

SPACE BIOREACTOR

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Microgravity offers new ways of handling fluids, gases, and growing mammalian cells in efficient suspension cultures. Some conceptual designs for a zero-g fermentor were developed as early as 1969. In 1976 bioreactor engineers designed another system using a cylindrical reactor vessel in which the cells and medium are slowly mixed. The reaction chamber is interchangeable and can be used for several types of cell cultures. NASA has methodically developed unique suspension type cell and recovery apparatus culture systems for bioprocess technology experiments and production of biological products in microgravity. The first Space Bioreactor has been designed for microprocessor control, no gaseous headspace, circulation and resupply of culture medium, and slow mixing in very low shear regimes. Various ground-based bioreactors are being used to test reactor vessel design, on-line sensors, effects of shear, nutrient supply, and waste removal from continuous culture of human cells attached to microcarriers. The small (500 ml) Bioreactor is being constructed for flight experiments in the Shuttle Middeck to verify systems operation under microgravity conditions and to measure the efficiencies of mass transport, gas transfer, oxygen consumption and control of low shear stress on cells.

Introduction

For several decades, many important pharmaceuticals and other biological products have been obtained from large scale culture of microbial cells. However, the mass cultivation of mammalian cells is much more difficult because of their delicate nature and their stringent environment requirements (1). In fact, contemporary culture technology is often inadequate in attempts to provide the proper conditions for optimum human cell growth and cell secretions. During the past 10 years the need for major technological improvements in human cell culture has increased because of the expanding demand for human cell products, such as hormones, enzymes, interferon, etc. to be used as pharmaceuticals.

Increased demand for large numbers of cells for virus and cancer research has also added impetus to develop practical systems for large scale culture of mammalian cells. Some improvements have been made, however, large scale culture systems still have major problems which make human cell culture very difficult and often uneconomical (2).

A great deal of excitement has resulted from the recent success in the genetic reprogramming of bacterial cells to produce human cell products such as growth hormones, human insulin, and interferon. However, the genetic

engineering of very complicated molecules is still several years from commercial practicality. Products are still contaminated with bacterial proteins and DNA fragments producing problems with allergenic or immune reactions to the preparation. To date only growth hormone, obtained from genetically engineered cells, has been approved by the Food and Drug Administration. Another limitation is the inability, so far, to genetically code for any glycosylated proteins. Certain human cell products which may not be practically produced by genetic engineering remain good candidates for production by culture of human cells whenever the technological problems are solved. Many diseases involving neoplasms, blood clots, hypertension, anemia, emphysema, growth disorders and others are now considered treatable with pharmaceuticals derived from cell culture if only the technology can be improved to make large scale cultures practical (3,4).

In the NASA bioprocessing laboratories at the Johnson Space Center we have evaluated several of the conventional and more recent methods for culturing human cells. Our studies involved: 1) the definition of the environmental conditions that must be controlled to insure successful growth and maintenance of human cells under microgravity conditions, 2) the recovery of cultured cells and cell products for postflight research, 3) the initial isolation or first step in down-stream processing which may be used as a feeder step to the purification of products by Continuous Flow Electrophoresis (CFE) or Recirculating Isoelectric Focusing (RIEF), and 4) the design features which must be included to maintain precise control of culture conditions under microgravity conditions (5).

In order the design proper control systems it was essential that we obtain performance data on various component elements of the culture apparatus. Special emphasis was placed on designs for fluid handling, temperature distribution, gas/liquid phase separation, etc., under microgravity conditions. We approached these problems by first identifying physical phenomena and operational principles of suspension culture which are gravity dependent. Then scientific workshops were held to determine which phenomena may be altered in microgravity to produce significant advantages or disadvantages in the culture process (6,7). It became apparent that many physical and biophysical interactions within cell culture systems are poorly understood. Several areas appeared to need additional research to better understand the importance of particular physical interactions which occur in suspension culture systems (see Table 1).

TABLE 1. CELL CULTURE RELATED RESEARCH AREAS (DATA IS NEEDED BOTH TO IMPROVE CULTURE TECHNOLOGY ON EARTH)

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Microgravity Related Research Needed:

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Upper limits of shear stress on cells  
 Minimum shear required for adequate mixing and mass transport  
 Details of required microenvironment surrounding the cell  
 Effects of shear on gas transport and cell membranes  
 Alterations (if any) of transport when bubbles don't coalesce  
 Mass transport in the absence of thermal convective mixing  
 Causes for attachment dependence and cell energy diverted to this behavior

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## Evaluations of Earth-based Cell Culture Systems

Classic industrial and research methods of growing human cells have been limited to systems which grow and maintain cells in a monolayer. This is largely due to the anchorage dependence of mammalian cells. It appears that virtually all normal human cells must be firmly attached to some suitable surface before they will grow, multiply, and eventually secrete any products. Attempts to grow large numbers of cells in the inside surface of roller bottles (8) and multi-plate propagators (9) has permitted moderate scale mammalian cell cultures.

