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FEASIBILITY OF COMMERCIAL SPACE MANUFACTURING

Production of Pharmaceuticals

FINAL REPORT

Volume II
Technical Analysis

MCDONNELL DOUGLAS ASTRONAUTICS COMPANY-ST. LOUIS DIVISION

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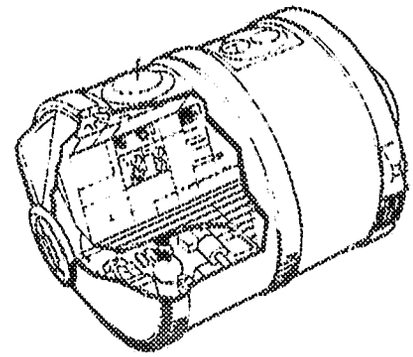

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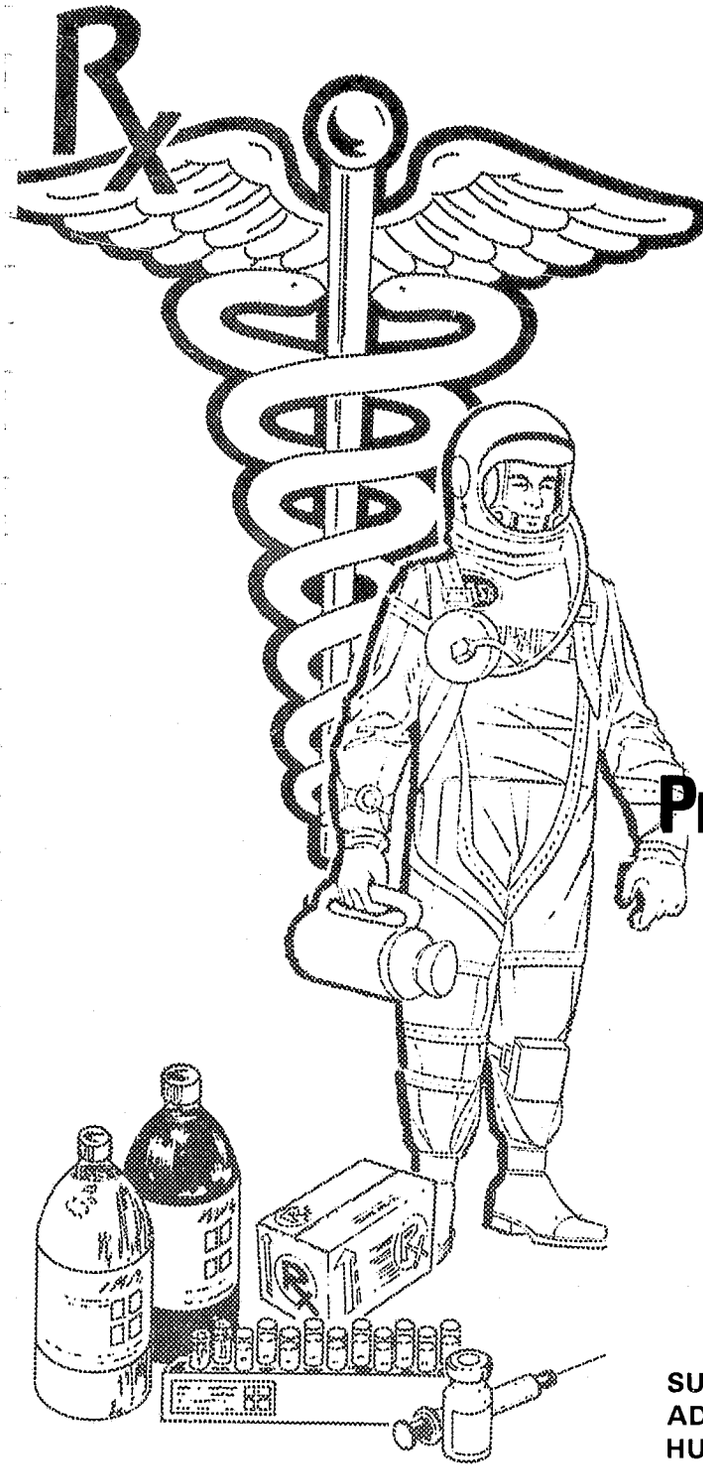

FEASIBILITY OF COMMERCIAL SPACE MANUFACTURING

Production of Pharmaceuticals

FINAL REPORT

Volume II
Technical Analysis

SUBMITTED TO NATIONAL AERONAUTICS AND SPACE ADMINISTRATION MARSHALL SPACE FLIGHT CENTER HUNTSVILLE, ALABAMA UNDER CONTRACT NO: NAS 8-31353

MCDONNELL DOUGLAS ASTRONAUTICS COMPANY-ST. LOUIS DIVISION

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MCDONNELL DOUGLAS CORPORATION

PREFACE

This report describes the study results achieved over two periods of related activity, June-December 1977 and March-October 1978. It is organized in three volumes to meet the needs of different audiences. The first, an executive summary, serves as an overview aimed at those responsible for committing public and private resources to new ventures. The second recounts the activities of the study and presents fundamental lessons learned. This volume is intended to serve two groups: those in the aerospace industry who may wish to have a model for their efforts to attract participation in space processing by other industries; and those in nonaerospace industries who want to learn more about the possibilities of space processing. The third volume contains the detailed product data collected and reviewed to support the activities in Volume II.

The report has been organized to take the reader through the chronology of the study process. Although many of these steps were accomplished simultaneously, we have -- for simplicity and clarity -- organized them into discrete segments, moving first through the plan established to target and contact pharmaceutical companies, then the laboratory work needed to support the expanding company-to-company cooperation and technology interchange, through the literature search and analysis of potential products and finally through the production engineering analysis. The report then summarizes the study by identifying the lessons learned during the course of its execution. Before the enormous potential benefits of space processing can reach the public -- the basic goal of NASA's Materials Processing in Space program -- industry must be willing to participate in the development of processes. Such investment, however, will not follow until industry itself is made aware of the promise of space processing and is supplied with hard data supporting such promises. It is to this purpose that we have directed our efforts.

This report is submitted under NASA Contract Number NAS8-31353. The work was performed by McDonnell Douglas Astronautics Company - St. Louis Division under the direction of William R. Marx and Dr. Ronald A. Weiss, Study Managers during the first and second periods of the study respectively. This contract was administered by the NASA Marshall Space Flight Center, Huntsville, Alabama.

This report was prepared under the direction of Dr. Ronald A. Weiss with the assistance of the principal contributors listed below:

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FOREWORD

As the principal result of this study activity, the McDonnell Douglas Astronautics Company - St. Louis Division, elicited and fostered the participation of several pharmaceutical firms, to varying degrees, in exploring the potential benefits which may accrue from processing pharmaceuticals in space.

One of the conditions for their participation, however, was that the companies not be linked with any potential product or process because of the highly competitive nature of the industry. With NASA concurrence, therefore, and participating company approval, we have deleted the names of any company associated with this study in order to be able to emphasize the important product data and technology interchange achieved with them.

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1.0 INTRODUCTION

The environment of space holds great promise for new manufacturing processes which take advantage of the absence of such earthbound phenomena as natural convection and sedimentation. Using these processes, space manufacturers cannot only produce products superior to those produced on the ground, they can produce entirely new classes of products. Though characteristics of space -- including high vacuum and radiation -- can be duplicated on earth, the most important characteristic, weightlessness, can be achieved only for an extremely brief period. In the microgravity of space, molten materials can be suspended without containers -- eliminating a major source of contaminants. More importantly, in space we can escape gravity-induced convection. Convection currents -- which are caused by the thermal gradients in fluids -- can lead to undesirable structural differences in the solid materials produced. Having escaped the problems posed by these currents, space manufacturers will be able to grow crystals of great purity with highly controllable characteristics; they will find it much easier to mix and homogenize liquids, to cast metals, and to separate and purify the elements of mixtures.

The question immediately arises, why is not industry actively pursuing opportunities to develop materials and processes in space? The first reason is that industry is not generally familiar with the potentials of space. NASA and key aerospace organizations are working continually to rectify that situation. The second, and by far the dominant, reason is that observation of basic phenomena with potential application is only the start of the industrial process. A major body of data on applied research into processes and materials characteristics, material applications potential, potential markets and their probable growth, and the characteristics of production systems and logistics must be developed as a vital decision base. Before private industry will invest the money required to begin such untried processes, it must be reasonably confident that the product will have a high value, that the benefits of processing in space will be substantially greater than processing on the ground (i.e., capable of producing less expensive, more useful products, or producing products that cannot be made on earth). The investor must also be reasonably confident: that the space process can be developed in a given time at an affordable cost; that a market exists at a price which assures a reasonable return on investment; and that this market will

not disappear because a new product appears and captures the market, or because a breakthrough in the technology occurs that permits competitive ground production.

Because these risks are so difficult to assess, and because the required initial investment is so large, most industries adopt a "wait and see" attitude. Until more data are available, industries find it extremely difficult to assess the potential of new processes and products.

To address this problem, we approached NASA with a proposal to study the feasibility of commercial manufacturing of pharmaceuticals. The goal of this undertaking was to induce pharmaceutical firms to participate actively, on a continuing basis, in exploring the possibilities of using the unique environment of space to produce new products. The MDAC-St. Louis' approach was, first, to secure the initial commitment of these firms by providing key management and technical executives with preliminary data and forecasts of the business and technical potential of space processing. The second aspect of the approach was to foster the initial commitments by establishing continuing technical and management exchanges with the interested pharmaceutical companies to our mutual benefit.

Our enthusiasm for space processing focused on the promise shown by our company funded efforts with electrophoresis. In order to accomplish the facets of this goal, we had to expand the data base we had developed -- including significant laboratory work and an awareness of the state-of-the-art -- and we had to target companies potentially interested in the benefits of the process.

In our early company funded work with electrophoresis, we learned how to separate relatively large quantities of test materials. We also experienced the adverse effects of gravity on the process -- causing the vertically flowing stream to collapse on itself (if the sample were denser than the carrier fluid) or to ball up and float to the top of the chamber (if the sample is less dense than the carrier fluid). On the basis of these experiences we began developing, with MDAC-St. Louis funds, our own mathematical models of these effects so that we could predict effects of design changes and operating conditions, and ultimately forecast the benefits of operating in space. We also ran company funded preliminary mass balance calculations; these activities assured us that we could define

and demonstrate the types of requirements needed to characterize conceptual space and ground production systems, with their requisite logistics capabilities, in presentations to NASA and industry.

Under the contract, we addressed the problem of targeting pharmaceutical companies. Our first step was to engage the services of Price Waterhouse and Company to provide important drug industry data. The overall drug industry analysis provided by Price Waterhouse included: detailed assessments of the top twenty companies in the industry, focusing on their apparent commitment to innovation, their research and production emphasis on products having high potential for space production, and the prominence of their executives. Price Waterhouse also helped us prepare the presentation to be made to these companies, recommending a "businessman to businessman" approach.

Letters were written to the selected companies. These letters gave an overview of the feasibility study, listed some of the potential benefits to pharmaceutical manufacturing by processing in space, and requested an opportunity to make a presentation. Ten of fourteen companies requested the presentation.

Although the pharmaceutical company personnel were generally skeptical at first, once they understood the benefits of microgravity, the implications of the preliminary results of continuous flow electrophoretic separation, and the potential of an integrated space pharmaceutical production system manufacturing products of great value, they became increasingly intrigued. As a result of these initial contacts, six companies responded positively to our invitation for assistance and cooperation in this study. Two companies agreed to participate actively in the form of laboratory testing a product of specific interest to themselves. Four additional companies agreed to participate in a more passive mode by suggesting products, providing marketing information and reviewing the analysis of results. One of the conditions for their participation, however, was that the companies not be linked with any potential product or process data because of the highly competitive nature of the industry. With NASA concurrence, therefore, and participating company approval, we have deleted the names of any company associated with this study and, instead, emphasized the important product and process information obtained from them.

This report describes our method of obtaining pharmaceutical company involvement, the development of protocols with two of these companies, laboratory results of the separation of serum proteins by the continuous flow electrophoresis process, the selection and study of candidate products, and their production requirements. From the twelve candidate products discussed with, or suggested by, the visited pharmaceutical companies, six were selected for further evaluation; antihemophilic factor, beta cells, erythropoietin, epidermal growth factor, alpha-1-antitrypsin and interferon. Production mass balances for antihemophelic factor, beta cells, and erythropoietin were compared for space versus ground operation. Selection of the best mode of operation for these three representative products permitted a conceptual description of a multiproduct processing system for space operation. Production requirements for epidermal growth factor, alpha-1-antitrypsin and interferon were found to be satisfied by the system concept.

In the technical interchanges that occurred with these pharmaceutical companies, significant data were generated and many valuable lessons were learned. These data and lessons, detailed in this report, are intended to serve others interested in exploring the possibilities of space processing.

2.0 INTRODUCTION

The aspiration of this task was to obtain the participation of one or more prominent ethical drug manufacturers in this study and gain their evaluation of product and process commercial feasibility. The industrialization of space, and any resultant social benefits, would not be realized at any early date without the cooperation, endorsement, and initiative of commercial industry. Thus, the ultimate effect of securing the initial participation of ethical drug producers and establishing a foundation for their continued involvement would be to assure the timely commercialization of pharmaceutical processing in space.

The approach to this task consisted of an analysis of the drug industry and identification of primary candidate companies for potential study participation. Communications were then established with a number of these companies for the purpose of gaining the assistance of one or more of them in product selection and evaluation.

Some ethical drug companies chose to participate in different ways ranging from suggesting candidate products to actual laboratory participation. The unanimous condition, however, for their participation was, as noted in the Foreword and Section 1, that company names be in no way linked with products or processes. In order to focus on our technological exchange with these firms and on the joint learning process through which we passed, company names are avoided in this and following sections.

2.1 COMPANY SELECTION

Selection of potential pharmaceutical company participants was limited to domestic United States ethical drug producers. The task was principally accomplished through subcontract with the St. Louis office of the business consultant firm of Price Waterhouse and Company. This firm has considerable knowledge of the pharmaceutical industry, its key personnel and the environment in which they operate. After preliminary discussions between both the MDAC-St. Louis marketing and aerospace medical representatives and the subcontractor, an overall approach to this selection process was established. This selection process is summarized in Figure 2.1-1. The work was accomplished in two steps. First, a preliminary screening

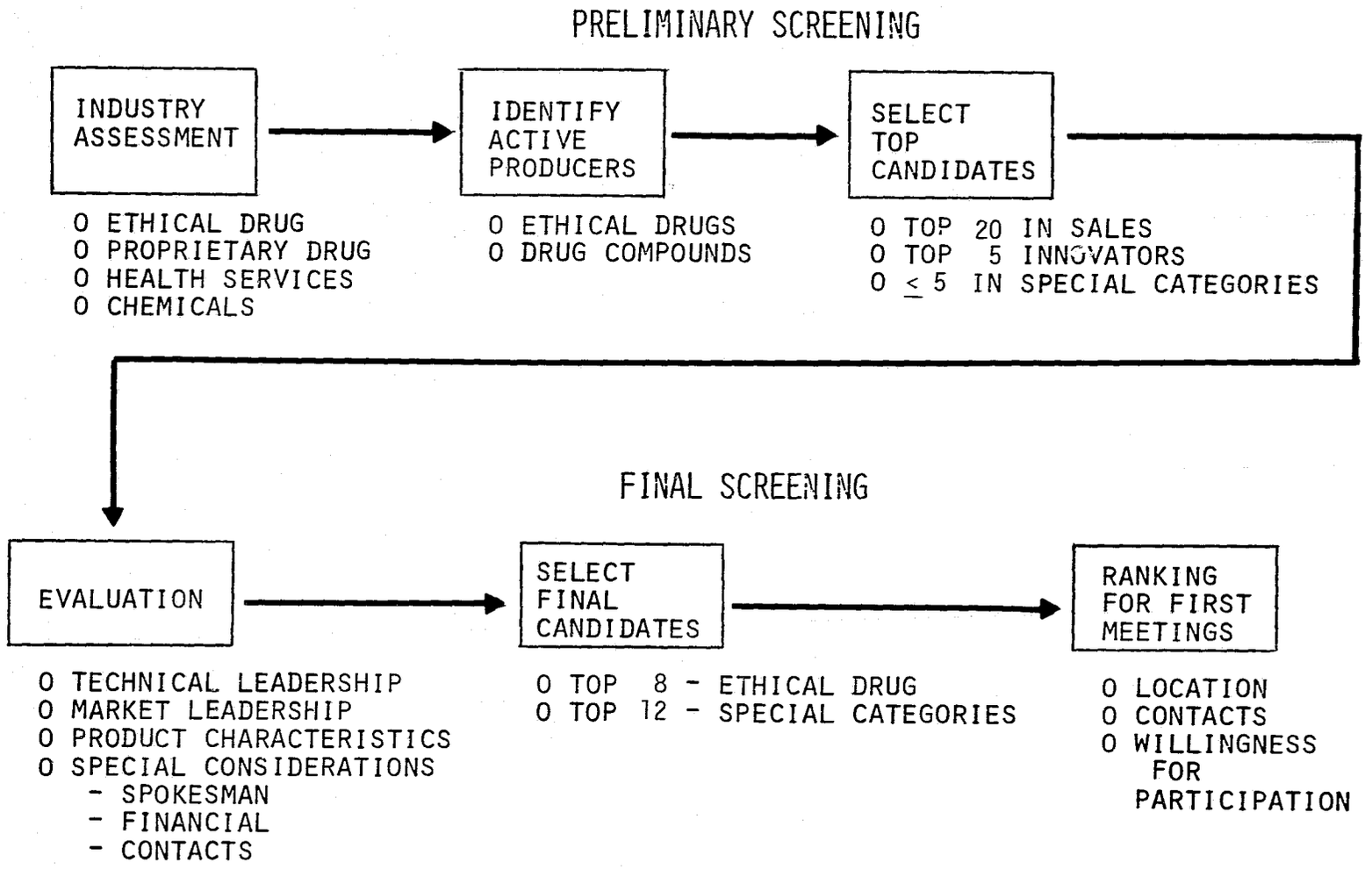
ETHICAL DRUG COMPANY SELECTION PROCESS
PRELIMINARY SCREENING
INDUSTRY ASSESSMENT
O ETHICAL DRUG
O PROPRIETARY DRUG
O HEALTH SERVICES
O CHEMICALS
IDENTIFY ACTIVE PRODUCERS
O ETHICAL DRUGS
O DRUG COMPOUNDS
SELECT TOP CANDIDATES
O TOP 20 IN SALES
O TOP 5 INNOVATORS
O ≤ 5 IN SPECIAL CATEGORIES
FINAL SCREENING
EVALUATION
O TECHNICAL LEADERSHIP
O MARKET LEADERSHIP
O PRODUCT CHARACTERISTICS
O SPECIAL CONSIDERATIONS
- SPOKESMAN
- FINANCIAL
- CONTACTS
SELECT FINAL CANDIDATES
O TOP 8 - ETHICAL DRUG
O TOP 12 - SPECIAL CATEGORIES
RANKING FOR FIRST MEETINGS
O LOCATION
O CONTACTS
O WILLINGNESS FOR PARTICIPATION

FIGURE 2.1-1

process identified the American drug firm candidates based principally upon ethical drug sales. A final screening process ranked 20 for initial contact (within the limits of the study travel budget) based upon evaluation criteria including technical and market leadership and product characteristics, among others.

During preliminary screening, a file of basic financial and "line of business" data were collected. It consisted of nonproprietary and publicly available information concerning individual major pharmaceutical companies as well as the ethical drug industry as a whole. The 100 companies which came under consideration were grouped according to the following Standard Industrial Classifications:

2801 - Major Chemical Producers
2802 - Intermediate Chemical Producers
2803 - Specialty Chemical Producers
2834 - Pharmaceutical Preparations
2835 - Ethical Drugs
2836 - Proprietary Drugs
2837 - Drugs-Medical and Hospital Supply
2899 - Chemical Preparations

Active producers of ethical drugs were identified through analysis of product line and related business data found in company annual reports, "10-K reports," and other publicly available sources of information. This analysis resulted in a reduction from 100 to 42 potential candidate companies. The ten largest pharmaceutical firms (in terms of 1976 consolidated net sales) were then identified. The largest firms were selected because: 1) prominent names associated with this effort would provide visibility and recognition; and 2) major companies would more likely have the resources to conduct experimentation and initial financial investment.

Additional drug or chemical company candidates were also identified for other reasons such as: prior established contact with MDAC management, location, and/or known company interest in space processed pharmaceuticals.

The 42 drug company candidates for study participation identified during the preliminary screening process were evaluated in a final screening for the purpose

of first selecting, then ranking, the candidates for initial contact. A final list of 20 candidate companies was compiled using several different selection criteria. Among these were quantifiable factors of comparison such as industry sales position, profitability, research and development expenditures, various financial ratios and growth rates. Subjective criteria were utilized as well to evaluate each company's leadership in new product development and other factors including top management commitment to innovation.

This analysis of the 20 candidate pharmaceutical companies revealed that all were potentially acceptable as participants in the study. However, time and travel budget constraints prevented inclusion of all 20. Based on the ranking, companies were contacted to offer a presentation on space bioprocessing until the 10 alloted trips were scheduled. Fourteen companies of the twenty ranked companies were contacted. The method by which these companies were approached is found in Section 2.2.

The Price Waterhouse and Company report also included a brief overview of the ethical drug industry and detailed information on the pharmaceutical companies recommended as candidates for presentations. This information included line of business data, results of operations and financial position, specific information concerning ethical drugs currently produced, biographical data on key individuals within the company, and preferred initial contacts at each company. Throughout their consultantship, Price Waterhouse and Company stressed the need for MDAC-St. Louis to initiate our contacts and communicate with pharmaceutical firms strictly on a "businessman-to-businessman" basis. Only in this way could we succeed in establishing the rapport which would permit us to address potential areas of mutual business interest, in a common language and with a mutual understanding of our technical and business goals.

2.2 ESTABLISHMENT OF COMMUNICATIONS

Once 20 pharmaceutical companies had been identified for contact in rank order of desirability, a program was established to develop the most effective method of communication with them and eliciting their participation in this study. This program, in summary, consisted of the following elements:

Identification of persons to be contacted
Selection of initial contacts
Introductory letters
Follow-up telephone calls
Formal presentation material preparation
Formal presentations at the drug company
Establishment of follow-up communications.

Publicly available biographical information on each company's top management personnel was reviewed in order to identify individuals, who, due to organizational position or prominence within the pharmaceutical industry, would be pivotal in persuading their company, and perhaps the drug industry, to participate in commercial space development.

Letters were prepared and sent to each selected pharmaceutical company contact. These letters, from the Vice President-Space Programs, MDAC-St. Louis, introduced the concept of pharmaceutical space processing, outlined potential benefits to the drug industry, and stated the importance of their participation in the study.

Approximately one week after the letters were sent, telephone calls were made to each of the pharmaceutical company officials who had received the letter. The purpose of these telephone calls was to establish dates for formal presentations on pharmaceutical space manufacturing. These follow-up calls were successful in setting up meeting dates with 10 of the first 14 companies. The other four were either already involved with NASA Space Processing or were uninterested in a briefing on the subject.

The formal presentation was organized into two principal sections. The initial section of the briefing established the meeting's objective, described MDAC-St. Louis' product line and involvement in space as well as defined the NASA space program and objectives. It also outlined the programmatic importance of early involvement of drug industry participants to assure the success of commercial space development. The second section of the presentation described technical and business objectives of the pharmaceutical study and the process system concept, described in detail the MDAC-St. Louis in-house experimental work accomplished to

date on continuous flow electrophoresis for use in space bioprocessing, defined the benefits of space improved biological separation, and identified candidate products for space processing.

A series of vu-graphs were developed to support the formal presentation. In addition, brochures incorporating these vu-graphs were prepared for distribution at the conclusion of each drug company presentation. This brochure allowed the drug company participants to review the material at their leisure after the formal presentation, discuss it with their colleagues and come to a conclusion about further participation.

During the course of this study, presentations were made to 10 pharmaceutical companies at their facilities. After an introduction by an MDAC-St. Louis Director of Marketing, the two sections of the formal presentation were made by the MDAC Space Processing Program Manager and Bioprocessing Study Manager, respectively. A member of the MDAC-St. Louis Aerospace Medical Department was also present to comment on the processing system preliminary concept and its intended space utilization during the informal question and answer period. The tone of the presentation was that of a progress report on an MDAC-St. Louis space bioprocessing program with solicitation of the drug company's participation if they felt it would be in their best interest.

In order to maintain an openness and flexibility of approach, no formal contractual agreements for drug company participation were solicited. However, during post-briefing discussions with drug company attendees, they were asked to send a letter from drug company management to MDAC-St. Louis stating their decision to participate, defining their limits of study participation, and identifying the contact within the drug firm for matters concerning the study. As a participation starting point, assistance was requested in defining market potential for products previously defined as candidate products by NASA and MDAC. For this purpose, an information sheet was prepared and distributed at the conclusion of each presentation.

About two weeks after the formal presentation, a telephone call was made to the drug company contact. The purpose of this call was to determine if any participatory action was to be forthcoming and whether the company needed any additional

information from MDAC-St. Louis. If the response was affirmative, a two way dialogue was developed between MDAC-St. Louis and the interested pharmaceutical company. This dialogue was accomplished through exchange of correspondence, telephone calls and facility visits.

2.3 DRUG COMPANY RESPONSE AND PARTICIPATION

Typically, the officials of each pharmaceutical firm were initially skeptical of the benefits of space processing, but they were willing to listen. Following the description of the MDAC-St. Louis laboratory experiments of continuous flow electrophoresis and how microgravity may enhance the separation of cells or protein substances in such a continuous flow electrophoresis system, most of the technically oriented officials perceived the potential capability of space processing to produce higher purity and/or unique pharmaceutical products. Discussions concerning this subject were fruitful and generally resulted in allaying of initial skepticism.

Highlights of significant comments and concerns expressed by the drug company officials during these presentations and subsequent follow-up communications are presented in Figure 2.3-1.

Several significant accomplishments were achieved by these meetings. Probably the most far reaching action was making the technical people present at these meetings aware of the effects of gravity in their current and future chemical processing activities. For one company in particular, this became an obvious answer to an existing chemical processing problem. Because of this initial exchange, they requested that another pharmaceutical division of their company also receive the same presentation. Another major accomplishment was making these corporate decision makers aware of the existence of a space program in which they could participate for the benefit of their company. They were under the impression that the space program was conducted primarily for astronomical research, political and military reasons. The characteristics of space applicable to both a research and manufacturing environment had never been elucidated for them until the formal presentation.

COMMENTS OF DRUG COMPANY BRIEFING ATTENDEES

OVERALL OPINION OF SPACE PROCESSING OF PHARMACEUTICALS	INITIALLY SKEPTICAL, BUT FOLLOWING AN UNDERSTANDING OF THE BENEFITS OF MICROGRAVITY, BECAME INCREASINGLY INTRIGUED WITH POTENTIAL.
CONTINUOUS FLOW ELECTROPHORESIS	SPACE - A UNIQUE PROCESS POTENTIALLY CAPABLE OF PRODUCING A MULTIPLICITY OF PRODUCTS. ABILITY TO SEPARATE, ISOLATE, AND PURIFY PARTICULAR STRAINS OF CELLS AND PROTEINS IS MUCH IMPROVED OVER CURRENT CAPABILITY. GROUND - ALSO INTERESTED IN SEPARATION POTENTIAL OF GROUND UNIT FOR COMMERCIAL, RESEARCH, AND TO GAIN BETTER UNDERSTANDING OF THE EFFECTS OF GRAVITY UPON THEIR PROCESS.
TISSUE CULTURE PROCESS IN SPACE	INTERESTED IN ANY PROCESS WHICH ACCELERATES THE LIFE CYCLE OF CELLS.
TYPES OF PRODUCTS, PRODUCT POSSIBILITIES MENTIONED:	CONCENTRATION OF ANTIGENS, VETERINARY PRODUCTS, ANTISERA, GROW AND SEPARATE VIRI, DIAGNOSTIC MATERIALS, COMMERCIAL ASSAY DEVICE IN SPACE.
INTERESTS IN OTHER MATERIALS:	SIGNIFICANT INTEREST IN ORGANIC CRYSTALS.
SPACE OPERATIONS	SOME CONCERN EXPRESSED ABOUT HIGH COSTS, TRANSPORTATION COSTS, AND INVOLVEMENT WITH GOVERNMENT.
PROPRIETARY PROTECTION	RETENTION OF PROPRIETARY RIGHTS IS VERY IMPORTANT. PATENT PROTECTION AND TRADE SECRETS ARE NOT SUFFICIENT FOR AN INITIAL ACTIVITY THAT WILL BE HIGHLY PUBLICIZED TO INTEREST OTHER PARTICIPANTS. EXCLUSIVITY FOR PRODUCTS AND PROCESSES IS REQUIRED TO ALLOW INVESTMENT RECOVERY AND A LEAD ON COMPETITION THAT DID NOT SHARE THE RISKS INVOLVED.
INTEREST IN ADDITIONAL INFORMATION	INTEREST IN SPACE EXPERIMENT RESULTS: CELL GROWTH, MAGNETS, METALS, ETC. SEVERAL REQUESTS FOR DESCRIPTIVE DATA ON MDAC-ST. LOUIS AUTOMATED MICROBIC SYSTEM.

FIGURE 2.3-1

A strong interest in the potential of continuous flow electrophoresis systems for separation and purification of pharmaceuticals was one of the major outcomes of the meetings. Taking a relatively old, research oriented process such as this, and applying a multi-disciplinary team to analyze, model and improve its potential for commercial bioprocessing was indicative to these pharmaceutical companies of the need for aerospace company participation in the total systems concept. A number of the drug companies contacted in this study did not have the multi-disciplinary talent to conduct such a systems analysis; they thought the MDAC-St. Louis "team" approach to space bioprocessing might be beneficial as an approach to many aspects of their company operations.

During the course of this study six of the ten companies contacted agreed to participate. Of the six, two companies agreed to participate actively in the form of laboratory testing a product of interest to themselves. More details of this participation will be found in section three of this report. The four other companies agreed to participate in a different mode by suggesting products, providing marketing information and reviewing the analysis of results. The products suggested can be found in Section 4.3. Several weeks after the formal presentation, a seventh company informed us that they had started investigating the space processing feasibility of four products of interest to them. They indicated that the results of this activity and a formal corporate decision to participate in the MDAC-St. Louis study would take time. During the course of this study, however, no formal comment to participate was forthcoming. Three of the companies contacted chose not to participate.

The approach taken to obtain drug company participation in the study resulted in the establishment of communications with the drug industry and general expressions of interest. The reasons for this success are many, but of greatest importance was the contribution of the in-house MDAC-St. Louis continuous flow electrophoresis laboratory test results to the basic understanding of the potential benefits of microgravity to the pharmaceutical industry. Other reasons include: 1) an obvious commitment of MDAC-St. Louis to the program; 2) careful selection of contacts and; 3) the fact that no immediate financial commitment was required of the drug company participants. Their initial willingness to listen to the presentation was probably tempered by curiosity about why MDAC-St. Louis was seriously

considering space processing of pharmaceuticals. Also, an influence could be the decreasing number of new pharmaceuticals being introduced; any process or concept which could produce multiple new and innovative products must be investigated.

The reasons why three companies have decided against participation are generally related to a combination of the extremely long development time frame (10-15 years), long term commitment with unanswered proprietary right retention questions, and the competition from alternative products and processes for limited corporate research and development resources. This latter factor was of paramount importance in two companies' responses.

The principle conclusion to draw from the decision of the three drug firms not to participate in the study is that despite its promise, not enough is known about potential space processed products to allow solid forecasts of how they would compete with other currently promising product alternatives which can be developed along more traditional lines.

Active participation, in the form of cooperative laboratory testing, indicates company interest in applied scientific research in addition to the feasibility analysis. Such an approach can be extended to space in the form of an applied research/development laboratory analogous to a ground development laboratory, that can serve to stimulate further drug company participation in space applied research.

3.0 INTRODUCTION

The initial interactions with pharmaceutical companies stimulated several of them to participate in the study to various degrees. Two companies in particular wished to cooperate with us in developing a better understanding of products of interest to them. Therefore, we established the laboratory test goal of developing the data base necessary for intercompany technological exchange. In this fashion we served the study goal by enhancing the cooperating companies' level of participation.

Initial protocols for cooperative investigations were prepared for the two companies based on data previously obtained during in-house work at MDAC-St. Louis. Specific cooperative experimental activities with antihemophilic factor VIII (AHF VIII) were then accomplished to expand our data base on this material and to exercise one of the test protocols. The second, multiproduct protocol will be implemented during 1979 under company funded auspices. Throughout these interactive processes a wide range of technology interchanges occurred between MDAC-St. Louis and the pharmaceutical companies, particularly in the areas of product characteristics, assay methods, state-of-the-art product research, sample handling logistics, and day to day technical procedures.

3.1 PRECONTRACT COMPANY FUNDED RESEARCH

Prior to entering into this study for NASA, considerable work was conducted with a first generation electrophoresis chamber during which methods were established to study the effects of gravity on the free flow electrophoresis process. These studies showed, among other things, that protein and cell samples whose specific gravities varied significantly from that of the carrier buffer could not be processed at flow rates required to separate materials having small differences in electrophoretic mobility. These data, documented in previous MDC publications (20,21) were used to demonstrate to the drug companies that laboratory test data were available concerning proteins and cells which illustrate the limit that gravity imposes on their processing by free flow electrophoresis.

In addition to these gravity effect studies, preliminary tests with AHF VIII were conducted using cryoprecipitate as the starting material. Processed samples were

sent to a local St. Louis hospital blood coagulation laboratory for evaluation. The results of these preliminary tests were encouraging because they showed that the protein materials in cryoprecipitate could be separated from one another and that AHF VIII activity was found with the fastest moving protein peak. Additional preliminary tests during this time with other partially purified materials showed that AHF VIII could be separated from other proteins present even in the most purified commercial products available.

3.2 PROTOCOL DEVELOPMENT

After pharmaceutical company participation was obtained, a series of follow up meetings with laboratory personnel from both MDAC-St. Louis and the participating drug companies ensued. The choice of material to be tested, assays to be used and the frequency of testing were established. Protocols were based on using previous MDAC-St. Louis company funded research, the capabilities of the laboratories, logistics of sample transport, and company policies concerning management of proprietary data. One of the major considerations of the protocols was control of the release of information. The protocols emphasized that the pharmaceutical company would have final say as to how the data would be presented.

A preliminary protocol was sent to the first company for review. It was promptly returned to MDAC-St. Louis with minor changes and a list of several tests they would perform on the electrophoretically separated materials sent to them. After their suggested changes were incorporated, the protocol was approved jointly by both laboratories. A copy of this test protocol is included as Appendix A.1 of this report.

Because the second company is multiproduct oriented, it has suggested participation in the exploration of several possible candidate products. Beta cells, pituitary growth hormone and granulocyte stimulating factor were submitted for their consideration. A preliminary protocol, Appendix A.2, was prepared based on these products. The final protocol will be prepared after the final selection of candidate products has been made by the drug company. Interlaboratory testing will begin in 1979 under company-funded auspices.

3.3 SEPARATION OF ANTIHEMOPHILIC FACTOR VIII (AHF VIII)

Initial interlaboratory tests with the pharmaceutical company were conducted with cryoprecipitate as a starting material. Protein assays and clotting times on the electrophoretically separated cryoprecipitate were initially conducted at MDAC-St. Louis. Early tests in which the separated samples were sent to participating companies for antigen assays showed deterioration due to problems associated with logistics. The cooperating company recommended and assisted MDAC-St. Louis in establishing an MDAC-St. Louis capability to carry out the required antigen assays eliminating delays and other factors tending to compromise test results. During these early cooperative tests we determined that the many variabilities of cryoprecipitate made it unsuitable as a research tool for free flow electrophoresis. Variable parameters included lipid and protein content, AHF VIII content, degree of solubility, and specific gravity. Consequently, the more uniform commercially available product, Hemophil^{R}, was chosen for laboratory testing. Cryoprecipitate, however, remains a viable candidate for space bioprocessing since these variabilities would be insignificant in large scale processing.

Hemophil^{R} contains approximately 250 units of AHF VIII in 700 mg of solids, of which about 50% is extraneous protein. The AHF VIII concentration is therefore approximately 0.086 μg per mg of protein. Samples were prepared by dissolving 0.5 to 0.7 mg of Hemophil^{R} in 0.0025M diethylbarbituric acid/sodium diethylbarbiturate, pH 8.3. The same buffer was used as the carrier fluid for the electrophoretic separation. A typical separation of Hemophil^{R} is shown in Figure 1. In this run sample concentration was 0.7 mg/ml, carrier flow was 20 ml/ min, and sample flow was 0.13 ml/min. Samples collected during sample streaming with no voltage applied showed that all protein materials were exiting through tubes 19 and 20 of 96 outlet tubes. A field strength of 48.19 volts/cm was then established in the chamber by applying 400 volts across the width of the chamber. The system was allowed to stabilize for 30 minutes, after which time samples were collected in tubes 20 through 56. Each tube was analyzed for total protein, AHF VIII clotting activity, and AHF VIII antigen activity.

The solid black line in Figure 1 represents the total protein content (in μg/ml) of each fraction collected. The dotted line represents AHF VIII clotting activity in seconds for each fraction. The black triangles along the abscissa indicate

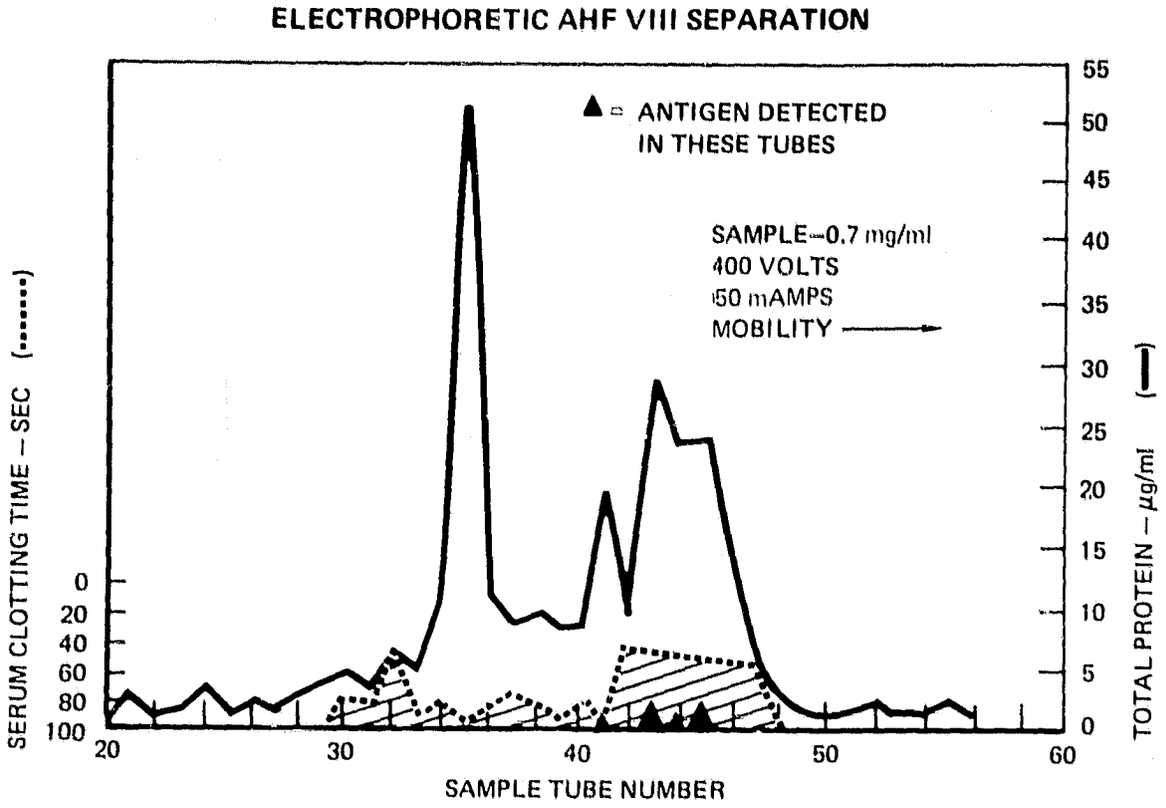

FIGURE 3.2-1

those tubes in which AHF VIII antigen was detected. Subsequent runs resulted in separation curves in which the principle features were similar to those shown in Figure 1.

The results of these runs showed that AHF VIII migrated in the electric field and that the antigen moiety and the bulk of the clotting moiety moved with the fastest moving protein peak.

The wide distribution of clotting activity indicates that this moiety has dissociated from the native AHF VIII molecule. As a result of this dissociation, various molecular forms of the clotting moiety appear to have separated due to their different electrophoretic mobilities. It is a well recognized characteristic of the AHF VIII molecule to undergo such dissociation. In order to determine the degree of dissociation of the material which may have occurred during processing, radioimmunological and radioautographic assays are being established at MDAC-St. Louis for future company funded studies.

Native AHF VIII possesses at least two characteristic activities which can be measured by analytical procedures. These are the clotting activity and antigen activity. After electrophoretic separation as shown in Figure 1, both antigen and clotting activities appeared in tubes 41, 43, 44, and 45.

A small peak of clotting activity occurred in tube 32. This represented only a small fraction (about 5%) of the total clotting material present in the sample prior to processing. However, it is a highly purified material with regard to extraneous protein content. It is possible that such a highly purified, although dissociated, clotting moiety might provide an important source of material for preparation of highly purified antibody to the AHF VIII clotting moiety.

The major protein in commercial AHF VIII preparations is fibrinogen. Since AHF VIII antigen and the bulk of the clotting activities moved with the largest protein peak, it is deduced the AHF VIII moves electrophoretically with fibrinogen. Although this has not been verified, it is reasonable to hypothesize that this is the case. Identification of fibrinogen as well as other materials in the starting product are planned as part of the on-going company funded MDAC-St. Louis R&D program.

3.4 HUMAN PLASMA AS A SOURCE OF AHF VIII

Since normal human plasma (including cryoprecipitate) is the only practical source of AHF VIII, the ability of free flow electrophoresis to separate the various human plasma fractions was investigated with company funding as an adjunct to the contract effort. For these studies a commercially available human plasma product, Standard Normal Plasma (SNP), was obtained from Dade Diagnostics, Miami, Florida. The sample was diluted in the same buffer as used for the AHF VIII studies at concentrations of about 0.8 mg/ml. A typical plasma separation is shown in Figure 2. The plasma protein was continuously collected in 45 fractions. In this case, ten distinct protein concentration peaks appeared, at tubes numbered 22, 24, 27, 30, 34, 42, 46, 52, 56, and 60. Shoulders appeared at 19, 40, and 55. The peak at tube number 46 was identified as albumin. Traces of alpha-1-globulins were found in tubes 44 and 45. These materials were positively identified by immunological procedures. Identification of other peaks is planned as part of the MDAC-St. Louis company funded R&D program.

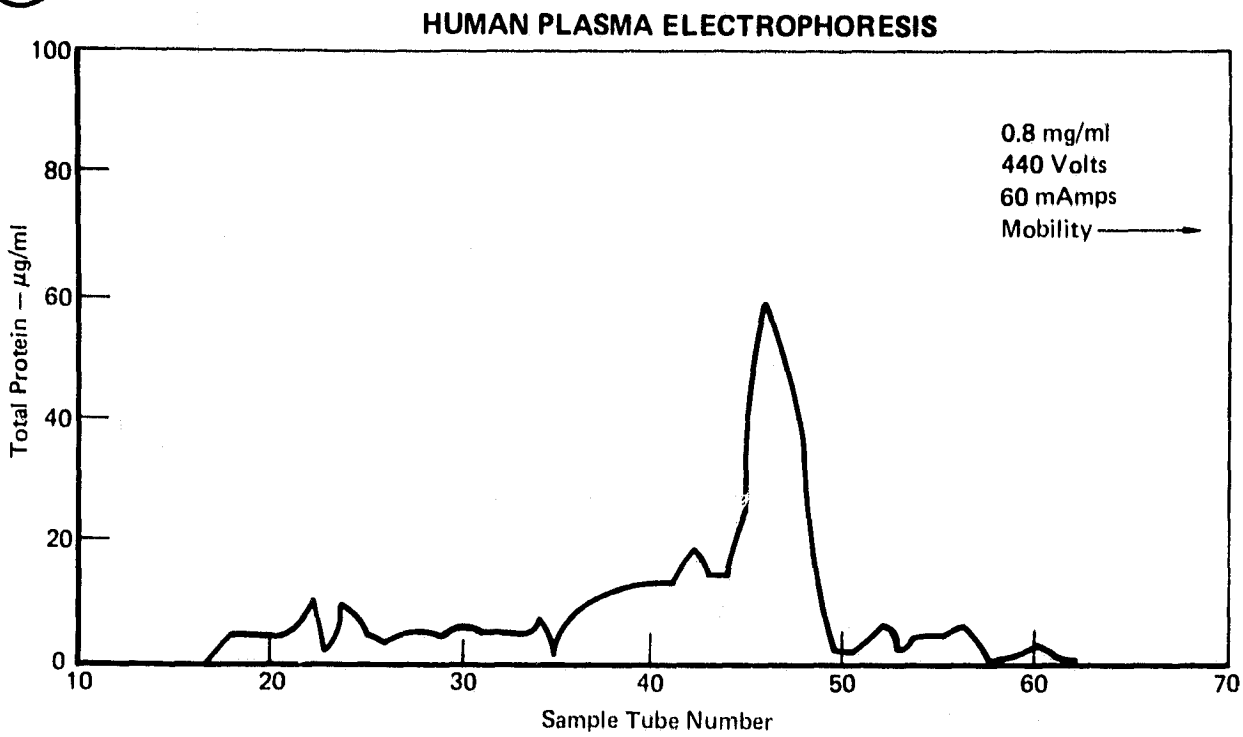

FIGURE 3.2-2

Dilution of plasma AHF VIII by the carrier buffer in these early electrophoresis procedures lowered AHF VIII concentration at the outlet port significantly below levels detectable by standard procedures. Therefore, the location of AHF VIII in the sample collections was deduced by relating its mobility to that of a key plasma component. This rationale is based on the observation that the bulk of the AHF VIII in Hemophil^{R} moved with the major protein peak of that purified preparation. Since fibrinogen is known to be the major protein component of Hemophil$_{R}$, it was reasonable to assume the working hypothesis that AHF VIII moved with, or near, fibrinogen in the plasma separations. Then the mobility of human fibrinogen was determined empirically under the same conditions as used for human plasma. Assuming that under identical separation conditions, fibrinogen in purified form or in the plasma will migrate approximately the same distance, the probable location of AHF VIII could be predicted for a given plasma separation. Samples collected in this location could then be tested using high sensitivity techniques.

