space freighter is an assembly station in space, manned by a construction crew. In the zero gravity of orbit, the lightweight structure of the powersat can be safely deployed with a minimum of effort and all the parts can be installed, connected, and checked out.

Completed power generation modules would be efficiently transferred to a stationary orbit at 35 405.6 kilometers (22 000 miles) by electric rockets drawing electric power from the modules. The low thrust of the electric rockets would not overstress the lightweight structure and could effect the transfer in about 3 months. Small conventional rockets would assist the electric rockets in keeping the power module pointed at the Sun. The electric rocket engines and their controls would be returned to the assembly station to be used again and again. Final assembly of the powersat occurs in the operational orbit by joining the modules together and attaching the microwave transmitter, transported by one of the power generation modules, at its operating position.

The power transmission system must efficiently move the electric energy obtained from the Sun over 35 405.6 kilometers (22 000) miles of space to the Earth's surface for use. Efficiency is critical. To maintain the same useful power output on the ground if transmission efficiency is reduced 10 percent, the entire powersat must be made 10-percent larger.

Wireless transmission of power, rather than just signals, has been foreign to our thinking. However, the possibility was originally recognized by Nikola Tesla about 75 years ago. Recent experiments at Raytheon and the JPL have demonstrated overall link efficiencies greater than 50 percent. Still to be accomplished are the major engineering developments of precision electronic phase control for beam forming and further conversion element efficiency improvement to reach the projected 70-percent transmission efficiency.

The transmission system would use amplitron radiofrequency generators much like microwave oven tubes, and a wavelength of approximately 12.7 centimeters (5 inches). Maximum beam strength at the center of the receiving antenna is approximately 484.4 W/m^2 (45 W/ft^2). At the antenna edge, the beam strength is well below public exposure standards.

SPACE TECHNOLOGY HISTORY AND POTENTIAL

It is instructive to contemplate the history of space technology when one is attempting to imagine the potential of its future. Still vivid are images of the early space vehicles of the late 1950's climbing into the Florida sky, all too often ending in a brilliant display of flames, fragments, failure, and frustrations. And just a decade later, the ponderous Saturns rose slowly into the same sky on Earth-shaking thunder carrying men to leave ageless footprints in the sterile dust of another world.

The history of "modern" space technology really goes back to Goddard's early experiments, beginning with his first liquid-rocket flight in 1926. The stage was quickly set for development, over the next 15 years, of the basic liquid rocket, which culminated in the 10.9-megagram (12 ton) German V-2 war rocket of 1942 to 1945. The next 15 years saw performance improvements, increases in size, and implementation of staging, all of which resulted in vehicles that could attain orbit. Early Russian spectaculars in space led to the American Apollo program. Steady improvements in reliability and safety, increases in size and complexity, and use of high-energy fuels put man on the Moon only 12 years after Sputnik I was launched.

Costs, as well as the achievements of space flight, became spectacular. The current thrust of space technology is toward lower costs through reusable hardware and cost-optimized design. This trend is on the path to economic practicality of the powersat. But a powersat program cannot be moved up to in one big all-encompassing step. A series of steps are needed, beginning with studies, concepts, and technology experiments

(see fig. 5). Space tests onboard the shuttle pertaining to fold-out structure, deployment devices, microwave experiments, and the like (see fig. 7) will proceed through pilot-plant prototype hardware ground tests and design studies of a space freighter. An orbiting subscale pilot-plant powersat will prove out the technical features and capabilities needed to make the full-size powersat an economically practical reality. Then we will be ready for the big step to an operational powersat program.

DEVELOPMENT PROGRAM

How long would it all take? Is this a next-century solution to a this-century problem? Boeing's preliminary studies have indicated that an orderly three-phase development program (see fig. 7) could place the first operating powersat in service in approximately 15 years. This prediction is entirely consistent with the historical fact that significant advances in aerospace technology require from 12 to 15 years to reach fruition.

The first phase is a detailed engineering analysis and design-to-cost optimization study. Supported by tests and experiments in critical areas, this phase would yield selection of most design features, and higher confidence in system economics and would provide firm direction and guidelines to the succeeding advanced development phase.

The second or advanced development phase would develop and prove out, by subscale flight demonstration, all the critical elements and features of the entire system except the space freighter. Flight experiments and pilot-plant assembly and operation could be a principal mission for the space shuttle in the early- to mid-1980's.

The third phase or full-scale development program would begin with design definition and long-lead development of the space freighter. High confidence in its performance, cost, and operational characteristics would be available when results from the advanced development program merited a final decision to proceed with the entire powersat system. The development phase includes design and construction of the ground and space facilities required to deploy one 10 000-megawatt satellite per year.

Forecasts indicate that by the 1990's the United States will need to add between 30 000 and 70 000 megawatts of new generating capacity per year (see fig. 8). The Boeing powersat economic analyses conservatively assumed capture of about one-fifth of this market. A greater powersat addition rate could be implemented if needed.

CONCLUDING REMARKS

Solar energy has bathed the Earth throughout its history. The ancient Greeks had begun to recognize some of its potentials for man. Small machines using solar energy were built and exhibited during the 19th century, but they were mostly curiosities. Solar energy powered Skylab; it has powered most of the United States spacecraft. Now, in the latter part of the 20th century, its potential value is beginning to be perceived as a limitless, permanent source of energy.

Coal, "combustible rock," was known to antiquity. Its use on a commercial scale began in England during the 16th century. Petroleum was discovered in the 19th century, and petroleum-based liquid fuels soon came into commercial use. During the 20th century, fossil sources of energy have been being depleted a million times faster than their creation rate. As a result, the age of petroleum is ending; but coal will be a principal source of fuel until at least well into the 21st century. Also during the

20th century, development has been initiated on two alternative sources of energy representing "permanent" solutions — nuclear (particularly fusion) and solar. The need for energy to provide power for civilization is so critical that all promising sources should be pursued.

Boeing's studies of the powersat have resulted in their consensus that the most practical way to use solar energy for electric power on a large scale may well be to collect it in space, without hindrance by darkness and weather. It is believed that the powersat should be considered as a promising long-range energy system and that current technology is sufficiently mature to initiate the beginning steps toward its development.

The time for a decision of major commitment is not now. The decision recommended now is to begin in-depth engineering and economic study of power from space. Many incremental decision points, each dependent on successful accomplishments, will lead to full-scale deployment. The promising long-range potential of the powersat is there; now is the time to take first steps.
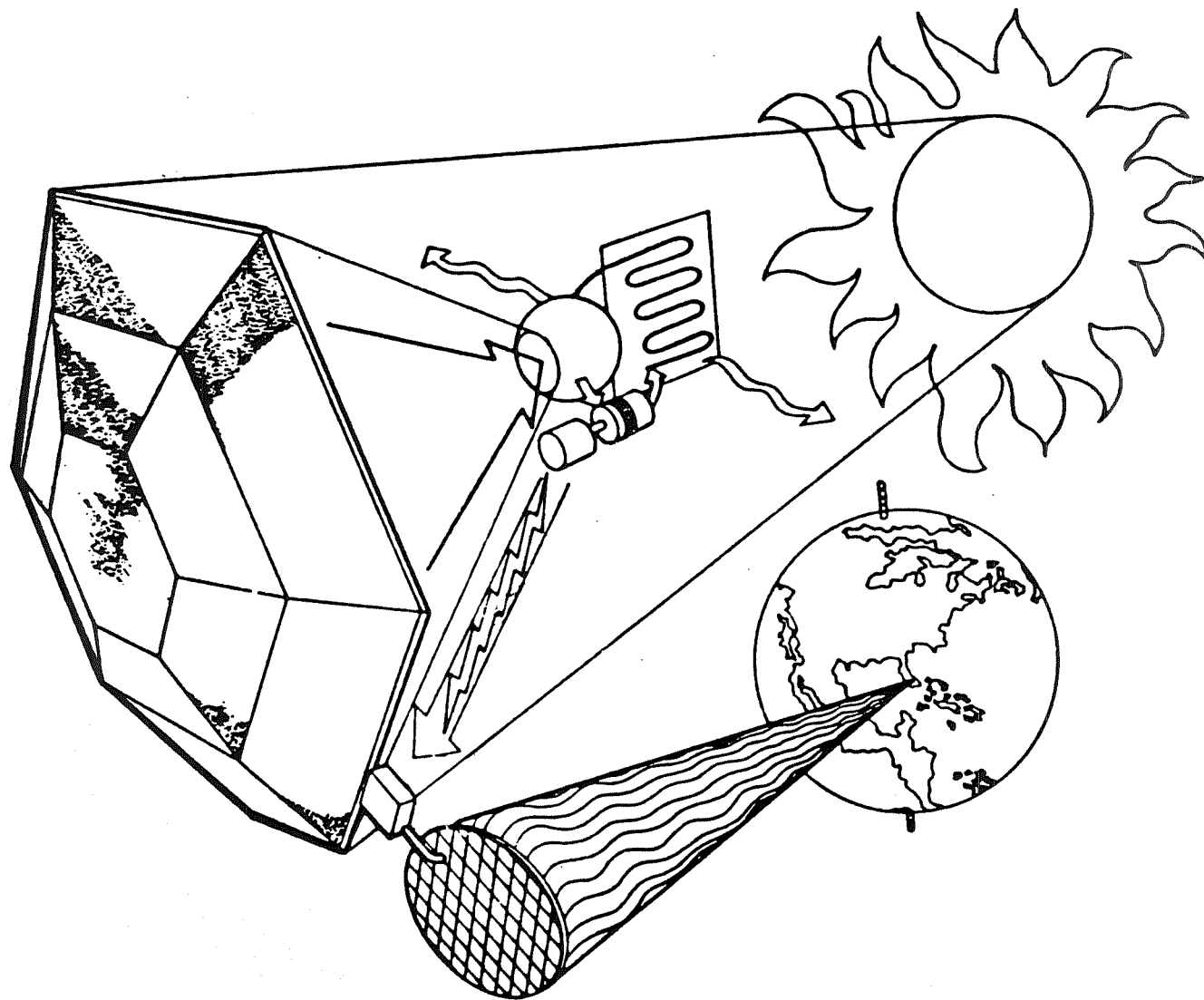
Figure 1.- Powersat in geosynchronous orbit beaming energy to Earth.
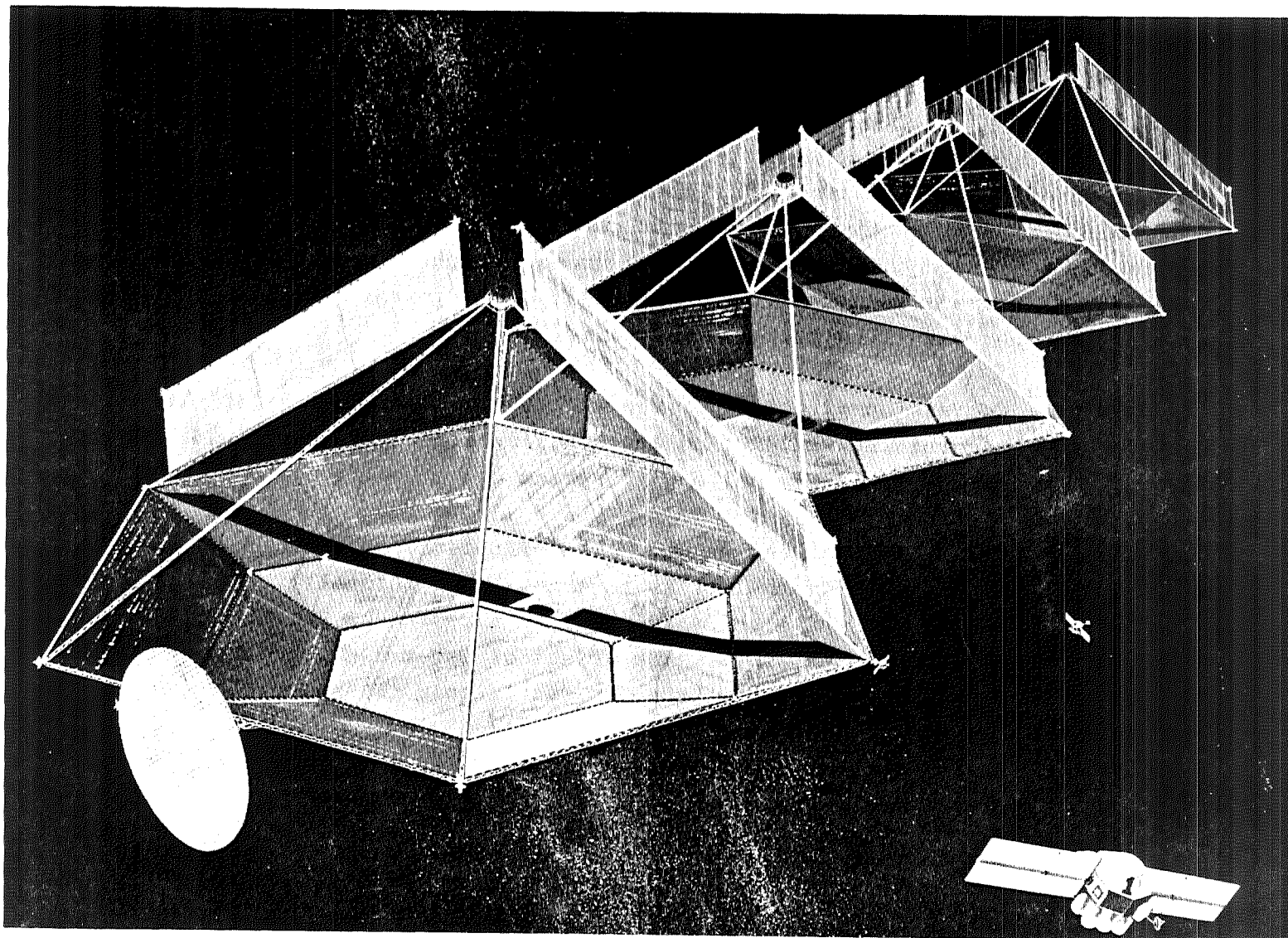
Figure 2.- Powersat configuration.