Some additional success has been achieved from growth cells attached to spiral wound membranes and to the inner surface of hollow-fiber dialysis bundles, in which nutrients can be supplied through the permeable membrane from media circulated on the opposite side of the membrane or hollow fiber (10). However, these techniques are still greatly restricted by poor surface-to-volume (culture medium) ratios. Some of the major problems associated with monolayer cultures; namely, 1) growth in only two dimensions (surface area), 2) cell proliferation limited by depletion of oxygen and nutrients, 3) difficulties with media circulation and volume to cell number ratios, 4) cell/product recovery, and 5) contamination control are more readily solved by using suspension culture. Unfortunately, almost all human cells of pharmaceutical interest grow quite poorly in modern suspension culture systems. Even when suspension culture of mammalian cells is accomplished on a small scale the maximum density of cells varies between  $5 \times 10^5$  to  $3 \times 10^6$  cells per  $\text{cm}^3$ . This concentration is orders of magnitude less than the number achieved in microbial cultures. Since the amount of product formed under proper physiological conditions, is directly proportional to the number of the cells, high cell concentrations are critical to successful commercial cultures.

In addition, the necessity to maintain the cells in a rather dilute nutrient media allows sedimentation to occur easily which in turn requires stringent mixing. Often the cells cannot tolerate the severe shear forces caused by even the best designed agitation systems. Prolific cells growth is also limited by the oxygen supply. Sparging of air is usually the best method to supply oxygen, however, the bubbles rise and coalesce so quickly that dissolution of oxygen is often incomplete during the bubbles' brief residence in the culture media. Vigorous bubbling of air causes high shear rates and too often produces uncontrolled foaming. Foaming in turn carries cells up out of the suspension where they dry out and die. Toxic waste products, cell fragments, and autolysis enzymes then fall back into the media where they poison the culture. Surfactants can be used to reduce foaming, however, their application is limited because they themselves are deleterious to living cells.

In an attempt to overcome the surface to volume disadvantages small (150-180 micron) Sephadex or DEAE cellulose beads were developed as microcarriers for cultured cells (11,12). Cells are allowed to attach to the surface of the bead then the beads are suspended in the culture vessel. This technique helped solve the attachment dependence problems of human cell culture, however, the particular surface of the bead must generally be custom designed to the requirements of the specific cell to be grown. Sometimes the surface

coating also acts as a repository for accumulation of cell waste products which in turn limits cell longevity.

We have compared several of the new cell culture technologies with the well established monolayer culture systems using several types of mammalian cells. This experience gave insight into selected features of each technique which may be combined into a suspension culture system for mammalian cells. We also have tested all of the commercial microcarrier beads (Cytodex, Superbeads, etc.) now on the market, however, sedimentation is still a problem especially after the cells are attached to the beads. Foaming problems from sparging of oxygen also remain. Often the mechanical mixing required to keep the beads suspended is so severe that cells are damaged and dislodged from the bead surface by high shear or bead-to-bead collisions.

Comparisons of commercially available microcarrier beads and discussions in cell culture workshops indicate that there may be many reasons to study cell culture technology under conditions of microgravity (6,7). The elimination of gravity dependent sedimentation of cells and bubble buoyancy may provide new insight into careful control of culture environments. Gentle mixing to insure adequate mass transport could be achieved while maintaining precise control of local temperature, pH, dissolved oxygen, shear, nutrient mixing, etc. in a manner heretofore not possible in Earth based laboratories. Figure 1 is a list of studies and workshops conducted by the Johnson Space Center to identify research topics and experiments which are needed to support the development of a suspension type Space Bioreactor to be used for precision controlled cell culture studies in microgravity.

#### Related Space Experiments and Cell Culture Systems

Studies of potential advantages of suspension cell culture technology in micro-G will only be valid if any direct effect of this environment on cell function is well understood. Early experiments with cells growing under weightlessness indicated that the cell density of S. typhimurium was significantly higher than the one obtained in the ground control experiment (13). The most noteworthy of several interpretations include the random distribution of cells in the culture liquid resulting in enhanced efficiency of nutrient transfer into, and waste transport from, the cells. There also could have been some improvement in the oxygen supply due to differences in gas-liquid mixing. Only one well controlled experiment has been carried out in growth of human WI-38 cells during the Skylab mission (14). Normal growth was observed along with normal mitotic index and subcellular structure. There was a small change in glucose utilization, however. This was considered inconclusive by the investigators.

Experiments on Spacelab 1 showed over a 95% reduction in the ability of human lymphocytes to respond to ConA mitogen (15). A change in glucose consumption was also noted but not considered significant until experiments could be repeated with an on-board one-G control. Other experiments showed an increase in proliferation of Hela cells, chicken embryo fibroblasts and mitogen stimulated lymphocytes of up to 30% due to hypergravity of 10-G while glucose utilization rate was unchanged from one-G (16). Experiments on Shuttle missions 7 and 8 were performed to determine the attachment efficiency of normal human kidney cells to collagen-coated microcarrier beads.