Human fibrinogen was obtained from Sigma Chemical Company and dissolved in the same buffer used in the previous studies at a concentration of 0.5 mg/ml. This sample was processed under the same conditions as the human plasma samples and the results are shown in Figure 3. In this test, with no voltage applied, the sample

HUMAN FIBRINOGEN ELECTROPHORESIS

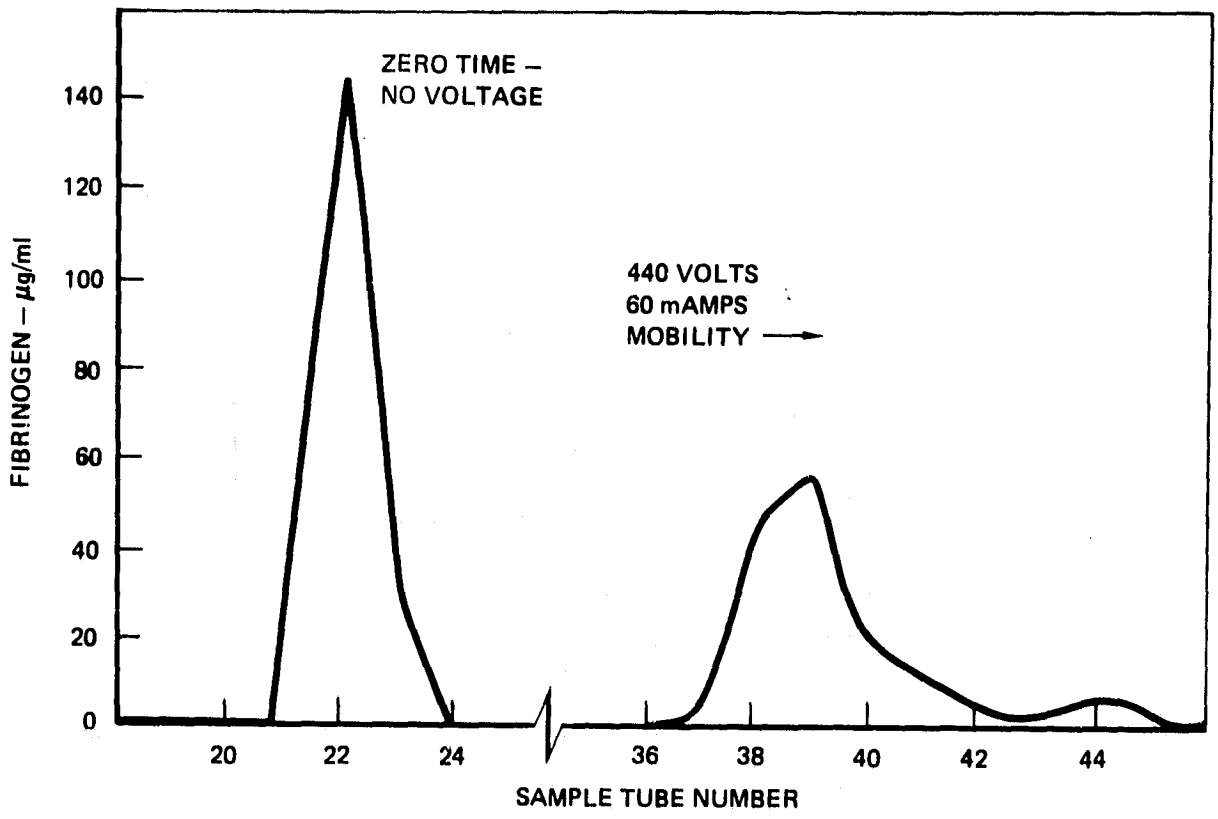

FIGURE 3.2-3

exited primarily in tube 22. After electrophoresis the bulk of the sample was found to have moved a distance of 17 tubes. The superimposing of this distance on the plasma separation curve indicated, according to the above rationale, that plasma fibrinogen would occur in tube 37 or 38, and that AHF VIII should be found in about the same tubes.

Immunological techniques for detecting and identifying fibrinogen and other plasma components, and sensitive radioimmunological and radioautographic assays for detecting position and output of plasma AHF VIII are being established in our laboratories as part of the MDAC-St. Louis continuing company funded R&D program.

3.5 SUMMARY AND CONCLUSIONS

A data base concerning AHF VIII and other blood components was developed on which MDAC-St. Louis established a technology interchange with the participating pharmaceutical companies. As a result of this exchange a preliminary characterization of AHF VIII and other important blood components by free flow electrophoresis was accomplished. This in turn, we believe, has enhanced the level of interest of those companies in the potential offered by processing pharmaceuticals in space.

Two test protocols were developed, one of which led to a technological exchange of AHF VIII analytical test data with a participating drug company. Interlaboratory testing in conjunction with the second protocol will begin in-house during 1979 with several additional products.

The results of intercompany communication and laboratory testing provided data of interest both to the pharmaceutical companies and to MDAC-St. Louis concerning AHF VIII and other important blood components. The exchange of technological information with the companies resulted in the following findings.

- o AHF VIII can be separated from a portion of the unwanted proteins found even in the most highly purified commercial preparations. This is of interest to the pharmaceutical companies because additives (required in their purification procedure, and which may cause liver damage) are not needed in the electrophoresis process.
- o Although molecular dissociation of AHF VIII occurs, the clotting properties are still intact. Since the patient with classic hemophilia lacks only the clotting moiety, its dissociation is not a deterrent to further investigation of this product. Both participating pharmaceutical companies expressed interest in a purified clotting moiety as a possible product.
- o A small amount of highly purified, slow moving material showing clotting activity was noted in typical AHF VIII separations of Hemophil^{R}. This fraction is of particular interest to one of the companies since it provided a unique form of clotting materials which could be used to study AHF VIII by new immunological techniques.
- o Preliminary tests revealed that use of cryoprecipitate as a product source will require development of special handling procedures to assure day to day uniformity of sample. This does not pose a critical technology obstacle.

- One sample sent to the participating laboratories was concentrated at MDAC-St. Louis by lyophilization. The resultant rehydrated sample contained no antigenic activity, indicating the possible removal, during processing, of a cryoprotective material. We are now investigating this loss of activity in-house by recombining several separated fractions. This finding highlighted the logistical problems to be encountered in transporting materials from one laboratory to another for conducting specialized tests. It emphasizes the need for MDAC-St. Louis to establish a broad base biological capability for complete in-house product evaluation of separated materials.
- Starting with human plasma as a product source, it may be possible to obtain all of the important blood fractions of medical importance in purified form in a single continuous process. Gamma globulins, used to protect against bacterial and viral diseases, are slow moving materials whereas albumin, used as a plasma expander, is very fast moving. These materials are currently obtained commercially by precipitation methods, but in such methods many important components, such as AHF VIII, other clotting factors, alpha antitrypsin, etc., may be neglected, lost or denatured by the process. A process in which all blood components can be obtained in a single step is of significant interest to both participating companies from the standpoint of economics as well as expansion of their product line.

4.0 INTRODUCTION AND SUMMARY

The objective of this phase of the study was to identify pharmaceutical products that might uniquely benefit from space manufacturing, select six to serve as "models" for further indepth analysis and then determine bioprocessing system requirements for a multiproduct space manufacturing facility needed to commercially produce them. Several candidates for space production were designated by NASA, MDAC-St. Louis, the pharmaceutical industry, and the academic community. The field of candidate products was narrowed to twelve over the length of the contract. Detailed technical and marketing analysis of each product was performed to provide information essential to an optimally defined multiproduct process.

Results of this activity included the six "model" products chosen, production quantities, product characteristics, product technical requirements and marketing data. In selection of the six model products, considerations for additional influences were incorporated: humanitarian needs, adaptability to a multiproduct facility and short timespan for research and development. The market evaluation established market need and market characteristics, as well as projected market value and associated market risk. Each of the six selected products were evaluated separately in order to provide the technical and marketing data as described. These market data provide the basis for determination of the production rate.

The six model products selected were anti-hemophilic factor (AHF), erythropoietin, beta cells, epidermal growth factor, alpha-1-antitrypsin and interferon. (Growth hormone, urokinase, immunoglobulins, somatomedin, granulocyte stimulating factor and transfer factor were the candidate products not chosen.) The analyses and evaluations conducted characterized each product sufficiently to allow definition of production system requirements.

4.1 APPROACH

The purpose of this task was to gather a list of candidate products, evaluate their potential for space processing and select six to serve as models for further indepth analysis.

A list of potential products, assembled by MDAC-St. Louis from many sources and coordinated with the participating drug manufacturers, for each major area of space bioprocessing including pharmaceuticals, cells for transplantation, diagnostic components, and basic research were evaluated with respect to humanitarian benefits, technical aspects and market needs. These products consisted of pharmaceutical products with no Earth processed substitute for a treatment regimen, improved pharmaceuticals for the diagnosis of disease, and products with significant, but indirect benefits such as those for medical research or agricultural use. Six representative products were then selected according to the following criteria: 1) the six selected products must represent the major types of products that would be produced in space, 2) the products must be improved in space, and 3) the selected products should have a major identifiable market. These products serve as the baseline pharmaceutical group for the purpose of this study. Technical aspects associated with process development for the selected products were also considered to identify possible roadblocks to early space processing implementation. The extent of the market for each selected product was then assessed to determine production rate morphology for a baseline system.

4.2 ANALYSIS AND RESULTS

To make a rational choice of six model products, a systematic selection procedure was used. First a list of candidate products was assembled and then reduced to a manageable group for evaluation purposes. Technical and economic data was then compiled for each product followed by a comparative numerical ranking of the candidates. This numerical ranking, when combined with the requirement to exercise the processing system in differing modes of operation (e.g. tissue culture, protein separation, etc.), allowed the final choice of the six model products to be made. Finally a market analysis was performed for each selected product. The following sections describe this selection procedure.

4.2.1 Candidate Products

The candidate products to serve as our analytical models for this study came from a number of sources. Starting with eight candidate pharmaceutical products compiled primarily from the results of previous NASA studies, this initial list was expanded during discussions with the company flight surgeon, local practicing physicians, faculty members at St. Louis University Medical School and Dr. Leonard Naeger at

the St. Louis College of Pharmacy. (Dr. Naeger is the editor of a biweekly national pharmacology newsletter sent to physicians for the purpose of disseminating new trends in pharmaceutical products.) This initial list of candidate products included blood coagulation factors VIII and IX, erythropoietin, growth hormone, granulocyte stimulating factor, pancreatic beta cells, stem cells, thymic hormone complex and urokinase. Additions to the list included immunoglobulins, glucagon, prolactin, epidermal growth factor, alpha antitrypsin, interferon, transfer factor, somatomedin, heparin and a number of hypothalamic releasing factors. A review of the medical literature of the last ten years then resulted in a shortened and more pertinent list of products. The thymic hormone complex, glucagon, prolactin and stem cells were eliminated, primarily because of a lack of readily available data on, and urgent clinical need for, these products.

Initial discussions with the various pharmaceutical companies visited confirmed the commercial interest in the shortened list of products. Other products were suggested by these companies and are listed in Section 4.3. They could not be included in this initial analysis due to limitations of time and manpower.

The final list of products is given in Table 4.2-1. A brief summary of the characteristics of each product is included in this section of the report. More detailed information on each product can be found in Volume III of this report.

TABLE 4.2-1 FINAL CANDIDATE PRODUCT LIST

Alpha-1-Antitrypsin	Immunoglobulins
Antihemophilic Factor VIII	Interferon
Epidermal Growth Factor	Pancreatic Beta Cells
Erythropoietin	Somatomedin
Granulocyte Stimulating Factor	Transfer Factor
Growth Hormone	Urokinase

Alpha-1-Antitrypsin

Alpha-antitrypsin probably functions as a major control protein against the tissue damaging effects of both endogenous and exogenous enzymes. It is the major inhibitor of two leukocyte enzymes having the ability to destroy human pulmonary tissue

resulting in emphysema. About 800,000 people in the United States have been diagnosed as having emphysema or chronic bronchitis. Because of the wide disparity in the literature with regard to patients afflicted with both alpha-antitrypsin deficiency and emphysema or chronic obstructive pulmonary diseases, we have conservatively estimated that approximately 100,000 people of this group have a severe alpha-antitrypsin deficiency, usually less than 20% of normal circulating blood levels. It has been suggested that raising the circulating blood antitrypsin levels to about half that of normal values might be useful in arresting the further progression of emphysema. Alpha-antitrypsin is administered intravenously in a dosage of 20 mg/Kg body weight at four to six day intervals. The metabolic half-life of this protein allows spacing between treatments similar to the clinical regimen for antihemophilic factor treatment. Previous attempts at treating patients with alpha-antitrypsin contained in whole plasma created side effects which necessitated discontinuance of the procedure. It is expected that purification of alpha-antitrypsin in large quantities will rejuvenate this treatment method and control the progression of the disease. No other specific treatment now exists for the treatment of emphysema.

Three companies produce alpha-antitrypsin for laboratory research and diagnostic purposes at $0.17 to 0.20/mg. This material is from botanical sources and is not considered acceptable for clinical purposes. Human material as an alpha-antitrypsin standard can be obtained from the American subsidiary of Berhingwerke, Darmstedt, Germany. If human plasma were the sole source of this product, in its clinical form it would require the simultaneous extraction of both alpha-antitrypsin and antihemophilic factor from almost all the blood collected annually in the United States.

This product is a single chain glycoprotein produced by hepatocytes. Twenty-four alleles of alpha-antitrypsin have been determined by electrophoresis, each with its own mobility. Because of the number of variants the isoelectric point ranges from 4.5 to 4.9. The molecular weight is about 50,000. The chemical composition of three variants can be found in Volume III, Section A.1. The molecule is unusual in that it has more than one carbohydrate side chain, probably as many as four. Alpha-antitrypsin is soluble in many solvents but it can be salted out. It

loses its biological activity when in acid solutions below a pH of 5.0. The protein can be lyophilized and stored for 12-18 months at 5°C. To our knowledge, human hepatocytes have not been cultured for the purpose of producing this protein. It may be possible to do so, however. Reports of both human and animal liver cell cultures of this product in both monolayer and suspension form are described in Volume III, Section A.1. A detailed procedure is presented for a monolayer culture that was viable for 14 months at the time it was reported.

There are two major methods for the ground based isolation and purification of this product. Each depend on the use of dialysis, "salting out" phenomenon and affinity chromatography to remove impurities. The main impurity is albumin which has a very similar solubility and electrophoretic mobility to alpha-antitrypsin. The usual laboratory yield is about 22% of the alpha-antitrypsin found in crude plasma.

Antihemophilic Factor VIII

Approximately 20-25,000 people in the United States suffer from a disease in which their blood does not clot readily when blood vessels are injured. The disease is called hemophilia. We have assigned a disease severity index value of 6. The major problem usually is not externally caused bleeding such as that from contusions, puncture and lacerations (all of which can be life threatening in themselves), but rather simple tissue injury due to movement of joints during normal walking, playing, etc. Blood loss from these simple activities can cause major hemorrhage into the surrounding tissue resulting in shock, permanent damage to the joint, immobilizing the patient and possibly death. The disease is hereditary, being passed by the genes of the mother but almost exclusively affecting the male children. Spontaneous development rather than heredity is now becoming a significant source of new hemophilics. It is only since the second world war that children afflicted with this disease can expect to reach young adulthood. Many of the oldest hemophilics have not yet reached their fortieth birthday.

The disease is due to a deficiency in one or more of thirteen different substances that must sequentially interact to stop the bleeding. The two most common deficiencies are antihemophilic factor VIII and plasma thromboplastin component IX. Only AHF VIII is considered for this analysis. The usual prophylactic treatment

is 250 units of AHF every day for life for severe or mildly severe hemophiliacs. The dose is also dependent on the rate and quantity of blood lost as well as external weather conditions and age; rapid alterations in environmental temperature will cause capillary bed hemorrhaging, requiring more AHF, and the older the subject the less product is required because he will tend to take better care of himself. During surgery or treatment for hemorrhaging as the result of an injury, a severe hemophiliac can consume about 17,000 units per episode. The unit of product is defined as that amount of substance found in one milliliter of blood of a normal person. The half life of AHF VIII after infusion is approximately 12 hours necessitating frequent replacement as a preventive procedure.

The only source of AHF is from human plasma. It is species specific and has no competitive products to share the market. Approximately 9,000,000 pints of blood are collected annually by the Red Cross, five pharmaceutical producers of AHF and those hospitals treating this disease in order to supply the deficiency factors needed by about 20,000 patients. AHF is a protein of about 195,000 molecular weight but its amino acid sequence has not been identified. McDonnell Douglas Astronautics Company has determined an electrophoretic mobility value for the product of 8.6×10^{-5} cm^2/volt-second. The current ground based production method utilizes the development of cryoprecipitate followed by the modified Cohn fractionation procedure I at very low temperatures. An elaboration of the production method can be found in Volume III, Section A.2. The actual site of AHF synthesis in the human body is not known. Therefore production by tissue culture is not relevant at this time. The current assay technique uses a wet chemistry procedure which measures the time required for the sample to cause clotting of AHF VIII deficient serum.

Epidermal Growth Factor (EGF)

This product is a single chain polypeptide produced in the submaxillary glands. It can also be found in the urine. Its normal physiological function is to stimulate the production of ectodermal tissue. It is currently undergoing investigation as a pharmacological agent to increase the rapidity of autologous skin replacement in those areas damaged by burns or injury. The annual number of patients with third degree skin burns covering at least five percent of their body surface is estimated to be at least 14,000. We have assigned a disease severity index value

of nine to this product category. There is no competitive product to serve the function of this protein. Because of "foreign" tissue rejection problems historically inherent in the use of skin grafts to cover the burn areas, the potential capability of EGF for this pathological condition may eventually make it the drug and procedure of choice. The dosage used for treatment has neither been established nor published by the two companies conducting clinical research with EGF. For the purpose of providing a rough order of magnitude comparison dosage with other products in this report, we have chosen a value of 20 nanograms/cm^2 of damaged skin area. It has been reported in the literature that this concentration of EGF causes epidermal cells in tissue culture to proliferate and spread into a fibroblast network.

Besides the clinical aspects of burn and wound healing of the skin, EGF appears to have many uses for the production of cells in culture. Investigators have reported that this product lengthens the life of cell cultures from 50 to 150 generations above controls and also drastically reduces the need for fetal calf serum as a nutrient in tissue culture. Use of EGF to supplement a reduction in fetal calf serum requirements for tissue culture could stretch the current annual supply of serum about five hundred fold.

Collaborative Research, Inc. is the only company commercially supplying the material for nonhuman research purposes. Their current market price for EGF is $495/mg.

Epidermal growth factor has been biochemically characterized and the amino acid sequence established. Using male mouse submaxillary glands as the source, this protein molecule has a molecular weight of 6045 based on 53 amino acid residues. When EGF is extracted from human urine, the molecular weight is 5400 with 49 amino acid residues. The chemical composition and amino acid sequence can be found in Volume III, Section A3. It has been separated for analytical purposes by various electrophoretic techniques and its rate of mobility depends on the pH of the buffer used. While its upper solubility limit in water has yet to be established, a concentration of 0.4% in 0.01M sodium acetate buffer has been reported. The product can be lyophilized and is stable at room temperature in that form. It is subject to bacterial and enzymatic degradation but is stable to boiling water.

Heating in either dilute alkali or acid will destroy EGF. Research has focused on the effect this product has on other cells in culture and not how to produce it in large quantities. Three methods of assay are used for the detection of EGF. They range from an in vivo assay involving the precocious eyelid opening of new born mice in the presence of EGF to the competitive binding radioimmunoassay technique capable of detecting the product in one nanogram per ml quantities.

The current ground based production method involves affinity chromatography and density gradient chromatography techniques. This procedure usually takes 7-10 days and yields about 700 micrograms of EGF from 10 grams of a benzoic acid/acetone extract of human urine.

Erythropoietin

This product is a natural glycoprotein hormone of the kidney cortex. A deficiency in its production results in a severe anemia that could be corrected on a temporary basis by red blood cell transfusions at regular biweekly intervals. These transfusions, however, are not used as a continuous treatment regimen because of the potential for virus hepatitis infection. We have assigned a disease severity value of 6 to this product category. The anemia is primarily found in patients with kidney failure. There are approximately 20,000 people in the United States afflicted with frank kidney failure and the anemia secondary to this disease.

At the present time erythropoietin is not commercially available as a pharmaceutical product for human use because of the current inability to purify the product from extraneous organic material. No known pharmacological substitutes are available to compete with this product. Based on extensive laboratory test data collected from a number of animal species including man, it is anticipated that the pharmacological dose of erythropoietin will be 20-200 units per kilogram body weight given once each week for the life of the kidney failure patient. In the less severe, chronically anemic patients, the dosage may be the same but the rate of medication may be once or twice per year.

Erythropoietin in a partially purified form is available for research purposes from the Connaught Laboratories in Toronto, Canada. Their current price for Step III partially purified hormone is $0.83 per unit or about $69,000 per year when applied to a 176 pound adult. A second commercial source of erythropoietin is from the Erythropoietin Collection Center in the Department of Physiology of the School of Medicine, Northeast University, Corrientes, Argentina. Neither organization has filed an Investigational New Drug (IND) application for clinical trials with the United States Food and Drug Administration. Most researchers in this country, however, obtain erythropoietin in small quantities from the urine of aplastic anemia patients and attempt to purify it themselves.

Erythropoietin is a glycoprotein of between 27-100,000 molecular weight. Sialic-acid is the carbohydrate moiety which must be present for stimulation of red blood cell production in-vivo but not in marrow tissue cultures. The amino acid content of the protein moiety has been elucidated but not the sequence. Erythropoietin has been separated electrophoretically into two active but diffuse bands between albumin and $alpha_1$ globulin. Subsequent electrophoretic separation of the two active bands has resulted in the elucidation of six bands suggesting depolymerization of the hormone into smaller but still active units. These electrophoretic techniques have concentrated the pharmacological activity to 8,300 units/mg. It is anticipated that further purification can concentrate this activity to even higher levels per unit weight. Erythropoietin is highly soluble (18%) in water and also soluble in relatively mild acids.

The hormone is known to be produced in the kidney cortex possibly by the cells located in the glomerular tufts. These cells have been separated electrophoretically and also grown in monolayer and suspension tissue cultures for periods up to several years. Production of tissue culture is feasible and may eventually be the method of choice for commercialization of this product. At the present time, however, the far simpler method for small research quantities of the material is to extract it from the urine of either anemic people heavily infested with hook worm parasites or sheep that have been made anemic artificially using whole body radiation followed by phenylhydrazine injections. Details of the extraction and partial purification procedure can be found in Volume III, Section A.4. The standard technique for quantitative analysis is the starved rat bioassay calibrated against the values obtained from erythropoietin international reference standard B.

Granulocyte Stimulating Factor

The function of granulocytes is to control and remove tissue damaging material from the mammalian organism. Agranulocytosis, a condition in which these cells are present in the body in abnormally low concentrations, can be caused by radiation therapy, chemical toxicity and stressful situations; e.g., massive infections. A natural protein found in human urine and produced in vitro from lung, embryonic kidneys, macrophages and spleen tissue cultures is able to stimulate the production of these granulocytes from marrow stem cells to restore them to their normal physiological levels. Estimates of the annual number of potential recipients who could benefit from this "granulocyte stimulating factor" vary from about 500,000 receiving radiation and chemotherapy for cancer, to about 2 million undergoing surgery. It also has potential for treating coronary thrombosis and the massive sepis of burns. A severity index value of 7 has been assigned to the above diseases. The use of granulocyte stimulating factor is still in the experimental phase and therefore the clinical dosage has not been established. Effective treatment for the diseases indicated above requires a circulating blood granulocyte concentration of 1000 cells/microliter (see Volume III, Section A.5). This usually can be attained with a dose of 2.5×10^8 units of granulocyte stimulating factor. With an intravascular survival rate of about 6-10 hours, the dose would have to be administered several times per day until the causative condition of the agranulocytosis is resolved or the patient dies.

Granulocyte stimulating factor is not available commercially for research purposes or clinical investigation. At the present time, all granulocyte stimulating factor is produced in the laboratories of the research scientists intending to use it. As a pharmaceutical product, granulocyte stimulating factor will be a direct competitor of the recently developed procedure for the transfusion of granulocytes obtained from matching human donors through continuous flow leukaphoretic centrifugation. Reactions to both the donor and recipient as well as the large number of matching donors required for a course of treatment by the latter method would make use of tissue culture produced granulocyte stimulating factor the drug of choice.

Granulocyte stimulating factor is believed to be a protein but it is not certain if carbohydrate side chains are present. Using Sephadex chromatography, four

fractions have been obtained. Their molecular weights are approximately 79,000, 40,000, 23,000, and 2,000 daltons. The 79,000 dalton fraction may be a dimer of the 40,000 dalton fraction. The material readily moves in an electrophoretic field showing a 50-70% recovery of biological activity when using polyacrylamide gel. A specific activity of 680,000 units/mg can be obtained with preparative gel electrophoresis as contrasted to 4,364 units/mg observed with hydroxyapatite chromatography. Granulocyte stimulating factor is stable in the pH range of 6.5-10 and temperatures of 56°C for 30 minutes. It is unstable upon freezing and thawing unless 0.1% gelatin or 0.2% mg/ml albumin is added. It is resistant to trypsin and neuraminidase but is sensitive to subtilisin, chymotrypsin and periodate. Assay for the product is done by counting the number of colonies (greater than 40 cells) formed ten days after the granulocyte stimulating factor is added to a culture of about 200,000 human marrow cells. Cells producing granulocyte stimulating factor also have been electrophoretically separated in space during the Apollo-Soyuz flight.

Growth Hormone (GH)

This product is a natural protein hormone of the anterior pituitary gland. A deficiency in its production results in dwarfism. Its normal physiological function is to stimulate growth in children of short stature. The hormone is currently undergoing investigation as a pharmacological agent to treat osteoporosis and stress ulcers. Estimates of annual potential recipients who could benefit from this product have ranged as high as 840,000 people. We have assigned a disease severity index value of 5 to this product category. At the present time only 1000-1500 children are being treated annually because of a lack of the raw material from which the hormone is isolated. GH is species specific and must come from other humans thus limiting the source of supply. There are no competitive products to serve the function of this hormone. In the treatment of dwarfism the usual dosage of growth hormone is 1 mg every other day over a several year period. In the experimental treatment of stress ulcers the dosage is 10 mg/day over a 4-18 day treatment period. The lack of material becomes obvious when the acetone-extracted human anterior pituitary gland can only provide about 3-5 mg of the unpurified protein.

There is one commercial company plus the National Institutes of Health National Pituitary Agency that are able to supply GH in 1978. Calbiochem has been approved by the FDA to market it with the trade name ASCELLACRIN. That company is currently charging $7.50/mg for the compound but its purchase requires acceptance of the patient for such treatment by Calbiochem physicians. Company acceptance is required primarily because of the short supply of the hormone and their goal of trying to prioritize the patients who could benefit most from its application. A second manufacturer, Serono, is expected to complete FDA clearance for marketing some time within the Spring of 1979. The National Pituitary Agency provides the hormone at cost for clinical and basic research. Because it is the national clearing house for the collection of human pituitary glands by the American College of Pathologists, it also distributes a portion of the extracted hormone material for sale as a pharmaceutical product and as a standard for diagnostic assays.

Growth hormone has been identified as a 191 amino acid protein with disulfide bonds and a molecular weight of 21,500. The amino acid sequence is shown in Volume III, Section A.6. It was the first hormone separated by static electrophoresis from homogenized pituitary material. The granules of the acidophil cells containing GH have also been separated by static electrophoresis. In neither case has the procedure been considered adequate for commercial production but rather a simple laboratory tool for identification and experimentation. The isoelectric point has been located at a pH of 4.9. Its solubility is very low, 0.03% in water, but it can be increased slightly in other solvents and also when additives, such as mannitol, are included. The current production method is acetone extraction yielding 4-8% dry weight of the pituitary gland and its stalk. Growth hormone has been produced by both monolayer and suspension tissue culture techniques for periods up to several years. The acidophil cells may produce two hormones simultaneously, i.e., GH and prolactin. A more detailed description of production and tissue culture methods for this hormone can be found in the appendix. Qualitative and quantitative analysis of GH is done by radioimmunoassy.

Immune Human Serum Globulins

Infectious agents generally induce the production of uniquely reactive proteins called antibodies, which appear in the gamma globulin fraction of plasma subjected to electrophoretic separation. Plasma rich in one or several specific antibodies

is referred to as disease specific immune globulin; for example, a plasma rich in specific antibody to chicken pox is referred to as "ZIG" or zoster immune globulin. This is not purified antibody but a plasma with high levels of anti-zoster antibody. Under limited circumstances it could be advantageous to have availability of highly purified preparations of specific antibodies against, e.g., hepatitis, rubella, rubeola, mumps, poliomyelitis, and others. As a general class we have assigned a disease severity index of 7 to this product category.

About 300,000 cases, representing all of the above mentioned diseases are reported to the National Center for Health Statistics annually in the United States. While a dose of specific immune serum globulins may be given to modify or minimize the effect of that specific disease in a person already afflicted, the usual procedure is to immunize all the people who have or may come into contact with the patient until the incubation period has been exceeded. Depending on the seriousness of the disease, the number of subjects receiving this passive immunity can range from 1-6 close contact family members for mumps up to many thousands of individuals threatened by serious epidemics. The number of patients given passive immunity from year to year will depend on many variables but primarily the number and location of people afflicted. This great annual uncertainty results in a large quantity of specific products being made, stored and destroyed when the product shelf life is exceeded if there is no threat of an epidemic.

The usual single dose of immune globulin given to a patient can vary with the disease; it can range from 3.6 to 330 mg per injection. In the extreme case where an infant is born with a gamma globulin deficiency he can receive up to 3.0 grams/ month. The average cost of a single dose of gamma globulin is between $3 and $5. The Physicians Desk Reference lists at least ten manufacturers in this small but competitive market. The American Red Cross and some local hospitals also may prepare specific gamma globulins for staff use from their own blood banks. At the present time there are no known substitutes for human gamma globulin with the ability to do the same protective function.

Antibodies are produced by plasmacytes and released into the blood stream. To minimize homologous protein antibody reactions, the source of human immunoglobins is from the gamma globulin fraction of human blood plasma. A "specific" gamma

globulin means that a person has been subjected to a disease, built up antibodies to that disease, and these antibodies appear among his protein gamma globulin fraction. These gamma globulins can be extracted from pooled plasma by cryoprecipitation and alcohol fractionation as well as electrophoretic separation. They have a very high solubility of 15 gms/liter. The standard assay technique is by immunoelectrophoresis against specific antibodies. A more detailed description of the processing procedures can be found in Volume III, Section A.8.

Interferon

This natural protein substance appears to be the modern day pharmacological panacea for almost all diseases of known viral origin as well as several other diseases in which viral involvement has been suspected but not proven. Its clinical use is still in the experimental stage with only a small part of its therapeutic potential explored. Interferon has been used in the treatment of various forms of cancer, lymphoblastic leukemia, influenza, common cold and viral infections. We have assigned a severity index value of 7 to this product as an average value for the variety of diseases showing response to interferon. If only specific diseases were used in the application of this index, the value would range from 10 for osteo sarcoma to 3 for the common cold and influenza. It is very difficult to estimate the number of patients that could benefit from this drug and its annual market value because the clinical trials have been small, therapeutic doses have not been firmly established and only a few of the potentially responding diseases have been tested. In the case of hepatitis A alone, it is estimated that 100 million people worldwide are suffering from this disease. Over 380 thousand people die annually in the United States from various forms of cancer. Prophylactic treatment with interferon for influenza epidemics would also involve hundreds of millions of patients. Each of these diseases has its own dosage schedule and treatment regimen (see Volume III, Section A.7 for more specific details).

Several competing antiviral drugs are already on the market including amantadine, vidarabine and idoxuridine, but are limited to the treatment of Herpes Simplex and prophylaxis of Influenza A. Radiation therapy and a number of chemotherapeutic products are already on the market for the treatment of cancer. While they

offer some pharmacological protection, many patients develop a physiological resistance to these compounds making them ineffective. A university research team is exploring the use of a nucleic acid that will induce interferon production endogenously without side effects.

Because of the tremendous market potential for this compound, approximately 100 industrial and university laboratories around the world are making interferon for their own research purposes. The current source of leukocyte interferon is the Interferon Department of the Finnish Red Cross Blood Transfusion Service in Helsinki. It is the largest supplier at the present time with an annual production of about 100 billion units. The Sloan-Kettering Institute has started a production facility in Switzerland and expects to match Helsinki production in 1979. The Wellcome Research Laboratories in England has also announced production of interferon from lymphoblastoid cell lines cultured in 1000 liter fermentation vats. They estimate their annual production will exceed that of Helsinki in 1979. HEM Research in Maryland and Calbiochem in California are the only commercial sources of any type of interferon in this country. While interferon from all of the above sources is only available for research and some FDA authorized human clinical trials, none of the material is available to the average private physician for treating pathology. There is some indication it may be an over-the-counter product in Russia (see Volume III, Section A.8). In the United States, all interferon for clinical research purposes must be obtained through the Interferon Working Group at the National Cancer Institute. While the present cost of interferon is $50/million units, continued clinical success combined with the interest and technical skills of the many involved research laboratories will ultimately increase production and lower unit cost.

Interferon is a glycoprotein substance produced by cells in-vivo and in-vitro when challenged by a virus. It is species specific and can be induced by normal and attenuated virus or any substance that is equivalent to a double stranded RNA. The current clinical trials have relied on only three tissues for interferon production: (1) the human leukocytes collected from blood donors, (2) fibroblasts grown in tissue culture from human infant foreskin obtained at circumcision, and (3) human leukocytes from a high yield lymphoblastoid Burkitt's lymphoma cell strain. Three types of interferon have been isolated from these sources with more

than one type coming from the same source. Molecular weight ranges from 15,000 to 21,000, depending on the type of interferon. They have not been isolated in sufficient purity and quantity to determine their molecular structure or amino acid sequence. The backbone of the interferon molecule is a folded polypeptide of one or more chains linked by disulfide bonds. One or more carbohydrate side chains containing sialic acid are attached to this backbone. A number of electrophoretic techniques have been used to purify interferon with varying levels of success. It is soluble in many solvents but it can be salted out. It cannot be dialyzed. Cells producing interferon can be cultured by both monolayer and suspension culture methods. These cells usually reach a peak interferon production level about 10 hours after induction with a virus and then become inhibited to further production for several days after the interferon is removed from the culture media. The standard method for bioassay is to measure the ability of interferon to protect cells in tissue culture against a challenge virus.

Pancreatic Beta Cells

The beta cells of the islets of Langerhans found in the pancreas produce a hormone known as insulin whose physiological function is to aid in the regulation of carbohydrate metabolism. A deficiency in production of this hormone results in a form of diabetes mellitus, often called juvenile onset or insulin dependent diabetes. We have assigned a severity index of 5 to this disease. For slightly over the last fifty years this disease has been brought under control by daily injection of insulin extracted from the pancreatic tissue of cattle and pigs. Essentially the extract of one animal pancreas is sufficient to treat one diabetic for one week at an over the counter cost of $3.00 per bottle. The present U.S. population of known insulin dependent diabetics is believed to be 1.5-3 million people concomitant with an additional 3.5-7.0 million adult onset (non-insulin dependent) diabetics who may eventually require insulin as their disease progresses to an insulin dependent state. An almost equal number of people are suspected of being undiagnosed diabetics in both forms. The projected expansion of both the American and world population has developed concern among pharmaceutical manufacturers due to their inability to meet the future demand for insulin when they are currently consuming almost the entire annual harvest of animal pancreatic tissue in the United States, Australia and Argentina.

Since there are no competitive products for this hormone in the treatment of diabetes mellitus, a great deal of laboratory investigation has been involved with transplantation of pancreatic tissue in whole or in part. The most promising concept to date involves isolating the islets of Langerhans and injecting them into the capillary circulatory system of the liver where they become wedged and start to function as a "replacement" pancreas. Several experiments of this nature have been conducted in both animals and humans with partially successful results. Within a week after injection of the partially purified islets of Langerhans, the recipients were able to greatly reduce and, in some cases, completely withdraw from supplemental insulin injections. The rate of successful transplantation in these studies may depend on the dose of islets or cells received and the ability to suppress or eliminate the immune response to "foreign" tissue. Estimates of the number of living islets required to cure one diabetic patient range from 60,000 to 200,000, a quantity difficult to obtain from one adult donor using current laboratory isolation methods. It is hoped that further improvement in beta cell production will result from continuous flow electrophoretic techniques of separation. The cost of the transplant would be extremely high in comparison to the current cost of insulin, but available evidence strongly suggests that a beta cell transplant would protect diabetics from the devastating complications of their disease far better than does insulin therapy.

The use of bacteria to produce insulin through the process of gene splicing and recombinant techniques is also being investigated. A number of pharmaceutical companies and research investigators have told us it will take 10-15 years to perfect this process but it does not pose a threat to space bioprocessing because the insulin must be separated from the other metabolic contaminants of the bacterial cultures.

Insulin is a double chained protein of 6000 molecular weight. Its sequence of 51 amino acids is known and differs slightly from species to species. There is enough similarity however that human, bovine, and porcine insulin can all be used as human medication. The similarity among islet cells or beta cells of different species is not known and therefore human adult and fetal pancreatic tissue are being investigated to minimize homologous tissue reactions. The electrophoretic mobility of both beta cells and islets is unknown but it is expected that they will migrate in an electrophoretic field similar to all other cells. The beta

cells and islets have been separated by enzymatic disruption from the surrounding acinar tissue in very cold temperatures to prevent enzymatic digestion of the desired cells. The cells have been cultured in monolayer cultures for long periods of time. The method of extracting and culturing islets of Langerhans can be found in more detail in Volume III, Section A.9. The current method of choice for assaying insulin is the radioimmunoassay procedure.

Somatomedin

This growth factor stimulates the incorporation of amino acids into cells for the production of protein and a gain in weight. There is some evidence that it can operate independently of growth hormone. A number of pharmaceutical companies have suggested that periodic injections of the substance into feed lot livestock might increase feed utilization by these animals. If such a situation could be produced pharmaceutically the total amount of meat produced per pound of feed consumed would be significantly increased. In the United States alone we slaughter approximately 123 million cattle, sheep and hogs each year. While research along these lines is being conducted in at least one industrial laboratory, the dosage of somatomedin required has not been established. To provide a comparison dosage with other products of this report we have arbitrarily chosen one half the dose of growth hormone or one unit (0.5 mg)/40 kg body weight given three times per week until the animal reaches maturity.

Somatomedin is not available commercially for research purposes or clinical investigations. At the present time all of this material is produced in the laboratories of the research scientists intending to use it.

Three separate somatomedins have been identified by their biochemical characteristics and biological activities. Somatomedin A is a neutral peptide of about 7000 molecular weight which stimulates sulfate uptake by chick embryo cartilage. Somatomedin B is an acidic peptide with a molecular weight of approximately 5000. It stimulates the incorporation of thymidine into glial cells in tissue culture. Somatomedin C is a basic peptide of about 7500 molecular weight that competes with insulin for the same binding receptor on human placental membrane. The amino acid composition has not been identified for any of the three substances. Somatomedin C, however, is expected to have about fifty amino acid residues and be rich in

arginine. Biochemical similarities and differences among the three somatomedins has not been determined.

The site of somatomedin synthesis in man is not known but animal experiments suggest that the liver may be important. The current source of this product is from human plasma (Cohn fraction IV-b). The fractionated plasma is subjected to a series of acidic ethanol, acetone-alcohol and formic acid extractions followed by chromatographic separation on a Sephadex column. Twelve hundred liters of plasma yield about 240 micrograms of somatomedin. The product is dialyzable and has been separated by gel electrophoresis as well as isoelectric focusing with a sucrose gradient. It is relatively stable to heat in the range of 60-100°C but becomes partially denatured in the presence of mercaptoethanol. Besides the three assay methods used to identify the three types of somatomedin, the product also can be assayed by weight gain or tibial width change in hypophysectomized rats or the incorporation of sulfate into porcine costal cartilage.

Transfer Factor

After a human leukocyte in vivo has been exposed to an antigenic type of material, it has the ability to effect a delayed hypersensitivity to that material when exposed on subsequent occasions. If these leukocytes or their dialyzable polypeptide-polynucleotide extract are injected into another person, the delayed-type cellular immunity is transferred to the recipient. This "transfer factor" is immunologically specific for the challenging agent. This transferred immunity resides in the recipient for long periods of time, greater than two years in some instances as compared to most antigen-antibody reactions which last only several days to several months. The clinical effects of transfer factor are currently being explored in many pathological conditions including multiple sclerosis, leprosy, malignant melanoma, breast cancer, and immunodeficiency diseases. In most of these studies only preliminary results on a small number of patients have been obtained. The treatment regimen for each of these diseases is different and has not been established as to optimal dosage making it difficult to predict annual market requirements. Multiple sclerosis is the most common neurological disease next to strokes in the United States affecting approximately 535,000 people. While leprosy is found in only 130 new patients each year in this country, about 15 million are affected in tropical countries worldwide. We have assigned a severity index value of 7 to this product resulting in an average value for the diseases treated.

Transfer factor is not available commercially for research purposes or clinical investigation. At the present time, all transfer factor is produced in the laboratories of the research scientists intending to use it. Transfer factor for a specific immunological agent can be obtained from circulating human leukocytes in the following ways: (1) concentrates of viable leukocytes, (2) leukocyte extracts prepared by distilled water lysis or by repeated cycles of freeze-thawing, (3) leukocyte extracts prepared by treatment with enzymes, (4) antigen liberation, and (5) dialysis. Transfer factor in whole leukocyte preparations is stable at -20°C for at least four years. Lyophilized transfer factor can be stored for at least five years at 4°C without loss of potency.

It is still not known whether transfer factor is a single entity or a class of closely related substances. It is believed to be a polypeptide-polynucleotide complex that is different from interferon in chemical properties and biological action. Several methods have been used for the isolation and purification of this product including various forms of electrophoresis, chromatography and dialysis. Isoelectric focusing has resulted in at least 17 subfractions with isoelectric points scattered over a pH range from 3.5 to 10.2. The molecular weight of transfer factor is between 3,500 and 10,000. The amino acid composition is listed in Volume III, Section A.11. Human lymphocytes in tissue culture are capable of producing transfer factor after induction with a specific immunological agent. There is only an eighty percent chance of induction. Once the cultures are producing they can be cut back to one percent of the final cell concentration at the end of a three to five week period and recultured for another transfer factor harvest without using a second induction period. It is believed that this ability to produce transfer factor will last as long as the cell source is still viable in tissue culture. The method for induction and culturing of transfer factor producing cells is described in Volume III, Section A.11.

The most sensitive assay method for transfer factor at the present time is done in-vivo on humans showing a negative reaction to a specific antigen when they are challenged by that antigen. In vitro assay techniques have been besieged with problems and reflect the detection of new receptors on the surface of the cell.

Urokinase

An estimated one percent of the U.S. population annually is subjected to surgery, broken bones and accidents damaging the vascular system. They are all potentially subject to blood clot formation and movement as a result of this tissue insult. If a clot plugs critical vascular pathways, the result can range from a slight area of tissue necrosis through eventual amputation of a limb or organ to almost instant death. We have assigned a disease severity value of 7 to this product category. Urokinase is a fibrinolytic enzyme activator used to break up and dissolve these blood clots disseminated throughout the body. National studies of the pharmacological activity of urokinase have been conducted for the past ten years under the auspices of the United States National Institutes of Health and European country members of NATO. The recommended dosage of urokinase for dissolving existing pulmonary clots was established at 4400 CTA (Committee on Thrombolytic Agents) units per kilogram of body weight per hour for 12 hours followed by heparin administration at 22 units per kilogram of body weight per hour for 14 days. Myocardial infarction was treated with 7200 units/Kg body weight given intravenously followed by half that dose hourly for 18 hours. The total quantity administered and duration of urokinase therapy may vary with the location and severity of the clot but it is believed that a Lee-White Clotting Time of greater than 25 minutes should be used as a criterion for optimum therapy rather than dosage of itself.

Three companies, Abbott Laboratories, Serono and Sterling-Winthrop, filed Investigational New Drug (IND) forms with the U.S. Food and Drug Administration in the late 1960s. In April of 1978, Abbott Laboratories was licensed to manufacture and sell urokinase under their brand name of Abbokinase. A recent telephone communication to the medical staff of Sterling-Winthrop has indicated that they do not expect New Drug Application approval for their brand of urokinase through next year. Serano has withdrawn their application in the United States. Abbott is currently marketing urokinase in the United States at a cost of $700 per million units. A Japanese company, Green Cross Corporation, has marketed the substance in its own country with 1977 sales reported to be 40 million dollars. These excessively high costs (about $3,500 for a 12 hour treatment of urokinase alone) and intense competition with much cheaper anticoagulants and fibrinolytic agents, e.g., heparin, warfarin, fibrinolysin, streptokinase, streptodornase, coumarin, etc., producing the same relative effect have greatly reduced the potential market for urokinase.

Urokinase is produced by cells in the kidney cortex and can be found in human urine. Its protein structure and amino acid sequence are unknown but the molecular weight is estimated to be about 33,000. It is nonantigenic and can be treated at 60°C for 10 hours to inactivate any contaminating virus present. Urokinase in plasma has been separated by electrophoretic techniques in laboratory quantities. This material is either mixed with or complexed to α_2 macroglobulin. The urokinase producing cells have also been separated by electrophoretic techniques and the resultant separated cells subjected to monolayer and suspension tissue culture where they can survive and produce the enzyme over long periods of time. A more detailed description of the protein extraction and tissue culture technique can be found in the appendix. Commercial production of urokinase from cultured kidney tissue is presently difficult because only a small fraction of the kidney cells, some 5-10 percent, actually produce the enzyme. Production of urokinase from urine is equally difficult, because the product is present in such minuscule quantities that huge volumes of urine must be processed to obtain a usable yield.

4.2.2 Product Evaluation

Once adequate information was obtained on each of the candidate products, they were evaluated with respect to several different parameters using a numerical "value index". The objective of the evaluation was to rank the candidate products with respect to their humanitarian value for the general population while considering the applicability of space processing to their production. The equation for the value index was:

$$VI = \frac{P \cdot SI \cdot IF \cdot C}{T}$$

where VI represented value index, P represented the number of patients treated, SI represented the disease severity index, IF represented the improvement factor anticipated by space processing, C represented the pharmaceutical competitive factor and T represented the number of baseline electrophoresis production systems required for annual production to meet anticipated markets.