50-megawatt closed-cycle turbomachine developed in West Germany

Projected 300-megawatt powersat generator

Current Size

Microwave power transmission tests
1969 - tens of watts
1975 - several kilowatts
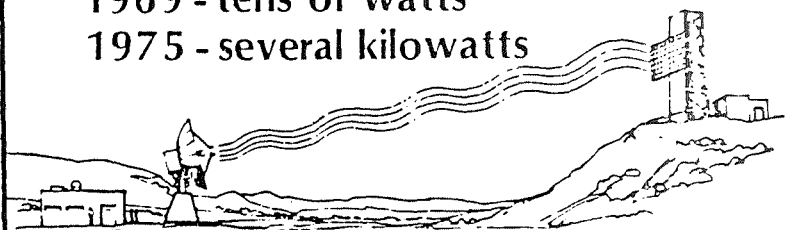
Lightweight mirrors built and tested by Boeing
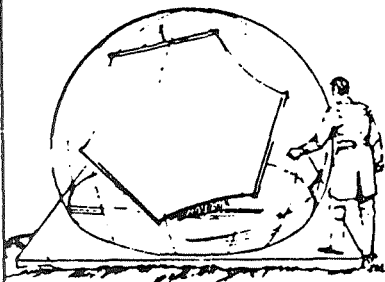
Decline in payload-to-orbit costs indicates space freighter feasibilty

Vanguard	Thor	Saturn	Shuttle
$500,000 /lb	$10,000 /lb	$600 /lb	$1.50 /lb
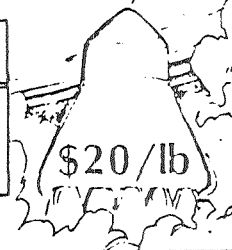
$20/lb

Man in space for assembly
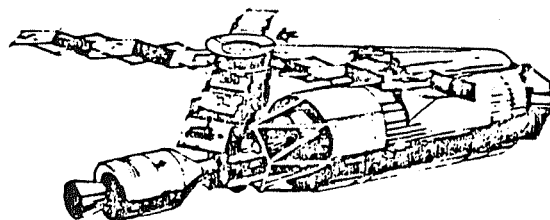
The Big Challenge
...Size!

Figure 3.- Feasibility based on existing technology.

LEO FREIGHTER

- 500,000 LB PAYLOAD
- FULLY REUSABLE
- LESS THAN $ 20/LB

ORBITAL ASSEMBLY

ORBIT TRANSFER

EFFICIENT ORBIT TRANSFER CONCEPT USES SELF-POWER

- SATELLITE MODULE GENERATES ELECTRIC POWER
- ELECTRIC ROCKET TUGS USE POWER TO GENERATE ORBIT TRANSFER THRUST

Figure 4.- Critical elements of power from space (common to any space-based system).

Figure 5.- Global space systems evolution.

Figure 6.- Space Shuttle: key to space solar power.

Figure 7.- Three-phase powersat development.

Figure 8.- Projected U.S. electrical power demands.

BENEFICIAL USES OF SPACE

By H. L. Bloom, General Electric Space Division, Valley Forge,
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ABSTRACT

Paper summarizes Bioprocessing – related results of recently completed three-year, three phase study, "Identification of Beneficial Uses of Space", for NASA-MSFC under Contract NAS8-28179.

First phase of study elicited over 100 ideas for Space Processing. Of the elicited ideas, more than 20% involved processing of biologicals, or related medical and life sciences applications. Among these were High Purity Separation of Isoenzymes, and Development of Biorhythms applications data.

Second phase of study focussed on program planning for four products, for which required experimentation and testing resulted in definition of nearly 70 series of tests in ground-based laboratories, sounding rockets, etc., and Space Shuttle. Development schedules established timing and interrelationships of decisions involved in carrying these products to the point of production.

Final phase of study determined potential profitability of the four products. Resources needed to achieve full scale production included use of Shuttle for transportation, for which a cost apportionment model was developed. R&D resources for the four products totalled $46,000,000 with Isoenzymes requiring the smallest expenditure, $4,000,000. A computerized profitability model (INVEST) was used to determine the measures of profitability of each product. Isoenzymes exhibited relative commercial attractiveness (e.g., up to 30% return on investment, $12,000,000 present value, and breakeven at 7 years from first major investment).

Conclusions are that, while specific Space Processing Applications so far identified may not be those that are ultimate "payoff" from space, results build confidence that there will be a payoff.

INTRODUCTION

At the General Electric Space Division we have recently completed a 3 year, three-phase study "Identification of Beneficial Uses of Space" (Figure 1) under contract NAS8-28179 from NASA's Marshall Space Flight Center.

This effort was aimed at defining and exercising a methodology for:

- Initially, identifying a community of potential Space Processing Users - specific organizations represented by specific individuals, who are involved in solving specific earth-based technological problems which can be related to specific knowledge and capabilities obtainable in spaceflight

- Secondly, involving such Users in defining the plans of specific technical steps and administrative processes required in carrying out such projects

- Finally, continuing the Users involvement in contributing to realistic assessments of (1) the resources required to carry out the planned projects; (2) the returns which may be realized from such a venture; and (3) its resultant profitability.

Where such steps indicate a good business potential, entrepreneurial users will eventually follow.

With the enthusiastic support of Messrs. R. Spencer and K. Taylor, MSFC's Contracting Officer's Representatives, and with the cooperation of over 400 Key Individuals from 80 organizations participating in the Study, we have developed a successful methodology, which includes survey and User contact methods, planned presentations and dialogs, as well as documents and formats for eliciting and maintaining the interest, support and aid of potential Space Processing Users. This methodology also includes operational analyses for comparison and selection of key technical alternatives, and the integration of that data with management planning data. Furthermore, our methodology provides several key commercial business analysis and evaluation techniques, adapted from ground processing business methods, for assessing market data, estimating R&D and production needs, and comprehensively analyzing the business worth of products.

The primary results of the Study have evolved from application of the aforementioned methods and techniques to the above-noted potential User organizations, and to their ideas for potential space processes, products, and services. Those results are briefly summarized in this paper, organized according to the three Phases of the Study.

PHASE I OF THE STUDY

As noted in Figure 1, Phase I has concentrated on User Identification. The development of peer-level, personal contacts; trial and error evolution of an introductory, educational idea-stimulating kick-off presentation; establishment of an overall dialog plan; and promotion of mutually supportive analyses have been the major methodologies in this Phase.

As a result of these efforts, our search for potential space-developed products, processes and/ or services initially uncovered over 100 ideas. The complete list of these ideas, together with the goals and objectives sought by the potential Users is given in Reference 1.

A sampling of those ideas that are of possible interest to this Bioprocessing Colloquium are shown in Figure 2. Observation reveals the wide spectrum of User interests. Typical products for which Users anticipated possible improvements due to the so-called "zero gravity" of spaceflight include such biologicals as high specificity isoenzymes and high purity insecticides; and such medical electronics materials as large germanium crystals and low-defect silicon crystals. Users also felt that processes such as thinner, defect-free coating of implantable sensors, and "enzyme engineering" using some adaptation of affinity chromatography might also accrue from weightlessness and lack of convection. Nor was research neglected - bone growth in "zero gravity" was viewed as an area of possible investigation for treatment of major fractures and for bone surgery, while the potential synergistic effects of "zero gravity" and space radiation on the mutation of micro-organisms (including those utilized in the dairy industry and in producing antibiotics) were of interest to several participants.