It was expected that the attachment rate would be reduced after mixing in microgravity, since the only opportunity would be random collisions while the cells and beads floated free in the culture medium. However, the results showed a significant increase in attachment for the flight samples vs. the ground control experiment (17). Within 3 hours, the cell attachment (based on the average number of single cells per bead) was 50% greater in microgravity than on Earth. Once attached, both the flight and ground control cells grew at the same rate during the first 25 hours. Cell-to-cell attachment was also greater among the cells free-floating in weightlessness. Future experiments are planned to give greater insight into the attachment mechanisms. However, practical implications include the possibility of seeding microcarrier cultures in microgravity and the knowledge that cells could reattach to microcarrier if they come off the bead surface in slowly mixed space bioreactors.

### Considerations for Culture of Mammalian Cells in Microgravity

The basic approach of NASA scientists to determine what factors must be included in the design of detailed cell culture experiments was to have industrial bioreactor experts study the merits of suspension cell culture systems and their applications in space. The definitive study (18) was performed by Drs. Nyiri and Toth at Fermentation Design, Inc. in 1976. They recommended a perfusion reactor system with an external, cell free, medium circulation loop to provide resupply of oxygen, nutrients, and scavaging of waste products using hollow fiber dialysis type membrane systems. The studies specified that the development of a dedicated microprocessor control system was essential to the proper operation and data acquisition required during space flight. Further, they evaluated the commercial potential of such a system and identified several hormones and cell secretory products as valuable enough to warrant the extra costs of production in space.

Figure 2 summarizes the general approach taken by the NASA Bioprocessing program in the development of the first Space Bioreactor.

Based on the interest generated by these studies and the results of a 1976 Colloquium on Bioprocessing in Space (19) a formal proposal to develop a Space Bioreactor flight unit was approved by NASA in 1978. Thereafter, several workshops were held with researchers to define experiments and typical science requirements for the design engineers. Major conclusions of the cell culture workshops identified several areas wherein the absence of gravity dependent phenomena in the culture environment could provide unique insight into certain cell functions and interactions between the cell and its micro-environment. Figure 3 shows the various areas where scientists believed that cell culture in micro-G could provide new information on the basic biodynamics of mammalian cells.

The objectives of the demonstration flight experiments are shown on Figure 4. Growth of normal human cells was selected because they are anchorage dependent and extremely fragile. Therefore these cells are very difficult to grow on Earth in suspension culture systems. In space potential advantages of lack of sedimentation and bubble buoyancy could enable operations with very gentle mixing and minimum turbulence to reduce shear effects on the cells. The basic objective also included exploration of product harvesting

techniques and principles of basic cell culture operation under microgravity conditions.

NASA and academic scientists then began a research program to study the specific gravity related problems which affected traditional and new cell culture techniques involving biosynthesis of cell secretory products. Research reactors were developed at two institutions and differences in gravity effects on the process technology of culturing cells which grow freely in suspension were contrasted with problems related to the anchorage dependent cells. Figure 5 shows these steps and the basic consideration for the initial design of the Space Bioreactor. Figure 6 illustrates more details of the systems development and biological systems (enzymes of cells) used to test the efficiencies of the prototype devices throughout three phases of development. As refinements were made, sensors added and mixing techniques tested various industrial process control systems were tested. Cultures were maintained for up to 14 days with L1210 cells, however, commercial process control systems were found to be inadequate to maintain the precise control required by human cells.

### Space Bioreactor Development

In recent months the Bioreactor Test Unit (BTU) been designed and fabricated to be accommodated in a volume equivalent to two or three middeck lockers on the Shuttle. Current concepts call for an early flight test of the basic unit and sensor systems to verify operations with fast enzyme reactions before a major flight experiment is conducted with live kidney cells. Designs are also considering accommodations in the Spacelab racks or special middeck experiment racks.

The basic functional requirements for culturing human cells on microcarrier beads for 7 to 10 days on orbit are shown in Figure 7. Unique considerations include no gaseous headspace in the reaction vessel, sensors in the medium circulation loop (to eliminate turbulence in the reactor vessel) and flow/pressure controls to maintain pressures at or below 20 psia (to eliminate effects of pressure on cell functions). Practical considerations are also included based on our experience with limited access to biological experiments in the middeck while the Shuttle is on the launchpad.

Design and operational requirements for major components of the system are listed in Figure 8. The culture vessel design is based on an adaptation of two innovations by industrial researchers. We decided to use our own version of a spin filter type culture system originally designed by Thayer at Arthur D. Little Co. (20). This allows continuous removal of cell-free medium from the reactor vessel. We also decided to separate the mixing and spinning filter functions by using separate magnetic drives which allows independent control needed for very slow stirring during the flight portion of the experiment. The vessel volume was limited to 500 ml. because of the difficulty in maintaining a large supply of human epithelial kidney cells from the same lot to reduce inter-experimental variations. The target shear range for on-orbit operations was determined previously by researchers at Rice University in flow chamber studies of shear effects on kidney cells which secrete urokinase (21). The cell number to medium ratios must be kept within 125% of the most effective static cultures to insure that the cells will be able to condition

the medium for maximum viability and product secretions. The mixing device chosen was based on a recent design by Feder & Tolbert at Monsanto Co. (22) using flexible spiral vanes which helped to minimize turbulence and bead to impellor collision damage. Our designs call for a co-axial arrangement of the flexible vanes and the spin filter with special designs to allow high spin rates on the filter needed for high flow rates (80 - 100 ml./min.) of medium withdrawal from the reactor vessel. This unique design is complemented by provisions for alternating backwash routes using the medium return flow to keep the spin filter from clogging.