The number of patients (P) treated by each of the candidate products is considered to be the best estimate available based on the wide range of conflicting values appearing in the literature. The National Center for Health Statistics at National Institutes of Health indicated that the data needed for this feasibility

study is not compiled by the Center because their charter is primarily to tabulate communicable disease. Discussions with personnel in the marketing groups of pharmaceutical companies reported that the usual numbers of potential patients may vary by as much as ±50 percent from marketing surveys. Their usual source of information for potential sales volume of a new product is a company sponsored survey of several hundred physicians across the country. Because we did not have access to a survey group, our potential patient populations are based on available literature and discussions with personnel of state and national groups identified with the disease (e.g., National Hemophilia Foundation).

An attempt was made to balance the potential patient population with the severity of the disease being treated. If the disease was very severe and the product treated a large number of patients, it would have a higher value index than a mild disease that had a larger patient population. The classification of severity of the diseases to be treated was expected to be obtained from a nationally recognized pathological severity index. The Armed Forces Institute of Pathology indicated there was no nationally recognized scale and this was confirmed by a literature search at the National Library of Medicine. The usual method of evaluating illness is to assign a subjective evaluation of zero through four for an increasing level of illness. This method of classification does not take into consideration the concept of the illness being chronic or acute.

As a result of the deficiencies mentioned above we have created our own severity index (SI) for this feasibility study. The index and the diseases we wished to rank were presented to several local physicians and the Armed Forces Institute of Pathology. In general these physicians agreed within one index point on the severity assigned to each disease. The value assigned to each disease can be found with the candidate products in Section 4.2.1 and Figures 4.2-2 and 4.2-2A. The point value and its related descriptive terminology can be found in Table 4.2-2.

TABLE 4.2-2 DISEASE SEVERITY INDEX

POINT	SEVERITY
0	HEALTHY
1	COSMETIC
2	CHRONIC UNRECOGNIZABLE
3	ACUTE MALAISE
4	CHRONIC MILD
5	CHRONIC SEVERE
6	CHRONIC DEBILITATING
7	ACUTE DEBILITATING
8	CHRONIC PARALYTIC
9	ACUTE PARALYTIC
10	TERMINAL

The unique characteristics of space are expected to lead to new products as well as improve the purity and yield of currently existing products. Improvement in throughput over ground electrophoresis of the latter group of products could increase commercial feasibility. In the value index, the improvement factor (IF) was assigned a value of two if it was a unique product only available commercially as a result of space processing. A value of one was assigned to those potential products in which some improvement in purity, yield or throughput was anticipated over current earth based production techniques. A value of zero was inserted if no improvement was anticipated. This zero value would effectively eliminate unimproved products from consideration.

The competitive factor (C) takes into account the number of current or anticipated pharmaceutical products or medical procedures that would be in competition with the potential space processed pharmaceutical product during the first five years of sales. It was also modified by a value judgement on our part for a possible decrease in the projected market resulting from patient and physician hesitancy to try something "new" when a certain level of "amelioration" was currently provided by an existing treatment. The competitive factor was also modified in the opposite direction when it was anticipated that the potential product would obtain such a vast improvement in treatment that it would become the drug of choice for that particular pathological condition.

The number of baseline electrophoresis production systems (T) required to satisfy the need for a product to meet annual production of anticipated markets was found as follows: the total annual requirement for each potential candidate product was determined by the mathematical product of the number of annual patients for each disease to be treated with the candidate product, the number of doses required for cure or on an annual basis (if a chronic affliction) for each disease and the quantity of product to be prescribed for each dose. This total annual requirement was divided by anticipated annual production of the potential product using space bioprocessing techniques. The annual production was based on an assumption of a space bioprocessing production system consisting of forty continuous flow electrophoresis chambers working twenty four hours per day for 360 days per year. The sample flow rate was equivalent to 0.6 ml/minute through each chamber in the case of proteins and 0.017 ml/minute when cells were being processed. The quotient of the total annual market requirements divided by the annual production capability of an assumed configuration production system presented an approximation of the size of the production facility required by that particular potential product. An extremely high value for this quotient would reduce the feasibility of space processing of that product because of the tremendous logistics involved (ignoring tissue culture). This evaluation of market and production for each product is summarized in Figures 4.2-1 and 4.2-1A.

When the value index was applied to each potential candidate product, numerical values were obtained as seen in Table 4.2-3. (The calculated values were divided by 10,000 and rounded to provide easier understanding.) The higher the value obtained, the more desirable the potential product would be as a candidate. This evaluation of the value index is summarized in Figures 4.2-2 and 4.2-2A.

4.2.3 Product Recommendation and Rationale

While the value index provided a basis for comparing each candidate product's relative potential for space bioprocessing and benefit to the American population, additional subjective criteria had to be considered in a final selection and recommendation of the six model products. These subjective criteria considered whether the product would: a) exercise the analytical model in one or more different modes of operation and, b) provide comparison data between earth and space if available.

TABLE 4.2-3 VALUE INDEX APPLIED TO CANDIDATES

PRODUCT	V.I.
ALPHA-1-ANTITRYPSIN	24
ANTIHEMOPHILIC FACTOR	4
BETA CELLS	750
EPIDERMAL GROWTH FACTOR	18
ERYTHROPOIETIN	1700
GRANULOCYTE STIMULATING FACTOR	6
GROWTH HORMONE	10
IMMUNOGLOBULINS	72
INTERFERON	11
SOMMATOMEDIN	1
TRANSFER FACTOR	8193
UROKINASE	1

Alpha-antitrypsin may some day be used to replace the deficiency levels of this protein in individuals with an inherited antitrypsin deficiency. Such people are known to be more susceptible than others to emphysema. Alpha-antitrypsin is not clinically available today primarily because of the inability to isolate and purify the substance in commercial quantities. Other approaches to releasing alpha-antitrypsin from hepatocytes of people with a circulating deficiency of this protein have not been fruitful to the present time. Enough work has been accomplished on the ground to provide meaningful comparisons of space processing for the analytical model when it is exercised in the electrophoretic separation of protein mode. For these reasons the product has been chosen to exercise the analytical model.

Antihemophilic factor had a low score on the value index primarily because of the relatively small number of patients to be treated. It was considered a major choice for space bioprocessing because of its significant pharmaceutical value and large impact on the American population. To meet the needs of approximately 20,000 people afflicted with hemophilia, the production of AHF consumes almost 5-9 million pints from the 12-13 million pints of blood donated in this country annually. If space processing could double the yield of antihemophilic factor from each pint of blood at the same time as it increased product purity, about 2-4 million people less would be required to donate blood each year. The purified

PRODUCT MARKET AND PR

PRODUCT	- MARKET -				
	DISEASE	NUMBER OF PATIENTS	AVERAGE DOSAGE PER YEAR (UNITS)	TOTAL ANNUAL REQUIREMENTS (UNITS) (1)	EL PROD (ml/
ALPHA-ANTI TRYPSIN	o EMPHYSEMA	100,000	264,000 mg	$2.64\ 10^{10}$	
AHF	o HEMOPHILIA	20,000	25,000	5×10^{8}	
BETA CELLS	o DIABETES	600,000	3×10^{5} CELLS/ DOSE	1.92×10^{11}	
EPIDERMAL GROWTH FACTOR	o BURNS	14,000	.02	8.8×10^{6}	
ERYTHROPOIETIN	o MALFUNCTION-ING KIDNEY	20,000	78,000	1.56×10^{9}	
	o ANEMIA	500,000	1,200	600×10^{6}	
GRANULOCYTE STIMULATING FACTOR	o WOUND HEALING	500,000	1.05×10^{10}	5.25×10^{15}	
GROWTH	o DWARFISM	1,500	300 units	450,000	
	o STRESS ULCERS	100,000	60 mg	6×10^{6} mg	

FIGURE 4.2-1

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CT MARKET AND PRODUCTION SUMMARY

	- PRODUCTION -			
TOTAL ANNUAL QUIREMENTS UNITS) ①	BASELINE ELECTROPHORESIS PRODUCTION CAPACITY (ml/YEAR)(360 DAY) (000,000)	PRODUCT CONCENTRATION $\left(\frac{\text{UNITS}}{\text{ml}}\right)$ ②	PRODUCT PRODUCTION $\left(\frac{\text{UNITS}}{\text{YEAR}}\right)$	NUMBER OF BASELINE E UNITS REQUIRED (① ÷ ②)
$2.64\ 10^{10}$	12.44	3 mg/ml	3.7×10^{7}	7.1×10^{2}
5×10^{8}	12.44	30	3.7×10^{8}	1.34
92×10^{11}	0.311	3.75×10^{6}	1.16×10^{12}	0.2
8.8×10^{6}	12.44	5 mg/ml	6.2×10^{7}	0.14
$.56 \times 10^{9}$	12.44	250	3.1×10^{9}	0.5
500×10^{6}	12.44	250	3.1×10^{9}	0.2
25×10^{15}	12.44	6.8×10^{5} units/ml	4.2×10^{13}	1.25×10^{2}
450,000	12.44	2.0	2.5×10^{7}	0.02
$x\ 10^{6}$ mg	12.44	0.12 mg/ml	1.5×10^{6}	4.0

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PRODUCT MARKET AND PRODUCT

PRODUCT	- MARKET -				
	DISEASE	NUMBER OF PATIENTS	AVERAGE DOSAGE PER YEAR (UNITS)	TOTAL ANNUAL REQUIREMENTS (UNITS) (1)	BASEL ELECTROP PRODUCTION (ml/YEAR) ((000
IMMUNO GLOBULINS	o DEFICIENCY	5,000	59,400 mg	3×10^8	12
	o RUBELLA	45,000	4,500 mg	2×10^8	12
	o RUBEOLA	75,000	500 mg	0.38×10^8	12
	o INFECTIOUS HEPATITIS	60,000	750 mg	0.45×10^8	12
INTERFERON	o HEPATITIS	100,000,000	6.6×10^8	6.6×10^{16}	12
SOMATOMEDIN	o MEAT PRODUCTION	123,000,000	390 mg	4.80×10^{12}	12
TRANSFER FACTOR	o MULTIPLE SCLEROSIS	535,000	10.4	5.56×10^6	12
	o LEPROSY	2,300	30	6.9×10^4	12
	o MELANOMA	14,000	26	3.6×10^5	12
UROKINASE	o DISSEMINATED INTERNAL CLOTTING	1×10^6	4,000,000	4×10^{12}	12

FIGURE 4.2-1A FOLDOUT FRAME

PRODUCT MARKET AND PRODUCTION SUMMARY (CONTINUED)

	- PRODUCTION -			
TOTAL ANNUAL REQUIREMENTS (UNITS) ①	BASELINE ELECTROPHORESIS PRODUCTION CAPACITY (ml/YEAR)(360 DAY) (000,000)	PRODUCT CONCENTRATION $\left(\frac{\text{UNITS}}{\text{ml}}\right)$	PRODUCT PRODUCTION $\left(\frac{\text{UNITS}}{\text{YEAR}}\right)$ ②	NUMBER OF BASELINE E UNITS REQUIRED (① ÷ ②)
3×10^8	12.44	5 mg/ml	6.2×10^7	4.8
2×10^8	12.44	5 mg/ml	6.2×10^7	3.2
0.38×10^8	12.44	5 mg/ml	6.2×10^7	0.6
0.45×10^8	12.44	5 mg/ml	6.2×10^7	0.7
6.6×10^{16}	12.44	5 mg/ml	6.2×10^7	1.06×10^3
4.80×10^{12}	12.44	5 mg/ml	6.2×10^7	7.7×10^4
5.56×10^6	12.44	5 mg/ml	6.2×10^7	0.09
6.9×10^4	12.44	5 mg/ml	6.2×10^7	0.001
3.6×10^5	12.44	5 mg/ml	6.2×10^7	0.006
4×10^{12}	12.44	2,500	3.1×10^{10}	128.6

FOLDOUT FRAME

VALUE INDEX = NUM / PATI / NUMB

VALUE INDEX SUMMARY

PRODUCT	DISEASE	NUMBER OF PATIENTS (A)	SEVERITY INDEX (B)	SPACE IMPROVEMENT FACTOR (C)	COMPE	
					COMPETITIVE PRODUCTS (NUMBER)	COMP PRO (NU
ALPHA-ANTI TRYPSIN	EMPHYSMA	100,000	5	1	1	
AHF	HEMOPHILIA	20,000	6	2	NONE	
BETA CELLS	DIABETES	600,000*	5	2	3-INSULIN DIABINASE ORINASE	N
EPIDERMAL GROWTH FACTOR	BURNS	14,000	9	1	1	
ERYTHRO-POIETIN	o KIDNEY FAILURE	20,000	6	2	NONE	
	o ANEMIA	500,000	4	2	NONE	
GRANULOCYTE STIMULATING FACTOR	WOUND HEALING	500,000	7	2	NONE	N
GROWTH HORMONE	DWARFISM	1,500	5	1	NONE	
	STRESS ULCERS	100,000	5	1	NONE	

FIGURE 4.2-2

* 150,000 NEW CASES ANNUALLY + 20% OF ORIGINAL POPULATION OF DIABETES

BLACK FRAME

$$\text{VALUE INDEX} = \frac{\text{A: NUMBER OF PATIENTS} \times \text{B: SEVERITY INDEX} \times \text{C: IMPROVEMENT FACTORS} \times \text{D: COMPETITIVE FACTOR}}{\text{NUMBER OF BASELINE ELECTROPHORESIS UNITS REQUIRED (E)}}$$

NT (C)	COMPETITION			NUMBER OF BASELINE (E) UNITS REQUIRED (E)	VALUE INDEX (A)(B)(C)(D)/(E)	
	COMPETITIVE PRODUCTS (NUMBER)	COMPETITIVE PRODUCERS (NUMBER)	COMPETITIVE FACTOR $\frac{1}{(1 + a + b)}$ (D)			
	1	3	0.2	710	141	
	NONE	4	0.20	1.34	35,820	
	3-INSULIN DIABINASE ORINASE	NONE	0.25	0.2	7,500,000	
	1	1	0.33	0.14	297,000	
	NONE	1	0.5	0.5	240.000	1.7×10^7
	NONE	1	0.5	0.2	10,000,000	
	NONE	NONE	1	125	56,000	
	NONE	4	0.2	0.02	75,000	1.0×10^5
	NONE	4	0.2	4.0	25,000	

POPULATION OF DIABETES

4-31/32

BEST AVAILABLE FRAME 2

VALUE INDEX SUMMARY (

PRODUCT	DISEASE	NUMBER OF PATIENTS (A)	SEVERITY INDEX (B)	SPACE IMPROVEMENT FACTOR (C)	COMPE	
					COMPETITIVE PRODUCTS (NUMBER) a	COMPET PRODU (NUME
IMMUNO GLOBULINS	o DEFICIENCY	5,000	7	1	2	5
	o RUBELA	45,000	7	1	2	5
	o RUBEOLA	75,000	7	1	2	5
	o INFECTIOUS HEPATITUS	60,000	7	1	NONE	NO
INTERFERON	HEPATITIS	100,000,000	7	1	1	4
SOMATOMEDIN	MEAT PRODUCTION	123,000,000	2*	2	NONE	NO
TRANSFER FACTOR	MULTIPLE SCLEROSIS	535,000	7	2	NONE	NO
	LEPROSY	2,300	6	2	1	1
	MELANOMA	14,000	9	2	NONE	NO
UROKINASE	DISSEMINATED INTERNAL CLOTTING	1×10^6	7	1	3-HEPARIN -UROKINASE -COUMARIN	4

FOLDOUT FRAME

FIGURE 4.2-2A

VALUE INDEX SUMMARY (CONTINUED)

SPACE PROVEMENT FACTOR Ⓒ	COMPETITION			NUMBER OF BASELINE (E) UNITS REQUIRED Ⓔ	VALUE INDEX $\frac{Ⓐ Ⓑ Ⓒ Ⓓ}{Ⓔ}$	
	COMPETITIVE PRODUCTS (NUMBER) a	COMPETITIVE PRODUCERS (NUMBER) b	COMPETITIVE FACTOR $\frac{1}{(1+a+b)}$ Ⓓ			
1	2	5	0.125	4.8	900	7.2×10^5
1	2	5	0.125	3.2	12,300	
1	2	5	0.125	0.6	109,000	
1	NONE	NONE	1.0	0.7	600,000	
1	1	4	0.16	1.06×10^3	110,000	
2	NONE	NONE	1	7.7×10^4	6,400	
2	NONE	NONE	1	0.09	83,220,000	
2	1	1	0.3	0.001	91,100,000,000	
2	NONE	NONE	1	0.006	42,000,000	
1	3-HEPARIN -UROKINASE -COUMARIN	4	0.125	128.6	6,800	

FOLDOUT FRAME

product would minimize, if not eliminate, antigen-antibody reactions with the product providing a longer life span for the afflicted individuals. Government regulatory problems with this product would be considered minimal. Comparison data between ground and space production and purification can readily be obtained. Because the site of cell production of antihemophilic factor is not yet known, this product can only exercise the analytical model in the electrophoretic separation of proteins.

Antihemophilic factor is currently extracted from plasma cryoprecipitate. This same material contains some of the immunoglobulins. Using cryoprecipitate as the intial protein source, both products may be isolated and purified simultaneously using the continuous flow electrophoresis process.

It is evident that the production of beta cells would have a dramatic effect on the American population. Not only would it be used to treat a large number of the population ($\geq$ 2%) it could do so in a way that might result in a permanent cure for the recipient. The regulatory problems with federal agencies could be anticipated to be held to a minimum because the candidate product would be a natural component from the body and should be considered in the same category as a heart or kidney transplant. While it could not provide direct comparisons of cost, production and technical data between earth and space processing because of differing forms of the same pharmaceutical agent, the similarities are enough that valid comparisons could still be obtained. Beta cell production would exercise the analytical model in the mode of processing cells alone; i.e., electrophoretic separation of cells from extraneous pancreatic cells, their tissue culture and eventual separation of the cultured cells from nutrients and media by a second electrophoretic step.

Epidermal growth factor has a potential use for both the treatment of burns and as a supplement to tissue culture media replacing a large proportion of animal sera currently required in them. A natural product of the body, it has been purified and is currently being investigated by two pharmaceutical companies for its potential in burn treatment to supplement or replace skin grafting. Comparisons of data between ground and space processing could easily be made with the analytical model exercising it in both the electrophoretic separation of protein mode and in the

electrophoretic separation of submaxillary gland cells, their culture and finally the electrophoretic separation of the product from the culture media.

While erythropoietin is commercially available in minute quantities for laboratory research, it has not succumbed to commercial techniques for large scale production as a human pharmaceutical product. When available it would be of significant pharmaceutical value for a number of pathological conditions and show a large impact on the American population. Comparison data could be drawn between space and ground production. As with the other products reviewed in this study, being a natural product of the human body will minimize government regulatory activity. Erythropoietin can exercise the analytical model in two different modes: a) free flow electrophoretic separation of partially purified erythropoietin and, 2) electrophoretic separation of kidney cells producing erythropoietin, tissue culture of these cells and either return to earth of the cells for transplantation, or rupturing the cell membrane and continuous flow electrophoresis of erythropoietin from among the other cell constituents. Erythropoietin is a good model product for exercising the tissue culture process.

Granulocyte stimulating factor was another product considered for use in exercising our analytical model of space bioprocessing. While there is a potential clinical need for such a product, research related to its biological activity and clinical usefulness have not been given high priority status within the pharmaceutical production community. Not enough data is available to use the product to exercise our model for a comparison of ground versus space production of granulocyte stimulating factor.

Growth hormone, for its typical use, would ordinarily not be considered a life saving drug and certainly would not have a large impact on the population. When used in the treatment of stress ulcers, however, this product could be as important as beta cells. This latter treatment is still in the experimental stage but will require large quantities of the hormone for testing. Growth hormone is currently being marketed by one company and FDA approval for marketing by a second company is anticipated during the Spring of 1979. Therefore, regulatory problems would be greatly reduced if not eliminated. Because of current purity levels, growth hormone could be improved by space separation. Comparison data could

readily be obtained between ground and space conditions. Growth hormone could exercise the analytical model in two modes, a) electrophoretic separation of the anterior pituitary cells producing growth hormone and, b) electrophoretic separation of the cells, their tissue culture and continuous flow electrophoresis of protein hormone. Growth hormone would be a good candidate product, however erythropoietin has a higher value index for a product exercising the system in the tissue culture mode.

Immunoglobulins had a relatively low value index as the result of the large number of production systems required to meet market demands and the existing competition for this product. This annual market is transitory however. These immunoglobulins are primarily used to offer passive immunity to individuals who have been exposed to contagious disease that could reach epidemic proportions. In many cases these products are produced, stored and eventually destroyed when their expiration date is exceeded because the need for large scale immunizations does not materialize. The annual critical need for these products is not envisioned at this time. Sufficient isolation and purification of the various immunoglobulins may also be accomplished as by-products in the space processing of plasma cryoprecipitate to obtain antihemophilic factor, such that the market for routine individual immunizations may be satisfied. The immunoglobulins are currently extracted from human plasma in impure form and would offer comparison data between earth and space processing. Current ground based production is favorable for reducing any government regulatory problems. The immunoglobulins would exercise the analytical model in the mode of electrophoretic separation of proteins alone.

Interferon is probably the most investigated pharmaceutical product in the world today with about one hundred companies examining its pharmacological potential and production problems. The Food and Drug Administration has issued a number of Investigational New Drug Licenses to explore the clinical efficacy of interferon in a variety of diseases ranging from cancer to virus infections. Pharmaceutical companies contacted in this study have indicated that even if space processing could only purify a small quantity of the interferon, it would give them the start for characterizing the substance. This could lead to antibody production for diagnostic purposes and possibly the ground production of interferon through recombinant DNA techniques. The major stumbling block for production of interferon is the

tremendous quantity of cells that must be grown in tissue culture to provide a commercial product. Better than half of these cells are killed by the challenging virus required to produce the interferon. The surviving cells are quiescent for several days after the viral challenge, being unable to reproduce themselves as well as synthesize interferon. The logistics problems currently anticipated for cell culture and production of the product in space do not make this an ideal candidate for our analytical model in the cell culture mode. It is an excellent example, however, for the electrophoretic separation of interferon from contaminating products. Interferon could provide direct comparison of cost, production and technical data between earth and space processing.

The United States slaughters approximately 123,000,000 cattle, pigs and sheep annually to supply the protein nutritional requirements of its population. Pharmaceutical companies working in the veterinary market are interested in finding a method for animals to extract a greater amount of nutrient from fodder, thus ultimately improving the movement of calories through the food chain. Somatomedin is one such product considered to increase the absorption of nutrients from fodder to raise the weight of the animals. In view of the fact that very little information is known about the dosage and treatment regimen required for a given animal weight increase as well as the fact that this product does not have the highest priority for utilization within the next twenty years, it was not considered a good example for this analysis.

Preliminary clinical trials indicate that transfer factor has tremendous pharmacological potential for a number of crippling diseases affecting large portions of the world population. It has been investigated for at least thirty-five years. During this time the product has not been chemically characterized nor has it been determined if it is a class of products rather than single entity. The method of tissue culture production and harvest of the transfer factor for electrophoretic separation could exercise the analytical model in two of its three modes of operation. Unfortunately there is no basis for comparison of ground and space production. Therefore, even though this product had the highest value index of the twelve products considered, it was passed over for products with a greater depth of immediate technical knowledge.

Urokinase started clinical trials on a national scale in 1967. Abbott Laboratories received Food and Drug Administration approval to market this product during the spring of 1978. A second American company participating in these clinical trials has not received approval to market urokinase as of this writing. Japan and Switzerland are currently marketing this product overseas. Market penetration would be difficult for any new version of this product unless it was cheaper and/or purer. Intravascular blood clotting does affect a large portion of the American population when they are treated for other physiological insults, i.e., broken bones, surgery, cardiac infarcts or strokes. Urokinase would be an additional, slightly improved pharmaceutical product in the field competing with established leaders like streptokinase and steptodornase as fibrinolytic agents and heparin as an anticoagulant to limit clot growth. Some comparison data between ground and space production and costing could be obtained, but the actual ground costs have not been completely defined. The analytical model would be exercised in the electrophoretic separation of urokinase producing cells, their tissue culture and continuous flow electrophoretic separation of cell constituents including urokinase. Urokinase is not considered to be a good model product for this study.

Based on the above information, the following six products were recommended for further analysis: alpha-antitrypsin, antihemophilic factor, beta cells, erythropoietin, epidermal growth factor, and interferon.

4.2.4 Market Analysis of Selected Products

Preliminary market analyses of the six selected products (alpha-antitrypsin, AHF, beta cells, erythropoietin, epidermal growth factor and interferon) were performed for the primary purpose of determining coarse estimates of the market potential for each product. The derived market size estimates established production rate requirements for use in subsequent space manufacturing plant sizing tasks. A secondary purpose of analyzing each product market was to develop an initial understanding of the market forces and risks involved in achieving a targeted market share for space processed pharmaceutical products. A risk assessment was performed assigning subjective probabilities to each market area subject to risk at appropriate program milestones.

ALPHA-1-ANTITRYPSIN

Alpha-antitrypsin is a protein found in human blood that inhibits the destructive action of an enzyme, trypsin. In people genetically deficient in this protein, the uncontrolled presence of trypsin in the lung can result in tissue damage producing emphysema. People with this deficiency ordinarily have alpha-antitrypsin levels less than 20% of normal control values. The isolation and purification of alpha-antitrypsin through space processing may provide the first pharmacological approach to containing the damage of emphysema. The results of a preliminary market assessment of space processed alpha-antitrypsin are summarized in Figure 4.2-3.

Market Size - There are approximately 800,000 people in the U.S. currently afflicted with emphysema or chronic bronchitis. A steady increase in this patient population has been predicted by the Environmental Protection Agency caused by increasing levels of air pollution. About 100,000 members of this group are conservatively estimated to have a severe alpha-antitrypsin deficiency. This protein product would have its major, and most immediate, effect in these severely deficient patients. Alpha-antitrypsin may also present some therapeutic benefit to persons suffering from osteogenic imperfecta, Marfan's syndrome and cutis laxa. The number of patients in each of these categories is very low, probably less than several thousand.

The average adult receiving alpha-antitrypsin would require an annual dosage of 264 grams of the substance. Using a cost figure of $0.02/mg (one-tenth of the present ground cost of the plant origin material due to mass production in space), this would result in an annual market value of 528 million dollars, if all 100,000 severe patients were treated. This reduced cost for mass production can be justified if the plasma source used for this product can supply many products simultaneously. As a conservative estimate for this analysis, 30,000 patients with a severe alpha-antitrypsin deficiency were selected as the model baseline for annual treatment with space produced material. Figure 4.2-4 shows the projected alpha-antitrypsin market for years 1986-1991 and reflects a 1% per year population growth.

PRELIMINARY MARKET ASSESSMENT
ALPHA-1-ANTITRYPSIN

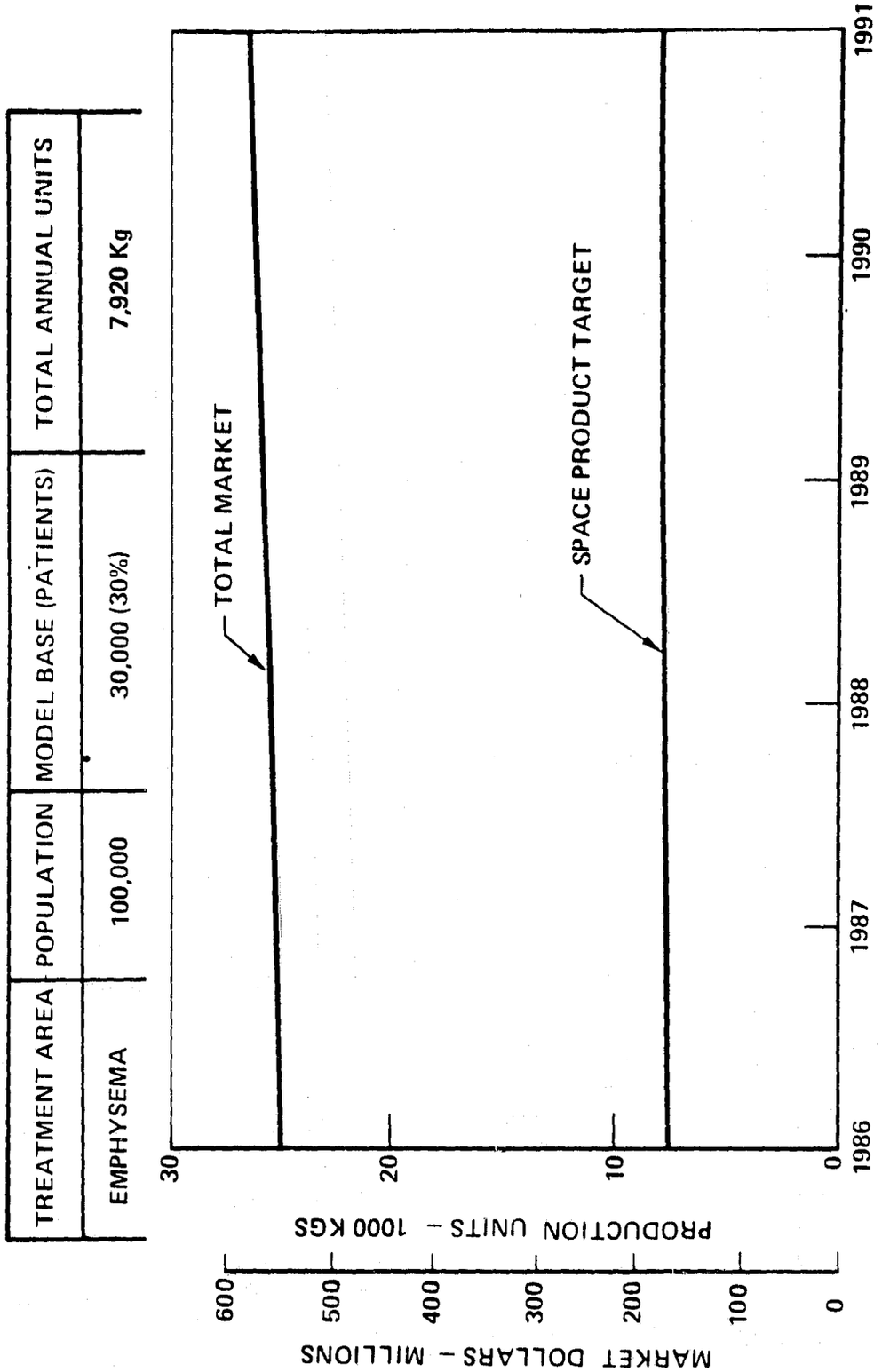
MARKET SIZE	COMPETITIVE PRODUCT	COMPETITIVE PROCESS	MARKET DEVELOPMEMT	REMARKS
o 100,000 WITH SEVERE EMPHYSEMA, 700,000 WITH MILD EMPHYSEMA NOT DIRECTLY REQUIRING SUPPORTIVE THERAPY o 26,400 KG OF A-AT REQUIRED ANNUALLY TO MEET NEEDS OF 100,000 SEVERE CASES o PRODUCT HAS POTENTIAL THERAPEUTIC VALUE FOR OTHER COLLAGEN DISEASES: CUTIS LAXA OSTEOGENESIS IMPERFECTA MARFAN'S SYNDROME BUT PATIENT POPULATION IS SMALL o CURRENT GROUND COST OF PLANT A-AT FOR RESEARCH PURPOSES IS $0.17/MG	o NO EXISTING PRODUCTS FOR TREATMENT OF A-AT DEFICIENCY AND CONTAINMENT OF EMPHYSEMA o LONG TERM RESEARCH PROGRAM TO DEVELOP COMPOUND TO RELEASE A-AT FROM HEPATOCYTES; NO SUCCESS TO DATE o LONG TERM RESEARCH PROGRAM FOR COMPOUNDS SERVING SAME FUNCTION AS A-AT; IN VITRO TESTS OF COMPOUNDS SHOW CYTOTOXICITY o RESULTING PATHOLOGICAL CONDITIONS RULE OUT ROUTINE HUMAN PLASMA TRANSFUSIONS o A-AT EXTRACTED FROM PLANTS NOT SUFFICIENTLY PURE FOR HUMAN USE	o TO DATE, NO GROUND PROCESS HAS SEPARATED A-AT ON COMMERCIAL SCALE FOR CLINICAL PURPOSES o VARIOUS CHROMATOGRAPHY PROCESSES WITH INTERMEDIATE DIALYSIS STEPS ARE TOO COSTLY, TIME CONSUMING, AND LIMITED TO SMALL BATCHES TO MAKE PRODUCT COMMERCIALLY VIABLE o POTENTIAL THREAT WOULD BE GROUND METHOD TO REMOVE AHF, ALBUMIN AND ANTITRYPSIN FROM SAME PLASMA AT A COST SLIGHTLY HIGHER THAN AHF SEPARATION NOW	o NO SPECIFIC TREATMENT NOW EXISTS FOR A-AT DEFICIENCY AND CONTAINMENT OF EMPHYSEMA o SOME PATIENTS WITH A-AT DEFICIENCY DO NOT SHOW CLINICAL SYMPTOMS REQUIRING CAUTION FOR LONG TERM USE OF PREVENTIVE THERAPEUTICS WITHOUT SUFFICIENT DATA o COST OF SPACE PRODUCT MUST BE KEPT REASONABLE	o LARGEST ANNUAL MASS REQUIREMENT OF ALL PRODUCTS ANALYZED

FIGURE 4.2-3

MARKET PROJECTION

(Alpha 1 – Antitrypsin)

TREATMENT AREA	POPULATION	MODEL BASE (PATIENTS)	TOTAL ANNUAL UNITS
EMPHYSEMA	100,000	30,000 (30%)	7,920 Kg



ASSUMPTIONS:

- 1% ANNUAL POPULATION GROWTH
- CONSTANT 1978 $
- UNIT (Kg) COST OF $20,000

FIGURE 4.2-4

Competitive Product - There are no known developed competitive products for the treatment of alpha-antitrypsin deficiency and the treatment and containment of emphysema. Three companies currently provide an alpha-antitrypsin of plant origin for biochemical research purposes. This plant material is not acceptable for clinical investigations because of its potential antigenicity. Two other approaches are currently being investigated that have long term potential competition. In one series of investigations, chemical compounds are being screened for their potential to release alpha-antitrypsin from hepatocytes. To date no compound has worked. In a second series of investigations chemical compounds are being screened for use as synthetic inhibitors of granulocyte elastase and trypsin. These in vitro tests are toxic to cells.

Competitive Process - While no large scale commercial ground processes capable of separating and purifying alpha-antitrypsin have been developed, by the late 1980s competitive processes may be available. If a method is developed to extract the alpha-antitrypsin from human plasma at the same time as albumin and/or antihemophilic factor is also being isolated, it will pose a competitive threat. The extent of this threat will depend on the purity of the isolated product. The various chromatography processes are not as serious a competitive threat because of the long intermediate dialysis times involved.

Market Development - No specific treatment now exists for a severe alpha-antitrypsin deficiency. The availability of a treatment for this disease would immediately open a market for alpha-antitrypsin. The market will be slow to develop, however, because some people with this protein deficiency do not show clinical symptoms of emphysema. Physicians and public health officials will be very cautious in the long term use of a prophylactic treatment for a disease that may probably develop. As sufficient data is collected demonstrating the safety of alpha-antitrypsin, its use as a therapeutic agent should continue to grow. The cost of the space produced material must be kept within the reach of potential users.

Risk Assessment - An analysis was made of the market risks associated with attaining the market share objective. This was accomplished by assigning a subjective probability of success (P_S) to the three market risk elements (competitive product,

competitive process, and market development) at four program milestones (today, completion of ground experimentation, completion of flight experimentation, and completion of the pilot plant demonstration). The results of this assessment are shown in Figure 4.2-5. At each milestone, the product of the P_S for each market risk element defines the overall chances of market success. This assessment shows that the chance of market success increases from one out of four today to eight out of ten following pilot plant demonstration.

ANTIHEMOPHILIC FACTOR (AHF)

AHF, or Factor VIII, is one of the 13 factors which act together in performing the blood clotting function. People whose bodies produce inadequate amounts of any one of these factors, hemophiliacs, have blood which does not clot properly. A significant fraction of these hemophiliacs have blood which will not clot at all unless they receive periodic treatment with large amounts of AHF. However, there are problems with this treatment regimen which include:

- Extremely high cost - patients with moderate hemophilia on home-care treatment may accumulate bills of $10,000 to $20,000 per year.
- Immunological reactions - approximately 10% of the hemophiliac population develop antibodies to current commercial AHF preparations.
- Large blood collection requirements - partially due to the relatively poor yield of existing AHF extraction processes.

The increased purity postulated for space processed AHF may eliminate the immunological reaction-producing impurities contained within existing preparations of AHF. This, therefore, should be an important element of space produced AHF market demand. In addition, because less blood plasma is required to process AHF in space, greater care in the selection of plasmaphoresis donors can decrease the probability of hepatitis virus contamination of the end product.

The results of a preliminary market assessment of space produced AHF are summarized in Figure 4.2-6. Market size information is presented as are principal market elements subject to risk. These include competitive products and processes and factors which may influence the rate of space product market growth.

MARKET RISK ASSESSMENT
ALPHA-1-ANTITRYPSIN

MARKET RISK

PROGRAM MILESTONES	COMPETITIVE PRODUCT P_S	X	COMPETITIVE PROCESS P_S	X	MARKET DEVELOPMENT P_S	= P_S EACH MILESTONE
SIGNIFICANT EVENTS IN DEVELOPMENT OF SPACE MANUFACTURING CAPABILITY	PROBABILITY OF SUCCESSFULLY COMPETING WITH OTHER PRODUCTS FOR MARKET SHARE		PROBABILITY OF SUCCESSFULLY COMPETING WITH GROUND PROCESS FOR MARKET SHARE		PROBABILITY OF DIFFERENTIATING THE SPACE PRODUCT AND SUCCESSFULLY DEVELOPING MARKET SHARE	THE PROBABILITY OF CAPTURING 30% OF THE 1986 MARKET
TODAY (1978)	0.75		0.50		0.70	0.26
COMPLETION OF GROUND TESTS (1982)	0.85		0.53		0.75	0.32
COMPLETION OF FLIGHT EXPERIMENTATION (1984)	0.93		0.88		0.83	0.68
COMPLETION OF FLIGHT DEMONSTRATION (1985)	0.95		0.90		0.95	0.81

FIGURE 4.2-5

PRELIMINARY MARKET ASSESSMENT
ANTIHEMOPHILIC FACTOR VIII

MARKET SIZE	COMPETITIVE PRODUCT	COMPETITIVE PROCESS	MARKET DEVELOPMENT	REMARKS
o CONSIDERABLE DISPARITY IN PUBLISHED MARKET SIZE ESTIMATES (HEMOPHILIA) - PREVALENCE RATES PER 10,000 MALES) QUOTED IN LITERATURE VARY CONSIDERABLY (1 TO 2.5) - DIFFERENT DOSAGE ASSUMPTIONS HAVE BEEN USED o A REASONABLE ESTIMATE FOR DOMESTIC AHF MARKET IS 500M UNITS o AHF PRICE/UNIT: - AMERICAN RED CROSS COST TO PATIENT 11¢ - HOSPITAL CHARGE (INCLUDING HEPATITIS TEST ≈ 17¢ - 1980 ESTIMATE ≈ 11.3¢ (1977 BOOZ, ALLEN & HAMILTON REPORT o ASSUME MARKET GROWS AT U.S. GROWTH RATE (1%/YEAR) o 1986 SPACE MARKET PROJECTION: - 30% AHF MARKET - 150 x 10^6 UNITS/YR	o NO SUBSTITUTE FOR AHF IS KNOWN o A POTENTIALLY COMPETITIVE PRODUCT WOULD BE THE IMPLANTING OF A TISSUE CAPABLE OF SYNTHESIZING AHF INTO A HEMOPHILIC. HOWEVER, TO DATE, NO AHF PRODUCING TISSUE CANDIDATES HAVE BEEN IDENTIFIED.	o MODIFIED FRACTIONATION o NEW (UNDEVELOPED) PROCESSES MIGHT ACHIEVE PURITY INCREASE RELATIVE TO COHN, BUT IT IS UNLIKELY THAT PURITY LEVELS POSTULATED FOR SPACE PRODUCED AHF WOULD BE DUPLICATED. o LONG TERM USAGE OF EXISTING AND NEW GROUND PROCESSES FOR MANUFACTURING AHF MAY ULTIMATELY RESULT IN ANTIGENIC REACTIONS BECAUSE OF IMPURITIES. o GROUND ELECTROPHORESIS MAY BE VIABLE COMPETITOR.	o FEATURES WHICH MAY PERMIT RAPID MARKET SHARE CAPTURE ARE: - GREATER PURITY WHICH ELIMINATES THE IMMUNOLOGICAL REACTIONS FROM GROUND PRODUCED AHF. o REACTIONS FROM COMPETITORS (e.g., PRICE CUTTING) COULD IMPEDE MARKET GROWTH FOR SPACE PRODUCT IN GENERAL AHF MARKET.	o YIELD INCREASES POSTULATED FOR SPACE PRODUCED AHF SHOULD BE CAUSE FOR AMERICAN RED CROSS AND NIH TO SPONSOR AND/OR PUBLICIZE BENEFITS

FIGURE 4.2-6

Market Size - An assessment was made of the potential AHF demand for the treatment of hemophilia. A review of the literature identified a considerable disparity in the reported prevalence rate of hemophilia. The reported prevalence rate varies from about 1(6) to 2.58(7) per 10,000 males in the United States. To establish a baseline, a prevalence rate of 1.5 per 10,000 males was selected because it is conservatively low. This selected rate results in an estimated 1986 annual market potential of about 500 million units of AHF when using the average annual AHF requirement of 31,200 units per hemophiliac as defined in reference (6). This correlates well with the projections in the Booz-Allen Report (7).

Due primarily to projected purity advantages, the space processed AHF should readily capture the AHF market segment made up of immunologically sensitive hemophiliacs. This assumes the purified space product minimizes or eliminates antibody reactions to contaminating substances in conventionally ground processed AHF and would thereby be readily prescribed. The space processed material may have to be priced higher than competitive products in order to absorb high transportation costs. Additionally, the annual supply of cryoprecipitate (which would be the raw material) is projected to be 3000 kg (7), which would contain only 30% of the required AHF. There is enough plasma collected however, to supply the necessary 10,000 kg of cryoprecipitate if that became necessary. Therefore, at best, the market share for space processed AHF probably would not exceed 30% of the total market.

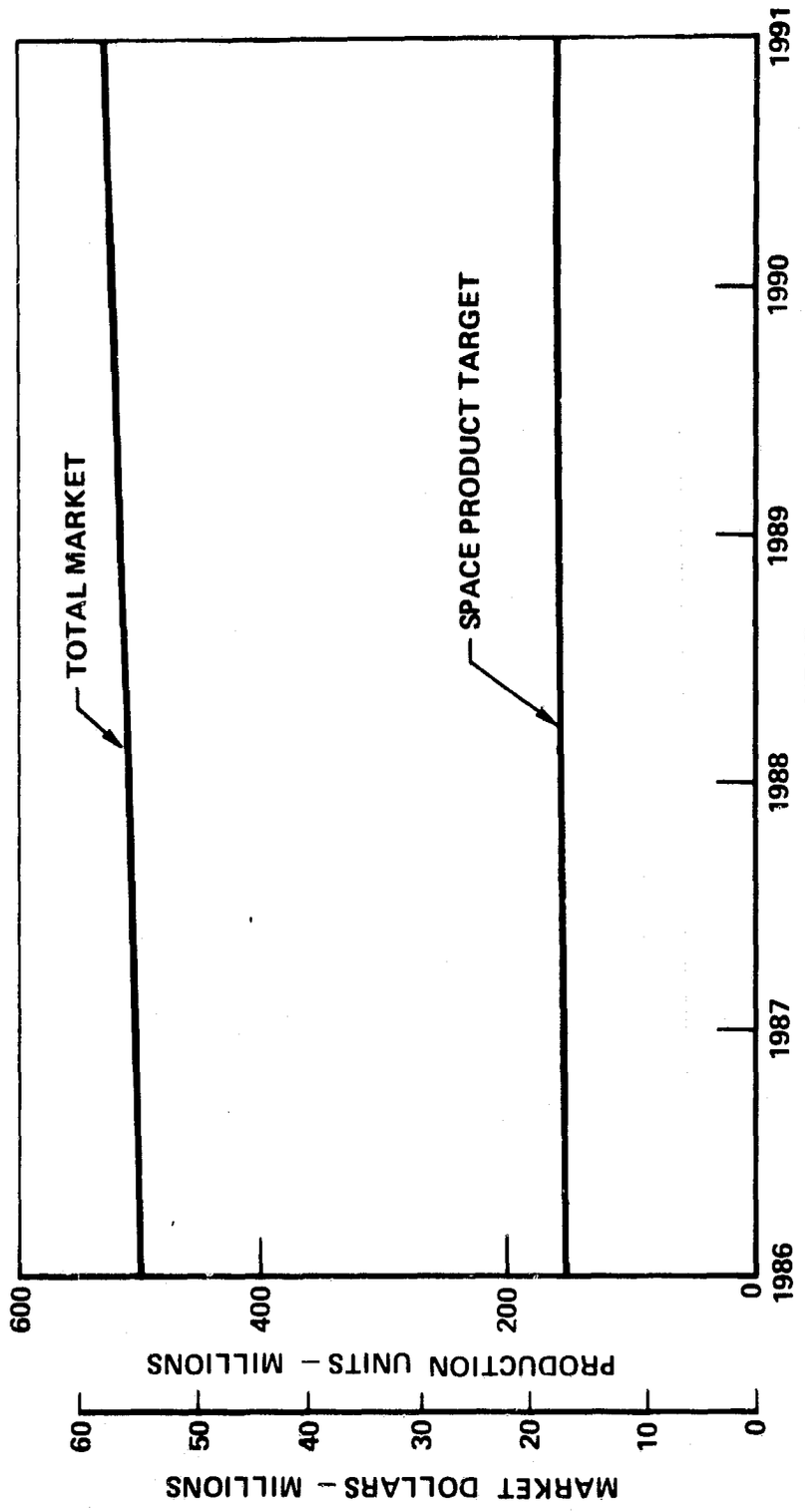
The AHF price per unit to the patient is currently 11 cents per unit (10) and is expected to increase by 1980 because of increasing plasma costs (7). Using this current 1978 price of 11 cents per unit results in a potential of $16.5 million for space processed AHF in today's terms. Extrapolating this potential to the late 1980s yields an annual market exceeding $17.9 million. Figure 4.2-7 presents market projection.