While almost all of the 100 ideas in this initial identification were considered as valid User needs, consultation with technology experts, analyses in various disciplines (both aerospace and non-aerospace), and various degrees of engineering judgement enabled us to extract 12 ideas with high potential for eventual implementation. These surviving ideas are shown in Figure 3, with details of the specific aims sought by the Users and the specific applicability of Space Processing. Twelve other ideas exhibited sufficient promise to warrant further evaluation at a later date. The remaining ideas were excluded from further study, due to conflict with Study Guidelines, overlapping objectives, analytical or empirical indications of scientific invalidity, etc.

Further Phase I effort on the 12 best ideas developed tentative experimental and operational alternatives for providing the required information, environments, and/or facilities found likely to meet the Users aims. This activity established early indications of the "mix" of ground and space operations involved in precursor experimentation and eventual commercial Space Processing.

Among the significant results of Phase I, shown in Figure 4, the early estimates of the $1 billion to $2 billion value of the 12 current ideas aroused considerable interest, but also raised questions as to the details of necessary pre-production research and development, and, perhaps even more important, as to legal, financial and administrative arrangements. Typical of these latter questions are those listed in Figure 5.

A major conclusion of this phase, therefore, was that further commitments by potential Users would require more information on ground rules and mechanisms which would govern the legal, financial and technical relationships between NASA and commercial industry, and which would, therefore, interact with key technical and administrative decisions and their timing.

PHASE II OF THE STUDY

These requirements helped formulate objectives for the second phase of study, in which we aimed to obtain in-depth technical planning data for typical products from Phase I, as well as program scheduling and decision information for management planning. Details of this phase of the study are found in Reference 2.

Due to timing and funding limitations, this phase of study was limited to the four products listed in Figure 6, and carried out with the support of the listed organizations, who aided the analyses of those products in Phase I.

For the four products, with the four participating User organizations, we evaluated nearly 130 alternative processing approaches prior to selecting those offering the best chances for successful development. With the Users, we then defined specific experiments and tests necessary to such development. This research and development program required a broad spectrum of facilities, both ground-based and spaceborne. Figure 7 lists the required facilities and number of test runs.

The large number of experiment runs in ground laboratories is indicative of the state-of-the-art in the listed areas, and acknowledges that a comprehensive ground-based program is a necessary part of the typical space processing program.

The program leading to high specificity separations of isoenzymes by large pore gel electrophoresis and/or isoelectric focussing calls for the largest number of experiment runs, although later work shows that it is the least costly program. For example, much of the testing, especially the centrifuge tests, centers around the effects of spaceflight operations on the large pore gels. It is important to understand the susceptibility of the gels, with and without separated specimens, to pre-launch handling, launch loads, and re-entry loads. Centrifuge data will answer many of the questions involved, at very low cost.

The details of the experiments and tests listed here were fitted into development timelines, which included all of the research, engineering, ground development tests, and flight testing (including Shuttle flights) to achieve a production capability in the early 1980's. These details included preliminary experiment and test protocols, estimates of equipment needs, as well as dates and duration. The resulting sequence and timing of technical tasks together with the indicated need for commitment of facilities, equipment and manpower enabled the Users, working in the Study Team, to formulate the flow of decisions necessary to implement each development program.

Figure 8 presents a typical decision flow for the Isoenzymes development program. Both technical and management decisions are shown. Their interrelationships are readily visible, as are key nodes in the flow of decisions. Major alternatives are indicated in the table, estimates (by the Users) as to the probability of each alternative are given, as are the preferred (by the User) alternative.

In summary, Phase II, produced a wealth of technical (processing approaches, experiment and test definitions, facility and equipment needs) and management (milestones, decisions, probabilities) results, Figure 9. However, many of the questions of Figure 5 were still being asked. Furthermore, while Phase II assembled data reflecting specific planning for evolving from concepts to experiments, to initiating commercial operations, a key element was not addressed in those phases... the business potential of Space Processing.

PHASE III OF THE STUDY

Can Space Processing be a profitable business venture? This question was the problem for Phase III. We continued working with the same four products and essentially the same Users to arrive at the answers.

The essence of our task in Phase III (reported in Reference 3) was to acquire sufficient technical and economic information to carry out the financial analysis pictured in Figure 10.

An examination of that figure reveals the scope of analyses we have carried out. For example, blocks listed as "Unit Price", "Total Market" and "Market Share" spell out the need for a comprehensive market analysis. "Unit Manufacturing Cost", and "Annual Plant and Equipment" reveal a requirement for thorough understanding of the commercial manufacturing flow and productivity (both ground-and space-based). "R&D Expense" calls for details of the precursor experimentation and development testing, with major emphasis on the timing and costs of facilities and equipment.

Typically, Figure 11 lists the major equipments needed to carry out the Isoenzyme Separation experiments and tests, briefly summarizes their development status, and notes quantities needed at various points in the development schedule.

A key part of our R&D analysis was the assessment of Shuttle/Spacelab utilization costs. This required construction of a cost allocation model.

Our cost model, given in Reference 3, provides for recovery of all operating costs, allocates costs on the basis of Shuttle resources utilized, and provides incentives (or dis-incentives) to encourage (or discourage) use of various resources. Using our recommended utilization rates, we show, in Figure 12, the cost rates by which a payload User may be allocated his fair share of a typical $10.7 million flight cost.

Similar depth and scope of analysis have been carried out for the production phase of each of the 4 products under study. Using life cycle market demands estimated for each product, in-depth conceptual design of equipments and payloads, comprehensive "throughput" analyses of each step in the production process, with the aid of the User participants, we generated the required data for the final profitability analyses.

The plots on Figure 13, for the Isoenzyme business venture represent the data generated for all four products. A detailed treatment is given in Reference 3.

Case A includes the User funding the total R&D program, $3.8M; nominal forecasted market and market share; conservative unit cost of·producing product; nominal selling price. Space production of high specificity Isoenzymes was not an attractive venture under these conditions.

For Case B, Mr. K. Taylor suggested that, since basic processes would have broader application than the individual products under study, it could be likely that the basic process feasibility would be proved under government funding. User, in Case B therefore, would only pick up those R&D costs that specifically provide prototype/pilot plant capability. Under these conditions, NASA (as the agent of the government) provides early R&D funds, and the Isoenzyme processing appears attractive.

In summary (Figure 14) for Phase III, our development planning data includes detailed formats describing and timing all Work Elements. Each element of work is backed by documentation of the human, facility, and materials resources required to perform that work, and the cost of such resources.

In Phase III, Users historical data and prognostications provided market forecasts, and the resulting needs defined production levels, which helped to establish size and performance requirements for processing equipment.

We identified resources required of the Space Program, such as the 85-600 kilowatt hours of energy required per flight for Space Processing R&D on the four products studied. Figure 14 summarizes such typical resources, and the costs of those resources for the R&D effort.

Finally, Figure 14 shows that Space Processing of Isoenzymes tends to be an attractive business venture, once the feasibility of large pore gel electrophoresis in space has been demonstrated. However, the long period before breakeven inhibits the attractiveness of Tungsten processing and Transparent Oxides. Reducing the unit manufacturing cost by 20% (rated a possibility, since several logical approaches for reducing on-orbit energy costs, have recently been brought to our attention by the Study C.O.R.) could reverse those conditions.

SUMMARY OF LESSONS LEARNED

It is appropriate, here, to review what we have learned from all phases of the Study.

Typical specific Lessons Learned from Phase I are briefly depicted in Figure 15.

●   The successful identification of Beneficial Uses of Space in Phase I, was based on gaining the interest of potential Space Applications Users through dialogs.

●   Considerable Study Team/User mutual education occurred during this Phase through the interchange of Aerospace/Non-Aerospace and Commercial/Government vendor data.

●   Since dialogs sometimes did not evoke immediate potential Users ideas, because of the novelty of the space environment to non-Aerospace organizations, we broadened and deepened our initial briefing data in order to lessen this effect.

●   Furthermore, the development of User concepts for Space Processing appears to be a time dependent process, and future studies should allow 6 months or more for the process of generating ideas.

Phase II, which carried the study from "identification" to "planning" also taught us some lessons. Figure 16 shows two conclusions for Phase II carried over from the earlier phase: one on User education and another on Legal/Financial issues.

● Phase II results verify that a mutually supportive effort, progressing toward specific concepts, maintains two-way communications between the aerospace community and non-aerospace industry.

● The wide spectrum of current technical unknowns evolving from the limited amount of available data, and the unpredictability of the state-of-the-art in the 1980's, are reflected in a necessarily broad scope of requirements for experiments and tests.

● Preliminary schedules of development programs are "comfortable" and can accommodate moderate redirection, where necessary.

As important as the results of Phase III are the lessons we have learned, Figure 17. Some simply verify or reiterate lessons from the preceding Phases (e.g., the value of dialogs, the requirement for blending aerospace/non-aerospace methods rather than imposing one on the other, etc.). Others are either new, or have become more apparent at this stage of effort, and, thus, bear some discussion.

● We note two key problems in Figure 17 for instance – that of acquiring a Space Processing Program User constituency, and a related need for a policy to determine the tariff for use of space facilities. In the dialogs with the commercial industry community, we have found that the prospect of deriving new or improved products through Space research and development does not supplant, but rather competes with prospects of current, low cost, often historically successful ground-based research programs. Thus, the technical competitiveness of Space Processing will have to be matched with economic competitiveness, and the combination used as a marketing "tool" to acquire the necessary User constituency.

● During the profitability analysis in this Study, two key space operations functions were found to exert profound effects on the production costs of the space products – (1) energy (primarily for heating and melting process steps), and (2) the launch of production facilities for each production run. As a result, one of our conclusions is that

a major effort must be undertaken to develop a low-cost, high power, in-orbit energy source

if space products which require high temperature heating and melting are to be profitable. In addition,

a long term, on-orbit automated, or semi-automated production facility will be required,

if repetitive production runs are to be performed without incurring the prohibitive expense of repeatedly launching the necessary processing equipment.

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1. Study for Identification of Beneficial Uses of Space (Phase I) Final Report, Contract NAS8-28179, GE Document No. 73SD4259, December 10, 1972 and April 23, 1973.

2. Study for Identification of Beneficial Uses of Space (Phase II) Final Report, Contract NAS8-28179, GE Document No. 73SD4281, November 1, 1973.

3. Study for Identification of Beneficial Uses of Space (Phase III) Final Report, Contract NAS8-28179, GE Document No. 75SD4281.
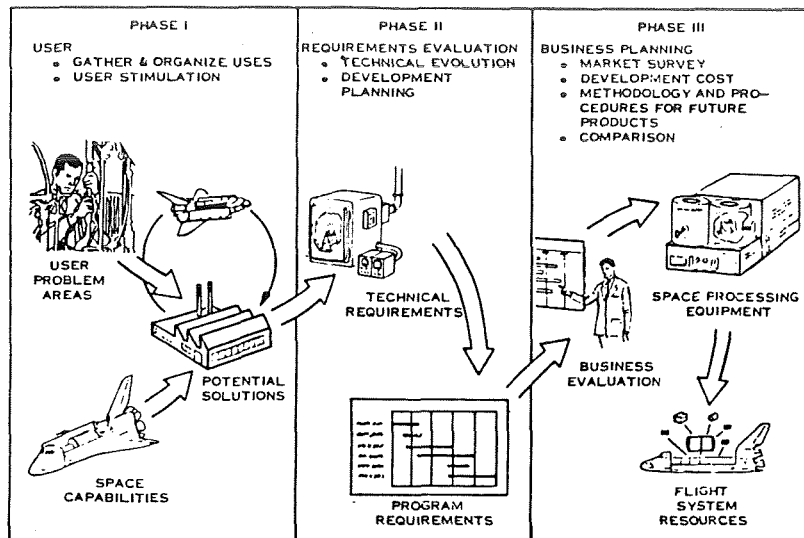
Figure 1.- Relationships of BUS study phases I, II, and III.