Process control considerations include a custom microprocessor control system to control the bioreactor to set point values, automatically log sensor data and provide minimum interface with the flightcrew. Our requirements include the capability to interface the process controller with a small personal computer (via RS-232) which in turn can operate off-line to perform statistical analysis of data, and prediction of adverse trend interception of alert or alarm set points. The capability to update the process controller with software commands from the "supervisory computer" has also been designed into the system.

The flow diagram of the reactor vessel, fluid loop, oxygenator and the protein concentration sideloop is illustrated in Figure 9. Detailed designs will be considered in later papers by Cross & Bowie, however, special mention should be made for position of the sensor blocks which allows calculations of cell metabolism from the difference in DO<sub>2</sub> and CO<sub>2</sub> levels in the input and the outflow from the reaction vessel. Provisions have been made to add concentrated culture medium, acid or base for pH control, dialysis type hollow fiber refeed system (which also can act as a repository for accumulating metabolic wastes). A microgravity bubble trap has also been included to remove any bubbles formed by gas dissolution during temperature or pressure changes and to trap any gas bubbles which may be injected into the medium circulation loop should the oxygenator membrane develop a leak during the flight. The function of the high molecular weight filter loop is to remove serum proteins from growth medium before changeout to maintenance medium when cells are confluent and to periodically (every 3 or 4 days) concentrate a sample of the circulating medium for product assays.

### Planned Flight Tests

The initial flight test requirements for the first two missions are shown in Figure 10. The selection of human kidney cells is based on some eight years of in-house experience with those epithelial cells which produce urokinase, one of the few pharmaceuticals approved for production from normal human cells grown in mass culture. Also our laboratories have extensive experience with detailed methods to screen cell lots to insure that they are normal, healthy, and hardy enough to withstand the rigors of space flight experiments. These cells have also been flown in space several times by our research group involved in separation of kidney cells in space by free-fluid electrophoresis techniques.

Several concepts for science objectives of planned early flight tests of the Space Bioreactor are listed in Figure 11. The major emphasis is on culture in environmental regimes which are impossible in one-G. These include cells

maintained in a very low shear field provided by slow gentle mixing which is possible in microgravity. Another tantalizing area is the culture of cells in the liquid phase of a controlled foam or bubbly culture medium comprised of oxygen bubbles which have no bouyancy nor do the coalesce readily in microgravity. Other suggested experiments involve studies of artificial oxygen carrier emulsions and specific cell physiology under quiescent culture conditions in space. More details are provided in the Appendix A, the Draft of the Space Bioreactor Science Requirements Document which was handed out for reference at this meeting.

Finally, it should be obvious that experiments involving human cell cultures and suspension bioreactors will provide basic engineering data on the design considerations required for most apparatus to culture cells in the absence of gravity. Once these principles are understood cell culture systems can be developed to provide live cells on orbit for cell biology and other types of bioprocessing related studies in microgravity. Should the expected advantages of micro-G be great enough it is possible that a Space Bioreactor could become the feeder facility in a multi-step bioprocessing system, wherein the cell products would be harvested from the bioreactor and fed to a Continuous Flow Electrophoresis System or other product purification device which can isolate pure pharmaceuticals direct from concentrated culture medium. Already the second phase of this type of bioprocess in space has been demonstrated by commercial CFES purifications on STS-4,6,7,8 and other flights. Such a multi-step bioprocess is a good candidate for commercially orientated research on the U.S. Space Station.