Competitive Product - There are no known developed competitive products to AHF. There are however, two extremely long range potential competitors. One is the possible implantation of cells capable of synthesizing AHF if this production source can be found. Another is the synthesizing of AHF itself after an amino acid sequence has been determined. Neither process is considered a deterrent to proceeding with space bioprocessing of AHF over the next 10-15 years.

MARKET PROJECTION

(Antihemophilic Factor)

TREATMENT AREA	POPULATION	MODEL BASE (PATIENTS)	TOTAL ANNUAL UNITS
HEMOPHILIA A	15,000	5,000 (30%)	1.5×10^8



ASSUMPTIONS:

- 1% ANNUAL POPULATION GROWTH
- CONSTANT 1978 $
- UNIT COST OF 11¢

FIGURE 4.2-7

Competitive Process - A rapid rate of market development will hinge on the ability to effectively exploit the purity advantages of the space processed material. Obviously, those hemophiliacs with developed immunological reactions to other preparations will become immediate users of the space product. For other hemophiliacs, demand for the space product will be sensitive to price relative to the purity. Some price cutting by existing AHF producers can be anticipated thereby affecting the rate of space product market growth. Assuming both the yield increases and by-products of the space process result in a cost advantage, then competitor reaction should only have a short term effect.

Risk Assessment - An analysis was made of the market risks associated with attaining the market share objective. This was accomplished by assigning a subjective probability of success (P_S) to the three market risk elements (competitive product, competitive process, and market development) at four program milestones (today, completion of ground experimentation, completion of flight experimentation, and completion of the pilot plant demonstration). The results of this assessment are shown in Figure 4.2-8. At each milestone, the product of the P_S for each market risk element defines the overall chances of market success. This assessment shows that the chance of market success increases from 11 out of 20 today to 19 out of 20 following pilot plant demonstration.

PANCREATIC BETA CELLS

Implantation of beta cells, processed and purified in space, may provide a long-term remedy, and possibly a cure for juvenile onset diabetes mellitus. The implanted beta cells produce the hormone, insulin, that is responsible for lowering glucose in the blood by promoting its absorption into the cells for metabolic activity. Elimination of the need for the current daily multiple insulin injections and the necessary patient handling of the drug should provide an incentive to purchase an alternative treatment regimen consisting of a single implantation injection which provides years and perhaps a lifetime of glucose control.

The results of a preliminary market assessment of space produced beta cells are summarized in Figure 4.2-9.

MARKET RISK ASSESSMENT
ANTIHEMOPHILIC FACTOR VIII

MARKET RISK

PROGRAM MILESTONES	COMPETITIVE PRODUCT P_S	X	COMPETITIVE PROCESS P_S	X	MARKET DEVELOPMENT P_S	= P_S EACH MILESTONE
SIGNIFICANT EVENTS IN DEVELOPMENT OF SPACE MANUFACTURING CAPABILITY	PROBABILITY OF SUCCESSFULLY COMPETING WITH OTHER PRODUCTS FOR MARKET SHARE		PROBABILITY OF SUCCESSFULLY COMPETING WITH GROUND PROCESS FOR MARKET SHARE		PROBABILITY OF DIFFERENTIATING THE SPACE PRODUCT AND SUCCESSFULLY DEVELOPING MARKET SHARE	THE PROBABILITY OF CAPTURING 30% OF THE 1986 MARKET
TODAY (1978)	0.85		0.72		0.91	0.56
COMPLETION OF GROUND TESTS (1982)	0.95		0.88		0.94	0.79
COMPLETION OF FLIGHT EXPERIMENTATION (1984)	0.97		0.94		0.97	0.88
COMPLETION OF FLIGHT DEMONSTRATION (1985)	0.98		0.99		0.98	0.95

FIGURE 4.2-8

PRELIMINARY MARKET ASSESSMENT
BETA CELLS

MARKET SIZE	COMPETITIVE PRODUCT	COMPETITIVE PROCESS	MARKET DEVELOPMENT	REMARKS
o CONSIDERABLE DISPARITY IN MARKET SIZE ESTIMATES (DIABETES) - 1 1/2 TO 20 MILLION U.S. DIABETICS o 150,000 NEW CASES EACH YEAR o BETA CELL DOSAGE REQUIRED IS APPROXIMATELY 300,000 CELLS o COMPARATIVE DOSAGE OF INSULIN: - 30 TO 80 UNITS OF INSULIN PER DAY - @ $0.0035/UNIT ($0.2/DAY) o MARKET SIZE ASSUMPTIONS - 2 MILLION EXISTING DIABETICS OVER A 5 YEAR PERIOD - 150,000 NEW CASES ANNUALLY - TOTAL 3.2M	o ASSUMING A SINGLE INJECTION FOR BETA CELLS PROVIDES A LIFETIME CURE FOR DIABETES, THEN THERE ARE NO EXISTING PRODUCTS COMPETITIVE WITH BETA CELLS o HOWEVER, THE SINGLE INJECTION CURE WILL COMPETE WITH PRODUCTS PROVIDING SUPPLEMENTAL TREATMENT. - INSULIN (ELDERLY CAN PROBABLY BE ADEQUATELY TREATED WITH DIABINASE) o CONTINUANCE OF THE HIGH (> $100M) GOV'T RESEARCH LEVELS INCREASES THE PROBABILITY OF COMPETITIVE PRODUCT DEVELOPMENT IN THE NEXT 10-15 YEARS	o TO DATE, NO GROUND PROCESS HAS SEPARATED BETA CELLS TO THE LEVEL OF PURITY POSTULATED FOR THE SPACE SEPARATION PROCESS o BIGGEST RISK IS THE GROUND DEVELOPMENT OF A WAY TO SEPARATE/PURIFY A SMALL QUANTITY OF BETA CELLS IN COMBINATION WITH A METHOD WHICH TISSUE CULTURES MARKETABLE QUANTITIES OF USABLE BETA CELLS	o FEATURES WHICH MAY PERMIT RAPID MARKET SHARE CAPTURE ARE: - SINGLE INJECTION WHICH PROVIDES LIFETIME CURE - ELIMINATION OF: ° DIET RESTRICTIONS ° FREQUENT INJECTIONS ° COST OF CONTINUOUS HEALTH CARE ° IMMUNOLOGICAL REACTIONS OF EXISTING PREPARATIONS o FOR CERTAIN OR MILD CASES OF DIABETES, & THE ELDERLY, THE INJECTION OF BETA CELLS MAY NOT BE ATTRACTIVE BECAUSE OF: - COST (SPACE SEPARATED CELLS + DOCTOR + OPERATION RELATED EXPENSES) - APPREHENSION (OF OPERATION)	o HIGH LEVEL OF PRESENT RESEARCH FUNDING ($100M) (GOV'T) WOULD SUPPORT THE BEST METHOD TO CURE DIABETES

FIGURE 4.2-9

Market Size - As previously described in Section 4.2.1, there is considerable disparity concerning the number of persons with insulin deficiencies. While the leading manufacturer of insulin, Lli Lilly and Company, estimates 1.2-3 million people require insulin injection, they also estimate there may be another 10 million with milder forms of glucose metabolism deficiency who are either unidentified diabetics or are being treated with controlled diets and/or synthetic pharmacological agents. A National Center for Health Statistics survey conducted in 1973 (see Volume III, Section A.9) indicates about 13% of the population over age of 17 (approximately 26 million persons) were suffering from some form of diabetes. Today's market for beta cell transplants will not likely exceed those requiring insulin injections, or about 2 million patients. Each year, however, there are an additional 150,000 new patients found with juvenile diabetes, or 1.2 million by 1986. The total market at that time, therefore, is projected to be about 3.2 million patients.

The next step of the analysis estimated a projected value for the space purified beta cells. A person with juvenile onset diabetes will require a daily treatment regimen of 30 to 80 units or more of insulin. At $0.0035/unit, this equates to $0.105 to $0.28 per day in 1978. For a 5-year period, a savings range of $192 to $511 would result from a single implantation of beta cells. A present value analysis of a benefit annuity equivalent to the annual savings for a 5 year period and discounted at 5%, resulted in a value range of $164 to $436 for the single implantation for each diabetic. The value of the entire known diabetic population on insulin medication is probably closer to the lower of the two dollar figures and would total over $525 million. Assuming a discounted benefit period of 25 years, this present value exceeds $1.4 billion.

A program to implant beta cells in all the nation's diabetics might be accomplished over a 5 year period. This appeared to be a reasonable assumption when compared with the joint government-commercial polio vaccination program which virtually eradicated polio within a shorter, three year period. The space produced beta cell market would then approximate 630,000 patients per year for a five year period. Assuming a lifetime cure results, implantations thereafter would be a function of new cases and would be based upon incidence of diabetes - pancreatic deficiency in

the newborn and aging population (150,000/yr). Figure 4.2-10 shows the projected beta cell market for the years 1986-1991 and reflects a 1% per year population growth. An average of 630,000 patients are treated annually with a corresponding discounted market value exceeding $100 million.

Competitive Product - There are no known competitive products under development which provide a multiyear control of insulin deficiency. As previously discussed, insulin deficiences can be offset by stringent diet control, insulin injections and sometimes other drugs. However, these remedies all involve significant measures of risk, inconvenience, and side effects not found in beta cells which naturally and automatically control insulin levels. Although significant improvements can be anticipated for insulin, including commercially scaled synthesization, it is unlikely that a multi-year treatment competitor to beta cells will be developed before 1986.

Competitive Process - Alternative ground methods for processing purified beta cells may be developed before space processed material is ready for the market place. Ground processing of beta cells using a continuous flow electrophoresis device in combination with a tissue culture system may pose the greatest threat to the space processed product. However, purity advantages may keep the space product price competitive.

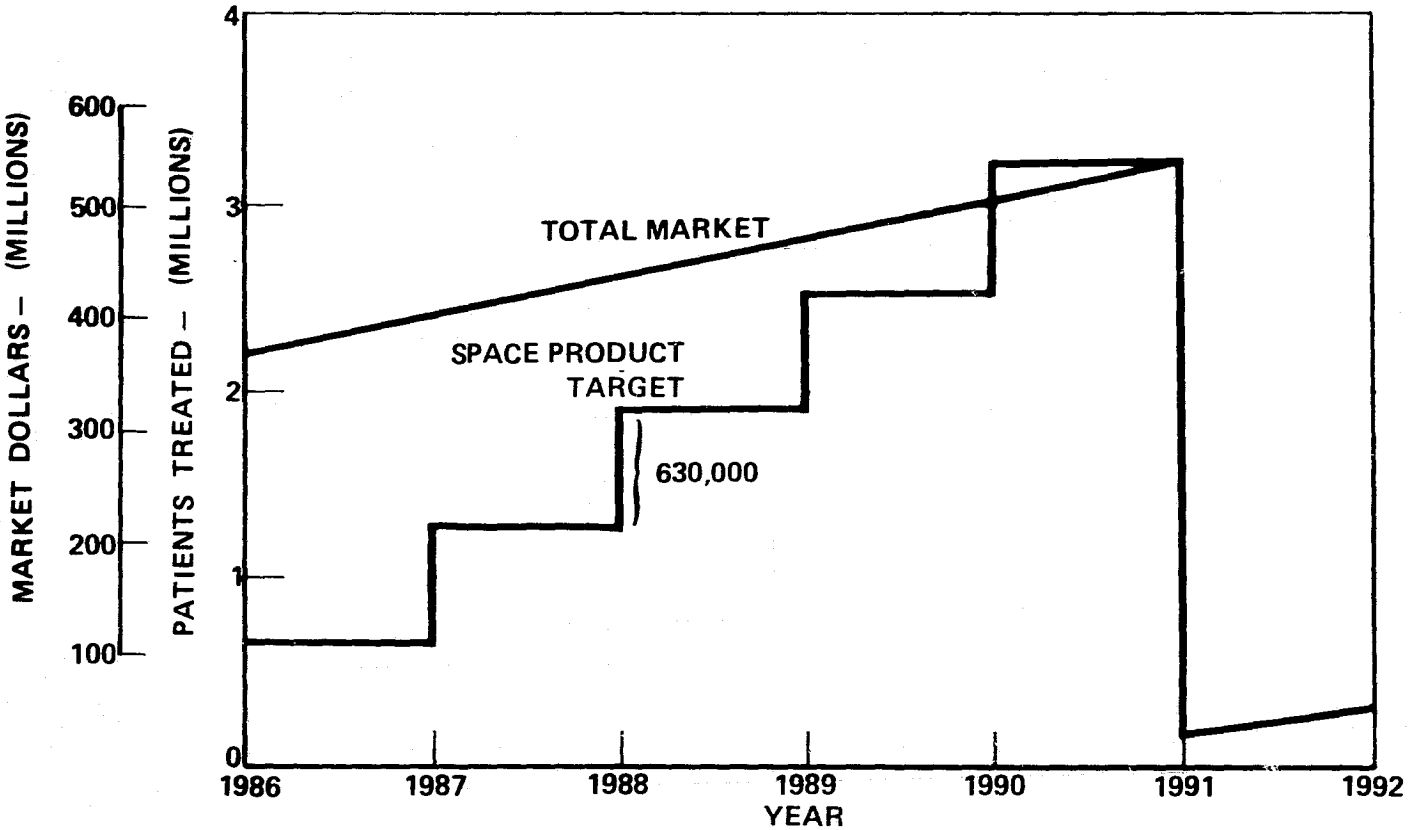
Market Development - With the capability for multi-year, and possibly a lifetime, cure of insulin deficiencies, there should be a good chance of achieving the targeted market for beta cells. The multi-year or lifetime cure will be strong motivators for all diabetics to obtain beta cell implantations. There would also be motivation for the government to sponsor a concurrent testing program to identify people with early signs of glucose metabolism deficiency. Also identified during this test program would be the large number of suspected diabetics who are not now undergoing treatment, yet are counted in the government statistics.

The greatest market threat would probably be from ground production of purified cells which would have similar product characteristics as the space processed beta cells.

MARKET PROJECTION

(Beta Cells, Islets of Langerhans)

TREATMENT AREA	POPULATION	MODEL BASE (PATIENTS)	TOTAL ANNUAL CELLS
DIABETES MELLITUS	3,200,000	630,000 (20%/YR)	1.9×10^{11}



ASSUMPTIONS:

- 1% ANNUAL POPULATION GROWTH
- 150,000 NEW JUVENILE ONSET DIABETICS ANNUALLY
- TREATMENT OF ALL EXISTING JUVENILE ONSET DIABETICS IN FIVE YEARS
- 3×10^5 CELLS/DOSE
- $170/DOSE

FIGURE 4.2-10

Risk Assessment - An analysis was made of the market risks associated with attaining the market objective for space processed beta cells. As previously described for AHF, this was accomplished by assigning subjective probabilities of success (P_S) to the three market risk elements at four program milestones. The results of the analysis are shown in Figure 4.2-11. This assessment shows that the chance of market success increases from four out of ten today to 9 out of 10 following pilot demonstration.

ERYTHROPOIETIN

Erythropoietin is a hormone produced by the kidneys, which controls the red blood cell production in the body. With an average lifespan of just 120 days, red blood cells must be continually produced to offset the loss of dying cells. Failure to maintain an adequate level of red blood cells results in anemia and, if sufficiently severe, eventual death. Major losses of red blood cells can be temporarily made up by blood transfusions. Because of the potential for hepatitis virus infection, however, these transfusions are not used as a continuous treatment regimen. Erythropoietin is not currently used clinically because of potential hazards associated with other protein components contained in the impure extract. If sufficient purification is achieved, as is postulated for space processed erythropoietin, then there would be an immediate extensive market.

The results of a preliminary market assessment of space processed erythropoietin are summarized in Figure 4.2-12.

Market Size - The market potential for space processed erythropoietin is defined by two distinct market areas. These are: 1) anemia due to kidney failure and 2) other forms of anemia responsive to erythropoietin treatment. Of kidney related disorders, kidney failure patients would have the greatest need for the purified erythropoietin and were thereby selected as the major market segment. It was assumed that, as for kidney dialysis, not all kidney failure patients can effectively utilize long term treatment. One third, or about 20,000 kidney failure patients were selected as a baseline. Based upon an average annual dosage of 83,000 units per patient, annual requirements of 1.7 billion units of erythropoietin would be needed. A cursory assessment of the various types of anemia and

MARKET RISK ASSESSMENT
BETA CELLS

MARKET RISK

PROGRAM MILESTONES	COMPETITIVE PRODUCT P_S	X	COMPETITIVE PROCESS P_S	X	MARKET DEVELOPMENT P_S	= P_S EACH MILESTONE
SIGNIFICANT EVENTS IN DEVELOPMENT OF SPACE MANUFACTURING CAPABILITY	PROBABILITY OF SUCCESSFULLY COMPETING WITH OTHER PRODUCTS FOR MARKET SHARE		PROBABILITY OF SUCCESSFULLY COMPETING WITH GROUND PROCESS FOR MARKET SHARE		PROBABILITY OF DIFFERENTIATING THE SPACE PRODUCT AND SUCCESSFULLY DEVELOPING MARKET SHARE	THE PROBABILITY OF CAPTURING 20% OF THE 1986 MARKET
TODAY (1978)	0.72		0.62		0.86	0.38
COMPLETION OF GROUND TESTS (1982)	0.83		0.74		0.94	0.58
COMPLETION OF FLIGHT EXPERIMENTATION (1984)	0.89		0.82		0.97	0.71
COMPLETION OF FLIGHT DEMONSTRATION (1985)	0.95		0.94		0.99	0.88

FIGURE 4.2-11

PRELIMINARY MARKET ASSESSMENT
ERYTHROPOIETIN

MARKET SIZE	COMPETITIVE PRODUCT	COMPETITIVE PROCESS	MARKET DEVELOPMENT	REMARKS
KIDNEY FAILURE (> 55,000/YR) 4.7×10^9 UNITS/YR (1.7×10^9 UNITS/YR, SPACE TARGET)	o KIDNEY TRANSPLANT o SYNTHESIS OF ERYTHROPOIETIN (10 TO 15 YEARS REQUIRED TO DEVELOP, TEST AND MARKET)	o NONE YET DEVELOPED o GREATEST RISK IS DEVELOPMENT OF GROUND PROCESSED ERYTHROPOIETIN.	o PRESENT TREATMENT IS VERY EXPENSIVE & WITHOUT A TRANSPLANT, IS ULTIMATELY TERMINAL. THEREFORE, SHOULD CAPTURE ENTIRE MARKET IN FIRST YEAR. o COST OF SPACE PRODUCT MUST BE KEPT REASONABLE.	
ANEMIA (1×10^6/YR) 2.4×10^9 UNITS/YR (350×10^6 UNITS/YR SPACE TARGET)	o SYNTHESIS OF ERYTHROPOIETIN	SAME AS ABOVE	o HIGH PRICE COULD BE A DETERRENT TO MARKET DEVELOPMENT.	
				o HAS POTENTIAL AS A HIGH ALTITUDE ACLIMATIZATION AGENT FOR MILITARY USE.

FIGURE 4.2-12

their associated patient populations resulted in the identification of 1 million patients per year for this potential market. Probably only 10-20% are severe chronic anemics and would utilize this therapy. With an average annual dosage of 2,400 units per patient, this represents an estimated 360 million units of erythropoietin. The total projected space product market for erythropoietin is estimated to be the sum of these, or 2.06 billion units/year. The space market value of $245 million per year is obtained by placing a value of $10,000, and $300 for the annual per patient costs of erythropoietin for kidney failure, and anemia, respectively. Figure 4.2-13 graphically shows the 1986-1991 market projection for space processed erythropoietin.

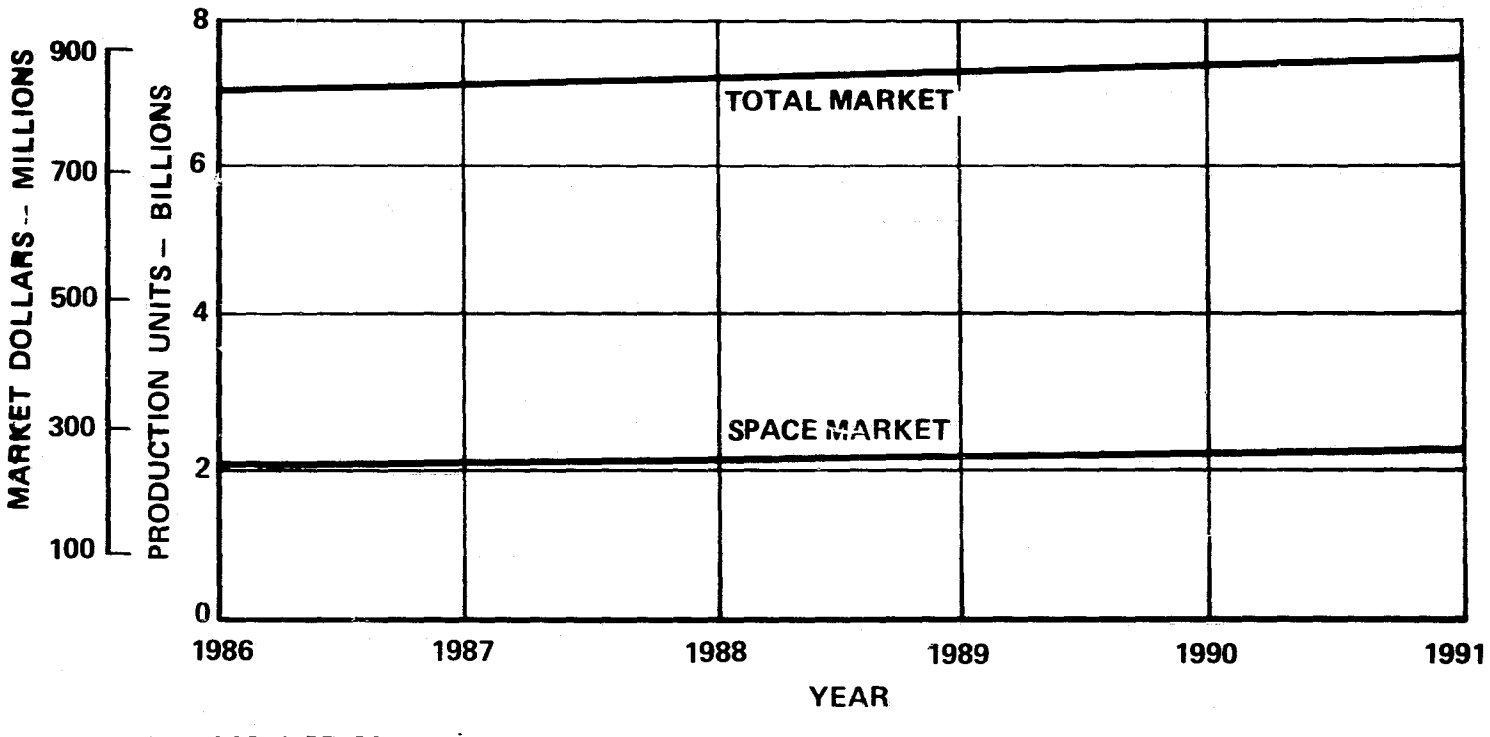
Competitive Product - There are no other known products with red blood cell production stimulation and control capabilities similar to the natural hormone erythropoietin. Since it has not yet been separated and purified sufficiently to determine its chemical structure, there is little risk that erythropoietin can be synthesized, extensive clinical tests completed, and marketed in the next ten years.

Competitive Process - While no large scale commercial ground processes capable of separating and purifying erythropoietin have yet been developed, by the late 1980s competitive processes may be available. Specifically, a ground system capable of purifying and tissue culturing ESF cells, and of separating and purifying the erythropoietin hormone in a fashion similar to the space processing concept may pose the greatest competitive threat.

Market Development - Cost consideration aside, there should be few obstacles preventing the attainment of the space processing erythropoietin market share. Erythropoietin is the natural stimulant and control hormone for red blood cell production and will have virtually no competition for the kidney failure and anemia market segments. Price may be a deterrent for the anemia market segment but those on dialysis may have little alternative. Ground produced erythropoietin of inferior quality may be available during the 1986-1991 marketing period. However, assuming the space product can be priced within reach of the potential users, there should be few constraints to quick market development. The biggest risk of market development is the ability of erythropoietin to be economically produced in space.

MARKET PROJECTION
(Erythropoietin)

TREATMENT AREA	POPULATION	MODEL BASE (PATIENTS)	TOTAL ANNUAL UNITS
KIDNEY FAILURE	55,000	20,000	1.7×10^9
ANEMIA	1,000,000	150,000 (30%)	3.6×10^8



ASSUMPTIONS:
- 1% ANNUAL POPULATION GROWTH
- UNIT COST OF 12¢
- CONSTANT 1978 $

FIGURE 4.2-13

Risk Assessment - An analysis was made of the market risks associated with attaining the market objective for space processed erythropoietin. As previously described for AHF and beta cells, this was accomplished by assigning subjective probabilities of success (P_S) to the three market risk elements at four program milestones. The results of the analysis are shown in Figure 4.2-14. This assessment shows that the chance of market success increases from two out of 10 today, to seven out of ten following pilot plant demonstration.

EPIDERMAL GROWTH FACTOR

Epidermal growth factor is a protein produced by the salivary glands. It has the fundamental property of stimulating the production of ectodermal tissue. In clinical situations where the skin is damaged by burns and wounds of various types, application of epidermal growth factor to the damaged areas could result in their rapid restoration to normal function. This substance also has a tremendous potential for both supplementing and partially replacing fetal animal serums in tissue culture systems. The results of a preliminary market assessment of space produced EGF are summarized in Figure 4.2-15.

Market Size - Discussions with directors of several hospital burn units throughout the country have resulted in a consensus of approximately 14,000 people annually receiving third degree burns over at least five percent of their body. The annual market requirement to treat these patients is assumed to be 8.8×10^6 mg. This is based on each patient receiving a daily treatment of 20 nanograms/cm^2 damaged surface area for a seven day period. The actual treatment regimen has not been published but this dose is known to cause epidermal cells in tissue cultures to proliferate and spread into a fibroblast network (see Volume III, Section A.3). In addition to burn patients, an eventual market may develop for its use in the rapid healing of all skin wounds. A third area of potential commercialization is the use of EGF to supplement a reduction in animal serum requirements in tissue culture. Its use could stretch the current limited supply about five hundred times. Even at the current ground based production cost of $495/mg for EGF, use of 2 nanograms/ml mixed with 0.02% fetal calf serum would tremendously reduce the cost of tissue culture production. It may even be the method of choice for culturing in space in order to reduce to a minimum the costs associated with the

MARKET RISK ASSESSMENT
ERYTHROPOIETIN

MARKET RISK

PROGRAM MILESTONES	COMPETITIVE PRODUCT P_S	X	COMPETITIVE PROCESS P_S	X	MARKET DEVELOPMENT P_S	= P_S EACH MILESTONE
SIGNIFICANT EVENTS IN DEVELOPMENT OF SPACE MANUFACTURING CAPABILITY	PROBABILITY OF SUCCESSFULLY COMPETING WITH OTHER PRODUCTS FOR MARKET SHARE		PROBABILITY OF SUCCESSFULLY COMPETING WITH GROUND PROCESS FOR MARKET SHARE		PROBABILITY OF DIFFERENTIATING THE SPACE PRODUCT AND SUCCESSFULLY DEVELOPING MARKET SHARE	THE PROBABILITY OF CAPTURING 30% OF THE 1986 MARKET
TODAY (1978)	0.82		0.38		0.60	0.19
COMPLETION OF GROUND TESTS (1982)	0.93		0.55		0.71	0.36
COMPLETION OF FLIGHT EXPERIMENTATION (1984)	0.96		0.70		0.78	0.52
COMPLETION OF FLIGHT DEMONSTRATION (1985)	0.99		0.85		0.84	0.71

FIGURE 4.2-14

PRELIMINARY MARKET ASSESSMENT
EPIDERMAL GROWTH FACTOR

MARKET SIZE	COMPETITIVE PRODUCT	COMPETITIVE PROCESS	MARKET DEVELOPMENT	REMARKS
o 14,000 PEOPLE RECEIVE THIRD DEGREE BURNS OVER MORE THAN 5% OF THEIR BODY SURFACE o ANNUAL MARKET REQUIREMENT IS EXPECTED TO BE ABOUT 8.8 x 10^6 mg o CURRENT GROUND COST IS $495/mg o PRODUCT HAS COMMERCIAL POTENTIAL AS A SUPPLEMENT TO PARTIALLY REPLACE FETAL CALF SERUM IN TISSUE CULTURE	o NO PHARMACOLOGICAL COMPETITION FOR TREATMENT OF BURNS o CURRENT TECHNIQUE IS TO USE SKIN GRAFTS OR WET STERILE WRAPS	o CURRENT RESEARCH QUANTITY PRODUCTION IS DONE BY VARIOUS CHROMATOGRAPHIC TECHNIQUES o ALREADY PURIFIED EGF MAY BE USED TO INDUCE LARGE SCALE PRODUCTION BY RECOMBINANT DNA TECHNIQUES	o BURN TREATMENT WITH EGF IS STILL IN RESEARCH STAGE o CLINICIANS WANT A METHOD FOR RAPIDLY COVERING AND RESTORING BURN DAMAGED SKIN	o PRODUCT HAS COMMERCIAL AS WELL AS CLINICAL SIGNIFICANCE

FIGURE 4.2-15

transportation of supplies. This analysis used a space produced market cost of $50/mg based on anticipated production competition once large commercial quantities became available for use. Figure 4.2-16 shows the projected EGF market for the years 1986-1991 and reflects a 1% per year population growth.

Competitive Product - The current practice for treating extensively burned patients is to cover the damaged areas with skin grafts, when available, or wet biocidal wraps. Biochemical means for inducing epidermal cell proliferation are not being used commercially at this time although two companies have already started clinical trials. If these trials show positive treatment advantages, EGF would be almost without competition in this field.

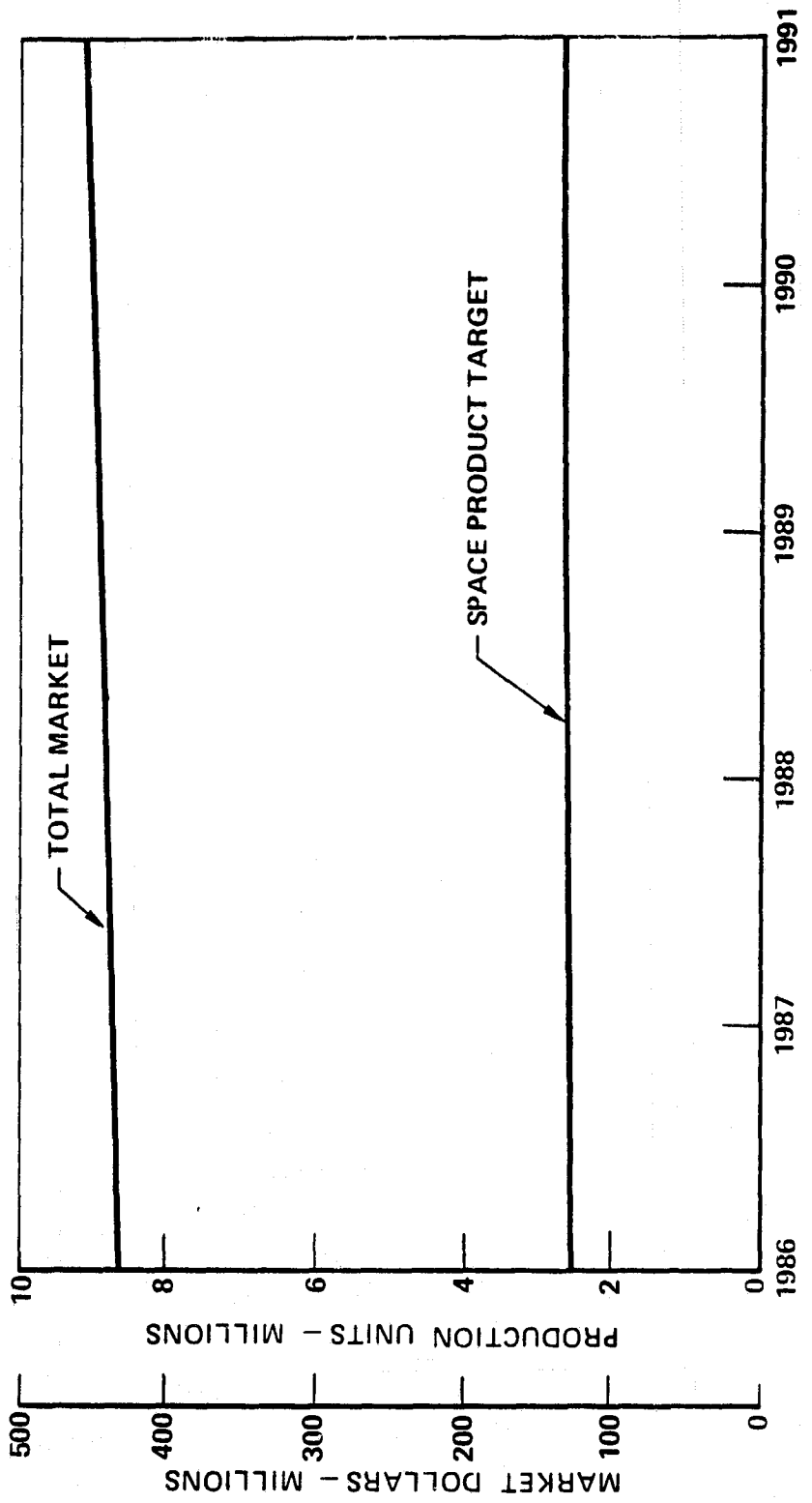
Competitive Process - Research quantities of EGF are currently isolated and purified by various exclusion and affinity chromatography techniques. They are costly and time consuming, usually taking about 5-10 days to produce 19 mg of the product. Processing the strained salivary gland extract or concentrated urine through a continuous flow electrophoresis device could considerably lower both the cost and time required for production. A major competitive threat to processing the material in space could come from the use of recombinant DNA techniques where genetically modified bacteria might produce the material in commercial quantities. Even though EGF has been biochemically characterized and its amino acid sequence determined, many researchers in this field as well as pharmaceutical companies contacted do not anticipate this process being a competitive threat for at least ten years.

Risk Assessment - An analysis was made of the market risks associated with attaining the market share objective for space processing. This was accomplished by assigning a subjective probability of success (P_S) to the three market risk elements (competitive product, competitive process and market development) at the four program milestones used with the other products analyzed. The results of this assessment are shown in Figure 4.2-17. This assessment indicates that the chance of market success for epidermal growth factor increases from slightly more than three out of ten today to seventeen out of twenty following pilot plant production.

MARKET PROJECTION

(Epidermal Growth Factor)

TREATMENT AREA	POPULATION	MODEL BASE (PATIENTS)	TOTAL ANNUAL UNITS
BURNS	14,000	4,200 (30%)	2.64×10^6 mg



ASSUMPTIONS:

- 1% ANNUAL POPULATION GROWTH
- CONSTANT 1978 $
- UNIT COST OF $50

FIGURE 4.2-16

2-2

MARKET RISK ASSESSMENT
EPIDERMAL GROWTH FACTOR

PROGRAM MILESTONES	COMPETITIVE PRODUCT	P_S X	COMPETITIVE PROCESS	P_S X	MARKET DEVELOPMENT	P_S = P_S	EACH MILESTONE
SIGNIFICANT EVENTS IN DEVELOPMENT OF SPACE MANUFACTURING CAPABILITY	PROBABILITY OF SUCCESSFULLY COMPETING WITH OTHER PRODUCTS FOR MARKET SHARE		PROBABILITY OF SUCCESSFULLY COMPETING WITH GROUND PROCESS FOR MARKET SHARE		PROBABILITY OF DIFFERENTIATING THE SPACE PRODUCT AND SUCCESSFULLY DEVELOPING MARKET SHARE		THE PROBABILITY OF CAPTURING 30% OF THE 1986 MARKET
TODAY (1978)	0.90		0.60		0.60		0.32
COMPLETION OF GROUND TESTS (1982)	0.93		0.75		0.85		0.59
COMPLETION OF FLIGHT EXPERIMENTATION (1984)	0.95		0.95		0.88		0.79
COMPLETION OF FLIGHT DEMONSTRATION (1985)	0.95		0.99		0.90		0.85

FIGURE 4.2-17

INTERFERON

Interferon is a protein substance produced by lymphocytes and fibroblasts challenged by a virus. This interferon offers temporary immunity to other cells against most challenge viruses when subsequently attacked. Preliminary human clinical trials indicate this natural body product has great therapeutic potential against many diseases of viral or suspected, but not proven, viral origin. It is probably one of the most sought after candidate products today with about one hundred pharmaceutical companies and universities actively conducting research and development related to its potential therapeutic commercialization. The results of a preliminary market assessment of space produced interferon are summarized in Figure 4.2-18.

Market Size - As reported in Section 4.2.1 and Volume III, Section A.7 dealing with interferon, this product may be a modern day therapeutic panacea. It has shown some clinical improvement or total remission in a number of diseases from cancer to the common cold. It is very difficult to estimate the number of patients that could benefit from this drug and its annual market value because the clinical trials have been small, therapeutic doses have not been firmly established and only a few of the potentially responding diseases have been tested. To simplify this analysis, only one disease, affecting a very large population, has been used for the calculations. If the patient population and annual dosage are in error by even twenty percent, the numbers used in the evaluation could readily be filled in with a patient load and annual dosage for another responding pathological condition. For this analysis infectious hepatitis was chosen because it is estimated to affect approximately 100 million people worldwide. To treat this large a population would require 6.6×10^{15} units annually. While the current ground production cost is fifty to seventy dollars per million units, we have reduced the cost to a conservative one dollar per million units. Even at this lower value the total annual world market for interferon to treat this disease alone could be 66 billion dollars. The one dollar per million units was chosen because it was felt both the quantity of the material needed annually and the tremendous industrial competition for a share of this huge market would drive the price close to this limiting level. A similar condition was seen in the expansion of the penicillin market after its discovery and introduction into the medical armamentarium.

PRELIMINARY MARKET ASSESSMENT
INTERFERON

MARKET SIZE	COMPETITIVE PRODUCT	COMPETITIVE PROCESS	MARKET DEVELOPMENT	REMARKS
o APPEARS TO BE MODERN DAY PANACEA FOR ALMOST ALL DISEASES OF VIRAL ORIGIN. DISEASES RANGE FROM CANCER TO COMMON COLD. o 6.6×10^{16} UNITS REQUIRED ANNUALLY TO TREAT 1×10^{8} HEPATITIS PATIENTS ALONE. o CURRENT GROUND COST IS $50/MILLION UNITS	o MANY COMPETITIVE PRODUCTS ON MARKET ALREADY BUT NOT ALWAYS EFFECTIVE o CONTINUANCE OF HIGH GOVERNMENT AND INDUSTRY RESEARCH LEVELS INCREASE PROBABILITY OF COMPETITIVE PRODUCT DEVELOPMENT IN THE NEXT 10-15 YEARS	o MADE FROM THREE DIFFERENT CELL LINES o LARGE SCALE CELLULAR BIOSYNTHESIS STILL BIGGEST PRODUCTION PROBLEM o RESEARCH CONTINUING FOR SUPER INDUCERS OF CULTURES o RESEARCH CONTINUING FOR CELL FREE INTERFERON SYNTHESIS o ALL ABOVE PROCESSES REQUIRE PURIFICATION OF FINAL PRODUCT o ABOUT 100 COMPANIES ARE PRODUCING INTERFERON FOR RESEARCH AND DEVELOPMENT PURPOSES	o PRESENT THERAPEUTIC TREATMENT FOR MANY OF THE ASSOCIATED DISEASES IS INEFFECTIVE o CLINICAL USE IS STILL IN EXPERIMENTAL STAGE o RESEARCH AND DEVELOPMENT IN MANY COMPANIES AIMED AT INTERFERON MARKET o FEATURES WHICH MAY PERMIT RAPID MARKET SHARE CAPTURE ARE: GREATER PURITY WHICH ELIMINATES IMMUNOLOGICAL REACTIONS FROM GROUND PROCESSED MATERIAL	o THOUGHT TO HAVE THE GREATEST THERAPEUTIC POTENTIAL BY ALMOST ALL COMPANIES QUERIED

FIGURE 4.2-18

Figure 4.2-19 shows the projected interferon market for the treatment of this one disease over the years 1986-1991 and reflects a 1% per year population growth.

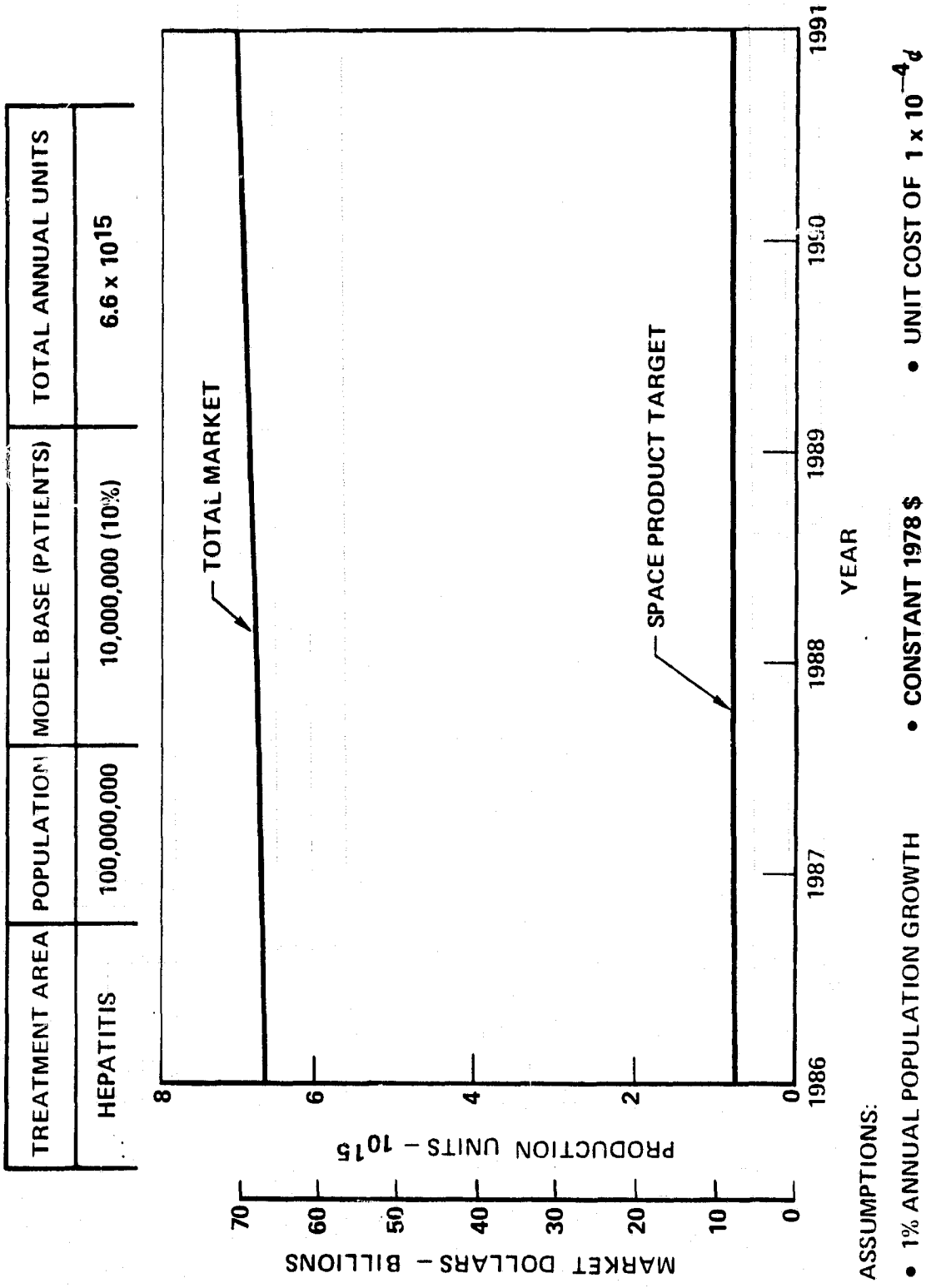
Competitive Product - Small scale clinical trials indicate that interferon is effective against many diseases of viral origin and suspected, but unproven, viral origin. In the case of cancer, radiation and chemotherapy are currently being used but they are not very effective. In other diseases, like hepatitis, there is no pharmacological treatment and interferon may rapidly capture the whole market. Since interferon has not been purified sufficiently to characterize its amino acid content and sequence, there is little chance that this product can be synthesized or made by recombinant DNA techniques within the next 10 to 15 years. Antiviral agents are gradually becoming available, although their scope of effectiveness is usually quite limited in comparison to that of interferon.

Competitive Process - Approximately one hundred pharmaceutical companies and university research laboratories are vigorously pursuing research and development with interferon. Large scale cellular biosynthesis is still the main production problem at the present time. Activity in this area ranges from monolayer production with roller bottles to use of dextran bead supports for suspension culture growth. The use of super inducers, e.g., poly rI:rC, has increased the quantity of interferon produced per cell. Current purification processes take up to ten days per research quantity batch. The use of the continuous flow electrophoresis separation process in space could readily capture this phase of the market if both the purification and throughput can be achieved as anticipated by theory. The largest threat to the dominance of space processed interferon is the development of a substance that will endogenously produce interferon in the patient. Such an approach has been tried several times before but was unsuccessful because of cytotoxicity side effects. Researchers at Johns Hopkins University claim they have developed a synthetic inducer that is metabolized several minutes after entering the patient, thereby circumventing cytotoxicity. They expect to start clinical trials in 1979.

MARKET PROJECTION

(Interferon)

TREATMENT AREA	POPULATION	MODEL BASE (PATIENTS)	TOTAL ANNUAL UNITS
HEPATITIS	100,000,000	10,000,000 (10%)	6.6×10^{15}



ASSUMPTIONS:

- 1% ANNUAL POPULATION GROWTH
- CONSTANT 1978 $
- UNIT COST OF 1×10^{-4} ¢

FIGURE 4.2-19

Market Development - Interferon should easily capture the lion's share of the market for treatment of viral origin diseases because present associated therapeutic procedures leave a lot to be desired. Preliminary clinical trials with interferon for these same diseases has shown tremendous potential therapeutic effect. The field is highly competitive. Anyone who can get on the market first with a highly purified product in sufficient quantity to satisfy even the smallest consumer demands will probably dominate the market for a long time to come.