- COATING OF IMPLANTABLE SENSORS
- PROSTHETIC MATERIAL FOR BONE GROWTH
- AFFINITY CHROMATOGRAPHY
- THIN FILMS FOR DIALYSIS
- HIGH DUCTILITY TUNGSTEN X-RAY TARGETS
- DEVELOPMENT DATA FOR PHONOCARDIOLOGY
- IMPROVEMENTS IN DAIRY PRODUCTS
- BONE GROWTH IN ZERO "G"
- LARGE GERMANIUM CRYSTALS FOR GAMMA RAY CAMERA

- HIGH QUALITY SILICON CRYSTALS FOR MEDICAL APPLICATIONS
- VIRAL INSECTICIDE MANUFACTURE
- LYOPHILIZATION
- BLOOD ANALYSIS SERVICE
- MUTATION AND GROWTH OF MICRO-ORGANISMS
- THERMAL CONDUCTIVITY OF LIQUIDS
- SEPARATION OF ISOENZYMES
- UTILIZATION OF BIORHYTHMS
- NEW ANTIBIOTICS
- CULTURING OF BIOLOGICALS

Figure 2.- User ideas of typical beneficial uses of space for bioprocessing.

PRODUCTS	BASIC SPACE PROCESSES REQUIRED
HIGH PURITY VACCINES	ELECTROPHORESIS
HIGH SPECIFICITY VIRAL INSECTICIDES	ELECTROPHORESIS (FREE FLOW)
MULTI-GIGAHERTZ FREQUENCY SURFACE ACOUSTIC WAVE ELECTRONIC COMPONENTS	LARGE CRYSTAL GROWTH AND VIBRATION-FREE LITHOGRAPHY
SINGLE CRYSTAL AND/OR EUTECTIC HIGH TEMPERATURE TURBINE BUCKETS	LARGE CRYSTAL GROWTH AND/OR CONVECTIONLESS SOLIDIFICATION
HIGH PURITY, DUCTILE TUNGSTEN X-RAY TARGETS	LEVITATION MELTING AND SUPERCOOLING
HIGH PURITY RADIOISOTOPES	PARTICLE MANIPULATION BY SMALL FORCES
LARGE, UNIFORM SILICON SINGLE CRYSTALS	LARGE CRYSTAL GROWTH AND/OR CONVECTIONLESS SOLIDIFICATION
UNIFORM GARNET SINGLE CRYSTAL FILMS	CONVECTIONLESS EPITAXIAL CRYSTAL GROWTH
TRANSPARENT METAL OXIDES	LEVITATION MELTING AND UNIFORM SUPERCOOLING
HIGH SPECIFICITY ISOENZYMES	ELECTROPHORESIS (LARGE PORE GEL) OR ISOELECTRIC FOCUSSING

Figure 3.- Twelve ideas with high potential.
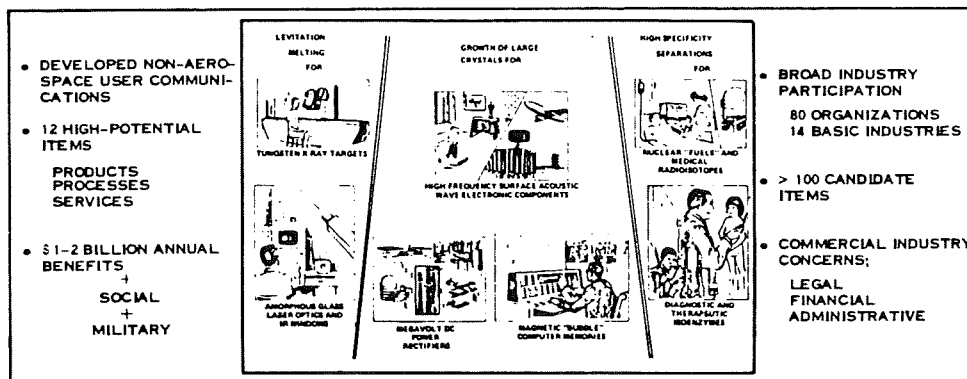
Figure 4.- Typical questions from phase I.

HOW WILL NASA HANDLE MY PROPRIETARY DATA (OR EQUIPMENT)?

WHAT RIGHTS WOULD NASA RETAIN ON MY DATA (OR PATENTS, OR PRODUCTS)?

WHO PAYS FOR SPACE EXPERIMENTS (OR TEST, OR EQUIPMENT) TO DEVELOP MY PRODUCT (OR PROCESS OR SERVICE)?

WHAT ROLE DOES NASA (OR GE) PLAY IN PROGRAM SUBSEQUENT TO B. U. S. ?

WHAT IS THE PROBABILITY THAT THERE WILL BE A SHUTTLE (OR SPACE FACILITY)?

WHEN DO DECISIONS TO GO AHEAD NEED TO BE MADE ?

HOW MUCH WILL IT COST TO RUN AN EXPERIMENT OR OBTAIN FACILITY SPACE?

Figure 5.- Products analyzed in phase II.

1. TUNGSTEN X-RAY TARGETS   GE - MEDICAL SYSTEMS, MILWAUKEE

2. HIGH SPECIFICITY   POLYSCIENCES, INC., WARMINSTER, PA.
   SEPARATION OF ISOENZYMES

3. SURFACE ACOUSTIC WAVE   GE ELECTRONICS LAB, SYRACUSE, N.Y.
   ELECTRONIC COMPONENTS

4. TRANSPARENT OXIDES   CORNING GLASS, CORNING, N.Y.
                                   (CONSULTING)

Figure 6.- Products analyzed in phase II.

IDEA / FACILITY	SEPARATION OF ISOENZYMES	TRANSPARENT OXIDE PROCESSING	HIGH PURITY TUNGSTEN X—RAY TARGETS	FABRICATION OF SURFACE ACOUSTIC WAVE COMPONENTS	TOTAL
GROUND LAB	8	6	4	4	22
CENTRIFUGE	2	--	--	--	2
ENGINEERING TEST LAB	2	1	1	1	5
DROP TOWER	--	1	1	1	3
KC—135	3	1	1	3	8
SOUNDING ROCKET	--	2	3	2	7
SPACECRAFT	7	--	--	1	8
SHUTTLE SORTIE LAB	4	3	2	2	11
TOTAL	26	14	12	14	66

EACH NUMERICAL ENTRY REPRESENTS A SERIES OF TESTS RANGING FROM 1 TO 120 RUNS

Figure 7.- Amount of experiments or tests needed.
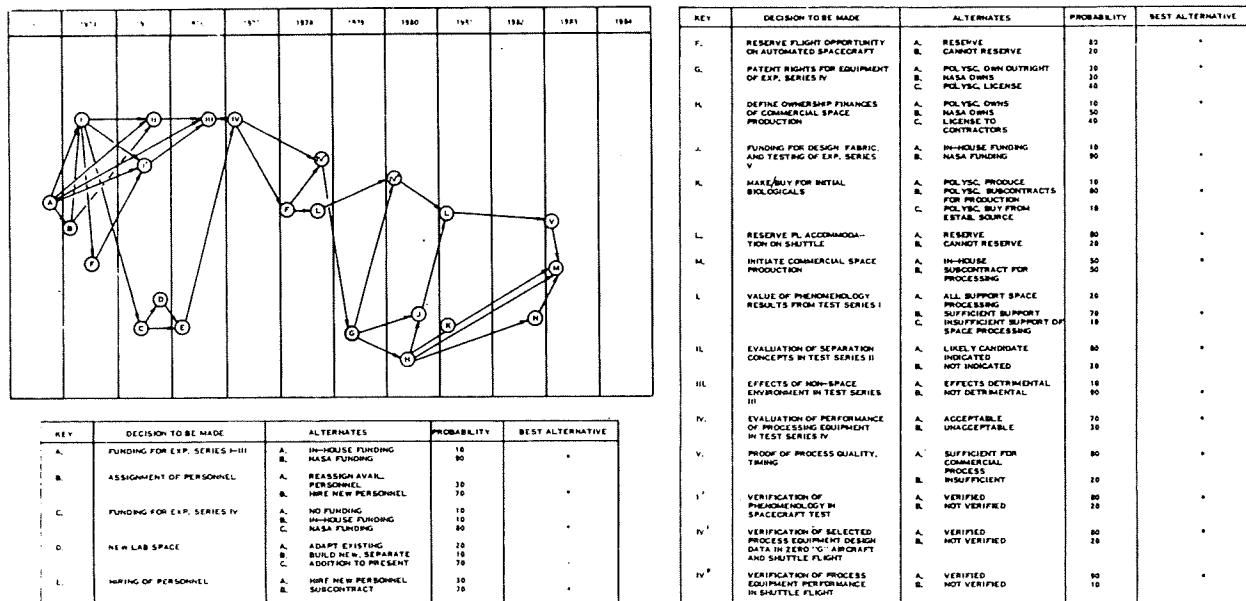
Figure 8.- Decision flow for isoenzymes.