## REFERENCES

1. Feder, J. and Tolbert, W. R., "Large - Scale Cultivation of Mammalian Cells", *Scientific Amer.* 248:36-43, 1983.
2. Tolbert, W. R. et al, "An Analysis of Factors Limiting Commercial Utilization of Bioprocesses Involving Non-conventional Organisms", Workshop on Bioprocess Scale-up, Battelle-Columbus, Ohio, 1983.
3. Morrison, Dennis R., "Cell Culture in Microgravity As A Source of Commercial Products, presented at the AIAA Annual Conference, St. Louis, Mo., Jan. 1981.
4. Glacken, M. W., Fleischaker, R. J. and Sinsky, A. J., "Mammalian Cell Culture: Engineering Principles and Scale-up", *Trends in Biotechnology* Vol. 1:102-108, 1983.
5. Morrison, Dennis R., "Cell Culture Research in Space", in NASA Symposium on Gravity and the Cell, Ames Research Center, Moffett Field, CA. Mar 20-21, 1975.
6. A. D. Little, Inc., "Feasibility Study for the Manufacture of Zero-Gravity Pharmaceuticals, Immunobiological and Viral Agents", Final Report NASA-CR-120424, August 29, 1974.
7. Thayer, P. and Broome, M. G., "Cell Culture Biosynthesis in Space", Final Report, NASA Contract NAS9-15322, 1978.
8. Kruse, P. F. Jr., and Patterson, M. K. Jr., eds. *Tissue Culture: Methods and Applications*, Academic Press, New York, 1973.
9. Weiss, R. E. and Schleicher, J. B., *Biotechnol. Bioeng.* 10:601-615, 1968.
10. Knazek, R. A., et al., *Science* 178:65-67, 1972.
11. Van Wezel, A.L., *Nature (London)* 216:64-66, 1967.
12. Levine, D. W. et al., "Microcarrier Cell Culture: New Method for Research Scale Application", *Somatic Cell Genetics* 3:149-155, 1977.
13. Mattoni, R. H.T., "Spaceflight Effects and Gamma Radiation Interaction on Growth and Induction of Lysogenic Bacteria," *Bioscience* 18:602-608, 1963.
14. Montgomery, P. O. 'B. et al., "The Response of Single Human Cells to Microgravity," *In Vitro* 14:165-173, 1978.
15. Cogoli, A., Tschopp, A., and Fuchs-Bislin, P. "Cell Sensitivity to Gravity" *Science* 225:228-230, 1983.
16. Tschopp, A. and Cogoli, A., "Hypergravity promotes cell proliferation," *Experientia* 39:1323-1329 (Basel), 1983.

17. Tschopp, A., Cogoli, A., Lewis, M. L. and Morrison, D. R., "Bioprocessing in Space: Human Cells Attach to Beads in Microgravity", J. Biotech. 1:287-293, 1984.
18. Nyiri, L. K. and Toth, G. M., "Space Biosynthesis Systems", Final Report of NASA Contract NAS9-14961, 1976.
19. Nyiri, L. K., "Some Questions of Space Bioengineering" in Morrison, D. R., ed. Proceedings of the Colloquium on Bioprocessing in Space, March 1976 NASA Technical Memorandum NASA TM X-58191, Jan. 1977 avail. NTIS.
20. Thayer, P., Arthur D. Little Co., Acorn Park, Cambridge, MA.
21. Stathopoulos, N. A. and Helms, J. D., "Shear Stress Effects on Human Kidney Cells In Vitro", Biotechnol. & Bioeng. 27:1021-1026, 1985.

BIOREACTOR STUDIES

- 1976-77 "Space Biosynthesis Systems" - Contract NAS9-14961 by Fermentation Design Inc., Bethlehem, PA. Investigator: L.K. Nyiri, Ph.D. and G.M. Toth, Ph.D.
- 1977 Proposal to develop experiments for multipurpose Space Bioreactor System. Proposal #770056, NASA A.O. No. AO-77-3, L.K. Nyiri, Ph.D. and Marvi Charles, Ph.D..
- 1978-79 "Development of a Space Bioprocessing System for Cell Culture". Contract NAS9-15619, Lehigh University, Bethlehem, PA. Marvin Charles, Ph.D. and L.K. Nyiri, Ph.D.
- 1979 "Cell Culture Biosynthesis". Contract NAS9-15322, A.D. Little Co., Cambridge, MA. Phil Thayer, Ph.D. and M.G. Broone, Ph.D.
- Jan. 1978 M.I.T. Workshop on "The Feasibility of Cell Culture Systems for Space Experiments". Proceeding summarized by A.D. Little, Inc. - Final Report NAS9-15322.
- June 1979 Salk Institute Workshop on "Microgravity Cell Culture Systems". Proceedings summarized by A.D. Little, Inc. - Final Report NAS9-15322.
- Jan. 1982 JSC/Conference on "Fluid Mechanical Aspects of Cell Culture", Rice University. Summary Report by Mike Reynolds, Ph.D.
- 1982 "A Microprocessor Interface for the Space Bioreactor System", conducted by Robert A. Snyder, Ph.D., (NRC Postdoc).
- Aug. 1985 JSC/ Workshop on "Space Bioreactor Science". A joint NASA, Academic and Industrial workshop on the development of a space bioreactor and potential flight experiments. Proceedings published in JSC Document No. 22264, edited by D. R. Morrison, Ph.D., Aug. 1986.