Risk Assessment - An analysis was made of the market risks associated with attaining market objectives for space processed interferon. As previously described for the other products, this was accomplished by assigning subjective probabilities of success (P_S) to the three market risk elements at four program milestones. The results of the analysis are shown in Figure 4.2-20. This assessment shows that the chance of market success increases from about one out of four today to almost nineteen out of twenty following pilot plant demonstration.

4.3 ADDITIONAL AREAS OF INTEREST

During our visit to the various pharmaceutical companies, several additional products were suggested as potential candidates for space processing. These are listed in Table 4.3-1. They are light weight proteins and would be readily marketable to a large segment of the population.

TABLE 4.3-1 ADDITIONAL AREAS OF INTEREST

THYMIC HORMONE COMPLEX
SOMATOSTATIN
SOMATOMAMMOTROPIN
HYPOTHALAMIC RELEASING FACTORS
POLYVALENT VACCINES
SPECIFIC IMMUNOGLOBULINS

A cursory look at thymus physiology and immunochemistory has indicated that this particular organ has the potential for producing many other pharmacologically active agents to enhance tissue and organ transplantation. It is thought to contain lymphocyte stimulating factor which may increase antibody production.

MARKET RISK ASSESSMENT
INTERFERON

MARKET RISK

PROGRAM MILESTONES	COMPETITIVE PRODUCT P_S	X	COMPETITIVE PROCESS P_S	X	MARKET DEVELOPMENT P_S	= P_S EACH MILESTONE
SIGNIFICANT EVENTS IN DEVELOPMENT OF SPACE MANUFACTURING CAPABILITY	PROBABILITY OF SUCCESSFULLY COMPETING WITH OTHER PRODUCTS FOR MARKET SHARE		PROBABILITY OF SUCCESSFULLY COMPETING WITH GROUND PROCESS FOR MARKET SHARE		PROBABILITY OF DIFFERENTIATING THE SPACE PRODUCT AND SUCCESSFULLY DEVELOPING MARKET SHARE	THE PROBABILITY OF CAPTURING 10% OF THE 1986 MARKET
TODAY (1978)	0.90		0.33		0.90	0.27
COMPLETION OF GROUND TESTS (1982)	0.95		0.70		0.93	0.62
COMPLETION OF FLIGHT EXPERIMENTATION (1984)	0.96		0.90		0.95	0.82
COMPLETION OF FLIGHT DEMONSTRATION (1985)	0.99		0.95		0.99	0.93

FIGURE 4.2-20

The pharmaceutical companies have expressed considerable interest in pharmacological agents that will regulate growth and increase body weight. Aside from the utilization of these compounds for alleviating human deficiencies, these manufacturers conceive of potentially large markets in the veterinary field to increase livestock size and weight to meet the world's anticipated food needs. Since we had already included growth hormone and somatomedin as some of the initial candidates for consideration in this task, they suggested there would be strong commercial interest in other growth factors including somatostatin, somatomammotropin, and possibly hypothalamic influencing factors.

Improved vaccines to provide immunity against specific diseases was also suggested by several companies. Vaccines, in general, have two major problems. Their relative impurity usually causes side reactions in approximately 10% of the recipients. They are difficult to put into solutions of sufficient concentration to permit injections of acceptable volumes of the material without precipitation. Electrophoretic processing in space may be the method of choice to increase the purity of the vaccine material and thus reduce the total amount of protein needed for each injection. In the livestock industry it is desirable to give each animal only one injection containing many different vaccines. The present technology (concentration of total proteins in a given quantity of fluid or development of polyvalent single protein antigens) will only allow three vaccines to be combined in one injection. Increasing the number of vaccines to 5 or 8 per injection is highly desirable.

An additional area of interest for the companies we contacted was the stability of crystallization procedures in space. The production of one pharmaceutical compound requires its crystallization as the final step before packaging. A change in its crystalline configuration results in different pharmaceutical activity. (One company has experienced three such crystalline changes in one of its major product lines.) Since the procedure does not revert back to the initial structure, production must be halted and FDA approval sought for the new crystal configuration. Because the approval cycle can take years, any process stability in space may cause them to use a space bioprocessing method rather than ground operations.

4.4 CONCLUSIONS AND RECOMMENDATIONS

We have explored a number of natural pharmaceutical products from a technical and a marketing viewpoint to serve as potential candidates for a space bioprocessing venture. This group of products was presented to the pharmaceutical industry as well as the medical and pharmacy academic community. With their concurrence, the list was narrowed down to twelve products: alpha-1-antitrypsin, antihemophilic factor, epidermal growth factor, erythropoietin, granulocyte stimulating factor, beta cells, human gamma globulins, interferon, urokinase, transfer factor, somatomedin and growth hormone. Literature reviews of each of these products showed that technical information was voluminous but of little direct value to sizing a processing system. Pieces of information were missing that would require technical assumptions, based on laboratory and bioengineering experience, in order to proceed with production system analysis.

All of the twelve biological products reviewed could exercise the proposed system in part or in its entirety thus supporting the concept of a true multiproduct system. Knowledge of the cell sources for erythropoietin, epidermal growth factor, growth hormone, interferon and alpha-antitrypsin production and an awareness that these cells and hormones can be separated by electrophoretic techniques, all support the concept of a full bioprocessing production system. Antihemophilic factor VIII and specific immune globulins are blood proteins with no defined site of production in a living organism. They, therefore, would only be able to exercise the space bioprocessing system in the separation mode. Islets of Langerhans (beta cells) and urokinase producing cells can be grown in tissue culture and also separated electrophoretically. Because the mobilities of these cells in an electrical field are lower than proteins, the separation chamber may have to be modified slightly in design or operational function. All twelve products could benefit through space bioprocessing by improvements in purity and throughput.

Based on economic and technical information, this task developed a value index of each product with respect to their humanitarian value and applicability to the concept of production through space bioprocessing. This index ranked the twelve products in descending order of importance: transfer factor, erythropoietin, beta cells, alpha-antitrypsin, epidermal growth factor, interferon, immunoglobulins,

growth hormone, antihemophilic factor, urokinase, somatomedin, granulocyte stimulating factor. In addition to this numerical evaluation, two additional subjective criteria were applied to each product, i.e. - would the product exercise the conceptual bioprocessing system in one or more modes of operation and could comparison data be attained between earth and space processing. This total evaluation resulted in a recommendation that erythropoietin, Islets of Langerhans (beta cells), antihemophilic factor, alpha antitrypsin, epidermal growth factor and interferon be used to exercise the model space bioprocessing system.

A preliminary marketing analysis of the six model products indicated that they would all be viable commercial candidates. Four of the products, erythropoietin and Islets of Langerhans (beta cells), alpha antitrypsin and epidermal growth factor would have minimal competition from earth based commercial sources. Space processed antihemophilic factor was projected as attaining thirty percent of the product market by 1986 as a result of its increased process yield and higher level of purity. Interferon would probably attain the majority of the market because of simplicity of production and quantity throughput in comparison to current ground based techniques. A risk assessment of obtaining these market objectives after pilot plant demonstration showed a minimum seventy percent chance of success. The widespread beneficial effect of these biological products on the average American would develop a new regard for space and an interest in its commercialization.

Discussions with a number of pharmaceutical companies resulted in a list of several other biologically active products for eventual consideration. These included: thymic factors, specific immunoglobulins, somatostatin, somatomammotropin and polyvalent vaccines. It is recommended that these products be explored in depth as a point of future communication and interest with the pharmaceutical companies.

5.0 INTRODUCTION

The purpose of this task is to assess the requirements for a multiproduct production facility to produce the selected products. Important considerations in this regard are the production rates relative to the needs defined in the Product Requirements and Analysis task and the capabilities projected for Shuttle and associated supporting hardware. These considerations along with the requirements for good manufacturing practices imposed by the Food and Drug Administration were used to formulate the process protocols for the selected products. These protocols and requirements will provide the basis for future conceptual design of a multiproduct pharmaceutical production facility. Such design studies performed in cooperation with ethical drug companies will provide information vital to future space manufacturing investment decisions by private industry.

5.1 BACKGROUND AND APPROACH

The rationale for this investigation of space processing of pharmaceuticals is that the microgravity environment of space may provide advantages to the production and purification of biological materials with attendant humanitarian benefits. Experiments conducted in space have already demonstrated the advantages of using static (14) and free flow (15) electrophoresis to separate biological materials in a microgravity environment. Cells separated during static electrophoresis showed increased production of urokinase and erythropoietin when subsequently subcultured in Earth based laboratories (16). Other studies, directed toward determining the effects of microgravity on a biological system, demonstrated increased cell density in cultures of the bacterium Salmonella typhimurium growing in space compared to ground control experiments. This increased density was attributed to improved transport of nutrients to, and waste materials from, the growing cell (17). Another report indicates that increased cellular growth rate and increased cell density may be obtained in space as a result of growing cells on carriers suspended due to lack of gravity (18).

Our approach to establishing process requirements is to evaluate current ground processes if any, assess how to best utilize space advantages through new or modified processes, and to define the process protocols on which the requirements will be based. The process protocols will reflect those existing Food and Drug Administration (FDA) requirements that will probably be applicable to the space

processing of pharmaceuticals. The key element of the process evaluation is to define mass flow balances for the space processes at production rates that satisfy the targeted market shares defined in the section on Product Requirements and Analysis. A multiproduct facility can then be defined to satisfy the combined requirements and product mix trade-offs can then be made in favor of those products which benefit most from space processing. In addition, establishing the scale of the process allows meaningful evaluation of man interface requirements. The results of the tradeoff studies will be a multiproduct process system description, and the protocol for the process.

5.2 ANALYSIS AND RESULTS

This section presents the results of the analysis defining the requirements for a multiproduct pharmaceutical production facility. Basic to this facility analysis is the selection of production methods described in Section 5.2.1, a consideration of applicable FDA requirements (Section 5.2.2), and the process evaluation (Section 5.2.3). The resulting multiproduct process system is described in Section 5.3.

Any of the six candidate products selected in Section four could be processed in the multiproduct facility in one of its three operational configurations, i.e., a) separation of protein alone, b) culture and separation of cells alone, and c) separation and culture of cells followed by separation of the protein products of those cells. Each of the products would use essentially the same facility components with only minor instrument setting changes for the particular product being processed. The three operational configurations are illustrated by in-depth process system requirements analysis for a product in each category. The three products employed in the requirements analyses are erythropoietin, antihemophilic factor, and pancreatic beta cells.

5.2.1 Production Methods

A biological production facility requires at a minimum the capability of separating pharmaceutical products from naturally occurring mixtures of cells and/or proteins. In addition, the production of such a system could be enhanced if it included the capability of culturing desired tissue strains. Such a system is shown schematically in Figure 5.2-1. Also included in the system is the assay capability required to ascertain that the desired products are produced. As shown in the figure, this

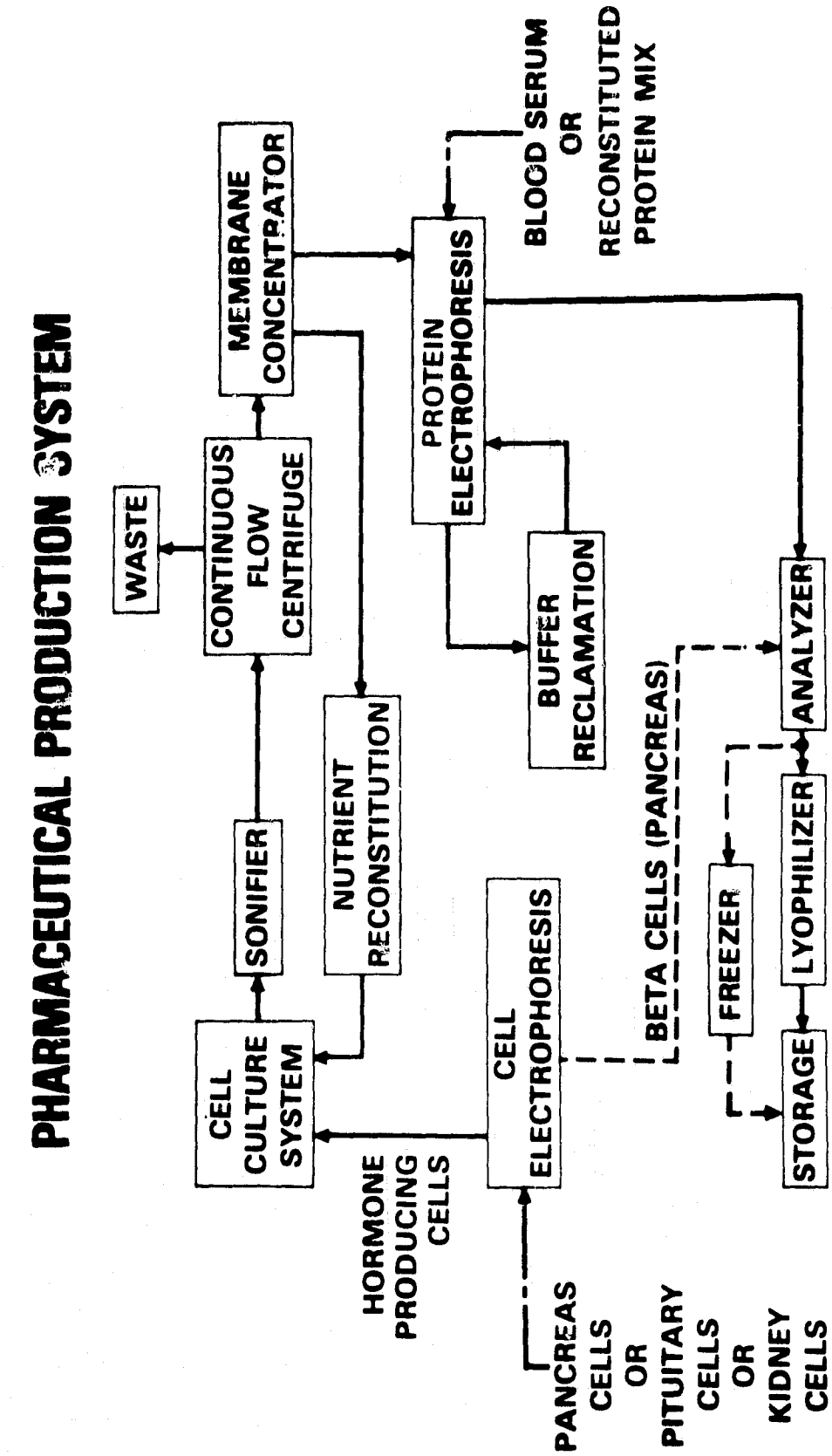

PHARMACEUTICAL PRODUCTION SYSTEM
WASTE
CONTINUOUS FLOW CENTRIFUGE
MEMBRANE CONCENTRATOR
PROTEIN ELECTROPHORESIS
BLOOD SERUM OR RECONSTITUTED PROTEIN MIX
SONIFIER
NUTRIENT RECONSTITUTION
BUFFER RECLAMATION
CELL CULTURE SYSTEM
CELL ELECTROPHORESIS
BETA CELLS (PANCREAS)
FREEZER
ANALYZER
LYOPHILIZER
STORAGE
HORMONE PRODUCING CELLS
PANCREAS CELLS OR PITUITARY CELLS OR KIDNEY CELLS
• GROWTH HORMONE
• BETA CELLS
• UROKINASE
• ERYTHROPOIETIN
• COAGULATING FACTORS

FIGURE 5.2-1

system has different entry points dependent on the raw material and the final product. For example, if the desired product is beta cells, it is possible to separate them directly from pancreatic tissue; however, production rates could be increased if these cells were subsequently cultured and separated from the culture media by a second cell electrophoretic procedure. For other applications, proteins are the desired products rather than cells. In these cases protein mixtures can be processed as in the case of blood serum or reconstituted lyophilized protein mixture. The separation of Antihemophilic Factor VIII from blood plasma or cryoprecipitate is an example of the direct separation of proteins. In other cases, protein production requires that specific cells which are high producers be separated and subsequently cultured to obtain usable yields of the final product. An example of this is the production of erythropoietin by kidney cells.

It is obvious that two processes at a minimum are required to demonstrate multiproduct capability, biological material separation and tissue culture. Possible methods of biological material separation are presented in Figure 5.2-2. The method most often used to separate proteins on the ground is precipitation, because of its inherent throughput advantage. However, it should be noted that except for throughput, the method of choice in terms of resolution, yield, and simplicity is electrophoresis. Therefore, when the throughput advantage for continuous flow space electrophoresis over ground electrophoresis (which is calculated to be at least two orders of magnitude) is considered, it becomes the method of choice. This choice is further reinforced when the separation of cells for culturing is considered, because space electrophoresis could have definite advantages in obtaining the required resolution.

In determining the method of tissue culture the method of choice is less clear. There are two possible methods, growth in monolayer and growth in suspension. In general, primary cells from the body grow only in monolayer form attached to a surface, so products like beta cell culture would require this method. It is possible that cells which are high producers of desirable protein products may present themselves as transformed cells which can be grown in suspension cultures. If cells can be grown in suspension, then growth is enhanced and the system can be simplified. Because of the uncertainties of advances possible in cell culture, conservative cell culture ground rules were based on densities obtainable either

GROUND SEPARATION TECHNIQUES

	ELECTROPHORESIS	OTHERS		
		COLUMN CHROMATOGRAPHY	PRECIPITATION	DENSITY GRADIENT
QUANTITY	3*	2	1	3
RESOLUTION	1	3	4	2
YIELD	1	2	2	3
SIMPLICITY	1	2	3	3

*RANK ORDER OF CHOICE

FIGURE 5.2-2

in monolayer on the surfaces of beads or in suspension. This approach eliminated sensitivity of the analysis to cell culture modes.

5.2.2 Applicable FDA Requirements

All of the candidate products evaluated in this study were either natural body substances or the products of natural body substances. Therefore, with the aid of a participating pharmaceutical company, contact was made with the Bureau of Biologics of the Food and Drug Administration to start exploratory discussions of the space bioprocessing concept, the candidate substances considered for production, and the applicable governing regulations. This agency was very cooperative and appreciative of being brought into our program at the conceptual stages. It gave them an understanding of what the goals of space bioprocessing were and our approach to achieving those goals. This awareness of the program then enabled them to provide meaningful guidance to our systems design and thus be in compliance with their good manufacturing practices. This guidance will be continued through meetings with Bureau of Biologics representatives from the divisions they consider pertinent to this concept. It has been made clear that these representatives could see no insurmountable problems with regard to space bioprocessing as outlined by us.

In determining a basis for applicability of FDA requirements to producing pharmaceuticals in space, many considerations emerge. Of primary importance was the category of the particular product. According to the requirements, products are classified as either biologic or drug. Furthermore, current good manufacturing practices, which are pertinent to the determination of applicable requirements, exist for both biological and drug products. Hence, it was necessary to either classify the three candidate space products into the biological/drug categories or select and analyze a specific current good manufacturing practice that would represent the applicable manufacturing practices or regulations for all three categories of the candidate products. The latter approach was adopted since the former required assistance from the FDA, in the form of their opinion, and had not been received at the time of this writing. The regulation analyzed was Current Good Manufacturing Practice for Finished Pharmaceuticals (see Appendix B) which was assumed to encompass all or most of the specific requirements found in the other regulations and current good manufacturing practices. This particular

regulation was judged most representative of the FDA regulations and would formulate a good basis for system definition constraints.

Categorization of the three selected candidate products into the biological/drug scheme was somewhat confused by the definition of a biological product. Biological products include any virus, serum, toxin, anti-toxin or analogous product. A virus is a minute living organism causing an infectious disease. A serum is a product obtained from blood by removing clotting components and cells. A toxin is a product containing a poisonous substance which can induce an immune response when injected in nonfatal doses. An anti-toxin is the immune response substance which counteracts the toxin. Only one of the three candidate products fits these definitions - antihemophilic factor is a blood component. The remaining two products, beta cells and erythropoietin do not fall into these classifications and thus cannot be classified as biological products at this time.

Additional considerations in determining applicability of requirements included those factors bearing on traditional space vehicle design and operation. Among others, weight conservation, cost optimization, reliability, weightlessness, minimal man interface, data volume and requirements, crew number and skill levels were the criteria used. Discussion of automated continuous processing arose often, which seems to indicate some degree of importance for incorporating this feature. Besides minimizing man interface and increasing reliability, automated continuous processing would also facilitate realization of FDA requirements by eliminating the human element.

The applicable portions of the regulations from current Good Manufacturing Practices for Finished Pharmaceuticals are tabulated in Figure 5.2-3. Appendix B contains a reprint of the complete regulation. Each regulation or portion is classified as "applicable," "applicable with interpretation" and "applicable as modified." The entries "subject to interpretation" include an interpretation posed for FDA consideration. Proposed modifications are further described in Figure 5.2-4 and are concerned with anomalies and discrepancies due to unique applications of the requirements to a space processing system.

APPLICABLE FDA REQUIREMENTS

BASED ON PART 211 - CURRENT GOOD MANUFACTURING PRACTICE FOR FINISHED PHARMACEUTICALS - FROM 1977 TITLE 21 CODE OF FEDERAL REGULATIONS

REGULATION SUB-PART	REGULATION PARAGRAPH	GENERAL SUBJECT	SUMMARIZED VERSION	RATIONALE/IMPACT	APPLICABILITY
A	211.10	PERSONNEL	(a) PERSONNEL RESPONSIBLE FOR DIRECTING THE MANUFACTURE AND CONTROL OF THE DRUG SHALL BE ADEQUATE IN EDUCATION, TRAINING & EXPERIENCE. ALL PERSONNEL SHALL HAVE CAPABILITIES COMMENSURATE WITH THEIR ASSIGNED FUNCTIONS AND ADEQUATE UNDERSTANDING OF OPERATION RATIONALE.	ON-ORBIT PERSONNEL MAY HAVE SPACECRAFT OPERATIONS DUTIES AS PRIMARY RESPONSIBILITY, BUT EFFICIENT UTILIZATION OF PERSONNEL MAY REQUIRE THEIR USAGE IN DRUG PROCESSING. THESE PEOPLE MAY NOT HAVE PRIMARY TRAINING IN DRUG PROCESSING BUT WILL COORDINATE WITH RESPONSIBLE GROUND PERSONNEL.	MOD
			(b) EXCLUDE FROM CONTACT WITH DRUG PRODUCTS, THOSE PERSONS WITH ILLNESS OR OPEN LESIONS TO AVOID CONTAMINATION.	CREW & ALL GROUND OPERATIONS PERSONNEL CONTACTING DRUG WILL HAVE TO BE SCREENED. SIMILAR CONTAMINATION OF ALL CONTACTED EQUIPMENT AND COMPONENTS WILL NEED TO BE PREVENTED. IF ILLNESS ON REMOTE, ENCLOSED, SPACECRAFT BREAKS OUT, OPERATIONS WILL HAVE TO CEASE AND FACILITIES MAY HAVE BE STERILIZED.	APP
B	211.20	CONSTRUCTION & MAINTENANCE OF FACILITIES & EQUIPMENT	BUILDINGS & FACILITIES SHALL BE MAINTAINED CLEAN AND ORDERLY AND SHALL PROVIDE ADEQUATE SPACE FOR STORAGE, HANDLING, PROCESSING, LABORATORY OPERATIONS, PACKAGING EQUIPMENT & MATERIALS. PROVIDE ADEQUATE FACILITIES TO MINIMIZE CONTAMINATION BY EXTRANEOUS ADULTERANTS, INCLUDING CROSS CONTAMINATION BETWEEN PRODUCTS AND FOR SUITABLE STORAGE CONDITIONS.	SPECIFIC REQUIREMENTS FOR BUILDINGS WHERE DRUG PRODUCTS ARE PROCESSED. AS STATED, DOES NOT INCLUDE OTHER ENCLOSURES AS WILL BE THE CASE FOR A SPACE VEHICLE.	MOD
B	211.30	EQUIPMENT	PROCESSING EQUIPMENT SHALL BE MAINTAINED CLEAN & ORDERLY AND SHALL BE OF SUITABLE DESIGN, SIZE AND CONSTRUCTION TO FACILITATE CLEANING, MAINTENANCE AND OPERATION. (a) CONTACTED SURFACES SHALL NOT BE REACTIVE, ADDITIVE, OR ABSORPTIVE SO AS TO ALTER DRUGS. (b) LUBRICANTS, COOLANTS, ETC, CANNOT CONTACT DRUG PRODUCTS. (c) CONSTRUCTION & INSTALLATION ALLOW ADJUSTMENT, DISASSEMBLY, CLEANING, & MAINTENANCE. (d) BE SUITABLE FOR ANY TESTING, MEASURING, MIXING, WEIGHING, OR OTHER PROCESSING OR OTHER STORAGE OPERATIONS.	STIPULATES SPECIAL DESIGN REQUIREMENTS FOR PROCESSING EQUIPMENT.	MOD

APP - PROBABLY APPLICABLE
INT - POSSIBLE APPLICABLE - SUBJECT TO INTERPRETATION
MOD - APPLICABLE AS MODIFIED - SEE SEPARATE CHART

FIGURE 5.2-3

REGULATION SUB-PART	REGULATION PARAGRAPH	GENERAL SUBJECT	SUMMARIZED VERSION	RATIONALE/IMPACT	APPLICABILITY
C	211.40	PRODUCTION AND CONTROL PROCEDURES	(a) EACH SIGNIFICANT STEP SHALL BE PERFORMED BY A COMPETENT/RESPONSIBLE PERSON AND CHECKED BY A SECOND PERSON; IF AUTOMATICALLY CONTROLLED, THEN CHECKED BY A PERSON. A WRITTEN RECORD SHALL BE RECORDED IMMEDIATELY FOLLOWING COMPLETION OF SUCH STEPS.	DEFINES MONITORING & RECORDING REQUIREMENTS DURING PROCESSING WHICH WILL NEED TO BE ADDRESSED IN THE SPACE PROCESSING SYSTEM DESIGN, REMOTE OPERATION & MONITORING WOULD REQUIRE GROUND CHECKING AND RECORDING.	MOD
			(b) ALL CONTAINERS, LINES & EQUIPMENT SHALL BE IDENTIFIED INDICATING CONTENTS & STAGE OF PROCESSING OF THE BATCH.	THIS CAN BE INTERPRETED TO APPLY TO CONTINUOUS PROCESSING SYSTEMS & WOULD FACILITATE CLEANING, MAINTENANCE & OPERATION.	INT B
			(c) TO MINIMIZE CONTAMINATION/ PREVENT MIXUPS, EQUIPMENT & CONTAINERS SHALL BE CLEANED AT SUITABLE INTERVALS.	PERIODIC CLEANING & REFURBISHMENT IS REQUIRED.	APP
			(d) APPROPRIATE PRECAUTIONS TAKEN TO MINIMIZE MICROBIOLOGICAL CONTAMINATION	REQUIRES PRECAUTIONS & PROCEUDRES FOR PREVENTION OF CONTAMINATION	APP
			(e) APPROPRIATE PROCEDURES TAKEN TO MINIMIZE CROSS-CONTAMINATION BETWEEN DRUGS.	REQUIRES PRECAUTIONS & PROCEDURES FOR PREVENTION OF CONTAMINATION	APP
			(f) TO ASSURE UNIFORMITY & INTEGRITY OF PRODUCTS, THERE SHALL BE ADEQUATE IN-PROCESS CONTROLS DONE AT APPROPRIATE INTERVALS.	REQUIRES IN-PROCESS CONTROLS WHICH MAY DICTATE DIFFERENT IMPLEMENTATION STRATEGIES FOR REMOTELY OPERATED EQUIPMENT.	MOD
			(j) PROHIBITS USE OF ASBESTOS-CONTAINING OR OTHER FIBER RELEASING FILTERS UNLESS NECESSARY AND BACKED BY A NONFIBER RELEASING FIBER.	SPECIFIC DESIGN CONSTRAINT.	APP
C	211.42	COMPONENTS	ALL COMPONENTS SHALL BE STORED & HANDLED IN A SAFE, SANITARY AND ORDERLY MANNER. COMPONENTS SHALL BE WITHHELD FROM USE UNTIL IDENTIFIED, SAMPLED, AND TESTED FOR CONFORMANCE WITH SPECIFICATIONS. COMPONENTS SHALL BE ROTATED IN STOCK, AND GUARDED AGAINST CONTAMINATION. APPROPRIATE RECORDS AND INVENTORIES OF EACH COMPONENT SHALL BE MAINTAINED. RESERVE SAMPLES SHALL BE BE MAINTAINED FOR SPECIFIED PERIODS.	REQUIRES SPECIAL HANDLING, SAMPLING & TESTING FOR ALL DRUG CONSTITUENTS BEFORE PROCESSING, THESE REQUIREMENTS WILL AFFECT GROUND OPERATIONS PLANNING. LIMITED NUMBER OF COMPONENTS WILL BE ON HAND DUE TO SPACE AND WEIGHT LIMITATIONS.	MOD
C	211.55	PRODUCT CONTAINERS & THEIR COMPONENTS	SUITABLE SPECIFICATIONS, METHODS, & PROCEDURES SHALL BE USED TO ASSURE CONTAINER, CLOSURES & PARTS ARE SUITABLE FOR INTENDED USE. CONTAINERS SHALL NOT BE REACTIVE, ADDITIVE, OR ABSORBTIVE SO TO ALTER THE DRUG PRODUCTS.	SPECIFIC CONTAINER REQUIREMENTS.	APP

APP - PROBABLY APPLICABLE
INT - POSSIBLE APPLICABLE - SUBJECT TO INTERPRETATION
MOD - APPLICABLE AS MODIFIED - SEE SEPARATE CHART

FIGURE 5.2-3 (Continued)

REGULATION SUB-PART	REGULATION PARAGRAPH	GENERAL SUBJECT	SUMMARIZED VERSION	RATIONALE/IMPACT	APPLICABILITY
C	211.58	LABORATORY CONTROLS	SPECIFICATIONS, STANDARDS, AND TEST PROCEDURES TO ASSURE THAT COMPONENTS, IN-PROCESSED DRUGS, AND FINISHED PRODUCTS CONFORM TO APPROPRIATE STANDARDS. ESTABLISH ADEQUATE RECORDS AND PROCEDURES FOR IN-PROCESS SAMPLING AND TESTING.	SPACE PROCESSING HARDWARE, OPERATIONS AND PLANNING WILL NEED TO ADDRESS THIS REQUIREMENT. IN-PROCESS SAMPLING AND TESTING MIGHT BE A FORMIDABLE TASK FOR AN AUTOMATIC REMOTE PROCESSING SYSTEM.	APP
C	211.62	EXPIRATION DATING	PRODUCTS LIABLE TO DETERIATION MUST MEET APPROPRIATE STANDARDS AT TIME OF USE BY EXPIRATION DATING RELATED TO STABILITY TESTING OF PRODUCT.	LONG TERM BULK STORAGE MAY CAUSE PRODUCT DETERIORATION. STORAGE CONTAINERS WILL BE LABLED WITH REGARD TO BULK PROCESSING DATE.	APP
D	211.80	PACKAGING AND LABELING	ONLY THOSE DRUGS THAT HAVE MET ESTABLISHED STANDARDS AND SPECIFICATIONS CAN BE DISTRIBUTED. MIX-UPS BETWEEN DRUGS SHOULD BE PREVENTED DURING PACKAGING/LABELING.	THIS REQUIREMENT WILL HAVE LIMITED APPLICABILITY SINCE FINAL PACKAGING AND DISTRIBUTION WILL OCCUR AFTER SPACE OPERATIONS. THE SPACE PRODUCED PRODUCTS (IN BULK UNITS) WILL BE ADEQUATELY PACKAGED AND LABELED AND HAVE MET STANDARDS.	INT
E	211.101	RECORDS & REPORTS	A MASTER PRODUCTION AND CONTROL RECORD FOR EACH PRODUCT SHALL BE PREPARED INCLUDING PRODUCT NAME, INGREDIENTS, WEIGHTS, ACTUAL AND THEORETICAL YIELDS, INSTRUCTIONS, PROCEDURES, SPECIFICATIONS, NOTATIONS, PRECAUTIONS, ETC.	DATA FROM REMOTE MONITORING DEVICES AND CONTROL PROCEDURES FROM MISSION DOCUMENTS WILL SUFFICE TO FULFILL THIS REQUIREMENT PROVIDED THESE DATA AND PROCEDURES INCLUDE APPROPRIATE INFORMATION PER THIS REQUIREMENT. "WEIGHTS" IMPLIES PREMEASURED GROUND WEIGHTS.	INT
			A BATCH PRODUCTION AND CONTROL RECORD FOR EACH PRODUCT SHALL BE PREPARED INCLUDING: A RECORD OF EACH SIGNIFICANT STEP, COGNIZANT PERSONS SIGNING PROCEDURES (OR CHECKING OPERATIONS), MAJOR EQUIPMENT, COMPONENTS AND PRODUCTS USED, IN-PROCESS & LABORATORY RESULTS, DATES OF PROCESSING, ETC.	THIS REQUIREMENT IS APPLICABLE AND EACH RECORD SHALL BE MAINTAINED FOR EACH MISSION WHERE BATCHES CAN BE EQUATED TO HARVEST OR RE-STOCKING PERIODS.	INT

APP - PROBABLY APPLICABLE
INT - POSSIBLE APPLICABLE - SUBJECT TO INTERPRETATION
MOD - APPLICABLE AS MODIFIED - SEE SEPARATE CHART

FIGURE 5.2-3 (Continued)

SUGGESTED MODIFICATIONS AND ADDITIONS TO

CURRENT GOOD MANUFACTURING PRACTICE REGULATIONS

BASED ON PART 211 - CURRENT GOOD MANUFACTURING PRACTICE FOR FINISHED PHARMACEUTICALS

(TITLE 12 CHAPTER 1 1977 CODE OF FEDERAL REGUALTIONS)

EXISTING SUB PART	REGULATION PARAGRAPH	GENERAL SUBJECT	CRITICAL PHRASE/SENTENCE	RECOMMENDATION	REASON
A	211.10	GENERAL PROVISIONS; PERSONNEL	(a) "THE PERSONNEL RESPONSIBLE FORDIRECTING THE MANUFACTURE AND CONTROL OF THE DRUG .... "ALL PERSONNEL SHALL HAVE CAPABILITIES COMMENSURATE WITH THEIR ASSIGNED FUNCTIONS, ..."	ADD SENTENCE "FOR REMOTE PRECISION AUTOMATIC MECHANICAL AND/OR ELECTRONIC PROCESSING SYSTEMS, THE COGNIZANT OPERATING PERSONNEL SHALL HAVE CONSTANT ACCESS WITH THE RESPONSIBLE PERSONNEL"	PARAGRAPH NEEDS TO ADDRESS THE RESPONSIBILITIES OF PROCESSING DIRECTION/ OPERATIONS DURING AN IN ORBIT PROGRAM REMOTELY CONTROLLED/ DIRECTED.
B	211.20	CONSTRUCTION & MAINTENANCE OF FACILITIES AND EQUIPMENT	"BUILDINGS SHALL BE MAINTAINED . . . . . AND LOCATION . . . . ."	ADD "AND FACILITIES" AFTER "BUILDINGS" AND REMOVE "AND LOCATION"	TO ALLOW MANUFACTURE AND CONTROL OF DRUG PROCESSING IN ENCLOSURES OTHER THAN BUILDINGS (e.g. SPACE OPERATIONS VEHICLE)
			(f) "PROVIDE FOR SAFE AND SANITARY DISPOSAL OF SEWAGE, TRASH AND OTHER REFUSE WITHIN AND FROM THE BUILDINGS AND IMMEDIATE PREMISES."	CHANGE (f)" ... FROM THE BUILDINGS, FACILITIES, AND/OR IMMEDIATE PREMISES." ADD SENTENCE "IN LIEU OF REFUSE REMOVAL, IRREVERSIBLE STORAGE AND ISOLATION OF REFUSE FOR PERIODS OF TIME IS PERMISSIBLE."	WASTE HANDLING IN SPACE OPERATION NECESSITATES ONBOARD STORAGE AND PRECLUDES IMMEDIATE REMOVAL TILL END OF MISSION OR REVISIT.
P	211.30	EQUIPMENT	ADDITION	(e) WHEN FEASIBLE, PERMIT RECYCLING, RECLAMATION, RECONSTITITION OR RE-USE OF FLUIDS OR CARRIER SUBSTANCES WHERE SUCH ACTION DOES NOT AFFECT THE SAFETY, IDENTITY, STRENGTH, QUALITY AND PURITY OF THE DRUG PRODUCT."	WEIGHT CONSERVATION AND EFFICIENCY CONSIDERATIONS DICTATE OPTIMUM UTILIZATION OF MATERIALS IN SPACE OPERATION.
			(c) "BE CONSTRUCTED AND INSTALLED TO FACILITATE ADJUSTMENT, DISASSEMBLY, CLEANING & MAINTENANCE TO ASSURE THE RELIABILITY.....	ADD SENTENCE "WHERE RELIABILITY OF EQUIPMENT IS NOT HIGH, REDUNDANT EQUIPMENT AND/ OR CONTROLS ARE PERMISSIBLE."	REMEDIAL MAINTENANCE OF SEMI-AUTOMATIC PROCESSING SYSTEM WOULD BE LIMITED TO MINOR REPAIRS DURING SPACE OPERATIONS WHILE IN-ORBIT REPAIRS OF AN AUTOMATIC SYSTEM WOULD BE ENTIRELY PRECLUDED.
C	211.40	PRODUCTION AND CONTROL PROCEDURES	(a) "EACH SIGNIFICANT STEP IN THE PROCESS .... SHALL BE PERFORMED .... AND CHECKED ... ; OR, IF SUCH STEPS IN THE PROCESSING ARE CONTROLLED BY PRECISION AUTOMATIC, MECHANICAL, OR ELECTRONIC EQUIPMENT, THEIR PROPER PERFORMANCE IS ADEQUATELY CHECKED BY ONE, OR MORE, COMPONENT AND RESPONSIBLE INDIVIDUALS. ... SUCH IDENTIFICATIONS SHALL BE RECORDED IMMEDIATELY FOLLOWING THE COMPLETION OF SUCH STEPS."	ADD SENTENCE "WHEN REMOTE AUTOMATIC CONTINUOUS PROCESSING SYSTEMS ARE USED, PROPER PERFORMANCE SHALL BE CHECKED BY CONTINUOUS MONITORING AND/OR REMOTE SURVEILLANCE OF PERTINENT STEPS AND KEY OPERATIONS AS PROVIDED BY SUFFICIENT DATA ACQUISITION DEVICES."	ONSITE, IMMEDIATE MONITORING OF REMOTE AUTOMATIC PROCESSING MAY NOT BE POSSIBLE. ON ORBIT MAN-INTERFERENCE SHOULD BE MINIMIZED.

FIGURE 5.2-4

EXISTING SUB PART	REGULATION PARAGRAPH	GENERAL SUBJECT	CRITICAL PHRASE/SENTENCE	RECOMMENDATION	REASON
			(f) "TO ASSURE THE UNIFORMITY AND INTEGRITY OF PRODUCTS, THERE SHALL BE ADEQUATE IN-PROCESS CONTROLS, SUCH AS CHECK WEIGHTS ....., THE HOMOGENEITY OF SUSPENSIONS, AND THE CLARITY OF SOLUTIONS. IN-PROCESS SAMPLING SHALL BE DONE AT APPROPRIATE INTERVALS USING SUITABLE EQUIPMENT."	ADD SENTENCE "STORAGE OF IN-PROCESS SAMPLES IS PERMISSIBLE IN THE CASE OF REMOTE AUTOMATIC PROCESSING SYSTEMS (STORED SAMPLES TO BE IDENTIFIED AND ANALYZED PRIOR TO PRODUCT PACKAGING) UNLESS SUCH STORAGE CAUSES DETERIORATION, DECAY, OR TIME ALTERATION OF THE DRUG OR ANY COMPONENT, THEN AUTOMATIC EQUIPMENT FOR THE IMMEDIATE ANALYSIS OF THE SAMPLES SHALL BE PROVIDED."	PERIODIC IN-PROCESS SAMPLING BY MANUAL PROCEDURES MAY BE PRECLUDED BY REMOTE NATURE OF SYSTEM. WEIGHTLESS CONDITIONS EFFECTIVELY CONTROL HOMOGENEITY OF SOLUTIONS IN SUSPENSION UNLESS SOME ORGANIC DECOMPOSITION IS OCCURRING. IN WEIGHTLESS CONDITIONS, PREWEIGHED MATERIAL WILL BE ADDED TO DEFINITE VOLUMES AND CAN BE CHECKED BY CHEMICAL ANALYSIS.
	211.42	COMPONENTS	(a) "EACH CONTAINER OF COMPONENT SHALL BE EXAMINED VISUALLY FOR DAMAGE OR CONTAMINATION PRIOR TO USE, INCLUDING EXAMINATION FOR BREAKAGE OF SEALS, WHEN INDICATED."	REMOVE "VISUALLY" AND ", INCLUDING EXAMINATION FOR BREAKAGE OF SEALS,"	TO EXPAND INTENT TO COVER OPERATION IN SPACE SYSTEM WHERE ON-SITE INSPECTION IMMEDIATELY PRIOR TO PROCESSING MAY BE POSSIBLE.
			(f) "(1) APPROVED COMPONENTS SHALL BE HANDLED AND STORED TO GUARD AGAINST CONTAMINATING OR BEING CONTAMINATED BY OTHER DRUGS OR COMPONENTS."	CHANGE (f) (1) "... BY OTHER DRUGS OR COMPONENTS UNLESS SUCH COMPONENT IS ADDITIONALLY USEFUL ELSEWHERE IN A CONTINUOUS SYSTEM AND CAN BE RECYCLED, RECLAIMED, RE-CONSTITUTED, OR RE-USED WITHOUT DELETERIOUS EFFECT ON THE SAFETY, IDENTITY, STRENGTH, QUALITY AND PURITY OF THE DRUG PRODUCT."	TO PERMIT RE-USE OF COMPONENTS IN A CONTINUOUS PROCESSING SYSTEM. THIS WILL CONTRIBUTE TO OPTIMIZATION OF WEIGHT CONSERVATION.
C	211.42	COMPONENTS	(f) "(2) APPROVED COMPONENTS SHALL BE ROTATED IN SUCH A MANNER THAT THE OLDEST STOCK IS USED FIRST.	CHANGES (f) (2) .... THE OLDEST STOCK IS USED FIRST, UNLESS SUCH ROTATION IS IMPRACTICAL IN A RE-CIRCULATING CONTINUOUS PROCESSING SYSTEM."	TO REMOVE RESTRICTION THAT DOES NOT APPLY IN CONTINUOUS PROCESSING SYSTEMS. NOT POSSIBLE IN WEIGHTLESS HOMOGENEOUS MIXTURE, FOR EXAMPLE.
			(g) "(3) AN INDIVIDUAL INVENTORY AND RECORD FOR EACH COMPONENT USED IN EACH BATCH OF DRUG MANUFACTURED OR PROCESSED."	CHANGES (g) (3) "... DRUG MANUFACTURED OR PROCESSED OR, IN THE CASE OF AUTOMATIC CONTINUOUS RECORD OF EACH COMPONENT AND RATE DURING PROCESSING."	TO MODIFY REGULATION FOR CONTINUOUS INVENTORY IN A CONTINUOUS PROCESSING SYSTEM.

FIGURE 5.2-4 (Continued)

Modifications were recommended in several areas. Where the operators and supervisors were required to have sufficient experience and training, a qualifying statement was added to permit Spacecraft crew operation and ground supervision. The requirments on facilities and equipment needed special consideration in order to handle waste in a space system, to allow recycling systems and to permit remote operation and monitoring of automatic processing systems. Weighing in space poses technical problems and thus, reference to any weighing operation had to be replaced with adequate substitutes. None of the FDA regulations were deemed major impediments to implementation of a space processing program.

In general, this regulation is organized into five subdivisions: general provisions, construction and maintenance of facilities and equipment, product quality control, packaging and labeling, and records and reports. The general provisions regulation allows use of automatic equipment and specifies certain personnel requirements for those persons involved in drug processing. The section headed construction and maintenance of facilities and equipment lists requirements for buildings and equipment. The product quality control section regulates quality procedures, requirements for components (constituents), specifications for containers, and laboratory controls, including in-process controls. The packaging and labeling section enumerates controls for packaging and labeling drug products and in-process components. The records and reports subdivision stipulates requirements for all production, control and distribution records.

The implications contained in these regulations are, by nature, subject to interpretation and conjecture. Since compliance to regulation includes meeting the intent as contained in these implications, sufficient understanding of these is important. The overriding consideration of the regulations is product safety as assured by adequate cleanliness, orderliness, and documentation before, during and after processing. A main overtone in the regulations dictates the desirable approach to processing drugs: dedicated and proper hardware, informed and trained operators, sufficient controls and monitors to act as checks and balances, appropriate documentation to ensure product conformance to established standards and traceability of distributed drugs, and orderly handling of products and components. Since the philosophies in the aerospace world are contiguous to this approach, compliance to drug regulations should not be insurmountable.

5.2.3 Process Evaluation

Evaluating the processes for the space production of the three selected pharmaceutical products requires definition of mass flow rates and capacities for the equipment required for the process. These mass balances are defined for three cases; 1) accomplishing the entire production cycle in space, 2) accomplishing the entire production cycle on the ground, and 3) the use of a combination of space and ground production that could be more advantageous than either of the previous alternatives. In order to make a meaningful comparison, consistent, reasonable ground rules and assumptions were established. These are described in the following paragraph. The mass balances for the production of erythropoietin, beta cells, and Antihemophilic Factor VIII are presented in subsequent paragraphs. Man interface requirements are discussed in Section 5.3. It is important to the optimization of a space production system for demonstration that the tradeoffs presented in the comparison of space-only production cycles, ground production cycles and space/ground combinations are used to re-evaluate the product production rates established in the Product Requirements and Analysis section. The results of this overall evaluation were used as the basis for the definition of the Multi-Product Process System described in Section 5.4 of this report.