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- MAINTAINED USER RAPPORT

- GENERATED ~ 30 MAJOR ALTERNATIVE PROCESSING APPROACHES (> 100 LESSER ALTERNATIVES)

- DEFINED REQUIRE-MENTS FOR ~ 70 TEST SERIES (IN-CLUDING 11 IN SPACE LAB)
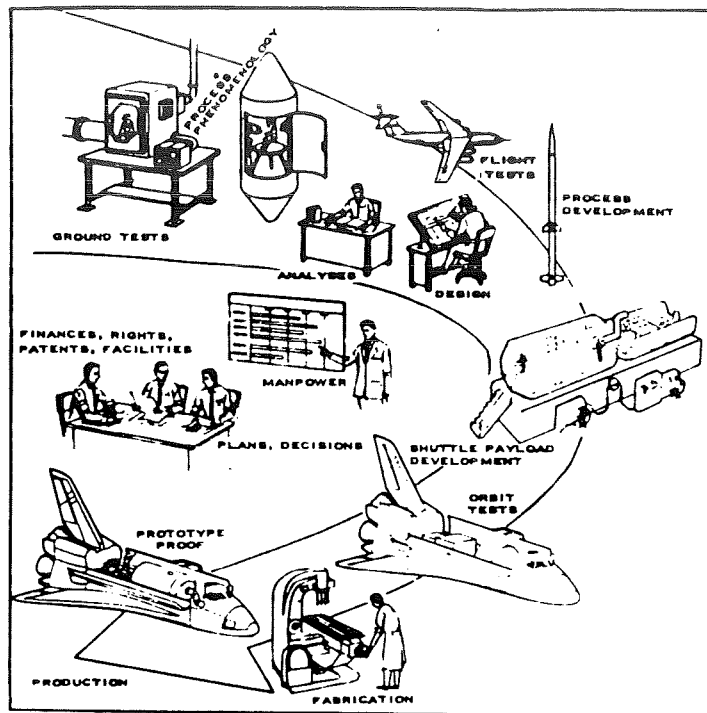
- DEVELOPED 10 YEAR PROGRAM TIMELINES AND MILESTONES

- CONSTRUCTED DECISION FLOW, IDENTIFIED DECISION NODES

Figure 9.- Phase II results.
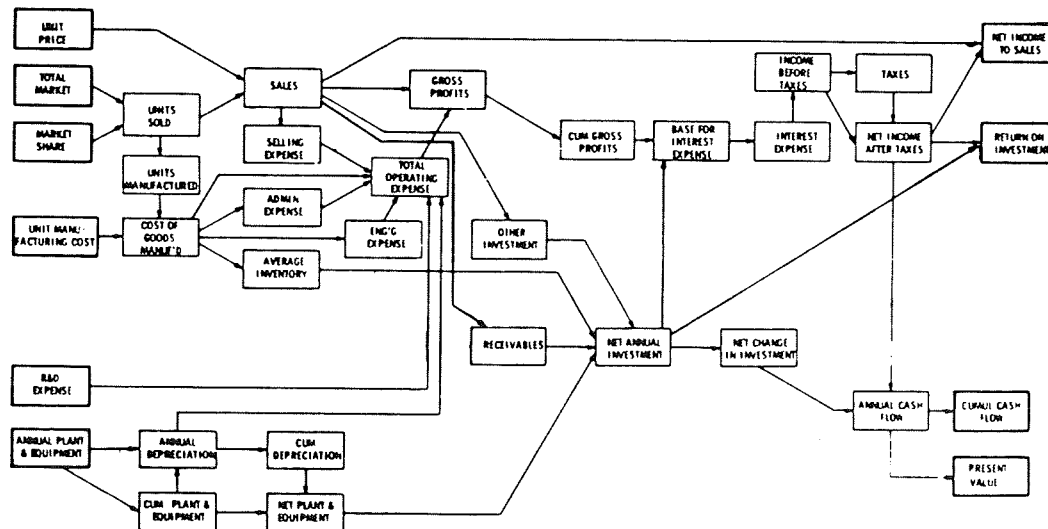
Figure 10.- Financial-analysis method for assessment of space processing opportunities.

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Item	Space Development Required?	Quantity Required Initial (Ground Test)	Quantity Required Prototype	Quantity Required Pilot	Quantity Required Production
Analytical 12 Col. Electrophoresis Separator	Yes	1	–	–	–
Preparative 1-Col. Separator	Yes	1	1	1	2
Power Supply, Electrophoresis	Yes	1	1	1	2
Cooling Bath (Circulating) & Pump	Yes	1	1	1	2
Copying Camera	No	1	1	1	–
Storage Refrigerator	Yes	1	1	1	1
Deep Freeze	Yes	1	1	1	1
Freeze Drying Unit	Yes	1	1	1	–
Vacuum Pump	No	1	1	1	1
Circulating Hot Water Bath	Yes	1	1	1	1
Thermocouples & Meters	No	1 set	1	1	1
Gas Chromatograph	No	1	1	–	–
Centrifuge (Clinical)	No	1	–	–	–
Homogenizer	No	1	–	–	–
Microscope	No	1	–	–	–
UV Spectrophotometer	No	1	–	–	–
Fraction Collector	Yes	1	1	1	1

Figure 11.- Equipment list for isoenzyme separation.

SHUTTLE RESOURCE UTILIZED	RATES UTILIZED IN STUDY*
Up-Transport Volume	$13,760/cubic meter
Up-Transport Weight	$108.81/kg
On-Orbit Energy	$1721/KWH
On-Orbit Crew	$6446/Man Hr
On-Orbit Data Transmission	$4286/MHz of RF Bandwidth
On-Orbit Data Processing	$2.36/word of Experiment Computer Storage
Down-Transport Weight	$184.44/kg.
Ground Operations, Mechanical Handling	$1,276/cubic meter
Ground Operations, Electronic Handling	$20.89/word of Experiment Computer Storage

*Based on C_M, Average per-mission Operational Cost = 10.7×10^6.

Figure 12.- Recommended user cost allocation rates for Shuttle/Spacelab.

	CASE A	CASE B
% NI/S (1992)	21%	21%
% ROI (1992)	71%	73%
PRESENT VALUE	$11M	$12M
USER R&D COST	$3.8M	$1.4M
NASA R&D COST	--	$2.4M
BREAKEVEN POINT	11 YRS	7 YRS
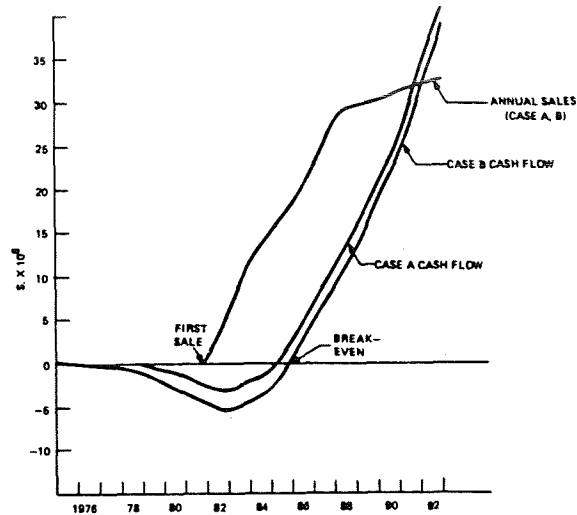
Figure 13.- Isoenzymes cash flow.

DETAILED DEVELOPMENT PLANS FOR SPACE PROCESSED TUNGSTEN, ISOENZYMES, SURFACE ACOUSTIC WAVE DEVICES, & TRANSPARENT OXIDES

R&D PROGRAM 1975 TO 1983
GROUND BASED EXPERIMENTS AND TESTS 1975 TO 1979
SHUTTLE-BASED EXPERIMENTS AND TESTS 1980 TO 1983

PRELIMINARY MARKET FORECASTS FOR THE FOUR PRODUCTS

TUNGSTEN TARGETS TO 31,200 TARGETS IN 1986; -- 40,000 IN 1888
ISOENZYME KITS TO 1,200,000/PER YEAR IN 1985; -- 5,000,000 IN 1992
SURFACE ACOUSTIC WAVE BANDPASS FILTERS TO 250,000 PER YEAR IN 1992
TRANSPARENT OXIDE OPTICAL ELEMENTS TO 5,000 PER YEAR IN 1985

RESOURCE REQUIREMENTS -- GROUND AND SPACE

ELECTRICAL POWER: R&D, 85-600 KW HRS PER FLIGHT; PRODUCTION, 2,300 TO 68,000 KW HRS/YR
SHUTTLE FLIGHTS: R&D 18; PRODUCTION 23/YR
GROUND FACILITIES: THREE EXPANDED, ONE NEW FACILITY (ISOENZYMES)
SPACE FACILITIES (PRODUCTION): 16 SPACE PROCESSORS

COST ANALYSIS

R&D: TUNGSTEN -- $9,000,000
     ISOENZYMES -- $4,000,000
     TRANSPARENT OXIDES -- $18,000,000
     S.A.W. -- $15,000,000

PROFITABILITY ANALYSIS

ISOENZYMES; RELATIVELY ATTRACTIVE, BREAK EVEN AT 7 YEARS
TUNGSTEN; RELATIVELY UNATTRACTIVE AT 9 YEAR BREAK EVEN. UNIT COST AND R&D COSTS ARE CURRENT PROBLEM
S.A.W.; RELATIVELY UNATTRACTIVE AT 13 YEAR BREAK EVEN. MARKET SIZE, R&D COSTS, ARE CURRENT PROBLEM
TRANSPARENT OXIDES; UNATTRACTIVE AT 9 YEAR BREAK EVEN. R&D COSTS, MARKET SIZE, ARE CURRENT PROBLEM

Figure 14.- Phase III results.

- A NON-AEROSPACE COMMUNITY WITH ACTIVE INTEREST IN SPACE PROCESSING EXISTS (80 PARTICIPANTS > 100 IDEAS)

- POTENTIALLY BENEFICIAL SPACE PRODUCTS, PROCESSES, SERVICES HAVE BEEN IDENTIFIED (> 12 IDEAS, VALUE ~ $2 X 10^9)

- THE DIALOG METHOD OF ELICITING POTENTIAL USER RESPONSE, WORKS (SPECIFICS MAKE THE DIFFERENCE)

- THE COMMERCIAL SECTOR OF INDUSTRY IS AS MUCH CONCERNED WITH LEGAL/ADMINISTRATIVE/ FINANCIAL PROBLEMS OF SPACE PROCESSING AS WITH TECHNICAL

- "IDENTIFICATION" INCLUDES FINDING THE REAL PROBLEM, REAL KEY INDIVIDUAL, REAL APPLICATION

- LIKE LIVING THINGS, COMMERCIAL SPACE PROCESSING IDEAS NEED GESTATION PERIOD

- NON-AEROSPACE USER COMMUNITY NEEDS DIRECTED SPACE PROCESSING INPUTS

Figure 15.- Lessons learned in phase I.

- IN MOVING FROM IDEAS TO CONCEPTUAL APPROACHES, USER RESPONSE STRENGTHENS (30 MAJOR ALTERNATIVE APPROACHES EVALUATED)

- PROJECTING TECHNOLOGY FOR ~ 1980 AND BEYOND IS SHAKY; JUDGEMENT AND "FEEL" VARY

    ∴ USER RESPONSES ARE QUALIFIED, EXPERIMENTS AND TESTS PROLIFERATE, ETC. (E. G. 66 TEST SERIES)

- NON-AEROSPACE USERS NEED EDUCATION AND EXPOSURE TO LOW-COST EXPERIMENT AND TEST METHODS

- 10 YEAR R&D PROGRAM IS "COMFORTABLE" (HINDSIGHT: MAYBE TOO COMFORTABLE?)

- USERS SEEKING EARLIEST INDICATIONS OF PROCESS FEASIBILITY (E. G. REQUIRED GROUND TESTS HEAVY IN '74)

- PROGRAM DECISION DATA (USER AND NASA) ARE INCOMPLETE (E.G., TECHNOLOGY GAPS, TEST OPPORTUNITIES, ETC.)