Figure 2

SPACE BIOREACTOR

APPROACH

- o CONCEPTUAL STUDIES BY ACADEMIC INSTITUTIONS AND INDUSTRY STARTED IN 1976
- o CELL CULTURE SCIENCE CONFERENCES - MIT, SALK INSTITUTE, JSC
- o INDUSTRIAL CONSULTATION WITH: MONSANTO CORP.; STANFORD RESEARCH INSTITUTE; MDAC-ST. LOUIS; BIOCHEM TECHNOLOGIES; FERMENTATION DESIGN, INC.; K. C. BIOLOGICALS; BIOREACTOR, INC.; TISSUE CULTURE ASSOCIATION; ALTON JONES CELL SCIENCE CENTER; ARTHUR D. LITTLE CO.
- o ACADEMIC MEETINGS WITH: MIT, PRINCETON UNIVERSITY, UNIVERSITY OF ROCHESTER, UNIVERSITY OF MISSISSIPPI, STANFORD UNIVERSITY, RICE UNIVERSITY, UNIVERSITY OF HOUSTON, WASHINGTON SCHOOL OF MEDICINE
- o JSC HAS ACCUMULATED 8 YEARS' EXPERIENCE WITH CELL CULTURES AND 4 YEARS' EXPERIENCE WITH BIOREACTOR BREADBOARD SYSTEM
- o JSC WILL DEVELOP TWO SPACE BIOREACTOR LABORATORY TEST UNITS WHICH WILL CONFIRM DESIGN REQUIREMENTS FOR FLIGHT UNITS AND CAN BE USED FOR HARDWARE VERIFICATION FLIGHT TESTS (DSO'S)

Figure 3

**BIODYNAMICS**

**MICRO-G ENVIRONMENT RESEARCH ON:**

- **SHEAR STRESS ON CELLS**
- **ANCHORAGE DEPENDENCE - CELL ATTACHMENT**
- **GAS TRANSFER / GAS - LIQUID FOAMS**
- **NUTRIENT / WASTE DIFFUSION**
- **INTERCELLULAR TRANSPORT**
- **GROWTH**
- **BIOENERGETICS**
- **PRODUCT SECRETIONS**

Figure 4

SPACE BIOREACTOR

OBJECTIVES

- o DEVELOP AND TEST PROTOTYPE SYSTEM FOR CONTROLLED GROWTH OF MAMMALIAN CELLS IN MICROGRAVITY
- o DEMONSTRATE ADVANTAGES
  - LACK OF SEDIMENTATION
  - LACK OF BUBBLE BOUYANCY
  - UNCONVENTIONAL CONTROLLED MIXING AT SLOW SPEEDS TO KEEP SHEAR AT MINIMUM
  - CULTURE OF EXTREMELY FRAGILE CELLS
  - PRECISE CONTROL OF GAS/LIQUID TRANSPORT, NUTRIENTS, AND WASTE
  - EVALUATE NEW METHODS OF O<sub>2</sub> CONTROL
- o DEVELOP CONTINUOUS CULTURE METHODS AND PRODUCT HARVESTING TECHNIQUES
- o PROVIDE A BASIC CELL CULTURE AND MAINTENANCE FACILITY FOR SUPPORT OF CELL BIOLOGY EXPERIMENTS (SHUTTLE, SPACELAB, AND SPACE STATION)
- o PROVIDE A TESTBED CAPABILITY TO EXPLORE OTHER TYPES OF CELL CULTURE INCLUDING HYBRIDOMA CULTURES, YEAST FERMENTATION, ETC.

Figure 5

BIOSYNTHESIS

GRAVITY PROBLEMS IDENTIFIED

COMPARISON OF CULTURE TECHNIQUES

- MONOLAYER/MULTIPLATE
- HOLLOW FIBER
- SPIN FILTER SUSPENSION
- MICROCARRIERS

DEVELOPMENT OF RESEARCH BIOREACTOR

- SUSPENSION CULTURES - L1210  
- HYBRIDOMA
- ATTACHED HUMAN CELLS - KIDNEY

DEVELOPMENT OF SPACE BIOREACTOR

- SPIN FILTER/MICROCARRIERS
- NO HEADSPACE
- MICRO-G FLUID HANDLING

SPACE BIOREACTOR

SYSTEMS DEVELOPMENT

BIOLOGICAL TEST SYSTEM

PHASE I - BREADBOARD

- SPIN FILTER SYSTEM - (NO HEADSPACE)
- SENSOR QUALIFICATION
- FLUID CIRCULATION LOOP
- TEMPERATURES CONTROL
- O<sub>2</sub> CO<sub>2</sub> - GAS INPUT SYSTEMS
- STERILIZATION

ENZYME REACTIONS

- NUTRIENT CONSUMPTION
- NUTRIENT SUPPLY
- CULTURE MEDIUM COMPONENTS

PHASE II - RESEARCH BIOREACTOR

- REFINED SENSOR DEVELOPMENT DATA LOGGING
- NUTRIENT CONSUMPTION
- GROWTH & UPPER LIMITS TO CELL MASS
- STIR/MIXING TECHNIQUES
- MICROPROCESSOR FEEDBACK CONTROL

- A. SUSPENSION CULTURE - L1210 CELLS
  - MAINTENANCE REQUIREMENTS LESS
  - LESS COMPLEX CULTURE MEDIA
  - EASIER FOR CELL COUNTING
- B. ATTACHED CELLS - MICROCARRIER BEADS

PHASE III - SPACE BIOREACTOR

- A. BIOREACTOR TEST UNIT (MIDDECK)
  - CULTURE CHAMBER + FLUID CIRC. LOOP
  - MINIMUM SENSORS RECORDED DATA ON ORBIT
- B. BIOREACTOR - FLIGHT UNIT
  - ALL UP SYSTEM/MICROPROCESSOR CONTROL
  - PRECISION CONTROL FOR HUMAN CELLS ATTACHED TO MICROCARRIER BEADS
  - 1 SPACELAB RACK OR 3 MIDDECK LOCKERS