Ground Rules and Assumptions

Ground rules and assumptions are required to make comparative evaluations of processing pharmaceutical products in space. Assumptions are required in the areas of tissue culture, electrophoretic separation, transportation of raw materials, cell handling, and recycle capability. Because of the variability of biological systems, rational and conservative assumptions were made with respect to the tissue culture requirements, applicable either to suspension culture or monolayer culture on beads. These ground rules are summarized in Table 5.2-1. These assumptions are: that cells can be inoculated at a concentration of 4.4×10^5 cells/ml. The nutrient required at this concentration is .019 g/ml/day. The doubling time of cells was assumed to be 35 hours. The concentration of cells during production was assumed to be 3×10^6 cells/ml. For those production cycles which require the culture medium, the medium was assumed to be 0.7% by weight solid protein. This culture medium corresponds to nutrient that is 10% fetal calf serum by volume. In growing cells as a product, it was assumed that primary cells grown to maximum density can be removed at a rate of one-half their concentration per

TABLE 5.2-1

PROCESS EVALUATION GROUNDRULES

- o CELL CULTURE
  - INOCULATE AT 4.4×10^5 CELLS/ML
  - CULTURE TO 3×10^6 CELLS/ML
  - NUTRIENT REPLACEMENT 0.019 GM/ML-DRY
  - MEDIA 0.7% PROTEIN (FETAL CALF SERUM)
  - DOUBLING TIME 35 HOURS
  - ERYTHROPOIETIN PRODUCTION RATE 9.5×10^{-6} UNIT/CELL-DAY
- o ELECTROPHORESIS
  - CELLS
    - GROUND - 1 ML/HR AT 1×10^6 CELLS/ML
    - SPACE - 1 ML/HR AT 1×10^8 CELLS/ML
    - COLLECTED BANDWIDTH - 50% FOR CULTURE INOCULUMS
      90% AFTER CULTURE
  - PROTEINS
    - GROUND - 18 ML/HR AT 0.1% PROTEIN
    - SPACE - 36 ML/HR AT 10.0% PROTEIN
- o GENERAL
  - TRANSPORTATION COST $700/KG
  - 1 KG TANKAGE/KG SUPPLIES
  - 0.6 KG PLASTIC BAGS/KG CRYOPRECIPITATE
  - FREEZE/THAW CELL VIABILITY LOSS 50%
  - 10% LOSS FOR WATER RECLAMATION
  - 360 DAY PRODUCTION CYCLE
- o MARKET GOALS
  - ERYTHROPOIETIN 30% (1.3×10^9 UNITS)
  - BETA CELLS 20% (1.9×10^{11} CELLS)
  - ANTIHEMOPHILIC FACTOR 30% (1.5×10^8 UNITS)

doubling period for 21 days before restarting the culture. In growing cells that generate protein as a product, it was assumed that the cells would have four production periods, each of which would be 14 days, for a total of 56 days per cycle.

Ground rules made with respect to electrophoresis chamber performance are summarized in Table 5.2-1. The sample flowrate for protein separation was assumed to be 36 ml/hr at a resolution quality of 0.3×10^{-4} cm^2/volt-sec. If it is found in future work that this resolution quality is not sufficient, then the number of chambers and the electrical power requirements changes will be inversely proportional to the change in resolution quality. Sample flowrates for cell separation were assumed to be 1 ml/hr. Allowable concentration of protein samples is assumed to be 10% (weight/volume) for space separation and 0.1% concentration for ground separation at comparable resolution. The corresponding cell concentrations were assumed to be 1×10^8 cells/ml for space separation and a reduction to 1×10^6 cells/ml for ground separation at comparable resolution. The 10^6 cells/ml ground limitation is the expected maximum concentration level that will allow sample streaming and subsequent separation in a one-g environment. Cells, however, can be readily concentrated to 10^8/ml without clumping and this concentration level is expected to be the microgravity limit. The higher concentration estimated for both cells and proteins is due to elimination of gravity effects on both buffer flow and sample flow. In addition, it is expected that for proteins in particular, higher allowable centerline temperature rise would allow doubling unit thickness with a corresponding increase in throughput. In separation of cells for initial culture the yield was reduced to 50% so that a corresponding increase in purity could be obtained assuming a normal distribution of cells over the outlet tubes. After culture, in subsequent electrophoresis, a wider fraction corresponding to a 90% yield was taken because of a predominance of the desired cell type.

These and other miscellaneous assumptions are presented in Table 5.2-1 including those regarding transportation. Shuttle capacity was assumed to be 29,400 kg and one kilogram of tankage was assumed for each kilogram of supplies, with the exception of the lightweight plastic bags for cryoprecipitate which have a mass ratio of 0.6 kg/kg. The cell viability loss from the freeze/thaw cycle was assumed to be 60% based on ASTP experience. Finally, in reclamation of water for space production a 10% loss is assumed.

ERYTHROPOIETIN MASS BALANCE

Space - The mass balance for the production of erythropoietin (ESF) in space is as shown in Figure 5.2-5. To satisfy 30% of the market for erythropoietin, 2.1×10^9 erythropoietin units are required per year. The number of high yield cells required to produce this amount can be estimated based on ASTP MA-011 data (16). The highest yield fraction and subsequent subculture was 14-2 which produced 5 units/ml after being on production media for 35 days. This subculture was grown in a 25 cm^2 T-flask in 10 ml of medium. What is not known, however, is the period of time that was actually required to reach 5 units/ml erythropoietin concentration since intermediate data were not taken. Assuming that erythropoietin production by the cells is inhibited by the concentration in the medium, then production possibly could have taken only one day or as many as the entire thirty-five. For purposes of sizing the system it was assumed that production required seven days. With this assumption and an assumed cell density, equivalent to 3×10^4 cells/cm^2 in monolayer on beads, we can estimate the erythropoietin production rate per cell per day to be:

$$\frac{5 \text{ Units/ml}}{7 \text{ Days}} \times \frac{10 \text{ ml}}{\text{Flask}} \times \frac{\text{Flask}}{25 \text{ cm}^2} \times \frac{\text{cm}^2}{3 \times 10^4 \text{ cells}} = 9.52 \times 10^6 \frac{\text{Units}}{\text{Cell-Day}}$$

and for 2.1×10^9 units we need 2.2×10^{14} cell-days. Because cells must first be cultured before production, production will not be continuous. It is assumed that primary cells can be cultured, put on production for 14 days, and then most of them discarded and the culture restarted. The cells would be restarted three times, that is for a total of four production periods. Each restart from about 10% of the former population would require about 7 days to reach production. The primary cell growth period would be 20 days however, because of the small inoculum obtained by cell electrophoresis. The resulting cycle is shown in Figure 5.2-6. As shown, the entire production cycle is 97 days. One year would have 3 complete cycles plus a partial cycle including 2 of the 14 day production periods, or a total of 196 production days. Therefore, the daily number of cells required is:

$$\frac{2.2 \times 10^{14} \text{ cell days}}{196 \text{ Production Days}} = 1.12 \times 10^{12} \text{ cells}$$

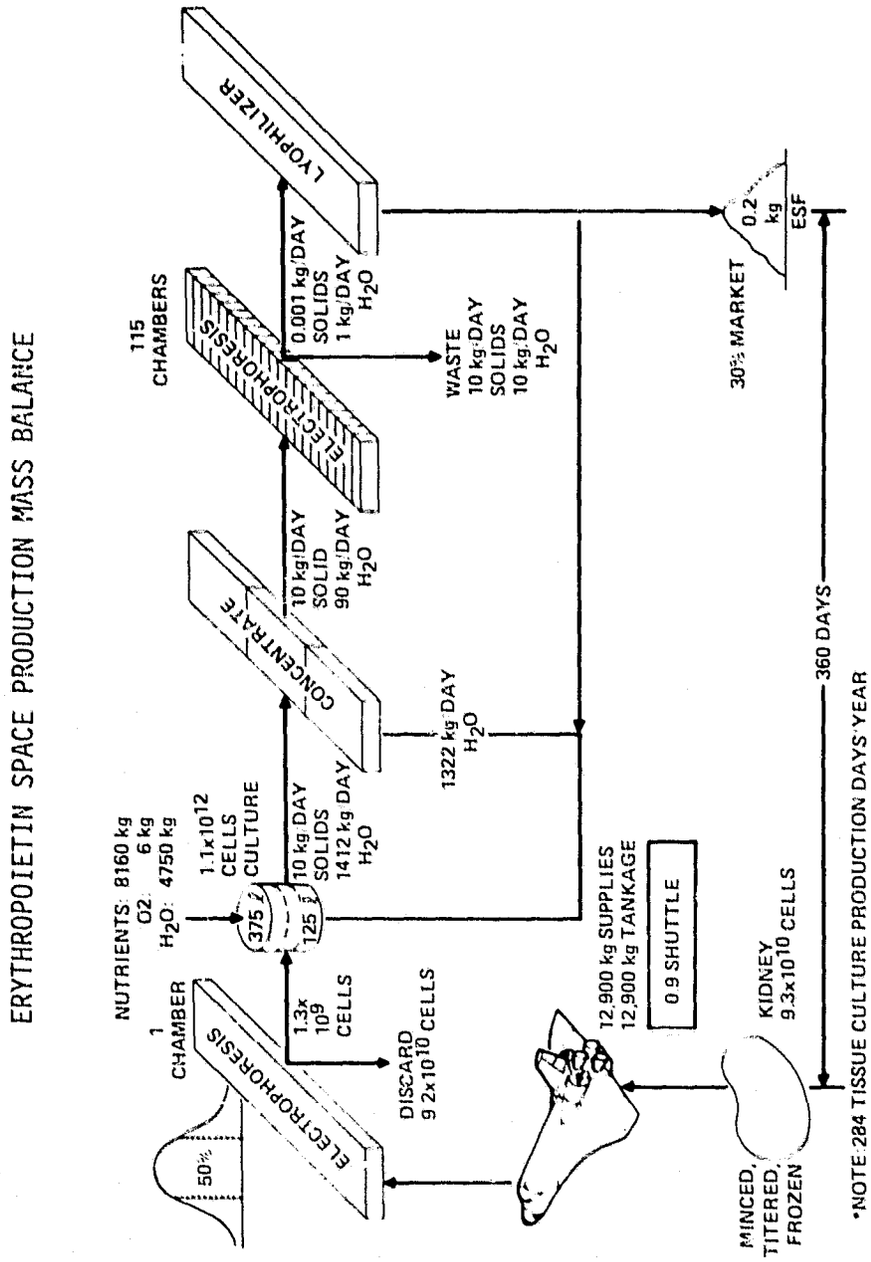

ERYTHROPOIETIN SPACE PRODUCTION MASS BALANCE
LYOPHILIZER
115 CHAMBERS
ELECTROPHORESIS
CONCENTRATE
1 CHAMBER
ELECTROPHORESIS
50%
NUTRIENTS 8160 kg
O2 6 kg
H2O 4750 kg
1.1x1012 CELLS CULTURE
375 ℓ
125 ℓ
0.001 kg/DAY SOLIDS 1 kg/DAY H2O
10 kg/DAY SOLID 90 kg/DAY H2O
10 kg DAY SOLIDS 1412 kg DAY H2O
1322 kg/DAY H2O
WASTE 10 kg/DAY SOLIDS 10 kg/DAY H2O
1.3x 10^9 CELLS
DISCARD 9 2x10^10 CELLS
12,900 kg SUPPLIES
12,900 kg TANKAGE
0 9 SHUTTLE
KIDNEY 9.3x10^10 CELLS
MINCED, TITERED, FROZEN
30% MARKET
0.2 kg ESF
360 DAYS
*NOTE 284 TISSUE CULTURE PRODUCTION DAYS/YEAR

FIGURE 5.2-5

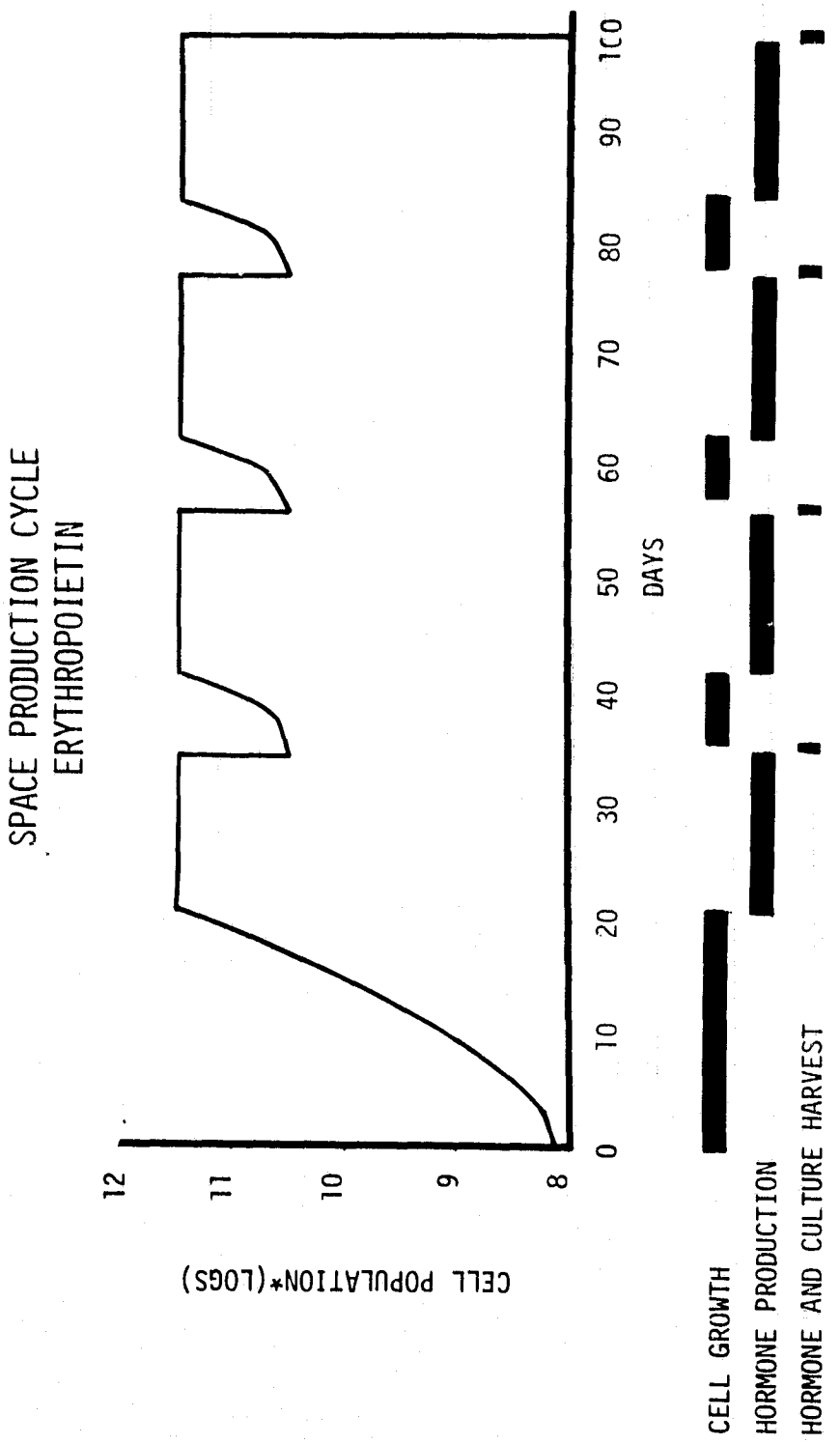

SPACE PRODUCTION CYCLE
ERYTHROPOIETIN
CELL POPULATION*(LOGS)
12
11
10
9
8
0
10
20
30
40
50
60
70
80
90
100
DAYS
CELL GROWTH
HORMONE PRODUCTION
HORMONE AND CULTURE HARVEST
* 125 LITER CULTURE
3 UNITS WORKING 3.3 CYCLES/YEAR

FIGURE 5.2-6

At a cell density of 3×10^6 cells/ml the culture volume required is 375 which would be divided into 3 tanks to provide a continuous electrophoresis. Initially, these tasks would require 20 days to reach 3×10^6 cells/ml of which 3 days is for startup, therefore for a 35 hour doubling time the initial inoculm is:

$$\left(\frac{1.12 \times 10^{12}}{3}\right) e^{-\left(\frac{17 \times 24}{35}\right) \ln 2} = 1.16 \times 10^8 \text{ cells}$$

As the initial inoculum should be at approximately 4×10^5 cells/ml, the initial culture volume should be about 290 ml and provisions would have to be made for the main culture tank and a 6 liter startup tank to have a volume variation of 20:1. Since only about 7.6% of kidney cells are high producers of erythropoietin and 60% are lost in the initial freeze/thaw cycle, 10% are lost in storage at 4°C, and initial cell electrophoresis has a yield of 50%, the number of kidney cells required is:

$$\frac{1.16 \times 10^8}{(0.076)(0.4)(0.9)(0.5)} = 8.48 \times 10^9 \text{ Cells/Cycle}$$

At 1×10^8 cells/ml the electrophoresis time required is 84.8 hours or about 4 days. Now since there are 360/97 inocula required per tank per year, the total kidney cells required for the initial electrophoretic separation are:

$$8.48 \times 10^9 \times \frac{360}{97} \times 3 = 9.4 \times 10^{10} \text{ cells}$$

And the yearly output of erythropoietin producing cells by the cell electrophoresis unit to inoculate the culture tanks is:

$$1.16 \times 10^8 \times \frac{360}{97} \times 3 = 1.29 \times 10^9 \text{ cells}$$

The culture medium and the protein must be removed from the tank at the concentration limit so that the erythropoietin concentration in the medium is maintained at 5 units/ml. Since two out of three culture tanks will be in production at any one time there will be $2/3 \times 1.12 \times 10^{12}$ cells producing erythropoietin and the production rate will be:

$$\frac{2}{3} \times 1.12 \times 10^{12} \text{ cells} \times 9.52 \times 10^{-6} \frac{\text{units}}{\text{cell-day}} = 7.108 \times 10^{6} \frac{\text{units}}{\text{day}}$$

At 5 units/ml the required flow is 1422 liters/day. This medium flow has about 0.7% by weight protein solids, most of which is the protein of the fetal calf serum used as nutrient. Therefore, to concentrate this medium to 10% protein by mass, the concentration outflow needs to be:

$$\frac{0.7\%}{10\%} \times 1422 \frac{\text{liters}}{\text{day}} = 100 \frac{\text{liters}}{\text{day}}$$

Since each electrophoresis chamber is assumed to have a throughput of 36 ml/hr the number of electrophoresis chambers required is:

$$100 \frac{\text{liters}}{\text{day}} \times \frac{\text{hr-chamber}}{.036 \text{ liter}} \times \frac{\text{day}}{24 \text{ hr}} = 115 \text{ chambers}$$

The water recycled directly to the culture is 1422 kg/day minus 100 kg/day or 1322 kg/day. Of the 100 kg/day undergoing electrophoresis, there is 10 kg/day lost and 80 kg/day of water recovered. About 10 kg of solids contain erythropoietin, most of which is waste. To support the level of tissue culture required it is estimated that 8160 kg of nutrients, 6 kg of oxygen, and 4750 kg of H_2 are required, for a total of 12,900 kg of supplies and a like amount of tankage. Together these masses constitute about 0.88 of a Shuttle payload capability.

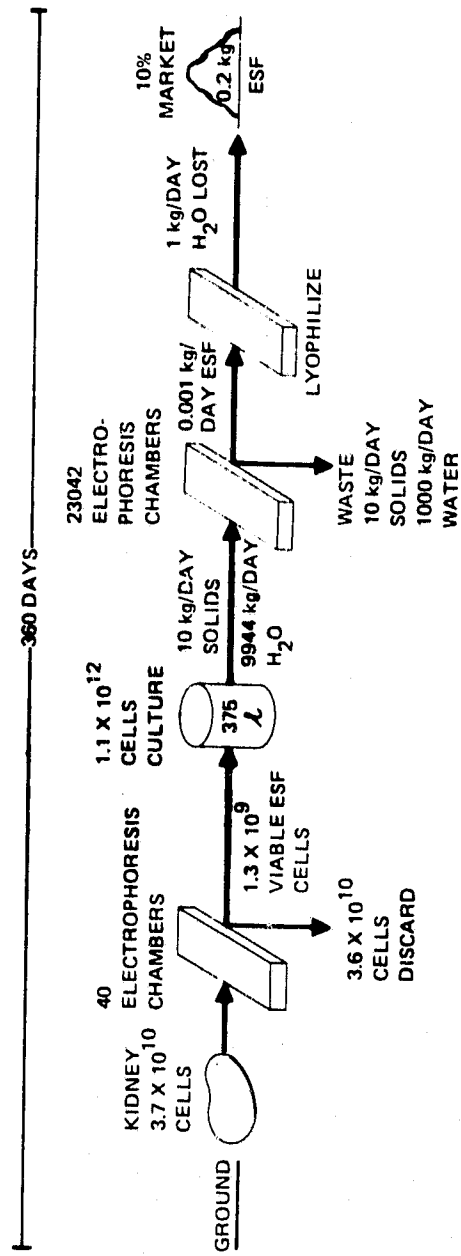
Ground - The mass balance for the ground production of erythropoietin (ESF) is as shown in Figure 5.2-7. The mass balance is for the production of 2.1×10^9 ESF units per year, satisfying 30% of the potential market. This amount requires the same 2.2×10^{14} cell-days of production calculated for the space mass balance. The production cycle derived for space operations would also be used for ground production. The principal difference between ground and space production is in the throughput for cell and protein electrophoresis. For space electrophoresis of cells the input stream can be concentrated to about 1×10^8 cells/ml, which is somewhat below actual limits of physical packing for typical cell sizes. The limiting concentration for cell electrophoresis on the ground is approximately two orders of magnitude lower or 1×10^6 cells/ml, for comparable separation quality. Another difference between the ground and space mass balance is that the 60% loss estimated for freezing and thawing of cells can be eliminated. Therefore the total number of kidney cells required per cycle is not the 8.48×10^9 cells required for space production, but 40% of that number or 3.4×10^9 cells. For the same cell electrophoresis time used during space production (84.8 hours), the number of comparable electrophoresis chambers required at a volumetric throughput of 1 ml/hour is:

$$\frac{1}{\frac{1 \text{ ml}}{\text{hr-unit}}} \times \frac{1}{84.8 \text{ hr}} \times \frac{3.4 \times 10^9 \text{ cells}}{1 \times 10^6 \frac{\text{cells}}{\text{ml}}} = 40 \text{ chambers}$$

This says that forty times as many chambers are required to obtain the same output of viable cells on the ground.

An even larger increase in the number of electrophoresis units occurs in the separation of erythropoietin from production media. For the space production case the medium, which contained about 0.7% protein solids by weight corresponding to 10% fetal calf serum by volume, was concentrated to 10% protein by weight. For ground electrophoresis the practical limit for protein concentration is about 0.1% by weight, therefore the medium must undergo a 7:1 dilution before electrophoresis. Just as for the production in space, the required outflow of medium to limit the concentration to 5 units/ml is 1422 liters/day. For ground production then the electrophoresis input rate including the 7:1 dilution is:

ERYTHROPOIETIN GROUND PRODUCTION MASS BALANCE



NOTE: 284 TISSUE CULTURE PRODUCTION DAYS/YEAR

FIGURE 5.2-7

$$\frac{0.7\%}{0.1\%} \times 1422 \frac{\text{Liters}}{\text{Day}} = 9954 \frac{\text{Liters}}{\text{Day}}$$

Since the protein throughput of the space electrophoresis chamber was estimated to be 36 ml/hr and the space chamber was double the thickness of the ground chamber, the corresponding ground chamber throughput would be 18 ml/hr. At 18 ml/hr the required number of electrophoresis units would be:

$$9954 \frac{\text{Liters}}{\text{Day}} \times \frac{\text{Hr}}{0.018 \text{ Liter}} \times \frac{\text{Day}}{24 \text{ Hr}} = 23042 \text{ Chambers}$$

Similarly, to support the required level of tissue culture, the same amount of nutrients are required for tissue culture, however water reclaimation is not necessary because of ready availability.

Space/Ground - A combination of space/ground production of erythropoietin is illustrated by the mass balance shown in Figure 5.2-8. Because the principal advantage of microgravity is the increased cell and protein electrophoresis throughput, and tissue culture in space requires transportation of nutrients, it is expected to be more desirable than either all-space or all-ground production. As illustrated by the figure, the transportation involved causes production to be a serial process consisting of cell electrophoresis in space, cell culture and production medium lyophilization on the ground, and protein electrophresis in space. Mission duration for the space proteins were selected to be representative of possible Shuttle on orbit operations, with a seven day period of cell electrophoresis and a longer, 30 day, period for protein electrophoresis, to minimize the number of chambers required.

The time remaining for tissue culture is 328 days and it was assumed that three tissue culture tanks would be used to obtain approximately constant input to the lyophilizer, with two tanks on production most of the time. Because initial startup would be staggered at 7 day intervals, the last-tank-started cycle would have 328 days minus 14 days or 314 days. Therefore, in all cases, there would be 3 complete 97 day production cycles. The remaining 23 days could then be apportioned for the ground logistics associated with the two Shuttle flights. Since

ERYTHROPOIETIN SPACE/GROUND PRODUCTION

MASS BALANCE

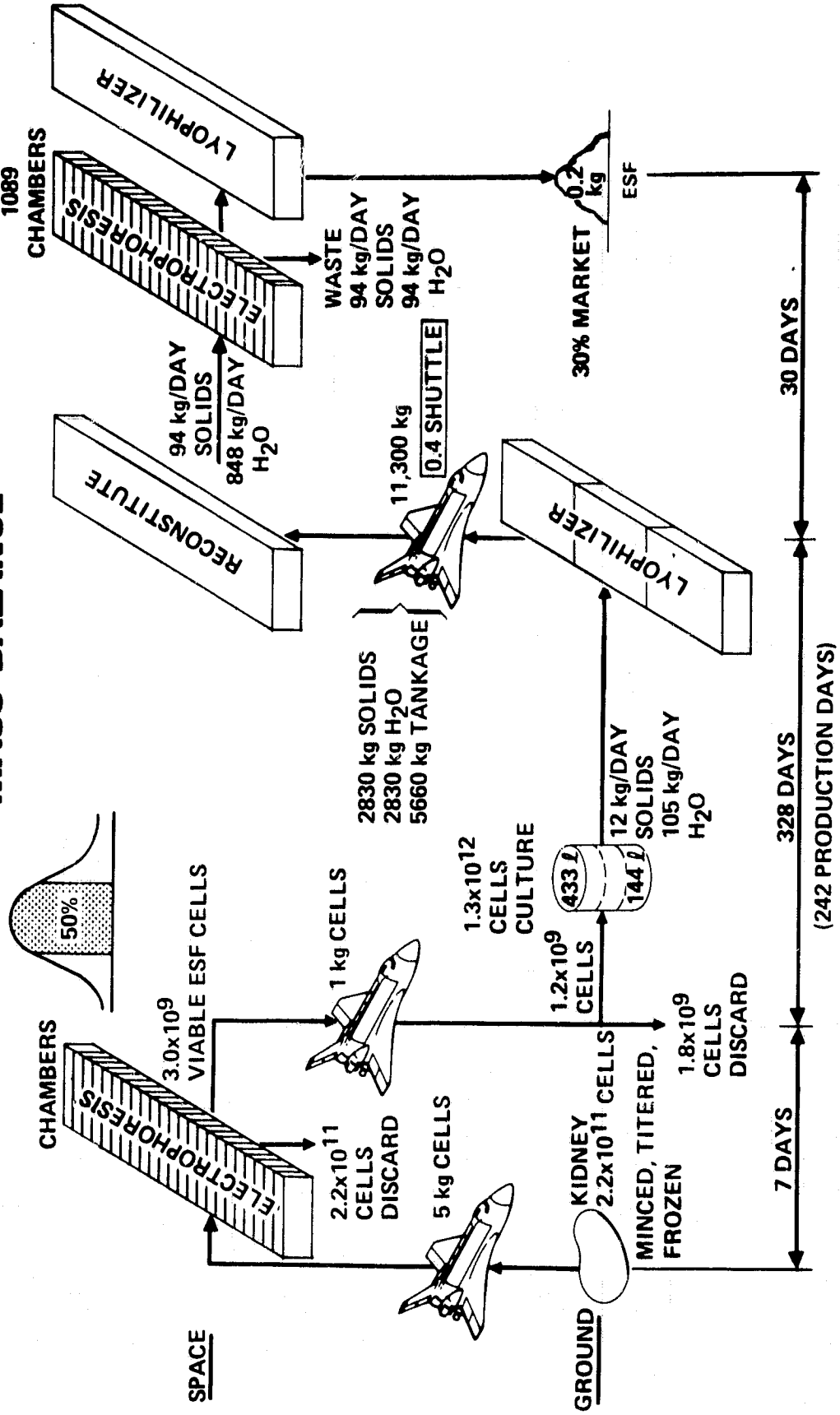


FIGURE 5.2-8

this cell production cycle has only 168 days, compared to 196 days for all space production, size of the culture tanks will have to be increased accordingly. As for space production 2.2×10^{14} cell-days of production are required and the number of cells per tank is:

$$\frac{2.2 \times 10^{14} \text{ cell days}}{168 \text{ production days}} = 1.3 \times 10^{12} \text{ cells}$$

And at a cell density of 3×10^6 cells/ml the culture volume required is 433 liters, which divided into 3 tanks is 144 liters per tank. Initially these tanks would require 20 days to reach 3×10^6 cells/ml of which 3 days is for startup, therefore for a 35 hour doubling time the initial inoculum is:

$$\left(\frac{1.3 \times 10^{12}}{3}\right) e^{-\left(\frac{17 \times 24}{35}\right) \ln 2} = 1.34 \times 10^8 \text{ cells}$$

As the initial inoculum should be at approximately 4×10^5 cells/ml, the initial culture volume should be about 335 ml and provisions would have to be made for a main culture tank and a 7 liter startup tank with a volume variation capability of about 20:1. Since only 7.6% of kidney cells are high producers of erythropoietin and there are two freeze/thaw cycles with a loss of about 60% each, in addition to 10% storage loss at 4°C, the number of kidney cells required including a 50% high resolution electrophoresis yield is:

$$\frac{1.34 \times 10^8}{(.076)(0.4)(0.4)(0.9)(0.5)} = 2.45 \times 10^{10} \frac{\text{cells}}{\text{cycles}}$$

At 1×10^8 cells/ml and 1 ml/hr the chamber time required is 245 hours, which exceeds the desired 7 day mission. Therefore about 1.46 units are required to compress the time to 7 days. In addition, there are 3 production cycles per tank and 3 tanks. Therefore, the total number of cell electrophoresis chambers required is:

$$1.46 \frac{\text{Chambers}}{\text{Cycle}} \times \frac{3 \text{ Cycles}}{\text{Year}} \times 3 \text{ Tanks} = 14 \text{ Chambers}$$

The effect of reducing the number of days available for product electrophoresis due to the serial nature of space/ground production is to increase the number of chambers required. Space production required 115 chambers operating for 284 production days or 32660 chamber-days. To do the same amount of separation in 30 days, the number of chambers would be:

$$\frac{32660 \text{ Chamber Days}}{30 \text{ Days}} = 1089 \text{ Chambers}$$

The amount of nutrients required for ground tissue culture is the same as for space culture, except that water is not reclaimed.

Comparison - Comparison of space production, ground production, and the space/ground combination is presented in Figure 5.2-9. The basis for comparison is ground production which would require 23,042 electrophoresis chambers to produce enough erythropoietin for 10% of the potential market. For space production, only 115 chambers would be required, but 26,800 kg of supplies and tankage would have to be transported. At a cost of $700/kg, the transportation cost would be $18.7M. The elimination of 22,927 chambers, with all the complexity, power consumption, and buffer loss makes all space production a better choice. However, the best choice is probably the space/ground combination, in which 974 more chambers are required with the same buffer loss and power consumption, but the added complexity of tissue culture in space is eliminated. In this case transportation of nutrients is eliminated in favor of lyophilized protein reducing transportation cost to $8.2M for 11,300 kg of supplies and tankage. That the space/ground combination is the best choice is even more clear, if the electrophoresis operation is made to be continuous over a multi-year period. Then the number of electrophoresis chambers required would be substantially reduced and be comparable to the space production case.

ERYTHROPOIETIN PRODUCTION COMPARISON

$264M VALUE	CULTURE SIZE	ELECTROPHORESIS CHAMBERS	TRANSPORTATION (SUPPLIES ONLY)
GROUND	375L	23042	
SPACE	375L	115	$18.7M
GROUND/SPACE	433L	1089	$ 8.2M

CONCLUSIONS:

- o GROUND, TOO MANY CHAMBERS
- o SPACE, STILL ONE SHUTTLE
- o SPACE/GROUND COMBINATION NOT ATTRACTIVE AT PRESENT TIME
- o ERYTHROPOIETIN PROBABLY NOT VIABLE EARLY PRODUCT
- o PRODUCTION RATE SHOULD BE INVESTIGATED

FIGURE 5.2-9

AHF (CRYOPRECIPITATE) MASS BALANCE

Cryoprecipitate is a serum product obtained by freezing whole serum and collecting the precipitate which fails to redissolve when the serum is allowed to thaw at 4°C. One pint of serum yields about 2 to 3 ml of material termed cryoprecipitate. This 2 to 3 ml contains approximately 125 units of AFH VIII activity (about 50 units/ml), and about 550 mg of total protein solids in addition to AHF. A typical analysis of this variable product shows these solids consist of Fibrinogen (31.8%), Albumin (6.8%), alpha globulin (0.9%), beta globulin (30.5%), gamma globulin (20.9%) and unidentified material (10%).

Separation of factor VIII from the other impurities consists of dissolving the cryoprecipitate in an appropriate buffer and processing it through a continuous free-flow electrophoresis unit. The concentration of protein solids can be greater during space electrophoresis due to the microgravity environment. For the purpose of comparing a ground vs space separation we have chosen 0.1% protein concentration limit for ground separation and 10% concentration for space separation.

Two cases are described, one in which cryoprecipitate is separated on the ground to yield 18 grams of AHF and the other to yield the same amount by processing in space. Both processes are based on a period of 360 days. By-products are also considered as they make up the bulk of the starting material. The starting material is 3000 kg of cryoprecipitate in both cases.

Space Process - The cryoprecipitate space process is shown in Figure 5.2-10. Frozen cryoprecipitate is flown to the facility via Shuttle where it is thawed, diluted 1:1 with buffer and electrophoretically separated at a rate of 36 ml/hour. The 3000 kg of product can be processed at a rate of 3000 kg/360 days or 8.33 kg per day. With 8.33 kg buffer added per day this requires processing 16.7 kg day through the electrophoresis separation or at 36 ml/hour the number of electrophoresis chambers is:

$$\text{Electrophoresis Chambers} = \frac{16.7 \text{ kg/day} \times \frac{10^3 \text{ml}}{\text{kg}} \times 360 \text{ day}}{36 \text{ ml/hr} \times \frac{24 \text{ hr}}{\text{day}} \times 360 \text{ day}} = 19.3$$

CRYOPRECIPITATE SPACE PROCESS

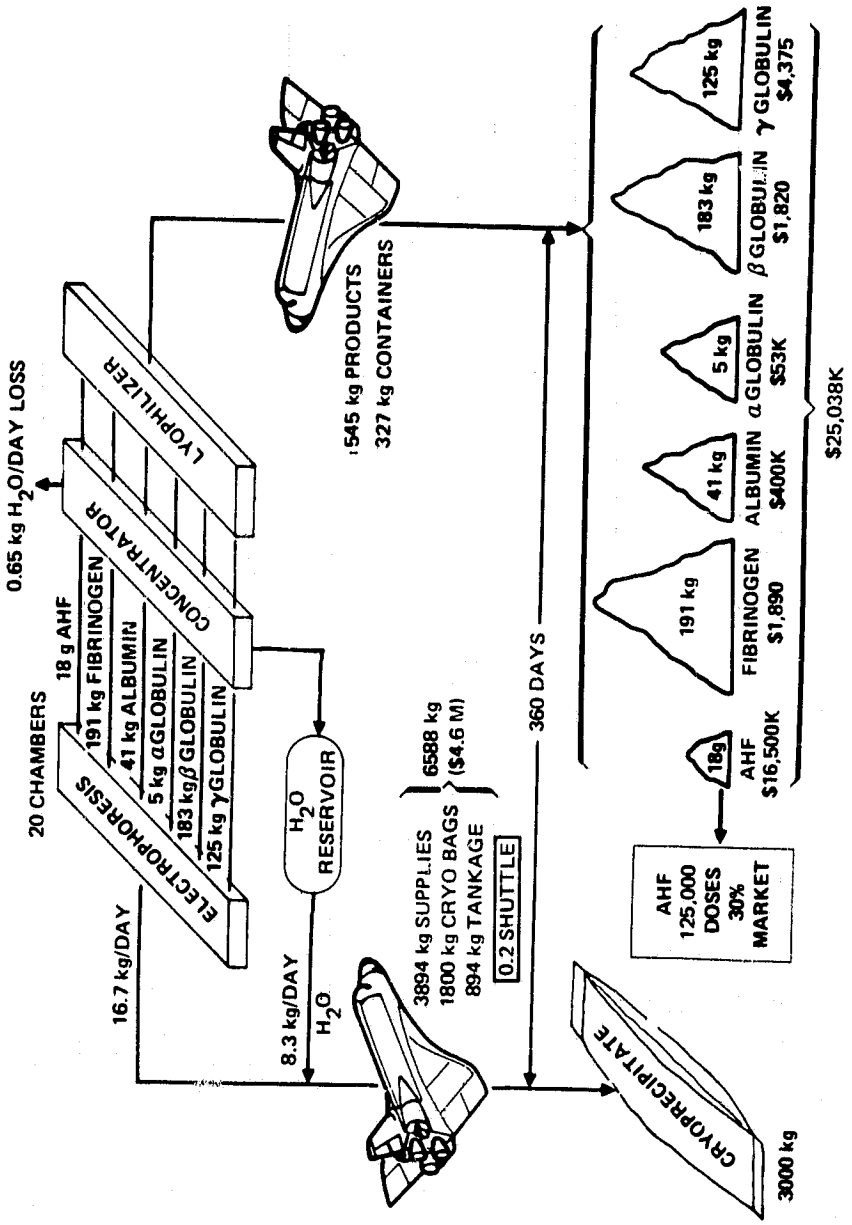


FIGURE 5.2-10

or about 20 electrophoresis chambers designed to operate at 36 ml/hr sample throughput. During this process the cryoprecipitate is separated into 6 individual fractions containing factor VIII, Fibrinogen, Albumin, Alpha globulin, Beta globulin, and Gamma globulin. These materials are passed through a membrane concentrator where most of the water is reclaimed for processing.

For this process, since cryoprecipitate is diluted 1:1 with buffer, a total of 3000 kg of buffer is required to process 3000 kg of cryoprecipitate. A portion of this can be claimed from the cyroprecipitate since it contains 78% water. Thus, 3000 x 0.78 or 2340 kg can be reclaimed. Assuming a 10% loss of water during reclamation, a total of 2340 minus 234 or 2106 kg is reclaimed. Thus, 3000 minus 2106 or 894 kg of water are required to be shipped and held in reserve for processing. At a process rate of 16.7 kg/day, we lose 1.5 kg/day of water and reclaim 13.3 kg/day, which is returned to the reservoir for further usage. The concentration materials are then lyophilized and returned to earth. During the lyophilization process, final traces of moisture are removed and this water is returned to the reservoir for reuse. This process requires the transport of 3000 kg cryoprecipitate, 894 kg H_2O, 894 kg tankage and 1800 kg of cryoprecipitate containers, or 6588 kg total transport weight. At \$700/kg this equals a transportation cost of \$700 x 6588 = \$4.6$\overline{M}$.

The amount of 18 grams of AHF VIII obtained will supply 30% of the total market for this product. The selling price of 11¢/AHF unit provides an AHF value of \$16.5$\overline{M}$. By-product values were determined from current catalog prices for research grade materials. By-product quantities were determined from the results of a single MDAC experiment. If these by-products were upgraded to injectables, the value would be substantially increased.

Ground - There are two major differences between separation of cryoprecipitate products on the ground as opposed to space processing. One of these is the size of the ground electrophoresis compared to that of the space chamber. The ground chamber is 1/2 the thickness of the space unit and thus has a throughput of 1/2 that obtained in space. We are assuming that a 10% sample concentration can be processed in space compared to a 0.1% concentration on the ground. The process for the ground separation is shown in Figure 5.2-11.

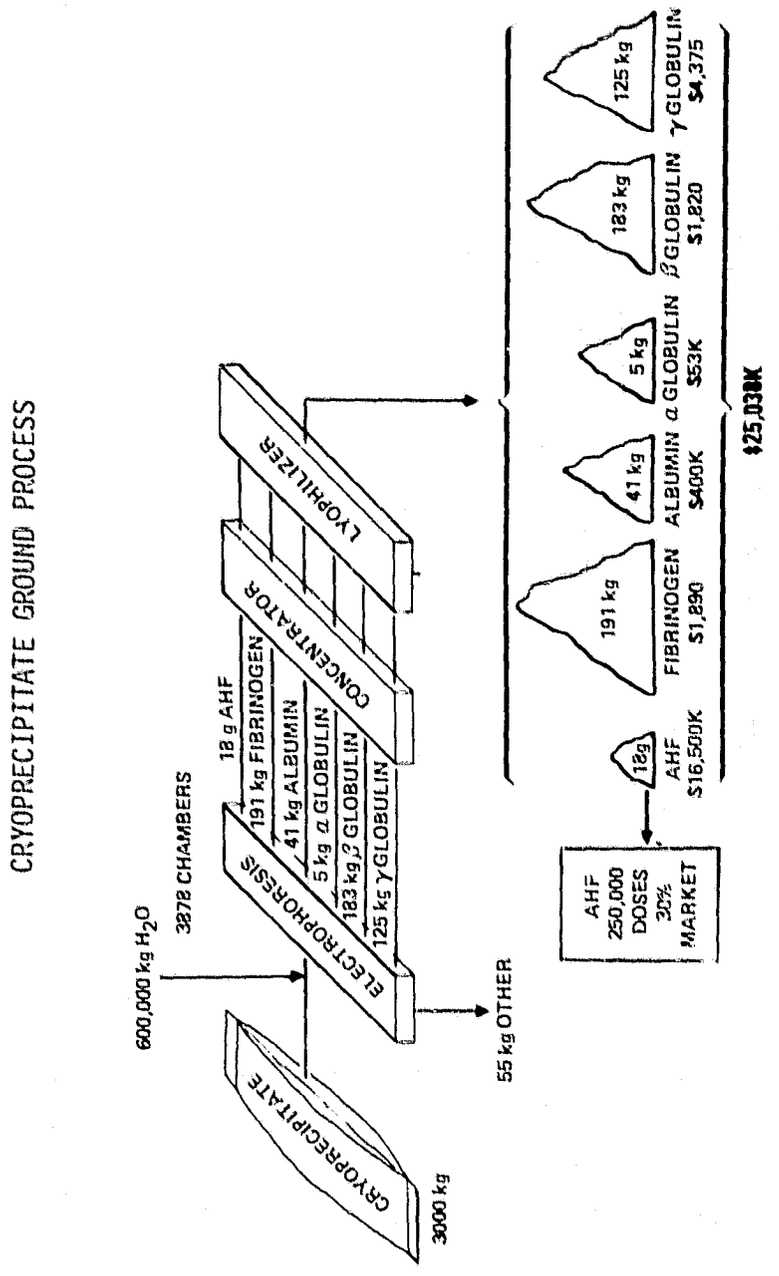

CRYOPRECIPITATE GROUND PROCESS
600,000 kg H_2O
3878 CHAMBERS
CRYOPRECIPITATE
3000 kg
55 kg OTHER
18 g AHF
191 kg FIBRINOGEN
41 kg ALBUMIN
5 kg α GLOBULIN
183 kg β GLOBULIN
125 kg γ GLOBULIN
ELECTROPHORESIS
CONCENTRATOR
LYOPHILIZER
18g
AHF
$16,500K
191 kg
FIBRINOGEN
$1,890
41 kg
ALBUMIN
$400K
5 kg
α GLOBULIN
$53K
183 kg
β GLOBULIN
$1,820
125 kg
γ GLOBULIN
$4,375
$25,038K
AHF
250,000
DOSES
30%
MARKET

FIGURE 5.2-17

As with space processing, 3000 kg of cryoprecipitate is used as the starting material. However, instead of diluting the sample at a ratio of 3000 kg cryoprecipitate to 3000 kg of water, which results in a protein concentration of about 10% separation, the dilution ratio is 3000 kg cryoprecipitate to 600,000 kg of water to give a concentration of about 0.1% protein for ground separation.

The number of electrophoresis chambers required to process this volume is:

$$\frac{600{,}000 \text{ Liters}}{0.018 \text{ Liters/Hr}} \times \frac{1}{360} \times \frac{1}{24} = 3877.3 \text{ Chambers}$$

or about 3878 electrophoresis chambers.

As in the space processing, the cryoprecipitate is separated into 6 major fractions of which the 18 gram yield of cryoprecipitate supplies 30% of the total market. By-product values are also as defined in the space process.

Comparison - Ground production of 18 grams of AHF would require 3878 electrophoresis chambers. For space production 20 chambers would be required, but 6588 kg of supplies and tankage would have to be transported. At a cost of $700/kg, the transportation cost would be $4.6M. The elimination of 3858 chambers, with all the complexity, power consumption and buffer loss may make space a better choice for AHF purification. An important driver may be the necessity for high purity AHF since higher purity can be obtained in space than on the ground. This consideration was not made in the analysis but should be considered in future efforts. Figure 5.2-12 illustrates the comparison.

ANTIHEMOPHILIC FACTOR PRODUCTION COMPARISON

$25M VALUE	CULTURE SIZE	ELECTROPHORESIS CHAMBERS	TRANSPORTATION (SUPPLIES ONLY)
GROUND	N/A	3878	
√ SPACE	N/A	20	$3.4M

CONCLUSIONS:

- o GROUND, TOO MANY CHAMBERS
- o SPACE REDUCES CHAMBERS REQUIRED
- o SPACE PRODUCTION BENEFITS AHF

FIGURE 5.2-12

BETA CELL PURIFICATION FROM PANCREAS HOMOGENATE

Three cases are treated for Beta Cell purification. They are 1) space culture and space electrophoresis, 2) ground culture and space electrophoresis, and 3) ground culture and ground electrophoresis. Each process is to require a one year (360 day) period.

In all cases a pancreas is minced, and the cells titered and suspended in a suitable buffer at the appropriate concentration for each process. Cell inoculum is about 4.4×10^5 cell per ml of growth medium and the cell population, when production cycles begin, is assumed to be about 3.0×10^6 cells/ml.