- LEGAL/FINANCIAL ISSUES ARE MAJOR NODES IN DECISION FLOWS

Figure 16.- Lessons learned in phase II.

- ONE-ON-ONE DIALOGS REMAIN THE WAY TO ACQUIRE SPECIFIC USER DATA

- DEVELOPMENT PLANS, R&D NEEDS, PRODUCTION ANALYSES REQUIRE INTIMATE BLENDING OF COMMERCIAL AND AEROSPACE METHODS — SOME COMPROMISES IN EACH.

- COMMERCIAL USERS CONSIDERATION OF NEW VENTURES IS PRIMARILY INFLUENCED BY ECONOMIC CONSIDERATIONS.

- KEY PROBLEMS IN 1.) ACQUIRING CONSTITUENCY OF USERS, 2.) POLICY ON USER CHARGES
  - SPACE FACILITY IS ONLY ONE R&D ALTERNATIVE (E.G. TUNGSTEN HAS 18 OTHER PATHS
  - A (PROMISED) BETTER MOUSE TRAP IS NOT SUFFICIENT. IT MUST FIT USER'S ECONOMICS.
  - A BETTER MOUSE TRAP MUST BE SOLD.
  - POLICY MUST NOT DISCRIMINATE AGAINST SMALL ENTREPRENEUR.

- MARKET FORECAST IS MAJOR INFLUENCE ON PROFITABILITY (MARKET SIZE, SHARE, UNITS SOLD, UNIT PRICE, LIFE CYCLE ARE AFFECTED). MORE INTENSE EFFORT REQUIRED HERE.

- SPACE POWER COSTS IS MAJOR INFLUENCE ON UNIT COST. DEVELOPMENT OF LARGE SOLAR CONCENTRATOR FOR HEATING WOULD BE MAJOR BENEFIT.

- 7-30 DAY SHUTTLE/SPACELAB AS COMMERCIAL PROCESSING PLANT LIMITS PROFITABLE PRODUCTS. LONG TERM, IN-ORBIT, AUTOMATED, INTERMITTENTLY MANNED FACILITY IS ANSWER. SHUTTLE FUNCTION IS MAINLY TRANSPORT OF RAW MATERIALS UP, FINISHED PRODUCTS DOWN, ALSO INSTALLATION, MAINTENANCE, REPAIR.

- THE FOUR PRODUCTS STUDIED CALL FOR MINIMUM OF 18 FLIGHTS FOR R&D, 23 FLIGHTS/YR FOR PRODUCTION.

- BASED ON FOUR PRODUCTS SMALL LOW POWER, SPACE-PRODUCED MATERIAL WITH HIGH MULTIPLIER FOR GROUND FINISHING AND HIGH $/LB PRICE IS BEST BET (E.G. ISOENZYMES). NASA SUPPORT OF PROGRAM TO FEASIBILITY STAGE, SHARING R&D COSTS AMONG MANY USERS, LOW COST POWER CAN ADD OTHER BETS (E.G. TUNGSTEN).

Figure 17.- Lessons learned in phase III.

WORKSHOPS

INTRODUCTION

By Dennis R. Morrison, Lyndon B. Johnson Space Center, Houston, Texas

One of the objectives of this colloquium was to establish the basis for a cooperative effort among Government and non-Government researchers to examine the potential of bioprocessing in space and to encourage active participation in the development of new concepts related to bioprocessing in space.

Following the general scientific sessions, four half-day workshops were held to allow participants to discuss new ideas, future biological research areas of interest, related gravity-induced problems, and new approaches to ways of using this new research tool — the unique space environment. The workshops were chaired by non-NASA scientist from both academic and industrial research institutions. Brief summaries of the workshop sessions included the following topic areas: (1) industrial biosynthesis — the discussions of potential space research involving cell modifications, processing problems and possibilities, biological recycling, and biochemical energy production; (2) pharmaceutical research — discussions including new possibilities for studying drug metabolism, physical pharmaceutical chemistry, and separation/purification processes; (3) biotechnology — discussions including biological sensor systems, space electrophoresis, and cell separation techniques; and (4) cell biology — discussions of fundamental research approaches involving the influence of space on cellular systems and cellular interactions.

It is noteworthy that one of the results of this colloquium and the ideas generated in the workshops has been the recent expansion of the NASA space-bioprocessing projects. The program now encompasses research to (1) extend the biomedical applications of electrophoresis in space, (2) develop other advanced separation techniques to isolate ultrapure, fragile biological substances, and (3) investigate cell culture and other biosynthesis techniques, in space, for manufacturing certain high-value biologicals that cannot be produced on Earth.

BIOTECHNOLOGY WORKSHOP SUMMARY

By John M. Walsh,[*] Beckman Instruments, Inc., Anaheim, California; and
Robert E. Allen,[†] Marshall Space Flight Center, Huntsville, Alabama

INTRODUCTION

The issues addressed by the Biotechnology Workshop were twofold: Discussions
were held on specific research areas of primary interest to the participants and also
on the equipment required to implement the experiments of others. This latter subject
is more the "how to do it" rather than the "what to do."

Initially, to orient the subsequent discussions, it was attempted to establish a
working definition of bioprocessing in space and distinguish between this activity and
the conduct of basic research in the life sciences. It was generally agreed that
research in bioprocessing is more directed and applied, because the results of such
research should have future economic utility. An example of such applied research
would be the production of biochemicals that might have clinical diagnostic value in
the detection of disease. Such products would have primary economic utility for their
life-saving or life-enrichment potentials. This would also be of interest to commercial
organizations considering the possibility of commercial utilization of space.

A brief synopsis of current activity in the design of space bioprocessing systems
was also given, both to demonstrate the potential benefits of space and to consider
the design constraints imposed by the space environment.

In response to the interests expressed by the workshop participants, the following
topic areas were addressed.

o Separation technology

o Biocompatible materials

o Experimental animals

o Remote biosensing

o Biorhythms

SEPARATION TECHNOLOGY

The topic of separation technology is pertinent in the consideration of bio-
processing systems because of a requirement for the isolation and purification of the
product of interest. This product might be a specific subpopulation of cells from a
complex mixture — discrete subcellular fractions, purified proteins, etc. — that are
useful in themselves or that could be used for the production of other materials —
antibodies, for example.

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[*]Manager, Life Sciences Advanced Technology Operations.
[†]Chief, Biotechnology Branch.

Extensive discussions were held on the various methods available for the separation of biological materials, including continuous preparative electrophoresis, isotachoelectrophoresis, affinity chromatography, and immunology-related separation systems. The relative merits of each were elaborated.

Concern was expressed that investigators might embrace the potentially enhanced separation capability of space too enthusiastically without first exhausting methods available on Earth. It was suggested that applications should be screened for validity to ensure that the environment of space would indeed offer potential benefits.

BIOCOMPATIBLE MATERIALS

Two topics related to biocompatible materials were discussed. The first of these stressed the importance of material selection in the design and construction of bio-processing systems. Because living systems — e.g., cells — will be used in many of the processes, it is essential to employ materials that will not compromise the viability of the cells or the utility of the end product. It was pointed out that this is a paramount design consideration and should not be minimized.

The use of the space environment for the fabrication of unique biocompatible materials was discussed briefly. Such materials would be useful in the fabrication of implantable prosthetic devices.

EXPERIMENTAL ANIMALS

It was suggested that NASA consider the use of Aves — such as chickens — as experimental animals in space because of the close relationship of avian and mammalian physiologic systems. Other potential advantages include their relatively light weight because of their bone structure, the high packing density that can be achieved, and their special suitability for certain studies — such as color perception, hearing acuity, and light/dark sensitivity.

REMOTE BIOSENSING

It was pointed out that microbial contamination would affect many bioprocessing systems and that the time required to ascertain the existence of contamination, with the use of current methods, is relatively long. It would be desirable to develop a remote biosensing capability to monitor microbial contamination, perhaps by employing microphotography and multispectral analysis.

BIORHYTHMS

The effect of biorhythms on the work performance of shuttle crewmen was discussed. It was the impression of the concerned participants that NASA had prematurely discounted any untoward effects of biorhythms altered as a result of orbital flight and that such phenomena should be investigated further. As an example, it was noted that altered biorhythms affect the tolerance of the body to certain drugs. This might have implications for in-flight therapy in the event of crew illness. It was felt that such research would be valuable in the maintenance of crew efficiency and would also be applicable in Earth-based industrial environments.

SUMMARY

Unrelated to any specific technology area, concern was expressed that excessive emphasis was being placed on the near-term economic justification of experiments because of the cost of a space flight. It was suggested that a "marginal-cost" concept might be useful for certain experiments. On a proposed flight, if space and facilities exist, provision should be made to conduct additional experiments for the marginal costs involved. This approach would lower the front-end costs to private organizations, provide more flexibility, and eventually expand the number of end users of the shuttle capability.

The benefits of this colloquium will not be — nor should they be expected to be — immediate. What was accomplished, however, was to expose a segment of the research and industrial communities to the capabilities available in the shuttle. The benefit will be derived in the future as these capabilities become available for the solution of specific problems or for opening new avenues of investigation leading to the development of new products.

CELL BIOLOGY WORKSHOP SUMMARY

By Jerry V. Mayeux, Bio Innovar, Inc., Storm Lake, Iowa; and Gerald R. Taylor, Lyndon B. Johnson Space Center, Houston, Texas

INTRODUCTION

Scientific investigations are often divided into two areas that overlap. These areas are basic and applied research. Basic research grows out of man's basic desire to know the how, why, what, and when of his existence and environment. Applied research grows out of man's desire to solve practical problems, to be able to do things that he is not presently capable of doing, or to provide things that will make his life more productive, pleasant, and enjoyable. The following point was recently made by Dr. H. Theorell of the Karolinska Institute of Stockholm, Sweden. After Dr. Theorell had requested funds for laboratory expansion, the president of the foundation to which Dr. Theorell had applied asked:

"Which disease do you intend to cure?"

Dr. Theorell replied, "None at all! Do you have a watch?"

Foundation President: "Yes."

Dr. Theorell: "What do you do in case it stops running?"

Foundation President: "I take it to a watchmaker."

Dr. Theorell: "Why do you take it to a watchmaker?"

Foundation President: "What do you mean?"

Dr. Theorell: "Well, you do it because the watchmaker knows how the watch is constructed and therefore how he can repair the watch. I try to find out how the living body is constructed; and, when we know that, we will be able to repair it."

Dr. Theorell's point is that all applied research is founded on basic research and that before the basic facts are known, it is a waste of money to support applications on nonexistent basic knowledge.