- A. ENZYME REACTION
  - FAST REACTION TO TEST SENSOR AND SET LIMIT TYPE FEEDBACK CONTROL
- B. SUSPENDED CELLS (L1210)
- C. ATTACHED CELLS
  - HUMAN KIDNEY CELLS ON BEADS
  - PITUITARY CELLS ON BEADS



Figure 7

BIOREACTOR

BASIC FUNCTIONAL REQUIREMENTS

SYSTEM REQUIREMENTS

BASIC DESIGN - CELLS ON MICROCARRIERS

NO HEADSPACE IN REACTOR VESSEL

CELL/BEAD FREE MEDIA CIRCULATION LOOP

- OXYGENATION
- RE-SUPPLY NUTRIENTS
- REMOVE TOXIC WASTE
- PRODUCT REMOVAL
- IN-LINE SENSORS

AUTOCLAVE ENTIRE CORE BIOREACTOR

PRESSURE ON CELLS <-- 5 PSI

SYSTEM PRESSURE - UP TO 15 PSI

CORE BIOREACTOR LOADED AT KSC/INSTALLED AT L-10 HOURS

SELF-SUFFICIENT PROCESS CONTROLLER - STANDARD OPERATIONS AND ALARMS

SEPARATE SUPERVISORY COMPUTER

TARGET CORE BIOREACTOR - 2 LOCKERS

PROCESS CONTROLLER/SUPPORT ELECTRONICS - 1-2 LOCKERS

Figure 8

MAJOR COMPONENT REQUIREMENTS

REACTOR VESSEL

- SEPARATE DEVICES FOR SPIN FILTER AND MIXING
- 500 ML CULTURE VOLUME
- MINIMUM INTERNAL COMPONENTS
- CONTROL MAXIMUM SHEAR 10-40 DYNES/CM<sup>2</sup>

APPROACH - BASIC TEST VESSEL - SPIN FILTER (MUST BE KEPT CLEAN)

- MARINE IMPELLOR VS. SPIRAL VANES
- ALTERNATING BACKWASH METHODS

PROCESS CONTROLLER

- ONBOARD CPU/OPERATING SYSTEM
- LOG ALL DATA - 12-24 HOURS
- MINIMUM TREND PROJECTIONS
- INTERFACE (RS-232) WITH SUPERVISORY COMPUTER
- MINIMUM CREW INTERACTIONS
- UPDATE WITH SOFTWARE COMMANDS