In each case cells undergo a lag period of about 3 days followed by logarithmic growth, at which time the cell population doubles every 35 hours. Once in the production phase, cells are harvested by removing 50% of the culture fluid every 35 hours and replacing the fluid with fresh nutrients and returned medium. Nutrients are replaced at a rate of 0.02 gm/ml/day. All cell cycles consist of a 7 day growth cycle and 21 days of production. At the end of the production cycle, the culture vessel is voided to about the original inoculum volume and those remaining cells are used as a fresh inoculum to start a new culture.

In case #1, a total of 12.8 such cycles is used, for (12.8 x 21) = 269 production days. For case #2, 12 cycles of (12 x 21) = 252 production days are required.

Sample flow rates used in the electrophoresis unit are assumed to be the same for earth and space, 1 ml/hour; cell concentration used for the electrophoretic process is 1×10^8/ml in space and 1×10^6/ml on the ground. The higher concentration separable in space is the gain due to the microgravity environment.

Beta cells will undergo two electrophoresis steps regardless of the particular process. Cell yields from the first electrophoresis are assumed to be 50% and that from the final electrophoresis is assumed 90%.

Cell loss due to freezing and thawing is considered to be 60%, and water lost during the process is assumed to be 10%. The cost of space transportation is computed at $700 per kilogram.

Space Culture and Space Electrophoresis - For space culture of Beta cells, the pancreas cells are suspended in buffer at a concentration of about 1.0×10^8 cells/ml of buffer, frozen and transported to the space facility via Shuttle. This process is shown in Figure 5.2-13 where 6.8×10^9 cells are processed through the separator. On thawing, only 40%, or 2.72×10^9 cells remain viable. This suspension of both live and dead cells are processed through the electrophoresis unit at the rate of 1 ml/hour requiring:

$$\frac{68 \text{ ml}}{1 \text{ ml/hr} \times 24 \text{ hr/day}} = 2.83 \text{ Days}$$

or about 3 days process time.

Only 7.5% of the total pancreas cells are Beta cells and only 50% are recovered by this first electrophoretic process. Therefore the yield of cells after the first electrophoresis process will be

$$2.72 \times 10^9 \text{ Viable Cells} \times 0.075 \text{ Beta Cells} \times 0.5 \text{ Loss} = 1.02 \times 10^8 \text{ Purified Beta Cells}$$

This amount of cells is inoculated into 231 ml of tissue culture medium in a vessel with a maximum capacity of 1900 ml at a concentration of 4.4×10^5 cells/ml. In 7 days, growth will have occurred through the lag and logarithmic phases and a cell concentration of about 1.5×10^6 cells/ml will have been obtained. During this period the culture volume will have been increased periodically to the production volume of 1900 ml. In another 35 hours the cells will again double, to 3.0×10^6 cells/ml, at which time 50% of the cells can be harvested every 35 hours and the volume replaced with fresh nutrients and returned medium. This culture cycle is shown in Figure 5.3-14.

Thus,

$$1900 \text{ ml} \times 0.5 \text{ Volume Removed} \times (3 \times 10^6 \text{ cells/ml}) = 2.85 \times 10^9 \text{ cells/35 hour, or}$$

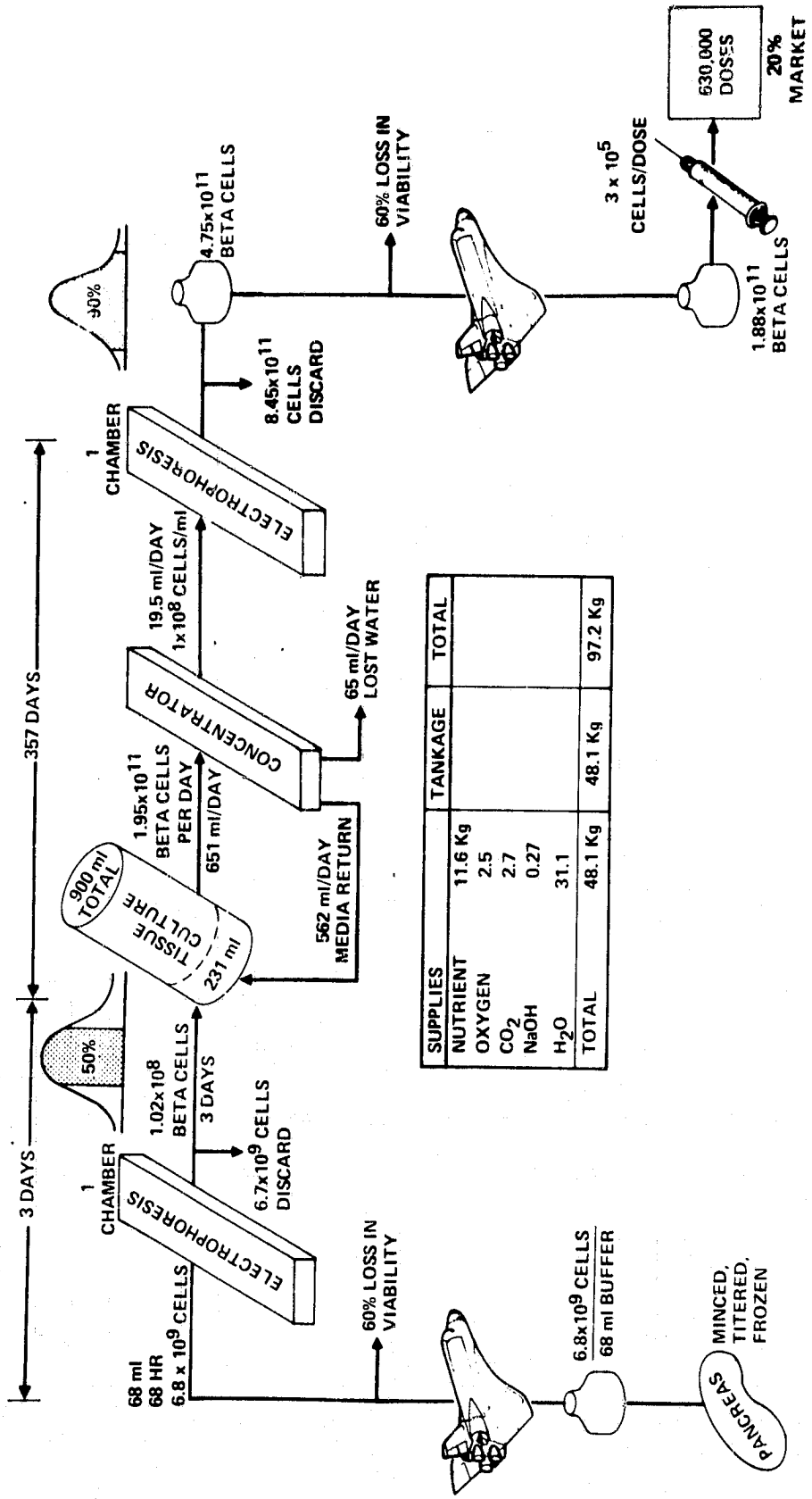

SPACE PRODUCTION - BETA CELLS
630,000 DOSES
20% MARKET
3 x 10^5 CELLS/DOSE
1.88x10^11 BETA CELLS
4.75x10^11 BETA CELLS
60% LOSS IN VIABILITY
8.45x10^11 CELLS DISCARD
90%
1 CHAMBER
ELECTROPHORESIS
19.5 ml/DAY
1x10^8 CELLS/ml
65 ml/DAY LOST WATER
CONCENTRATOR
1.95x10^11 BETA CELLS PER DAY
651 ml/DAY
900 ml TOTAL
TISSUE CULTURE
231 ml
562 ml/DAY MEDIA RETURN
357 DAYS
3 DAYS
50%
1.02x10^8 BETA CELLS
3 DAYS
6.7x10^9 CELLS DISCARD
1 CHAMBER
ELECTROPHORESIS
68 ml
68 HR
6.8 x 10^9 CELLS
60% LOSS IN VIABILITY
6.8x10^9 CELLS
68 ml BUFFER
MINCED, TITERED, FROZEN
PANCREAS
SUPPLIES | TANKAGE | TOTAL
NUTRIENT 11.6 Kg
OXYGEN 2.5
CO2 2.7
NaOH 0.27
H2O 31.1
TOTAL 48.1 Kg | 48.1 Kg | 97.2 Kg

FIGURE 5.2-13

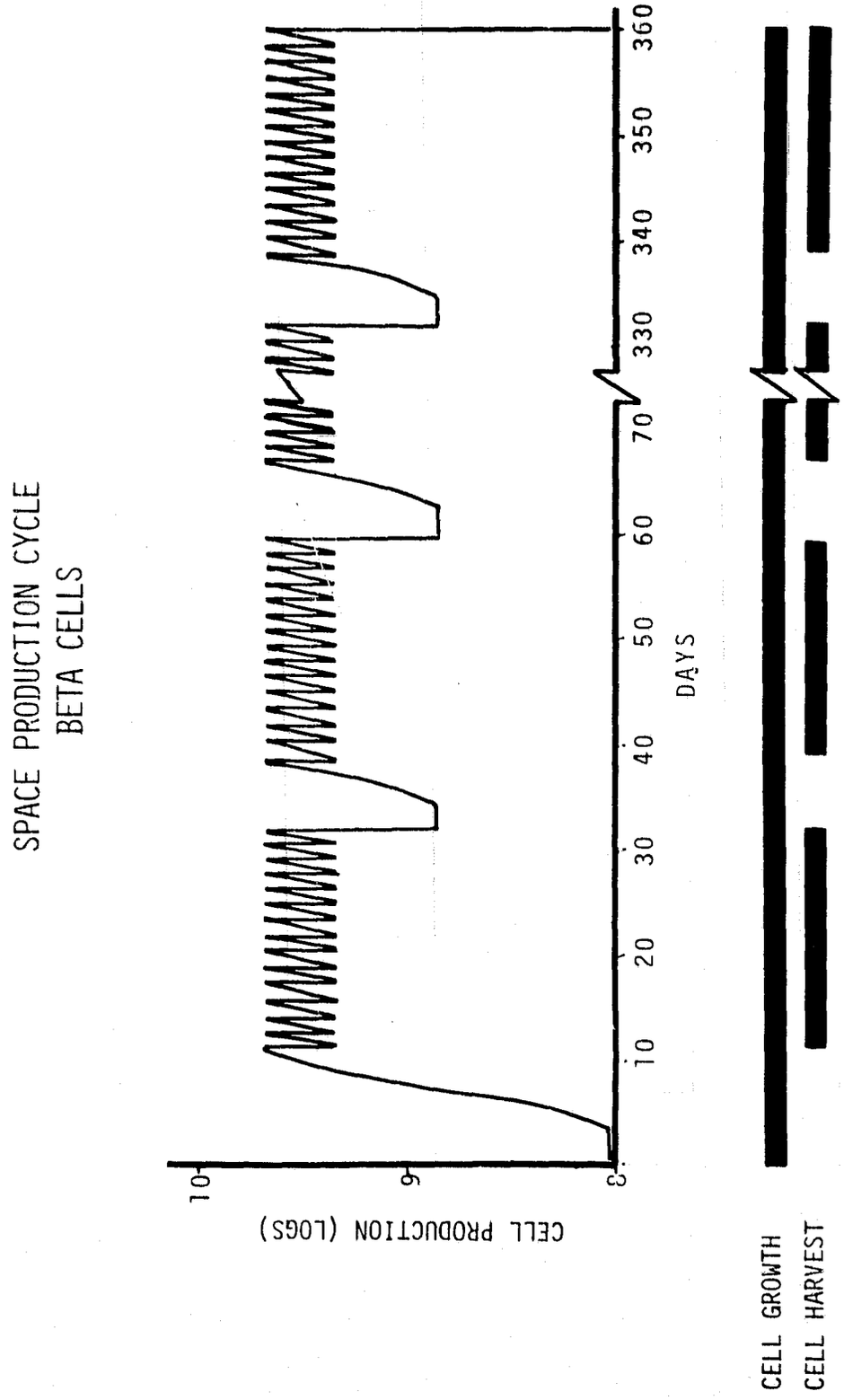

SPACE PRODUCTION CYCLE
BETA CELLS
CELL PRODUCTION (LOGS)
10
9
8
10
20
30
40
50
60
70
330
340
350
360
DAYS
CELL GROWTH
CELL HARVEST

FIGURE 5.2-14

$$2.85 \times 10^9 \times \frac{24}{35} = 19.5 \times 10^9 \text{ Cells/Day}$$

in a volume of

$$1900 \times \frac{24}{35} \times 0.5 = 651 \text{ ml/day}$$

These cells are concentrated to 1×10^8 cells/ml for electrophoresis, resulting in a volume of:

$$\frac{1.9 \times 10^9}{1 \times 10^8} = 19.5 \text{ ml/day}$$

to be processed through the electrophoresis unit. A total of $1.95 \times 10^9 \times 269$ production days = 5.2×10^{11} cells must be processed per year. At a process rate of 1 ml/hour per electrophoresis chamber, a single chamber will process these cells in:

$$\frac{5.2 \times 10^{11} \text{ cells}}{19.5 \text{ ml/day} \times 1 \times 10^8 \text{ cells/ml}} = 269 \text{ Days}$$

or 12.8 (7-21 day) cycles. The number of electrophoresis chambers required for space electrophoresis is,

$$\frac{5.2 \times 10^{11} \text{ cells}}{1 \times 10^8 \text{ cells/ml}} \times \frac{1}{1 \text{ ml/hr/chamber}} \times \frac{1}{269 \text{ day}} \times \frac{1}{24 \text{ hr/day}} = 0.8$$

thus, a single unit can process all the required cells.

A 10% loss of cells is incurred during electrophoresis and an additional 60% loss is incurred during the freezing process for their return to earth; thus the net yield of cells is

$$5.2 \times 10^{11} \times \frac{40}{100} \times \frac{90}{100} = 1.88 \times 10^{11} \text{ Cells}$$

at a rate of 3×10^5 cells per dose this represents about 630,000 doses or about 20% or the market.

During this cell harvesting and concentration procedure a portion of water is lost and a portion is returned. Removal of cells at the rate of 50% of the latest 1900 ml, or 950 ml every 35 hours equates to,

$$950 \text{ ml} \times \frac{24}{35} = 651 \text{ ml/day}$$

Of this volume 19.5 ml per day are lost since they are run through the electrophoresis unit. An additional 10% or 65 ml are not returnable due to unavoidable losses, leaving 562 ml/day to be returned to the tissue culture vessel. This medium is then mixed with fresh nutrients and returned therein.

In addition to water and nutrients, other materials required for maintaining the culture system include oxygen and carbon dioxide and sodium hydroxide for pH control. During the entire culture period the following amounts of materials are estimated to be required: Nutrients, (11.6 kg); oxygen, (2.53 kg); carbon dioxide (2.7 kg) and sodium hydroxide, (0.27 kg).

Each day 651 ml of water is removed from the tissue culture vessel of which only 562 can be returned; thus (651-562) = amount per day which must be returned. This equals (651-562) x 269 = 23,941 ml or 23.9 kg. Initial charging of the system will require an additional 7.2 kg. This water, nutrient and additional culture materials constitute a total weight of 11.6 + 2.53 + 2.7 + 0.27 + 23.9 + 7.2 = 48.2 kg at a cost of $700/kg, and assuming a 1/1 ratio for tankage the transportation costs equal $700 (48.2 x 2) = $67,480.

Ground Culture/Ground Electrophoresis - Ground culture and electrophoresis of Beta cells requires a starting concentration of 2.3×10^9 cells in 2300 ml of buffer. The cell concentration, (1×10^6 cells/ml) is two orders of magnitude lower than that processed in space due to the gravity effects. This concentrate is electrophoresed at a rate of 1 ml/hr. Assuming a 24 day Shuttle process time would require:

$$\frac{2.3 \times 10^9}{1 \times 10^6 \text{ cells/ml}} \times \frac{1}{1 \text{ ml/hr/chamber}} \times \frac{1}{24 \text{ day}} \times \frac{1}{24 \text{ hr/day}} = 3.9 \text{ Chambers}$$

Using four chambers, this can be accomplished in 24 days.

Of the 2.3×10^9 cells electrophoresed, 7.5% or 1.7×10^8 are Beta cells and 50% or 8.8×10^7 are recovered after the first electrophoresis. These are used to inoculate 200 ml of culture fluid contained in an 800 ml tank. The cells will attain a concentration of about 1.5×10^6 cells/ml on day 6, and after 35 more hours will attain a concentration of 3.0×10^6 cells/ml at which time the production cycle begins. At this point 50% of the total volume, or about 3.0×10^6 cells/ml x 400 ml = 1.2×10^9 cells every 35 hours. This equates to $1.2 \times 10^9 \times 24/35$ or about 8.2×10^8 cells/day.

For 12 (7-21 day) cycles this provides 252 days x 8.2×10^8 cells/day = 2.1×10^{11} total cells for separation by electrophoresis. These cells are adjusted to a concentration of 1×10^6 cells/ml. The number of chambers required to separate these cells is,

$$\frac{2.06 \times 10^{11}}{1 \times 10^6} \times \frac{1}{1 \text{ ml/hr/chamber}} \times \frac{1}{336 \text{ days}} \times \frac{1}{24 \text{ hr/day}} = 26.04$$

or 27 chambers to process the cells. During this process 10% of the cell are lost resulting in a net yield of $2.06 \times 10^{11} \times .9 = 1.85 \times 10^{11}$ cells. At a rate of 3×10^5 cells per dose this equals 630,000 doses or about 20% of the market. This evaluation is shown in Figure 5.2-15.

Ground Culture and Space Electrophoresis - Pancreas cells are suspended at a concentration of 3.6×10^{10} cells in 360 ml of buffer, frozen and transported to space via the Shuttle. The process is shown in Figure 5.2-16. On thawing, 40% or 1.44×10^{10} cells/360 ml remain viable. This suspension of both dead and live cells are processed through the electrophoresis unit at a rate of 1 ml/hour, requiring:

$$\frac{360 \text{ ml}}{1 \text{ ml/hr} \times 24 \text{ hr/day}} = 15 \text{ Days}$$

BETA CELLS - GROUND CULTURE/GROUND ELECTROPHORESIS

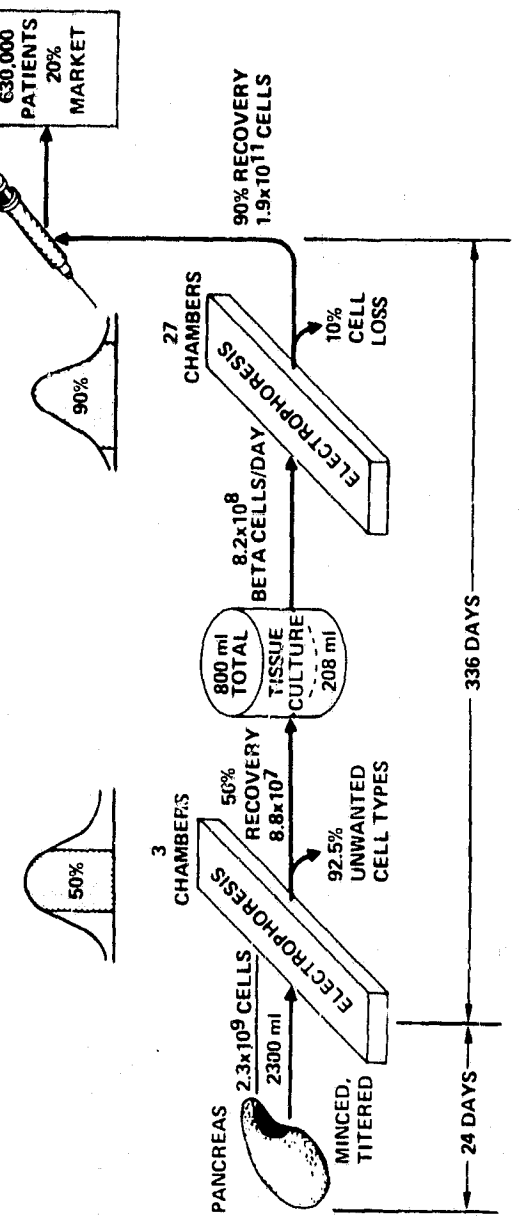


FIGURE 5.2-15

BETA CELLS - GROUND CULTURE AND SPACE ELECTROPHORESIS

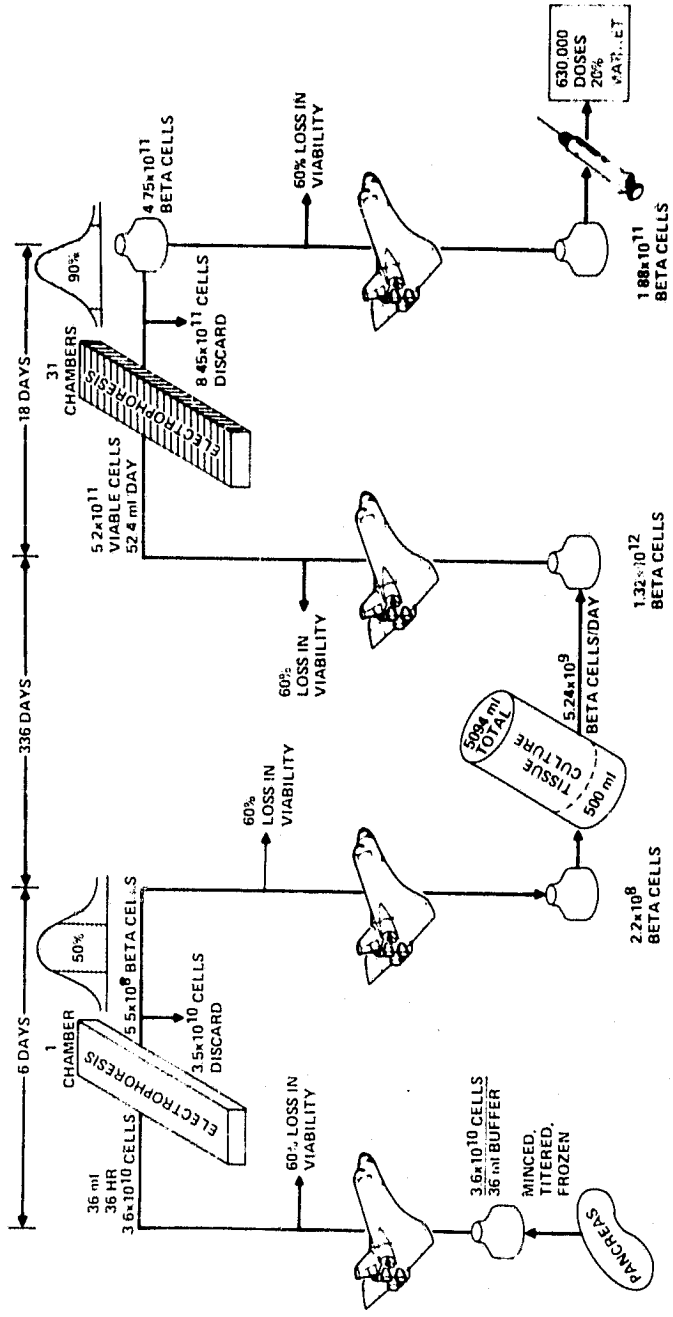


FIGURE 5.2-16

Using 3 electrophoresis chambers this process is completed in 5 days. Only 7.5% of the 1.44×10^{10} cells are Beta cells and only 50% recovery is anticipated from this first electrophoresis process. Therefore, the yield of cells after the first electrophoresis will be:

$$1.44 \times 10^{10} \text{ Viable Cells} \times 0.075 \text{ Beta Cells} \times 0.5 \text{ Loss} = 5.4 \times 10^{8} \text{ Beta Cells}$$

another 60% is lost when the cells are frozen for transport to the ground and rethawed; thus:

$$5.4 \times 10^{8} \text{ Cells} \times \frac{40}{100} = 2.2 \times 10^{8} \text{ Cell Yield}$$

This 2.2×10^{8} cells is used to inoculate 500 ml of tissue culture medium in a vessel with maximum capacity of 5094 ml at a concentration of 4.4×10^{5} cells/ml. In 6 days, growth will have occurred through the lag and logarithmic phases and a cell concentration of about 1.7×10^{6} cells per ml will have been attained. During this period the culture volume will be increased periodically to the production volume, 5094 ml.

In another 35 hours the cells will again double, to 3.0×10^{6} cells per ml, at which time 50% of the cells can be harvested every 35 hours and the volume replaced with fresh nutrients in returned medium.

Thus:

$$5094 \text{ ml} \times 0.5 \text{ Volume Removed} \times (3 \times 10^{6} \text{ cells/ml}) = 7.64 \times 10^{9} \text{ cells/35 hr period}$$

or

$$\frac{24}{35} \times 7.64 \times 10^{9} = 5.24 \times 10^{9} \text{ cells/day}$$

in a volume of $24/35 \times 5094 \times 0.5 = 1746$ ml/day.

These cells are concentrated to 1.0×10^8 cells/ml or a volume of 52.4 ml/day, frozen and transported to space for electrophoresis.

A total of $5.24 \times 10^9 \times 252$ production days = 1.32×10^{12} cells which must be processed each year. To process this number requires:

$$\frac{1.32 \times 10^{12} \text{ cells}}{1 \times 10^8 \text{ cells/ml}} \times \frac{1}{1 \text{ ml/hr/chamber}} \times \frac{1}{24 \text{ hr/day}} \times \frac{1}{18 \text{ days}} = 30.6$$

Thus, 31 chambers are required to process the 1.32×10^{12} cells. Sixty percent of the cells lose their viability during the freeze/thaw cycle and an additional 10% are lost during electrophoresis. The yield from electrophoresis is thus:

$$\text{Yield} = 1.32 \times 10^{12} \times 0.4 \times 0.9 = 4.75 \times 10^{11} \text{ Cells}$$

These cells are frozen and transported to earth for transplantation. During this process an additional 60% loss is incurred due to freezing, leaving a net yield of $4.75 \times 10^{11} \times 0.4 = 1.9 \times 10^{11}$ total cells. At a rate of 3×10^5 cells/dose this represents about 630,000 doses or about 20% of the market.

Transportation costs for this ground/space combination include the original pancreas, and taking up the ground-processed cells. These weights, respectively, are .36 kg and 132 kg or about 132.36 kg total. With tankage, a total of 265 kg is required and at $700/kg: 700 x 265 or $185,500 total transportation cost.

The cost for nutrients for the ground culturing is about $3,489 for a total of about $189,000.

Comparison - The least expensive process is the all ground process in which 4 chambers are required and no transportation costs are incurred. However, it is not likely that cells separated on the ground will be as pure as those separated in the space environment since better resolution can be obtained in the microgravity.

If purity is a requirement, then the ground/space process seems more advantageous since the space environment will be utilized to obtain higher purity. The costs of transportation may be negligible when compared to the benefits obtained from being able to process high purity cells. A disadvantage of the ground/space process is the number of chambers, 31, which must be used. The higher number is required since the space electrophoresis must be conducted in a shorter period of time, 18 days, than the ground process. This is required to provide only a single space transportation of the cells instead of several Shuttle launches spread throughout the year. With these considerations, even with the added transportation costs it appears beneficial to employ a total space process, thus eliminating all but one chamber and yet providing high purity cells for use on the ground. Figure 5.2-17 illustrates the comparison.

Summary Evaluation

A comparison of space production with alternative ground production or combinations is shown in Figure 5.2-17. For antihemophilic factor separations from cryoprecipitate, the advantages of space separation are obvious. For a transportation cost differential of $\$3.4\overline{M}$, the complexity and cost of 3878 chambers is reduced to that of 20 chambers in space. This cost is also a relatively small fraction of the $\$25\overline{M}$ value of the separated products. For beta cells, the product value is much higher at $\$100\overline{M}$, based on equivalent insulin injections; however, the amount of product and the scale of the process is smaller. Again, there is a large reduction in electrophoresis chambers required favoring space processing. For ESF production the small amount of product in the culture media required both large scale cell culture and electrophoresis. The large scale favors ground culture; however, the large number of chambers (23,042) required for ground separation favors a space separation/ground culture combination. Erythropoietin process requirements make it much less desirable as a product for the multiproduct system than either beta cells or antihemophilic factor.

5.3 MULTIPRODUCT PROCESS SYSTEM DESCRIPTION

System Requirements - The basic requirement for the multiproduct process system is the ability to produce the three selected candidate products: erythropoietin, beta cells, and antihemophilic factor VIII. The system mass flows required to produce the desired market shares for each product were presented in Section 5.2.3.

SPACE PRODUCTION BENEFIT COMPARISON

PRODUCT	CULTURE SIZE	ELECTROPHORESIS CHAMBERS	TRANSPORTATION (SUPPLIES ONLY)
AHF/CRYOPRECIPITATE ($25M VALUE)			
GROUND	N/A	3878	
√ SPACE	N/A	20	$3.4M
BETA CELLS ($100M VALUE)			
GROUND	0.8L	27	
√ SPACE	2.0L	1	$0.05M
GROUND/SPACE	5.0L	31	$0.03M
ERYTHROPOIETIN (NOT RECOMMENDED)			
GROUND	375L	23042	
SPACE	375L	115	$18.7M
GROUND/SPACE	433L	1089	$ 8.2M

FIGURE 5.2-77

Based on the space transportation requirements and a comparison with ground operations, it was determined that, generally, cell and protein separations should be space operations. Whether tissue culture should be a space operation was found to be dependent on the product. If the product is the cells being cultured, as in the case of beta cells, then the reasonable transportation requirements make it advantageous to culture in space, because the cell separations to get the starting inocula and to separate the products are best done in space. If the product is small amounts of protein from the cells in the culture media, as in the case of erythropoietin, then the excessive transportation requirements make ground culture followed by space electrophoresis of reconstituted, lyophilized media more advantageous. However, because of the excessive separation requirements along with many technical unknowns, erythropoietin is not included in the requirements for a multiproduct processing system at this time. The resulting system capabilities are heavily biased toward electrophoretic separation, but include tissue culture capability to the extent required for the culturing of cells as products, or limited investigation of protein production from tissue culture.

System Description - A schematic of the system is shown in Figure 5.3-1. Production of beta cells by tissue culture takes place in a 2 liter culture vessel. The culture is supplied with the required oxygen and carbon dioxide based on partial pressures sensed by the controllers. Medium pH is controlled by the addition of sodium hydroxide. Nutrients are stored dry in appropriate proportions for growth and production media and are mixed and supplied as required. To start the culture, the desired cells are separated by electrophoresis and maintained at low temperature ($\leq$4°C) in a startup tank until the initial inoculum is collected. The inoculum is then introduced into a small amount of growth medium in the culture tank, maintained at approximately 37°C and the volume of medium gradually increased as the cells multiply. When the cells have reached the desired concentration, the cells and medium are passed through a membrane concentrator. By returning most of the medium and none of the cells to the culture, the concentrator increases cell concentration to about 10^8 cells per ml to maximize the cell throughput of the electrophoresis chamber. At this point it may be necessary to perform counter current ion exchange to remove nutrient salts from the cell sample to ensure proper electrophoresis unit performance. Temperature of the fluid is maintained at $\leq$ 4°C.

MULTI-PRODUCT PROCESS SYSTEM

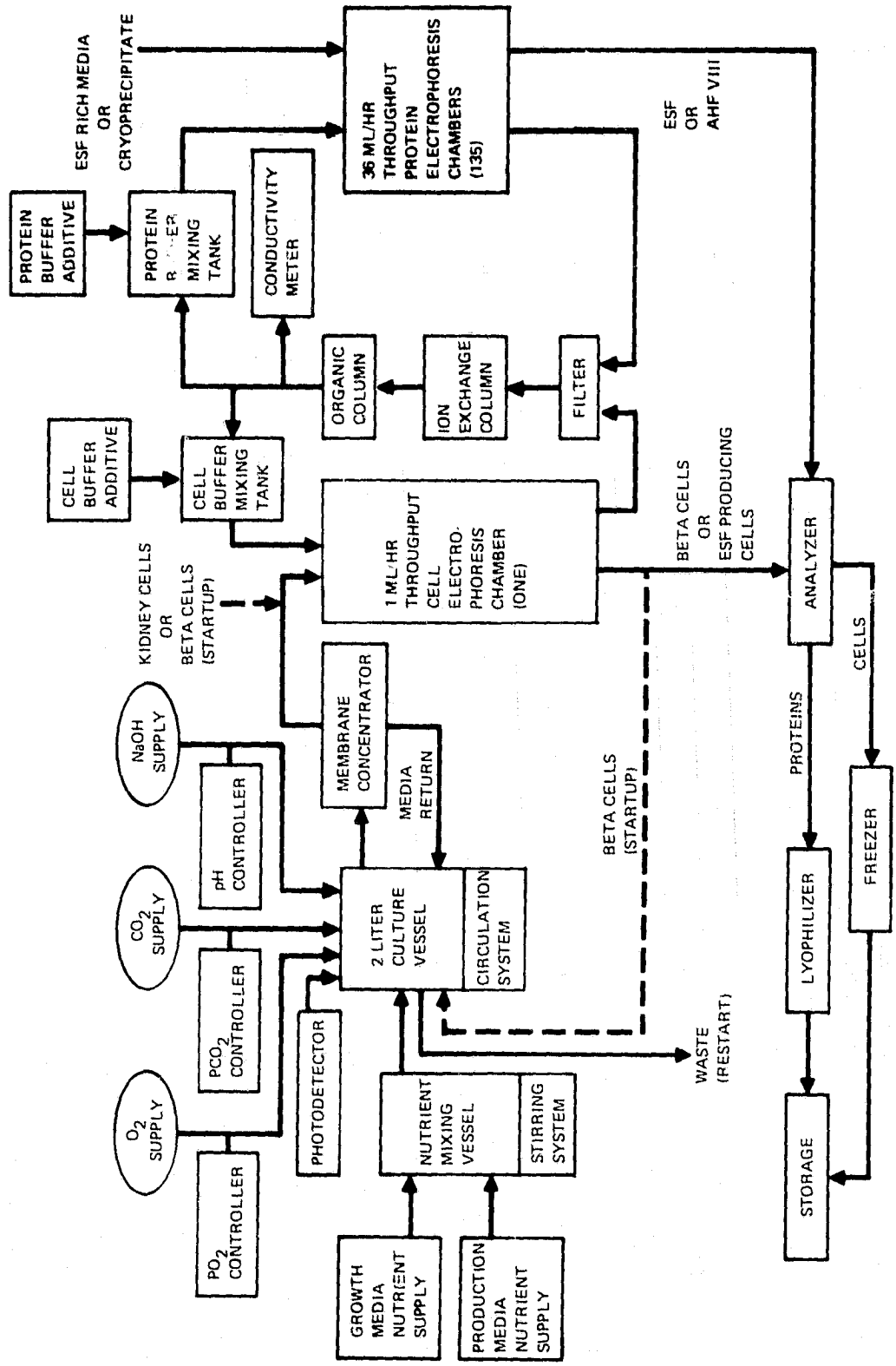


FIGURE 5.3-1

After separation, the output fraction is analyzed to ascertain that the desired cells were obtained and these cells are then frozen at a controlled rate and placed in a freezer at $\leq$-20°C for storage. Cell growth and separation can only continue for a few weeks and then the culture must be restarted. To restart the culture most of the cells and culture medium are removed along with the accumulated waste, and fresh nutrient medium is added. Then after the cells have multiplied to the desired concentration they once again can be separated.

Protein separation is accomplished in 20 electrophoresis chambers with a throughput of 36 ml/hr, each. One important aspect of this space operation is that buffer reclamation is required to maintain the space operating advantage. To reclaim the buffer, the cell or protein waste is filtered out and the fluid is passed through ion exchange and organic columns. The clarified fluid is then tested for conductivity and used along with makeup water to make the required buffer. The buffering components are stored dry and are added in buffer mixing tanks. The desired output fractions are then concentrated by removal of excess buffer and the fractions are lyophilized (freeze dried) for storage at $\leq$ 20°C.

System Protocols - Each of the three selected products exercise the system in a different mode, i.e. - AHF as a protein electrophoretic separation system, beta cells as a tissue culture and cell electrophoretic system and erythropoietin as a tissue culture, cell and protein electrophoresis system. The specific protocols for space production of the three products will be found in Appendix C. Each protocol defines in general terms the preflight and inflight operations required to produce the products.

6.0 INTRODUCTION

During this study a number of lessons have been learned about obtaining and fostering commercial producer participation in studies of space processing. These should be given due consideration in formulating plans for future studies of this nature. They are presented here in brief, and MDAC-St. Louis recommends that they be adopted as elements in the NASA model for exploring other market sectors considered for the commercialization of space.

6.1 INVOLVING PRODUCER COMPANIES

The key to involving industry in space processing is to establish a fully business-like footing for their participation. In most cases, the producer industry is relatively unfamiliar with the space environment, operations in space and the requirements and techniques of designing and integrating systems hardware to be flown in space missions. Dealing directly with NASA would involve them in a new form of governmental interface to which they are not accustomed.

The "Buffer Team"

In this situation, a "buffer" organization is needed to serve as the interface between NASA and the segment of industry NASA desires to interest. Aerospace companies are in a particularly good position to serve in this buffer capacity.

By establishing a buffer team between itself and the industry with which it desires to build participatory agreements, NASA can establish the businessman-to-businessman relationship so essential to nuturing commercial enterprise in space. By this approach, NASA was free to stand aside while, in the present instance, MDAC-St. Louis engaged and fostered the interest of pharmaceutical firms on a strictly business basis. MDAC demonstrated its own willingness to commit to space processing, thereby encouraging pharmaceutical concerns to make proportionate commitments to their potential future mutual benefit. As this approach applies in the pharmaceutical field, so it should apply in many other industries.

The aerospace company chosen for the buffer team should have established a competence in dealing with the particular process NASA wishes to advance as a candidate for production operations in space. Moreover, the company should have made a

significant commitment on its own, in terms of funds and manpower, to the development of that process before NASA chooses that firm to serve as the buffer team. Only if the aerospace company has made a real management commitment to the particular process and products of interest, can it convince candidate participating producer firms of the potential for timely returns on their investments.

The buffer team should also include an independent business analysis firm specializing in the particular industry to be approached. Most aerospace companies do not possess the personnel and experience, in a marketing area other than their own, to identify good prospective cooperating firms or perform the business analysis required in preparing to contact them. The right business analysis firm (such as Price Waterhouse Company vis-a-vis pharmaceuticals) not only knows the industries of interest, but is familiar with the particular environment in which the producers operate. It has access to business documentation resources beyond the aerospace horizon; and, most importantly, such a firm will know key management and technical personnel of these companies plus the correct business basis on which to approach them.

By choosing an aerospace company with a business commitment to both a particular process and class of products for space production and then linking to that company a first class business analysis firm specializing in business relations among producers and marketers of the products of interest, NASA will create the strongest possible team to establish a business basis on which producer industries may be induced to participate in space processing development.

Candidate Company Selection

The producer firms identified by the buffer team should be subjected to a penetrating business analysis by the business consultant member of the team. This analysis should include such factors as: the firm's annual sales and growth; the size of the company; the new products it has marketed; the tenure of the firm's senior officers; the surplus funds available for investment; the size of the firm's R&D budget; and the identifiable constraints on the firm's growth. In addition, there will be factors requiring evaluation which are peculiar to the specific class of industry being approached.

Choosing the companies to be contacted will involve weighing a variety of these factors. Here too, the business consultant member of the team will be of particular assistance. During the assessment of each candidate firm a number of weighting considerations have evolved. Companies demonstrating a relatively stable rate of growth will seek out new methods to retain it. Both small and large companies are more willing to take risks than medium size companies, because the former must be more agressive to succeed and the latter have more capital for expansion. The number of new products placed on the market and the size of a company's R&D budget are indications of its creativity and commitment to innovation. In the area of personnel, the position tenure of senior officers is also a measure of innovativeness; if these officials are changed frequently they will be less likely to make affirmative decisions involving risks of long range returns. As a final example, care must be taken to identify any constraints to growth for each company. In some instances we have found that company research was directed toward only one specific product or that a specific product was absorbing most of the development budget. Growth potential, based on a company's willingness to look at new ideas or risk investment in new processes, is minimized in such cases.

Prepresentation Preparations

Once the companies to be contacted have been chosen, the contacts should begin with an introductory letter transmitted at the corporate level. Corporate involvement in the first contact is essential to assure that the producer company will recognize the established commitment of the "buffer" company (e.g., MDAC-St. Louis) to space processing and will respond by poviding a management level audience. In follow-up telephone calls, arrangements should be made for a formal presentation at the producer's own facility. The audience will be more at ease on their "home ground", and it will give them the option of bringing in additional company personnel as the need arises during the presentation. Plenty of time should be allowed for the company being visited to complete a pre-meeting investigation of the "presenting company" to determine the presentor's technical and business interests, level of commitment and degree of competence.

Having made contact with the producer companies and established a degree of rapport with their business or technical management, the presenting company should tailor each presentation to the interests of the key people in each producer company, i.e., the corporate decision makers and senior technical personnel.

Presentation Team

A number of personnel and technical characteristics are highly desirable for the presentation team. Personnel making the presentation should be thoroughly familiarized with the segment of industry they will be visiting; at least one member of the team should have credible experience in that producer industry. All members of the team should be prepared to speak the vocabulary distinct to that industrial field of endeavor.

The presentation team should be a systems team that is capable of addressing all aspects of space processing to the audience's satisfaction. Not only must they be knowledgeable in the area of products and processes, but also familiar with space flight systems and how day to day activities in space are carried out. They must be thoroughly prepared, as well, to discuss resource requirements, costs and manpower, and schedules. Inclusion of a life sciences specialist in the team is highly desirable so that questions on man's contributions and requirements in space can be answered.

Presentation Approach

The presentation approach should reflect the businessman-to-businessman relationship desired between the buffer team and the manufacturing firm. MDAC-St. Louis' experience in this study illustrates this. Pharmaceutical firms, for example, are reluctant to become involved with government R&D funding; they desire to avoid any potential compromise of their patent right which might arise through such an involvement. Moreover, in a high risk venture such as commercial space processing, they desire a "buffer" industry that is familiar with space systems and operations as well as drug company operation. They are unfamiliar with the space environment and usually do not claim the engineering talent and diversity sufficient to make space experimentation worthwhile on their own. Finally, they would prefer not to add to their existing government interfaces.

Since the aerospace industry possesses the requisite engineering talent and the experience of working within the NASA administrative framework, they can serve very well in this "buffer" capacity. By the same reasoning, the aerospace industry is usually not familiar with the government regulatory agencies for the participating industry, e.g., the Food and Drug Administration for the pharmaceutical industry. In this situation, the presentation approach should reflect that a

joint working arrangement between the visited company and the aerospace industry would be mutually beneficial using the strengths of each partner to achieve new goals.

During the presentation, the question is invariably asked of the presentor, "Why should we manufacturers be interested in space processing?" Placing a good reason for their interest early in the presentation can forestall the inquiry. The reason can easily be developed identifying the visited company with a candidate product that has the potential of being produced in space and that also complements their already existing product line. We have found it is also essential to indicate very early in our presentations that the products discussed are of the very low volume-very high value type. Many of the proprietary pharmaceutical companies think in terms of large volume-low cost products which are not applicable in the space operations we visualize.

The presentation should deal with current reality and what can be accomplished within short term goals. It's focus should avoid the subject of long-range, large scale space facilities. Most companies do not plan more than three to five years in advance because of the rapid change in technology and the press of competition. The pharmaceutical companies seem to be one of the few exceptions to this rule in that they plan between five and fifteen years in advance. This long lead time is occasioned by the lengthy (3-11 years) regulatory approval cycle of a new product that their competitors must also go through.

One of the most important considerations is the attitude of the presentation. It should avoid talking down to the audience. Minute detail should be dropped if the audience is familiar with the product or process. The presentation must reflect the fact that the audience has the expertise in the field, and we as visitors are seeking help through their participation. The presentation team can show their depth of knowledge of the industry by answering technical questions posed by the audience.

The presentation approach must recognize the realities of business life. It must reflect that industry is profit rather than knowledge oriented; research must ultimately lead to increased corporate profit. By selecting products of particular

interest to that company and presenting relevant market and business forecasts, a profit potential can be demonstrated in a way to engage both the technical and management attention of the audience. Using a conservative approach in the presentation, especially with technical and business values familiar to the audience, will give individuals an excellent chance to contribute to the discussions and to realize that their experience and participation would greatly enhance the program.

The presentation should demonstrate the personal commitment of the speakers and their company to the program. It must convey the attitude: "We are deeply involved and would like you to join us" rather than "You tell us what we can do for you in space." If the presentation features working hardware, mathematical analyses and models, as well as preliminary product data with their related market and risk analyses, the audience will feel that the presenters have a strong corporate investment in the concept, both financially and in terms of manhours of effort.

Brochures of the presentation material should be left with the key men of the audience. This brochure should contain all the visual aids plus an explanation of each. It will serve as a memory aid for internal company discussions. In addition, a point of contact for both the presenting team and the company visited should be identified for follow-up telephone calls requesting help or clarification on either side.

Post Presentation Period

Results of the presentations will develop relatively slowly. The companies visited will take time to digest what is presented and investigate the claims made. Experience in this study has shown that this phase will take about three months. If results of the initial investigation are favorable, the company will present the concept to their corporate management. This second phase usually will take two more months. The formal development of participating documentation (e.g., protocols, agreements, etc.) and approval of budgets will ordinarily consume an additional six to twelve months. All during this time, routine contact between the two companies should be maintained with pertinent management and technological data exchange by both sides as required.

During this period, it is extremely important not to exploit the manufacturing company names or the products they have under consideration. Most industries are very competitive and maintain strict confidentiality regarding the potential products or processes they are investigating. If such information became generally known without the consent of the candidate participant, cooperation would probably be terminated. The privacy of a manufacturing company considering participation must be respected until the firm decides to announce publicly, for itself, its intent to participate in the exploration of space applications.

6.2 LABORATORY SUPPORT

Interlaboratory testing protocols take time to assemble and be approved by the management of both companies. It is imperative that responsible personnel be involved in working up the agreements. Detail must be provided with regard to both the specificity of the test and the level of sensitivity for that test. While both companies may be testing for the same end product, if each investigates the presence of a different characteristic of that product (or the same characteristic at different sensitivity levels) one of the laboratories may report negative analytical results even though the end product is present. At least two different analytical tests must be used to cross check if any compromise of the product integrity may be suspected.

When working with the bioprocessing of pharmaceutical products it is necessary to have the support of a broad based biological laboratory and its inclusive personnel. If a particular test is not within the analytical program of the laboratory, it will usually take about four months to obtain the requisite chemicals and instruments, become familiar with the test and develop the repeatability and accuracy required. By establishing, from the beginning, a diversified laboratory and staff capability, the time and effort to incorporate new procedures can be eliminated or drastically reduced.