GENERAL RECOMMENDATIONS

Participants in the Cell Biology Workshop feel that at this time, there is not sufficient information available on the behavior of cells in the space environment to identify cell-related bioprocessing applications beyond those already discussed at the meeting. There is a consensus that the space-flight environment permits carrying out basic research that cannot be accomplished in the presence of gravity and that will very likely have practical biological and biomedical applications here on Earth. The group strongly suggests that a meeting be organized to discuss and further explore biological and biomedical research needs and that the resulting objectives be used to determine prime space-flight experiment areas, constraints, and potential equipment requirements. The following statements summarize the general points of agreement arising from the Cell Biology Workshop.

o Cells are an integral part of many important bioprocessing procedures.

o This group currently lacks fundamental information about cell behavior and function in space flight and therefore cannot recommend new applications for the use of the reduced gravity of space for bioprocessing.

o We have defined space bioprocessing as the use of space flight as a critical step in some production process using cellular systems.

o We have identified certain basic research that must be conducted to understand cell processes well enough to be able to propose space-processing experiments. Research programs that could advantageously utilize null gravity are discussed in the following section.

o We propose the identification of a small group to further develop these research objectives and to act as advisors to the NASA in discussing space-processing experiments involving cells.

o We suggest that a similar meeting be conducted to discuss aspects of pure cellular and biomedical research in space.

SPECIFIC RECOMMENDATIONS

In addition to the general considerations listed previously, specific problem areas were identified as high-priority space research areas.

o Determine the influence of reduced gravity on cellular metabolic activities.

o Measure the effects of space flight on circulations within the cell (streaming and convection).

o Study intercellular and intracellular biological and metabolic transport functions in a convection-free environment.

o Determine the influence of null gravity on cell-cell interactions and embryological development.

o Determine the influence of null gravity on the reproductive process.

o Use the space-flight environment to study gravity-sensing mechanisms within cells.

SAMPLE RESEARCH AREAS

The following items are specific research activities that were discussed at the Cell Biology Workshop. If it is known, the name of the person(s) responsible for preparing each section is included for reference.

1. The selective mating and cloning of unicells in fluid media. (Jerry Mayeux) This technique, if it could be shown to be facilitated by null gravity, could greatly expand developments in agriculture and medicine. Selective mating could be used to transfer genetic characteristics from one cell type to another. This procedure could

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lead to development of unique strains of microorganisms for synthesis of hormones or new therapeutic drugs.

2. Cellular interactions. An area of critical interest to fundamental cell biology is the nature of interactions between and among cells that are required for generation or regulation of a specific differentiated response. For example, development of both antibody response and cell-mediated immune response in vitro involves interactions between several subpopulations of lymphocytes and macrophages. Little is known, however, about the physical requirements for establishment of such interactions or the consequences of establishing stable interactions that may be feasible only in conditions of null gravity or controlled convection. Experiments in space could profitably correlate morphological studies of lymphoid cell interactions by phase contrast microscopy with functional manifestations of antigen or mitogen stimulations of such cultures. Conversely, the space environment would be ideally suited to investigations of cellular aging in the absence of normal cell interactions that could be initially prevented and then subsequently promoted.

Additional specific areas of cell interactions in which regulation of differentiation should be investigated include fundamental problems in embryogenesis (either as isolated cells or intact cell systems) and the interactions among cells that may promote the capacity of cells undergoing neoplastic changes to metastasize to distant sites.

Because convection and gravity are two essential variables that may modulate or establish cell-cell interactions, it is believed that control of these essential variables, uniquely available in the space environment, will contribute significantly to new information in this area of fundamental biology.

3. Decompression sickness. (Joseph D'Arrigo and James Verlander) Decompression sickness can best be studied in the weightless environment of space, simply because the causative agent (i.e., bubble formation in the blood and body tissues) can be studied directly (visually) without having to rely on the indirect and often misleading means presently used (e.g., gelatin entrapment, Doppler detection) to monitor bubble formation in fluids. Such information would have immediate application to the development of improved decompression tables, both for the Navy diving program and the shuttle safety standards.

4. Biological transport (permeability phenomena). (Don Lee) The effects of space flight on the active transport activities in biological systems should be evaluated. Specific applications are outlined below.

Rate-limiting step in metabolism.

(1) To answer the reason for growth rate differences previously observed in outer space.

(2) To study the sequence of events that occur in metabolism in some cells (some active transport system phosphorylate or activated molecules as they move through the membrane — Roseman system of active transport).

Transport may be directly involved with the cyclic series of events that occur in cells.

Due to permeability differences between fertilized and unfertilized eggs, transport of materials and subsequent metabolic induction might be directly correlated to differentiation.

5. <u>Reproductive biology</u>. (Wallis Clark) It is essential that carefully designed studies of reproductive processes be performed in a weightless environment. These studies should include the following categories: gametogenesis (meiotic processes and gamete differentiation), gamete association, zygote formation, and zygote-fetal association with maternal tissues.

6. <u>Gravity sensing in cells</u>. (Bodo Diehn) Most plants exhibit geotropism, and some free-swimming unicells also seem able to sense and respond to gravity. Very little is known about their putative gravity sensors. What is the minimum force of gravity that will induce geotropism in higher plants? Does the absence of gravity lead to the growth of structurally weaker plants? If that is the case, can one, on the basis of the results of zero-gravity studies, interfere with the gravity-sensing system to grow structurally stronger plants in unit gravity? As far as unicells are concerned, do they really have a gravity sensor or have the investigators been observing the effects of surfaces on behavior? If there is a gravity sensor, is it inducible; i.e., can space-grown progeny sense gravity immediately? (Can the sensor be identified morphologically?) What are the interactions between gravity sensing and other stimulus/response systems, such as light-induced-movement behavior? Unicells may provide useful model systems for sensory processing in higher organisms.

7. <u>Embryological development</u>. (Andrew Webb) On the assumption of a long-term commitment — on the part of the U.S.A. and the NASA program — to interplanetary travel and the eventual colonization of space, the potential influences of the weightless environment on successful human reproduction will have to be assessed. It is imperative, therefore, that the preliminary experiments in embryology so far undertaken in the space program be extended to mammalian (for example, female rodent) material as soon as possible. Specific topics that should receive immediate attention include fertilization phenomena, blastula transport, and blastocyst implantation. There is good evidence to suspect that these initial phases of mammalian development, in particular, could be drastically affected by a "zero-g" environment.

8. <u>Biochemical evolution in space.</u> (David Deamer and Melvin Shelton) It has been understood since 1950 that primitive Earth atmospheres could evolve basic biomolecules (amino acids, hydrocarbons, purines, and carbohydrates) under electrical and ultraviolet (UV) radiation. Most scientists agree that the early Earth surface was probably an organic "soup" of these biochemicals. Much of this synthesis must have taken place in the upper atmosphere. However, as far as is known, direct exposure of primitive Earth conditions to solar flux has not been carried out. It is difficult to imitate solar UV flux on Earth, because specific elemental UV sources such as mercury arc lamps are used. These sources produce only a few bands. It would be of fundamental interest, and quite simple, to expose a number of different atmospheres to direct solar flux, followed by qualitative and quantitative analyses of synthesized components and quantum efficiencies. Such an experiment would add considerably to current knowlege of primitive Earth biochemical evolution.

9. <u>Effects of space flight on circulations within the cell (streaming and convection</u>. (John Kessler) The following questions should be asked.

How does the elimination of buoyancy affect the intercellular and intracellular streaming associated with cell kinetics?

Can the zero-gravity regime be utilized to elucidate fundamental aspects and mechanisms of cell transport?

Does the zero-gravity environment alter the frequency or other operating parameters and characteristics of cyclic enzyme processes?

Can the results obtained in this type of research be put to practical use in such separation, production, and processing schemes?

In consideration of these points, it should be remembered that streaming in an inhomogeneous active environment results from buoyant convection, gradients of inter-facial tensions, and, possibly, active transport. Thus, the elimination of buoyancy may be expected to reveal the role of the other components of the driving forces in intercellular convective transport and in the streaming of the cell interior.

10. Freeze-fracture methods in space. (David Deamer) Freeze-fracture electron microscopy is an important and widely used method for preparing biological samples. It is the only technique that permits visualization of unfixed specimens and that re-veals membrane structure at 2.0-nanometer (20 Å) resolution. On Earth, it is limited by vacuum technology and by throughput because only one to four specimens per hour can be prepared. It is also an expensive technique, requiring $50 000 or more initial investment.

Freeze-fracture is thus ideally suited to the space environment because it requires a noncontaminating vacuum and extremes of cold (173.15 to 73.15 K (-100° to -200° C)). The basic apparatus is simple: A rotating sample holder that can hold numerous speci-mens in the form of 2-millimeter disks, a sharp cutting edge to shave the specimen sur-faces, and a platinum-carbon electron gun shadowing device with approximately 1 cubic meter of accessible vacuum are also required.

11. Electron microscopy in space. (David Deamer and William Sanford) Present electron microscopic applications are limited in part by vacuum chamber size, by quality of vacuum, and by contamination of surfaces. Such problems could be resolved by con-struction of an electron microscope usable in space, that takes advantage of the space environment (noncontaminating, high vacuum, and weightlessness). An electron micro-scope, at least in principle, can be light and simple in construction. Much of the bulk of a microscope on Earth is produced by the pumps, column armor, and power supplies. These items would obviously be unnecessary in space. The basic requirements would be an electron gun, a set of lenses, a specimen holder, and a camera or video imaging de-vice. The lenses could be electrostatic or perhaps super-conducting magnetic and thus eliminate heavy power sources.

Three modes of electron-specimen interaction could be used.

Transmitted electrons could be imaged to provide cross-sectional information from sections or replicas.

In a scanning mode, which resolves surface topology, secondary electrons could be collected to generate an image displayed on a cathode ray tube (CRT).

Secondary X-ray emission from the specimen can be analyzed to provide quanti-tative analysis of elemental composition.

The following applications are envisaged.

Materials analysis. An example is the thin-film microcircuitry proposed for space production. The scanning mode could be used to image the circuit, and X-ray emission could be analyzed for dopant concentrations.

Biological specimens. It is possible that the space environment will enable the potential resolving power of electron microscopy (30.0 to 70.0 picometers (0.3 to 0.7 Å)) to be approached. This power is sufficient to resolve atomic dimensions and would enable the viewing of important biomolecules for the first time.

FUTURE ACTIVITIES

The members of the Cell Biology Workshop strongly proposed the formation of a smaller group to serve as advisors to the NASA and as a point of contact around which cell biologists could organize to consider space-flight experiments. These persons are listed below. It is hoped that each will communicate to the NASA/JSC contact, within 2 weeks of receipt of this report, his or her willingness to serve in this capacity. It is expected that all business will be conducted by telephone or through the mail.

Ad Hoc Advisory Committee on Cell Biology Studies in Space:

NASA/JSC contact: Gerald Taylor

Committee members: Bodo Diehn

                                      Beth Basen

                                      Wallis Clark

                                      Joseph D'Arrigo

                                      David Deamer

                                      John Kessler

                                      Don Lee

                                      William Sanford

                                      N. S. Shah

                                      James Verlander

INDUSTRIAL BIOSYNTHESIS WORKSHOP SUMMARY

By W. E. Brown, Squibb Institute, Princeton, New Jersey; and
Robert E. Sparks, Washington University, St. Louis, Missouri

INTRODUCTION

Our discussions of problem areas that might be better understood by experimentation in space can be grouped into six categories, as follows.