APPROACH - TEST COMMERCIAL SYSTEMS - NONE ADEQUATE

- BUILD CUSTOM SYSTEM
- BUY MDAC ECCM

SUPERVISORY COMPUTER

- OFF-LINE DATA ANALYSIS
- COMPLICATED TREND PROJECTIONS
- GRAPHICS

APPROACH - IBM-AT (GROUND ) AND LAP COMPUTER FOR FLIGHT

SPACE BIOREACTOR  
MAIN COMPONENTS AND FLUID LOOPS

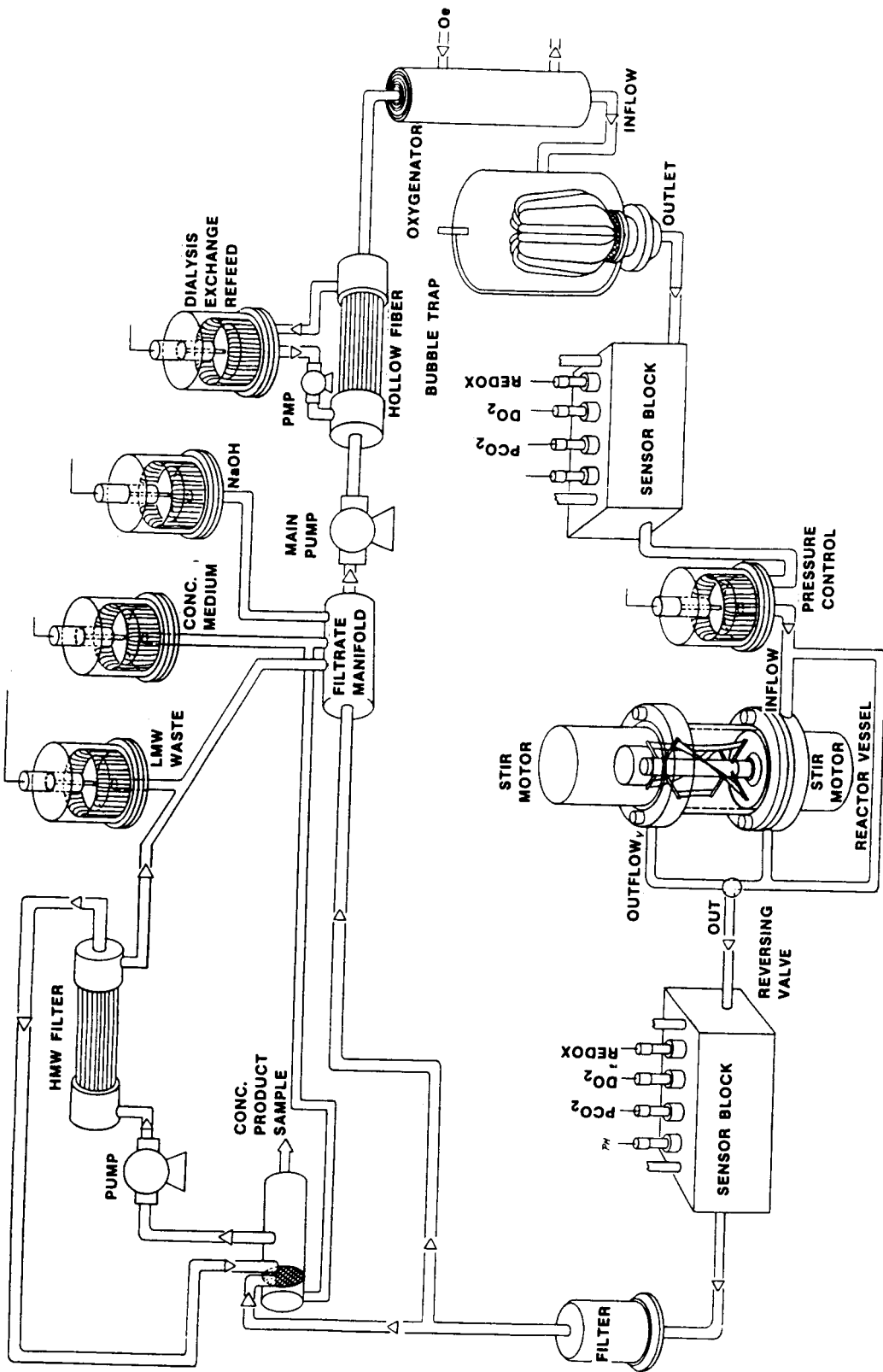


FIGURE 9

Figure 10

BIOREACTOR TEST REQUIREMENTS

- FIRST FLIGHT TEST
  - NO CELLS
  - MINIMUM CONTAMINATION CONTROL
  - TEST - FLUID SYSTEM
    - MIXING
    - SENSORS
    - HMW FILTER SYSTEM
  
- SECOND FLIGHT TEST
  - CULTURE FEASIBILITY DEMONSTRATION
  - 7-8 DAY MISSION
  - KIDNEY CELLS ON MICROCARRIERS
  - GROW ON BEADS BEFORE FLIGHT (GROWTH MEDIUM)
  - MAINTAIN CELLS ON UKPM (SERUM FREE)
  - EXERCISE HMW FILTER ON 4-DAY INTERVALS
  - REPLENISH MEDIUM WITH NEW MEDIUM OR DIALYSIS REFEEED
  
- LATER FLIGHTS
  - GROW CELLS ON MICROCARRIERS, THEN SWITCH TO SERUM-FREE MEDIUM
  - OTHER REACTION VESSELS - HYBRIDOMAS
    - FERMENTATION (YEAST)

EXPERIMENTS WITH HUMAN CELLS (ATTACHED TO MICROCARRIERS)

A. EFFECTS OF SHEAR STRESS

- LOW SHEAR 10-40 DYNES/CM<sup>2</sup>
- TARGET EXACT SHEAR RANGE TO STIMULATE UK SECRETIONS
- DIRECT MEASURE OF METABOLISM - FLUROMEASURE SYSTEM

B. CULTURE IN A CONTROLLED FOAM IN THE ABSENCE OF BOUYANCY

- CREATE STABLE FOAM OF O<sub>2</sub> BUBBLES IN CULTURE MEDIUM
- GROW CELLS IN LIQUID PHASE
- PROVIDE CONTINUAL & INTERMEDIATE ACCESS TO O<sub>2</sub> SUPPLY WITHOUT CELLS EVER BEING EXPOSED TO GAS PHASE
- COMPLETELY AVOID USE OF ANTI-FOAMING AGENTS

C. USE OF FLUOROCARBON OXYGEN CARRIERS IN ABSENCE OF DENSITY-DRIVEN SEPARATION

- USE OF PERFLUORINATED CARBON SOLVENTS (S.G. 1.87)
- MAINTAIN A LONG-TERM STABLE DISPERSION TO CARRY O<sub>2</sub> FROM OXYGENATOR TO THE CELLS IN REACTOR VESSEL
- EXPLORE USE OF SERUM IN MEDIA WHERE FOAMING WILL NOT BE PRESENT AS IN EARTH BASED SYSTEMS

OTHER EXPERIMENTS TO DETERMINE DIRECT CELLULAR EFFECTS

- ALTERATIONS OF CELL PHYSIOLOGY OR MORPHOLOGY
- CELL SECRETIONS IN MICROGRAVITY - GH, UK
- CELLULAR EFFECTS OF MAGNETIC FIELDS IN ABSENCE OF GRAVITY
- ALTERATIONS IN CYTOSKELETON, CELL MOTILITY