1. Cell modification

2. Processing problems and possibilities

3. Hormone production

4. Immobilized enzymes

5. Biological recycling

6. Energy production by photobiochemical reaction

CELL MODIFICATION

Continued Screening Studies

Experiments should be continued to determine whether the growth rate or production rate of enzymes or metabolites is increased in the space environment. This determination will permit assessment of the possible importance of large-scale cell processing in space.

An excellent test case would be a careful study of gluconic acid formation by Pseudomonas ovalis. The sequential steps and their kinetics are very well understood. This information would aid greatly in the interpretations of any abnormal observations made during a fermentation in space.

The screening studies for abnormal cell behavior in space should be extended to other cells, such as those of the invertebrates. It has been shown, for example, by Dr. Jack Cecil of Osborn Laboratories (New York Zoological Society) that sponge cells produce substances showing antibiotic and anticancer properties. Any augmentation of production of such promising molecules would be most attractive. Such cells are also very difficult to separate and would be promising candidates for electrophoresis studies.

Modification by Heavy, High-Energy (HZE) Particles

The availability of 40-million-electron-volt HZE particles in space cannot be duplicated at present on Earth, where maximum particle energy is 2 million electron volts. The effect of these HZE particles in causing promising cell modification should be studied. It might be possible to produce cell strains with higher production rates of desired products.

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In addition, a study of the effect of HZE particles on tumor cells might be of value. A more direct study, suggested by Dr. Martin Griffin (Oklahoma Medical Research Foundation), would be to orbit mice with carcinogens, or tumor-laden mice, for exposure to HZE particles. In a 10- to 30-day period, the effect of these high-energy particles might be apparent. It would be necessary to look for complete remission. For example, tumor antibody antigen release might alert the immune system to the abnormality of the cells and lead to their destruction.

PROCESSING PROBLEMS AND POSSIBILITIES

Gas-Liquid Contact

If fermentation were to be conducted at zero g, adequate gas-liquid contacting would be a formidable problem. The volume of air that must be used to supply oxygen (O_2) and sweep out carbon dioxide (CO_2) is many times the liquid volume. The formation of a stable foam or dispersion would bring the fermentation to a halt. Hence, a method would have to be devised to allow the gas to separate; a centrifugal field or passage through disengagement screens might be involved. This necessity would seem to eliminate any gas-liquid contact advantage in zero g.

Sterilization and Lyophilization

The ready availability of ultraviolet (UV) radiation from the Sun might be of considerable utility in sterilization. There might even by an economic advantage for preparing in space the sterile medium for fermentation. Differences should be borne in mind between UV sterilization and sterilization on Earth by other methods. A UV sterilization might call for replacement of degraded vitamin A and also might catalyze other unwanted reactions.

The ready availability of a high-vacuum source would make lyophilization in space very easy.

Handling of Anaerobes

The presence of pathogenic anaerobes has been recently demonstrated in human tissue previously classified as sterile — e.g., "sterile abscesses." Demonstration of the presence of the anaerobes required techniques for complete exclusion of oxygen, which is lethal to the anaerobes. They can be grown only in expensive, specially prepared pre-reduced media.

The vacuum and absence of oxygen in space would permit growth of these and similar oxygen-sensitive organisms for research purposes and possibly for the production of metabolites, yet to be identified. It is possible that cell fragments could be utilized as immunizing agents.

Similar considerations might make the space environment attractive for the use of hydrogenases, which are poisoned by oxygen.

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Processing of viruses in suspension could also benefit from availability of UV for sterilization and inactivation of the virus.

HORMONE PRODUCTION

Of the possible products discussed by Dr. Laszlo Nyiri in his presentation at the colloquium, only the hormones could stand the economic burden of processing in space.

To maximize safety of the final preparation, the hormones should be produced by noncancerous tissue. Dr. Steven Drew has shown that such tissue need not be grown attached to a surface. He has obtained growth in a weak agar suspension that holds the cells just off the surface. Splerules 5 to 10 millimeters in diameter are formed that resemble benign tumors, containing internal necrotic tissue, probably caused by oxygen limitation. The tissue used was murine leukemia tissue, which grows at approximately the same rate as normal organ tissue.

A zero-g environment could be of great value in enabling suspension growth of noncancerous tissue, because it would enable the use of normal, low-viscosity media. Under these conditions, cell-cell and cell-surface adhesion would be prevented and large-scale culturing of healthy cells would become possible.

IMMOBILIZED ENZYMES

Consideration should be given to the enzymatic modification of organic molecules to produce compounds not otherwise readily obtained. These transformations could be produced by cell cultures, enzyme-rich cells, or isolated enzymes. The latter two enzyme sources offer the possibility of conducting the biosynthesis at a relatively high concentration in short times. The use of immobilized enzymes is particularly attractive because of their stability and the fact that they can be reused many times.

On some substrates — e.g., Sephadex beads — the scale of this operation (and also the operation of gel permeation chromatography) is severely limited by the fact that the soft beads deform and restrict flow. In zero-g, this change would not occur and large-scale processing would be possible.

Dr. Steven Drew (Chemical Engineering Department, Virginia Polytechnic Institute) suggested that the low-convection environment of near-zero gravity may offer an advantage over Earth-bound environments for the enzymatic synthesis of biopolymers. Synthesis of biopolymers by immobilized enzymes may be subject to varying degrees of shear (convective shear), the entent depending on the size and shape of the polymer. For instance, enzymatic synthesis of dextran on Earth results in a family of biopolymers with an average molecular weight of approximately 20 000 and varying degrees of branching. Is the molecular weight distribution determined by shear effects? If so, then perhaps enzymatic synthesis of dextran in space could raise the average molecular weight to a much higher figure. This result could have considerable impact on the use of dextran as a blood plasma extender in the treatment of shock, etc.

Another example follows from recent developments in the field of genetic engineering. It has become apparent that some day in the not too distant future, we will be able to manufacture specific gene segments for incorporation into various organisms for the purposes of genetically modifying their characteristics. If complete genomes

are to be synthesized, the template must be copied without error. The absence of convective shear effects in space might reduce "transcriptional" error in cell-free synthesis systems and, at the same time, enable unrestricted growth of the linear molecule.

Experiments should be designed to determine the effects of shear on such sensitive aspects of biopolymer formation as error, branching, and ultimate chain length.

BIOLOGICAL RECYCLING

Biological recycling on a small scale has received considerable attention in the past, but this work has ceased because it seemed probably that recycling for space travel would not be needed before the turn of the century. The technical problems of urine recycle and algae growth (for CO_2 recovery, O_2 supply, and food augmentation) have been largely solved. However, if space bioprocessing is to be done on a large scale, water will have to be recycled in enormous quantities and biological recycling will be required for the work forces associated with processing and power generation in space.

Because a long leadtime is associated with large-scale development of biological recycling, it is urged that studies in this area be given a realistic priority.

ENERGY PRODUCTION BY PHOTOBIOCHEMICAL REACTION

Dr. Sidney Fox (University of Miami) has recently achieved success in producing adenosine triphosphate by UV irradiation of an aqueous solution of adenosine monophosphate (AMP), inorganic phosphate, and a pigmented thermal polyamino acid that appears to act as a photosensitizer. Conversions are 30 to 60 percent on the basis of AMP conversion in 20 hours of irradiation in a 1-millimeter cuvette. The source was an 85-watt GE mercury lamp placed 6 centimeters from the solution. These results have been reproduced by two people in over 100 runs.

It might be attractive to study this reaction in space, where a suitable orbit would permit continuous exposure to high-intensity solar radiation.

Scale-up of this reaction would permit the development of new energy generation techniques but would require innovative design of large-scale photochemical reactors.

CONCLUDING REMARKS

The space experiments considered to be of high value are as follows.

1. Cell modification by HZE particles

2. Suspension culture of noncancerous cells

3. Synthesis of large biopolymers and genomes

4. Photobiochemical energy production
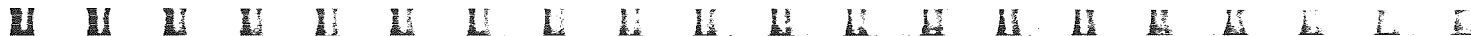
PHARMACEUTICAL WORKSHOP SUMMARY

By E. John Staba, University of Minnesota, Minneapolis, Minnesota;
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INTRODUCTION

Approximately 15 to 20 individuals participated in the session on pharmaceuticals.
Three broad subject areas were considered, as follows.

1. Separation and purification processes

2. Drug metabolism

3. Pharmaceutics

SEPARATION AND PURIFICATION PROCESSES

Suggestions made regarding separation and purification processes in space environ-
ments were as follows.

1. Possible improvement of separation processes presently being used, or develop-
ment of new processes, by controlled temperature-induced convections (Clusius Dickel
principle).

2. A more thorough investigation of the effects of forces such as magnetism, sound,
etc., on separation processes.

3. The use of high-performance centrifuges to drive liquids and to separate par-
ticles.

4. Separation and purification of protein drugs such as erythropoetin, enzymes,
allergens, hormones, antibiotics, etc.

5. Separation of high-product-producing plant suspension cells; i.e., those cell
that produce or biotransform steroids.

DRUG METABOLISM

With respect to drugs in man, the following suggestions were made.

1. The absorption, metabolism, distribution, and excretion of drugs commonly used
by subjects in space should be extensively studied; for example, those drugs used for
motion sickness, sedatives, and stimulants.

2. Consideration should be given to the preparation of blood volume expanders such
as human plasma, etc., for emergency use.

3. Effects of circadian rhythm and body sleep position on drug effect should be
studied.

A somewhat aside but intersting discussion developed regarding the discharge into space of toxic materials (carcinogens) and unwanted substances (radioactive) that result from space processes.

PHARMACEUTICS

The discussions on the effects of space environment on drug manufacturing resulted in the following suggestions.

1. Critical study of particle distribution patterns and mass transfer theory.

2. Study of solids with respect to their flow and ability to be compacted. A spin-off of this concept might be the preparation of highly efficient columns for gas chromatography, and the granule preparation of drugs.

3. Study of the evaporative processes from solid surfaces and fractional crystallization studies.

4. Performance of dissolution and diffusion kinetic experiments as they pertain to drug formulation, drug release, and drug chemical synthesis.

MISCELLANEOUS MATTERS

Discussion was also held on matters unrelated to pharmaceutical bioprocessing but of real concern to the participants. They were as follows.

1. Announcement of Opportunity or (compared to) Grant Proposal procedures as the best basis for the selection of future space experiments.

2. Cost per kilogram to private enterprise to perform space environment experiments.

3. The preparation of a useful bibliography for those interested in space bio-processing.

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APPENDIX B

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