Logistics is a very critical factor when dealing with biological products. They must be protected from the environment at all times with only very short temporal excursions beyond those limits permitted. While the most detailed logistics plans may be proposed on paper, the human element is involved in its execution. The larger the corporation and the further the distance between companies, the greater

the chance for mishandling the environment critical product during shipping, transportation and receiving. The experiences gained in this study will serve well for developing and executing product logistics support activities between a participating company and the space launching facilities.

The continuous flow electrophoresis system used as the heart of the space-bioprocessing system is a very powerful analytical instrument. Its resolving capabilities, in many instances, are greater than that observed by zone electrophoresis. Commercially prepared and certified pure protein samples present a single band pattern in zone electrophoresis. When these same purified samples were used to calibrate the continuous flow electrophoresis instrument or identify the location of that protein in a mixture of proteins being separated under the same conditions as that for the zone electrophoresis system, it was not uncommon to find more than one separation peak for the material. These smaller, secondary peaks observed may be trace impurities or they may represent dissociation products of the calibrating material. This latter hypothesis is unlikely, however, because when the main peak is collected, lyophilized, resuspended, and again electrophoretically processed, the secondary peaks do not appear.

A data base concerning a potentially profitable candidate product must be developed upon which a technology interchange can be established between the interfacing company and the candidate producing company. MDAC-St. Louis established such a technology interchange with the participating pharmaceutical companies. This in turn, we believe, has enhanced the level of interest of these companies in the potential offered by processing pharmaceuticals in space.

While mutually exploring candidate products with a potential space process, new product derivatives may be found that can be exploited jointly. The electrophoretic separation of AHF VIII from cryoprecipitate is an example. Although molecular dissociation of AHF III occurs, the clotting properties are still intact. Since the classic hemophilic patient lacks only the clotting moiety, its dissociation is not a deterent to further investigation of this product. Both participating pharmaceutical companies expressed interest in a purified clotting moiety as a possible product.

Preliminary tests revealed that the use of cryoprecipitate as a product source will require development of special handling procedures to assure day to day uniformity of sample. This does not pose a critical technological obstacle.

Starting with human plasma as a product source, it may be possible to obtain all the important blood fractions of medical importance in purified form in a single continuous process. Gamma globulins, used to protect against bacterial and viral diseases, are slow moving materials whereas albumin, used as a plasma expander is very fast moving. These materials are currently obtained commercially by precipitation methods requiring the use of unwanted materials in the separation process. In such procedures important components, such as AHF VIII, other clotting factors, alpha antitrypsin, etc., may be neglected, lost or denatured by the process. A process in which all blood components can be isolated in a single step is of significant interest to both participating companies from the standpoint of economics as well as expansion of their product line.

6.3 PRODUCT REQUIREMENTS AND ANALYSIS

Many potential products can be proposed for space processing by reviewing the literature and discussing the subject with professionals in the field of interest. Care must be exercised, however, when selecting the products. Many of the suggestions may be of interest scientifically for their own sake but will have little chance of being rapidly adopted by the workers in that field if they offer no substantial improvement over existing materials. Industrial manufacturers have marketing staffs and diversification groups that are continuously looking for new products. Their main criteria, however, is can the company make a profit from it in a relatively short period of time. If the companies do not see a significant return on their investment in research and development of a product they will ignore that product.

When products are attractive from a marketing standpoint, technical information is then gathered to assess the capability of producing the product and the extent of the competition to be expected. Literature reviews, both technical and marketing, on each of the products will generally show that the information is voluminous but of little direct value to sizing a processing system. Pieces of information are usually missing that would require technical assumptions based on laboratory and

production experience in order to proceed with a production system analysis. What may be relatively easy to do in a laboratory on a small scale may be fraught with problems when the process is magnified to meet commercial needs.

Judgments must be made concerning the potential of product competition from other technologies during the period of investment recovery. Most pharmaceutical companies contacted about space processing brought up the possible competition from the recombinant DNA approach to protein synthesis using bacteria. Their own study and analysis of that concept led them to understand that that process still has a long way to go to become practical. While the recombinant DNA process may be able to make the products on a large scale in the distant future, they would still need a process like the continuous flow electrophoresis system to isolate the product in sufficient purity to make that process commercially feasible. Thus, two possibly competitive processes may actually be complementary.

A hardware system that is capable of producing a variety of products with only minor operational changes, e.g., instrument settings, chemical substrates, and flow rates, would offer significant operational, logistics and cost advantages to a producer. All of the twelve biological products reviewed in this study could exercise the proposed system in part or in its entirety thus supporting the concept of a true multiproduct system. Knowledge of the cell sources for erythropoietin, epidermal growth factor, growth hormone, interferon and alpha-antitrypsin production and an awareness that these cells and hormones can be separated by electrophoretic techniques, all supported the concept of a full bioprocessing production system. Antihemophilic factor VIII and specific immune globulins are blood proteins with as yet no defined site of production in a living organism. They, therefore, would only be able to exercise the space bioprocessing system in the separation mode. Beta cells and urokinase producing cells can be grown in tissue culture and also separated electrophoretically. Because the mobilities of these cells in an electrical field are lower than proteins, the separation chamber may have to be modified slightly in design or operational function. All twelve products could benefit through space bioprocessing by improvements in purity and throughput. Additional analyses, such as that done by mass balancing would be required to show their economic feasibility.

Based on economic and technical information, this study developed a value index of each product with respect to their humanitarian value and applicability to the concept of production through space bioprocessing.

In addition to this numerical evaluation, at the current state of knowledge two additional subjective criteria must be applied to each product, i.e., would the product exercise the conceptual bioprocessing system in one or more modes of operation and could comparison data be attained between earth and space processing. The comparison data must be available, or arrived at by analysis, to show the potential of space processing to prospective producers.

Development of market data on products important to candidate participating firms is a key to securing their interest. A search of the literature, supplemented by consultations with clinical authorities will provide the information necessary to develop a picture of the current market open for the model products. Reasonable assumptions, based on the guidance of clinical research teams, will yield useful market projections for advanced clinical uses. Finally, appropriate business risk analyses should be employed to assess the market risks for processes and products as they move from initial R&D commitment through ground and flight experimentation to achieve successful flight production demonstrations. By offering these preliminary analyses to prospective participators, much useful data can be obtained during the presentation itself. Most important, the potential participating firms will be assured that the presentor has a business understanding and an investment attitude appropriate for cooperative endeavors for their mutual benefit.

Discussions with a number of pharmaceutical companies resulted in a list of several other biologically active products for eventual consideration. These included: thymic factors, specific immunoglobulins, somatostatin, somatomammotropin and polyvalent vaccines. It is recommended that these products be explored in depth as a point of future communication and interest with the pharmaceutical companies.

6.4 PROCESS SYSTEM REQUIREMENTS

While this study was done to assess the commercial feasibility of manufacturing pharmaceutical products in space, it serves as a model for those who wish to consider other processes or products in the same environment. The use of the mass

balances concept forces a delineation of what must be accomplished in the process for each product in a stepwise fashion. The calculated quantities of materials at each step will quickly determine if the process is feasible with current technology, where the areas of information must be obtained to fill in the gaps, and anticipated recurring transportation costs to haul the material to and from space. While it does not define the total cost of the system, it does give the prospective manufacturer and NASA a general idea of the size, power and weight of the processing equipment as well as the extent and type of storage requirements. The length of the missions will be defined to determine economic feasibility. This will have to be interwoven with the NASA program and schedules to determine if, and when, a vehicle capability will be available to support such a manufacturing facility. Legal regulatory considerations will also have to be defined.

After a relatively thorough review of the current commercial and laboratory methods for producing the final twelve candidate products, a conceptual design of a space bioprocessing system was verified. It could be used as a multiproduct process system with only minor instrument settings and fluids composition changes when different products enter into the system. A comparison of space production with alternative ground production, or combinations of the two locations was performed on three candidate products using the concept of mass balancing.

Based on the space transportation requirements and a comparison with ground operations, it was determined that, generally, cell and protein separations should be space operations. Whether tissue culture should be a space operation was found to be dependent on the product. If the product is the cells being cultured, as in the case of beta cells, then the reasonable transportation requirements make it advantageous to culture in space, because the cell separations to get the starting inoculums and to separate the products are best done in space. If the product is small amounts of protein from the cells in the culture media, as in the case of erythropoietin, then the excessive transportation requirements make ground culture followed by space electrophresis of reconstituted, lyophilized media more advantageous. Erythropoietin shows an excessive requirement for orbital separation systems along with many technical unknowns. However, it was included in the requirements for a multiproduct processing system at this time in order to provide a typical capability for applied research on marginal materials of this type. The resulting system capabilities are heavily biased toward electrophoretic separation,

but include tissue culture capability to the extent required for the culturing of cells as products, or limited investigation of protein production from tissue culture.

The use of mass flow balances for each candidate product were very helpful in clarifying the sizing, continuous support, and transportation requirements for the system. They were particularly useful in highlighting areas where new techniques are required. For example, it is essential that a major portion of the fluid materials required to operate the system in space be reclaimed. The cost of transporting these fluids, if not recycled, would eliminate the profit potential of the bioprocessing system. Moreover, the continuous loss of the fluid to the space environment surrounding the structure containing the bioprocessing system would preclude the possibility of other NASA activities external to that structure. Reclamation of these fluids is not now feasible and is a study area requiring immediate and concentrated investigation.

Another pertinent observation was that commercial production of a particular candidate product was only feasible by bioprocessing in space rather than by using a ground based facility. The cost of the large quantities of buffers to process erythropoietin in commercial quantities on the ground by electrophoretic techniques would be more than the annual expected sales of the product. More over, the total annual requirements for that buffer's constituents would be greater than that currently being manufactured world wide.

All of the candidate products evaluated in this study were natural body substances. Therefore, with the aid of a participating pharmaceutical company, contact was made with the Bureau of Biologics of the Food and Drug Administration to start exploratory discussions of the space bioprocessing concept, the candidate substances considered for production, and the applicable governing regulations. This agency was very cooperative and appreciative of being brought into our program at the conceptual stages. It gave them an understanding of what the goals of space bioprocessing were and our approach to achieving those goals. This awareness of the program then enabled them to provide meaningful guidance to our systems design and thus be in compliance with their good manufacturing practices. It has been made clear that these representatives could see no insurmountable problems with regard to space bioprocessing as outlined by us.

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APPENDIX A

TEST PROTOCOL-ANTIHEMOPHILIC FACTOR

TEST PROTOCOL
ANTIHEMOPHILIC FACTOR
12 December 1977
PREPARATION AND HANDLING OF ANTIHEMOPHILIC FACTOR VIII
SAMPLES FOR TESTING BY
(PARTICIPATING PHARMACEUTICAL COMPANY)

Purpose and Contents

The purpose of this protocol is to establish the basic guidelines for preparation, handling, and assaying samples which have been processed through the McDonnell Douglas Astronautics Company - St. Louis (MDAC-St. Louis) electrophoresis unit and sent to (participating company) for testing.

The contents of the protocol are arranged as follows:

1. Introduction
2. Electrophoretic Process
3. Preliminary Assay at MDAC-St. Louis
4. Assay of Samples at (participating company)
5. Sample Volume and Characteristics
6. Sample Disposal
7. Test Periodicity
8. Data Management
9. Technical Contact

1.0 INTRODUCTION

McDonnell Douglas Astronautics Company - St. Louis (MDAC-St. Louis) has requested the aid of (participating company) to conduct assays on material processed through an electrophoresis chamber. The samples will consist of biological materials contained either in cryoprecipitate or the commercially available Hemophil preparation, a more purified form of Factor VIII.

Samples will be sent to (participating company) frozen on dry ice per their specific requirements and will be accompanied by a sample history sheet providing any preliminary data acquired by MDAC-St. Louis personnel. After the assays are

completed, the samples will be disposed of at the discretion of the (participating company) personnel.

Data acquired from these tests will become the property of MDAC-St. Louis and will be shared with (participating company) and managed as described in Section 8.

The length of this arrangement is open. However, for planning purposes, a period of six months is anticipated for preliminary screening, after which time a more detailed arrangement may be in order. During this six month period, it is anticipated that one set of samples every two weeks will provide sufficient turnaround time to be able to apply acquired data to the conduct of the next sample acquisition.

2.0 ELECTROPHORETIC PROCESS

The electrophoretic process will consist of passing samples through a carrier buffer in the electrophoretic chamber in such a manner that laminar flow is attained. Under these conditions, when voltage is applied across the unit the materials in the sample will migrate to the anode or cathode depending upon their net charge. As they separate they can be collected in separate test tubes at the exit portion of the chamber. The collected samples can be assayed immediately and the remainder quick frozen at -70°C for future use.

During the electrophoresis process, sample temperature is maintained at 4-10°C. From the time sample is prepared for assay until the separate fractions are frozen will be about 30-40 minutes.

3.0 PRELIMINARY ASSAY AT MDAC-ST. LOUIS

All samples will be screened by MDAC-St. Louis personnel before shipment to (participating company). Upon collection of samples, 1.0 ml will be removed and assayed for Factor VIII by the coagulation test described in Appendix A, paragraph 1, and for total protein by a modified Lowry procedure described in Appendix A, paragraph 2. The remainder of the sample (about 4.5 ml) will be quick frozen in dry ice-acetone and then stored at -70°C until shipped to (participating company).

4.0 ASSAY OF SAMPLES AT (PARTICIPATING COMPANY)

Tests conducted by (participating company) personnel will depend on the number of samples sent. However, one or more of the following will be conducted, depending upon available personnel, time constraints, etc.

A. One-stage Factor VIII assay utilizing Factor VIII deficient plasma.
B. One-stage Factor VIII assay utilizing Factor VIII deficient plasma (barium chloride absorption).
C. Two-stage Factor VIII assay.
D. Fibrin split products.
E. Chromogenic assays for thrombin and plasmin.
F. Hepatitis B surface antigen.
G. Factor VIII antigen - Laurell method.

These assays will be conducted by procedures normally employed by (participating company). For the purposes of these preliminary tests, it is not necessary for their test methods to be disclosed.

5.0 SAMPLE VOLUMES AND CHARACTERISTICS

The volume of each sample sent to (participating company) for assay purposes will consist of about 4 to 5 ml. Each electrophoresis run will consist initially of about four tubes of zero time materials and as many as 14-16 tubes of processed or separated material. As described above, MDAC-St. Louis will provide the results of in-house assays for each tube with respect to AHF VIII clotting activity and protein concentration.

Once established that various activities are found in particular tubes, then it will not be necessary to perform all tests on all tubes. For example, we know that Factor VIII will be contained in only about four of the 14-16 tubes, but we do not know about the location of other materials such as the hepatitis antigen, proteolytic enzymes, etc. Thus, once established where the various activities occur, only the fractions of interest would be sent for assay. As such data become available, the number of "runs" and samples can be more clearly defined.

All samples will be safe for transport by common carrier and no particular safety procedures will be required by either laboratory. The only cautions that will prevail are those normally imposed for handling human serum cryoprecipitate or partially purified human plasma Antihemophilic Factor VIII.

6.0 SAMPLE DISPOSAL

All samples obtained by (participating company) will be assayed and discarded at their discretion. MDAC-St. Louis has no requirement for their return. Additionally, if so desired (participatiung company) personnel may subject the samples to additional testing not discussed specifically in this protocol. MDAC-St. Louis will be informed of such additional testing or usage.

7.0 TEST PERIODICITY

It is anticipated that data obtained from testing will result in the increase in knowledge of the potential of the electrophoresis process to separate biological products. Therefore, the results of each run should have some bearing on the nature of future experiments. A turn-around time for data reporting of two weeks would result in a process run and subsequent samples about every two weeks. Initially, then, the protocol will call for a sample run to be sent to (participating company) every two weeks. In order to use their data for the next run, MDAC-St. Louis should have test results within the next 7-10 days.

8.0 DATA MANAGEMENT

All data will become the property of MDAC-St. Louis, and can be used at their discretion for inclusion in contract reports, in-house documents and Independent Research and Development (IRAD) reports. This will include a free exchange of data with (participating company).

At the discretion of (participating company) their affliation may or may not be used in these reports. The nature of this affiliation can be detailed prior to any handling of data in reports which are sent to third parties.

Any open literature publications which may result as a result of these tests would include personnel of (participating company) at their discretion.

9.0 TECHNICAL CONTACT

All questions regarding the test protocol, laboratory procedures, shipping of samples, etc., should be directed to:

Dr. J. W. Lanham
McDonnell Douglas Astronautics Company
P.O. Box 516, Dept E423, Bldg 65
St. Louis, MO 63166
(314) 232-7923

Test Protocol
Appendix A

1. Assay of Antihemophilic Factor VIII

Reagents

a. Glyoxaline Buffer -
3.4 gm Imidazole in 250 ml distilled water.
186.0 ml 0.1 in HCl added to above
5.85 gm NaCl added to above
Bring volume to 950 ml
pH to 7.35
Bring volume to 1000 ml

b. Platelin plus Activator - DADE #B4218-1

c. Factor VIII deficient plasma - DADE #B234-8

d. 0.025M $CaCl_2$

e. Factor VIII Reference Standard - WHO Reference II - DATA #B4224-10

Procedure

a. Into 13 x 100 ml tubes add the following:
0.1 ml Reagent b.
0.1 ml Sample to be tested
0.1 ml Reagent a.
0.1 ml Reagent c.

b. Incubate at 37°C for 10 minutes

c. Add 0.1 ml Reagent d.

d. Begin timing immediately and measure time until clot forms. The time required for clot formation is compared to the time required when 0.1 ml of a Factor VIII Reference Standard containing 0.25 to 1.0 unit/ml is used in place of 0.1 ml of sample.

Results are recorded as percent of AHF VIII in normal serum.

2. Protein Assay

Reagents

a. 1.5 ml of 1.0% Potassium Tartrate in 150 ml of 2% Na_2CO_3. While stirring add 1.5 ml of 1% $CuSO_4$ (or 1.5% $CuSO_4 \cdot 5\ H_2O$).

b. Folin Reagent - Sigma Chemical Company #F-9252 - Dilute 1:1 with distilled water.

c. One N NaOH.

Procedure

a. Add 1 ml of sample to be tested to test tube.

b. Add 0.5 ml Reagent c.

c. Add 5.0 ml Reagent a. Incubate 10 minutes at room temperature.

d. Add 0.5 ml Reagent b. Let stand 30 minutes at room temperature.

e. Read in spectrophotometer at 700 nm.

f. Standard curve is prepared using 1.0 ml aliquots of known protein content from 0 to 320 μg/ml.

ANTIGEN ASSAY PROTOCOL - AHF

MATERIALS

1. Buffer - 9.76g Sodium Barbital
   2.47g Barbituric Acid
   5.78g TRIS
   } Dissolve in one liter of distilled water and dilute up to 2L.
2. Agarose - Purified Powder
   Dissolve 0.1g in 10 ml of above buffer
3. Antiserum - Behring Factor VIII Antisera
4. 55°C Water Bath
5. LKB Immunoelectrophoresis Apparatus
   Balance Plate
   Cooling Plate
   Anti-Condensation Cover
6. 8.4 x 9.4 cm Glass Plates
7. Coomassie-Blue R-250 Stain

PROCEDURE

1. Heat 20 ml of above buffer to 100°C.
   Dissolve 0.2g of Agarose power to yield 1% Agarose solution. Heat until solution boils.
2. Prepare Factor VIII standards using a stock concentration of 5 mg per ml of buffer and dilute serially.
3. Pour 10 ml of 1% Agarose solution into 10 ml test tube and place in 55°C H_2O bath until temperature of solution decreases to 55°.
4. Dilute Factor VIII Antisera and buffer 1/1.5 to yield 5.67% antibody concentration.
5. Add 560 λ of diluted antibody to 10 ml of 1% Agarose solution.
6. Heat balance plate and place glass plates on to warm up.
7. Carefully pour tube of Agarose solution containing Factor VIII antibody onto plate until all edges are covered.
8. Allow 15 minutes to cool.
9. Punch appropriate number of holes in agar to yield 3 or 10 volume holes using gel puncher and template.

10. Spot samples and standards to be tested.
11. Place plates on cooling plate.
    Soak paper wicks (5 layers thick) for 15 to 30 minutes. Cover edges of both sides of plate(s).
12. Cover with anticondensation lid.
13. Turn voltage on to yield 6.5 mAmps per plate.
14. Check voltage across gel; should be 2-5 V/cm.
15. Run for 18 hours.

STAINING PROCEDURE

1. Remove plate(s). Place absorbent paper on top. Cover with 1 kg jar to press gel to fine film.
2. Wash plate in 0.15 M NaCl solution - 15 minutes.
3. Press as in Step 1. - 15 minutes.
4. Repeat steps 2 and 3.
5. Wash in distilled H_2O - 15 minutes.
6. Press.
7. Dry in 50°C oven for 20 minutes.
8. Stain in Coomassie-Blue R-250 - 20 minutes.
9. Destain in three solutions of acetic acid destaining solution.
10. Dry in 50°C oven.

ANTIHEMOPHILIC FACTOR - Factor VIII

REAGENTS AND EQUIPMENT

1. Ames Prothrombin Heating Plate (Model #4500) 37°C
2. Ames Prothrombin Reaction Chamber (#4510)
3. Dade Normal Human Serum Lyophilized
4. Dade AHF Deficient Serum Lyophilized
5. 0.025M Calcium Chloride (made in H_2O)
6. Glyoxaline Buffer - 3.4 gm Imidazole in 250 ml H_2O
   - 186 ml 0.1N HCl
   - 5.85 gm NaCl
   - bring to one liter with H_2O and pH to 7.35
   - Keep at 4°C.
7. Normal Human Serum Standards
   Dilute in H_2O - 1:10, 1:20, 1:40, 1:80 and 1:160. Keep all samples in ice. Once serum is reconstituted, it is only good for approximately two hours.
8. Dade Cephaloplastin Activator

PROCEDURE

1. Preheat plate and reaction chamber to 37°C.
2. For standards or unknown add:
   Large side of chamber: 50 λ activator
   50 λ standard dilution or sample
   50 λ AHF deficient serum
   50 λ buffer
   Small side of chamber: 50 λ 0.025M $CaCl_2$
3. Let incubate at 37°C for exactly 10 minutes.
4. Mix two sides together and time with a stopwatch until clot is formed (performed at room temperature).
5. Stop if greater than two minutes.
   1/10 dilution = 100% activity
   1/20 dilution = 50% activity
   1/40 dilution = 25% activity
   1/80 dilution = 12.5% activity
6. Graph on two-cycle semilog paper.

APPENDIX A.2

TEST PROTOCOL-PANCREATIC ENDOCRINE CELLS

GROWTH HORMONE

GRANULOCYTE STIMULATING FACTOR

Test Protocol
2 August 1978
Preparation and Handling of Biological Samples
for Testing by (Participating Company)

Purpose and Contents

The purpose of this protocol is to establish the basic guidelines for preparation, handling and assay of samples which have been processed through the McDonnell Douglas Astronautics Company - St. Louis (MDAC-St. Louis) electrophoresis unit and sent to (participating laboratory) for testing.

The contents of the protocol are arranged as follows:

1. Introduction
2. Electrophoresis process
3. Preliminary assay at MDAC-St. Louis
4. Assay at (participating company).
5. Sample volume and characteristics
6. Sample Disposal
7. Data management
8. Technical contact MDAC-St. Louis

1.0 INTRODUCTION

MDAC-St. Louis is initiating an interlaboratory test program with (participating laboratory) to conduct tests which will aid in the selection of products as candidates for space bioprocessing. This test protocol will serve as a basic guideline for these tests and is amenable to changes and updates where necessary.

Present materials being considered are pancreatic beta cells, pituitary cells producing growth hormone, and cells producing granulocyte stimulating factor. Since living cells are the primary product, special handling is required not only during processing and assay, but during transport of the samples from one laboratory to another.

Where human cells are required and cannot readily be purchased by MDAC-St. Louis, (participating company) will attempt to provide the necessary organs for study.

Preliminary in-house data will be supplied to (participating company) along with samples to be tested.

All data resulting from this participation can only be used with the consent and discretion of the other participant. If the information is released, the use of corporate names witn the data is excluded unless additional consent is provided.

The length of this testing program is open. For preliminary purposes this test protocol will assume a one year relationship after which time progress will dictate follow-on tests.

2.0 ELECTROPHORETIC PROCESS

The electrophoretic process will consist of passing samples through a carrier buffer in the electrophoretic chamber in such a manner that laminar flow is attained. Under these conditions, when voltage is applied across the unit the materials in the sample will migrate to the anode or cathode depending upon their net charge. As they separate they can be collected in separate test tubes at the exit portion of the chamber. The collected samples will be assayed for cell counts, viabiity, hormone release and culture, and sent to the participating company for analysis and/or further testing. In general, temperatures in the electrophoresis chamber will be maintained between 4°C and 10°C. Processed samples will be shipped either on dry ice, refrigerated or at room temperatures, depending on the cell types and their specific requirements.

3.0 PRELIMINARY ASSAY AT MDAC-ST. LOUIS

Basically, MDAC-St. Louis personnel will conduct tests to determine the following: (1) ability to separate cells from debris and from one another, (2) viability of processed cells, (3) hormone produced by processed cells, and (4) ability to culture processed cells. Procedures used for these assays are described in Appendix A of this protocol.

Currently our in house capabilities include culture of the routine cell types such as primary kidney cells, suspensions of transformed cells, and lymphocytes. Insulin, glucagon and growth hormone assays are being developed and culture techniques for the specific cells are being investigated. Our tentative schedule

calls for completion of an insulin and glucagon assay procedure by early 1979, growth hormone and granulocyte stimulating factor during the second quarter of that year and state of the art culture capabilities by mid 1979.

4.0 ASSAYS OF SAMPLES AT (PARTICIPATING COMPANY)

Not Complete. Will be determined after (participating company) has reviewed protocol and final agreement is established as to the products to be tested.

5.0 SAMPLE VOLUMES AND CHARACTERISTICS

Sample volumes will be determined during processing. Since most processed samples will be cellular, they may be supplied as slurries or pellets. If the original volumes are maintained, as may be required for some samples, the volumes will be 4 to 8 ml. The number of samples to be tested by (participating company) will vary depending on the separation of products and the product(s) of interest in the separated sample. For example, if the sample separates over only a few tubes then only a few samples need to be assayed. However, if the sample separates over several tubes, then many samples will require testing from that particular process.

6.0 SAMPLE DISPOSAL

Samples sent to the participating company will be disposed of at their discretion after testing is completed. Additional tests may be conducted by the participating laboratory if they desire. However, MDAC-St. Louis will be informed of the nature of any additional tests and will have access to the results.

7.0 DATA MANAGEMENT

All data will become the property of MDAC-St. Louis, and can be used at their discretion for inclusion in contract reports, in-house documents and Independent Research and Development (IRAD) reports. This will include a free exchange of data with (participating company).

At the discretion of (participating company) their affliation may or may not be used in these reports. The nature of this affiliation can be detailed prior to any handling of data in reports which are sent to third parties.

Any open literature publications which may result as a result of these tests would include personnel of (participating company) at their discretion.

8.0 TECHNICAL CONTACT - MDAC-ST. LOUIS

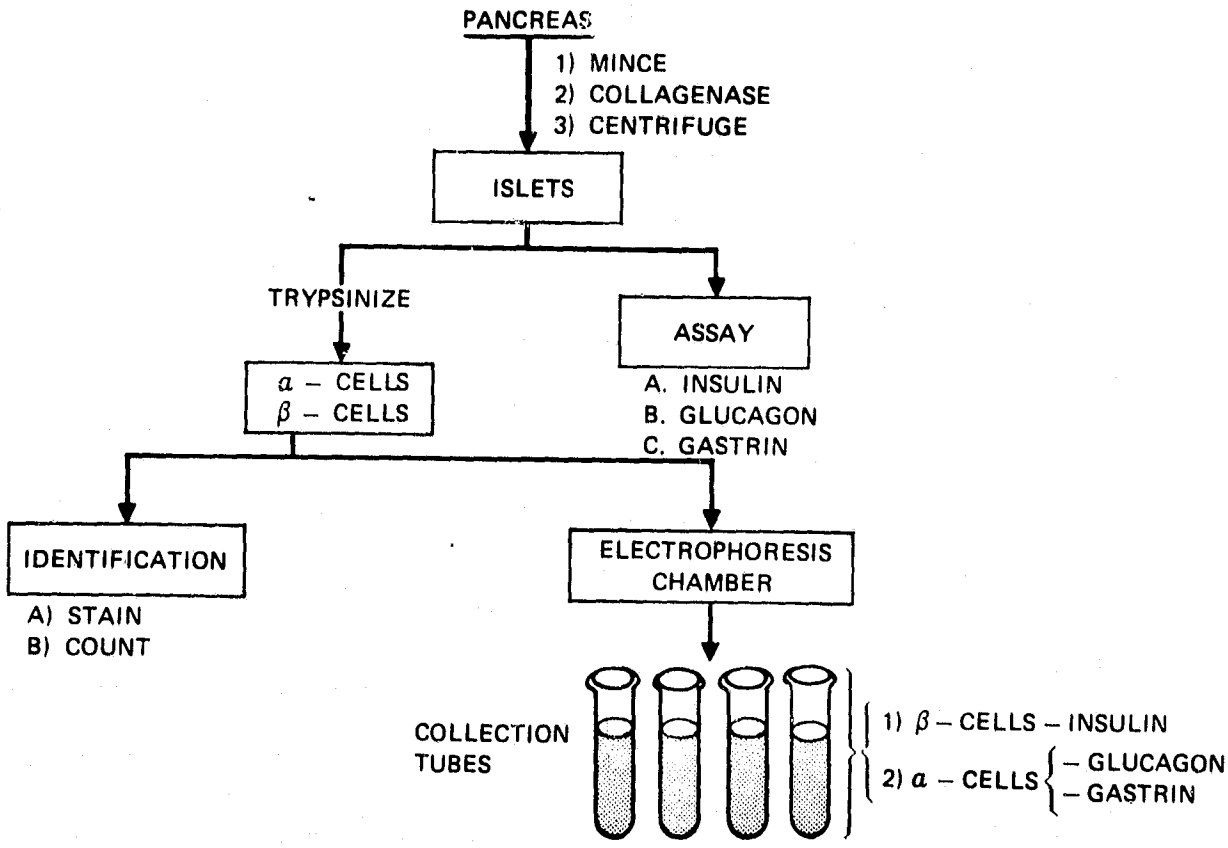
All questions regarding the test protocol, laboratory procedures, shipping of samples, etc., should be directed to:

Dr. J. W. Lanham
McDonnell Douglas Astronautics Company
P.O. Box 516, Dept E423, Bldg 65
St. Louis, Missouri 63166
(314) 232-7923

PANCREATIC ENDOCRINE CELL SEPARATION PROCEDURE

A. Pancreas minced.
B. Treat with collagenase.
C. Assay islet hormone production.
D. Trypsinize to obtain alpha and beta cells.
E. Stain and count cells.
F. Process through electrophoresis unit.
G. Assay products for cell count, hormones.

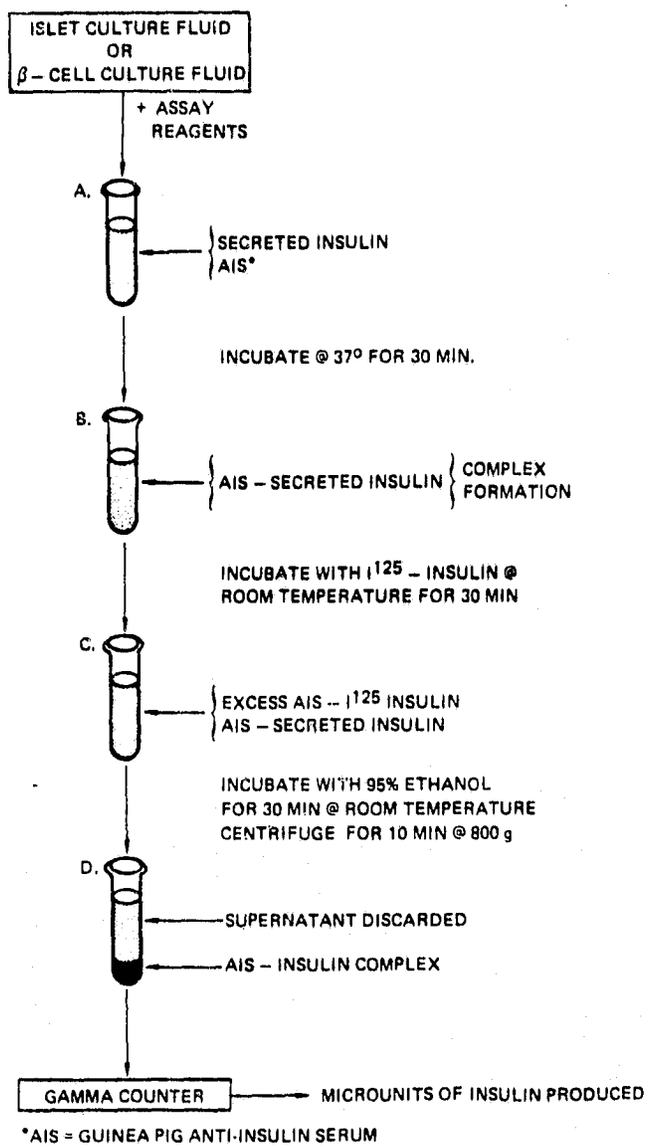
PANCREATIC ENDOCRINE CELL SEPARATION



INSULIN ASSAY PROCEDURE

A. Insulin culture fluid complexed with anti-insulin serum.
B. I^{125} insulin added - competition for anti-insulin serum.
C. Ethanol extraction.
D. I^{125} insulin - anti insulin complex counted in gamma counter.

INSULIN ASSAY PROCEDURE



*AIS = GUINEA PIG ANTI-INSULIN SERUM

GLUCAGON ASSAY PROCEDURE

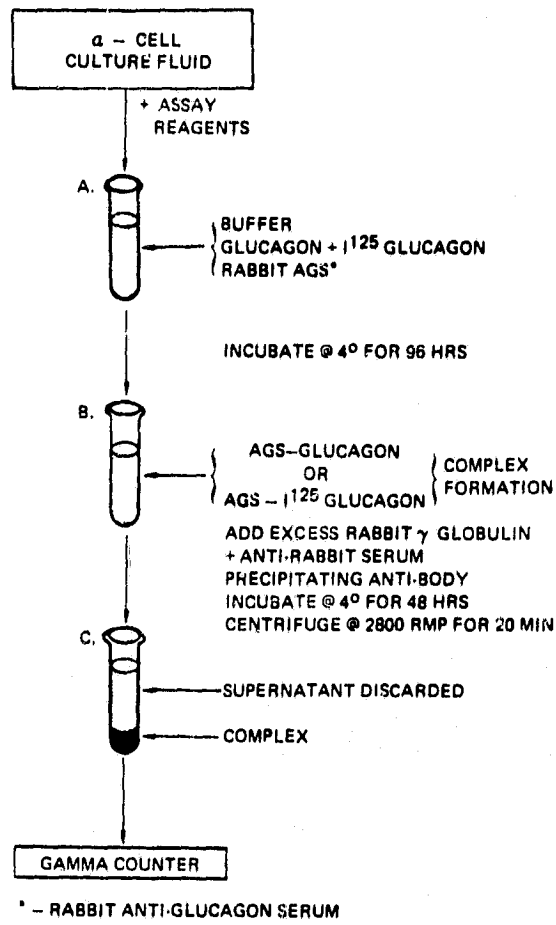
A. Glucagon culture fluid and I^{125} - glucagon - anti glucagon complexes formed.

B. Precipitating antibody added.

C. Complex formed.

D. I^{125} - glucagon complex counted in gamma counter.

GLUCAGON ASSAY PROCEDURE



INSULIN AND GLUCAGON ASSAY REFERENCES

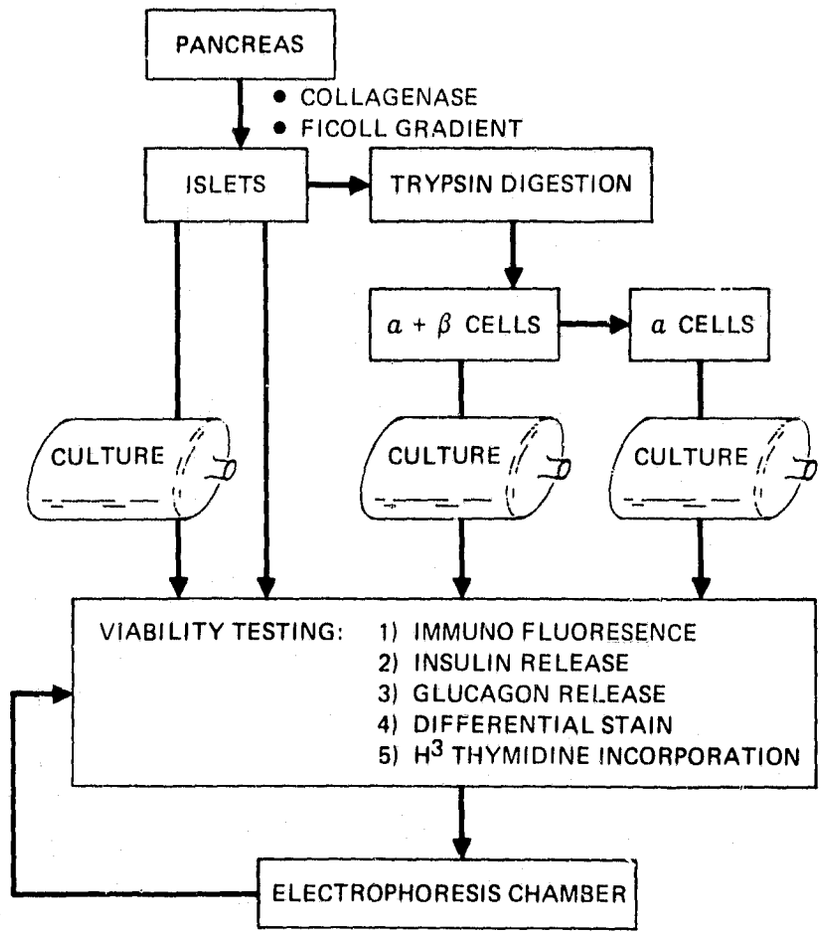
1. Diabetes, 17:537, 1968.
2. Diabetes, 18:660, 1969.
3. Diabetes, 20:33, 191.
4. Endocrinology, 81:226, 1967.
5. Handbook of Physiology/Vol. I - The Endocrine Pancreas, Williams & Williams, Baltimore, 1972.
6. Am. Zool., 13:613, 1973.
7. Glucagon Molecular Physiology, Clinical and Therapeutic Implication, P. J. Lefebvre and R. H. Unger, Pergamon, Oxford, 1972.
8. Br. Med. Bull., 30:18, 1974.
9. Biochem. J., 89:114, 1963.
10. BBA, 251:363, 1971.
11. ACTA Endocrinol, 54:527, 1967.
12. Handbook of Radioimmunoassay, G. E. Abraham (ed.), Marcel Dekker, Inc., NY, 1977.

PANCREATIC ENDOCRINE CELL CULTURE

A. Pancreas Distended and Minced (1)
B. Collagenase Digestion (2)
C. Ficoll Gradient (2)
D. Islets
   a. Culture (3, 4, 5) and Viability Testing (6, 7, 8, 9, 10)
   b. Electrophoresis and Viability Testing
E. Islet Trypsin Digestion (11)
   a. Alpha and Beta cells isolation (12)
   b. Alpha cells isolation (13)
F. Cells
   a. Culture (3, 4, 5, 12) and Viability Testing (6, 7, 8, 9, 10)
   b. Electrophoresis and Viability Testing

(Numbers in parenthesis indicate references for each procedure.)

PANCREATIC ENDOCRINE CELL CULTURE



PANCREATIC ENDOCRINE CELL CULTURE REFERENCES

1. Diabetes, 16:35, 167.
2. Transplantation, 16:686, 1973.
3. Endocrinology, 90:239, 1972.
4. Endocrinology, 96:637, 1975.
5. Tissue and Cell, 1(4):747,1975.
6. Endocrinology, 99(3):684, 1976.
7. Diabetes, 21 (Suppl 2):546, 1972.
8. American Journal of Clinical Pathology, 20:656, 1950.
9. Diabetologia, 12:115, 1976.
10. Diabetes, 22:687, 1973.
11. Proc. of Society of Exp. Biol and Med, 149:402, 1975.
12. Endocrinology, 84:208, 1969.
13. Pancreatic Beta Cell Culture, Workshop Conferences, HOESCHT/Vol 5, Exerpta Medica, Amsterdam, Oxford, 1977.

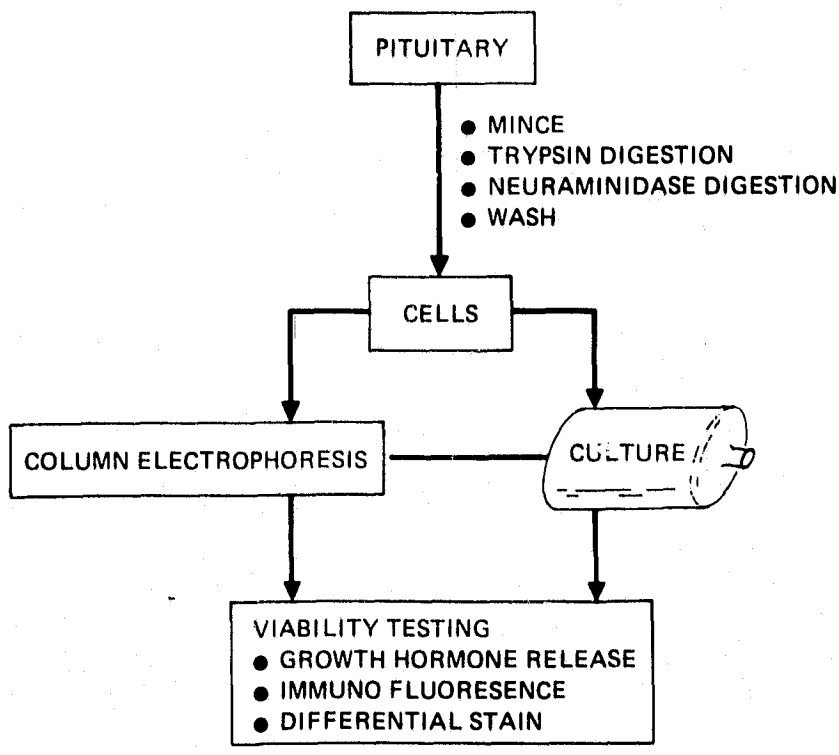
C-3

PRIMARY PITUITARY CELL SEPARATION AND CULTURE

A. Pituitary - Minced (1)
B. Trypsin Digestion (2)
C. Neuraminidase Digestion
D. Wash and Centrifuge
E. Cell Suspension
   a. Culture (3), or
   b. Electrophoresis
F. Viability Testing
   a. Growth Hormone Release (4, 5, 6, 7)
   b. Immunofluorescence (9)
   c. Differential Stain (8, 9)

(Numbers in parenthesis indicate references for each procedure.)

PRIMARY PITUITARY CELL CULTURE



PRIMARY PITUITARY CELL CULTURE REFERENCES

1. The Anterior Pituitary, Ultrastructure in Biological Systems/Vol. 7, A. Tixier-Vidal and M. G. Farquhar, Academic Press, NY 1975.
2. Journal Cell Biology, 59:276, 1973.
3. Journal of Clinical Endocrinology and Metabolism, 45:73, 1977.
4. Metabolism, 13:1135, 1964.
5. Biochem. Journal, 89:114, 1963.
6. Journal of Lab. Clin. Med., 70, 1973, 1967.
7. Methods of Hormone Radioimmunoassay, B. M. Jaffe and H. R. Behrman, Academic Press, NY 1974.
8. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd edition, L. G. Lune, American Registry of Pathology, 1968.
9. Selected Histochemical and Histopathological Methods, S. W. Thompson, Charles C. Thomas, Illinois, 1966.

GROWTH HORMONE ASSAY PROCEDURE

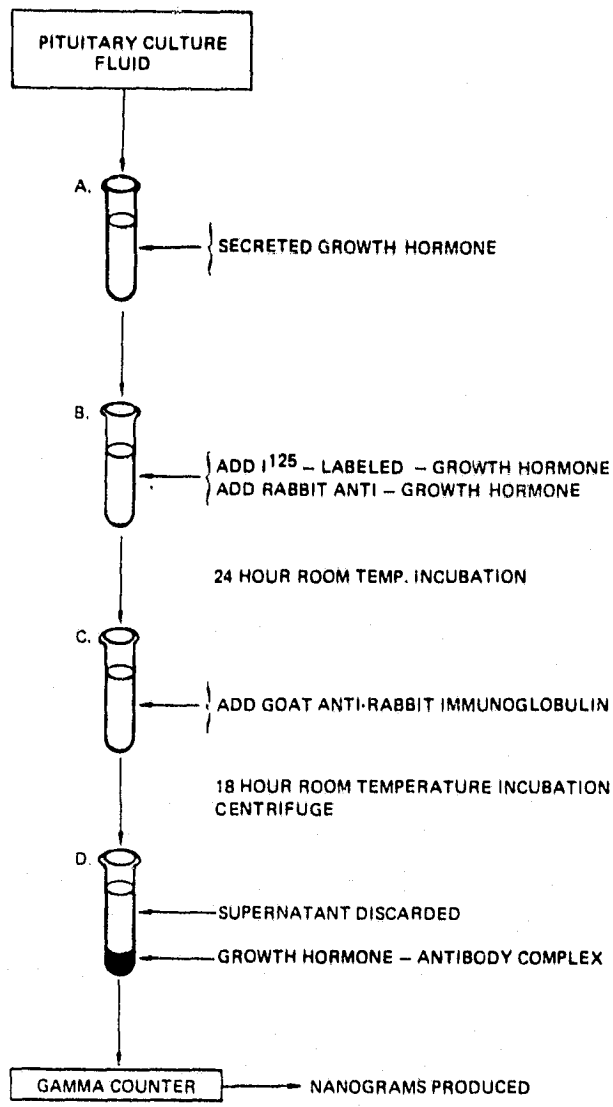
A. Growth hormone culture fluid and I^{125} growth hormone - antigrowth hormone complex formed.

B. Precipitating antibody added.

C. Complex formed.

D. I^{125} - growth hormone complex counted in gamma counter.

GROWTH HORMONE ASSAY PROCEDURE



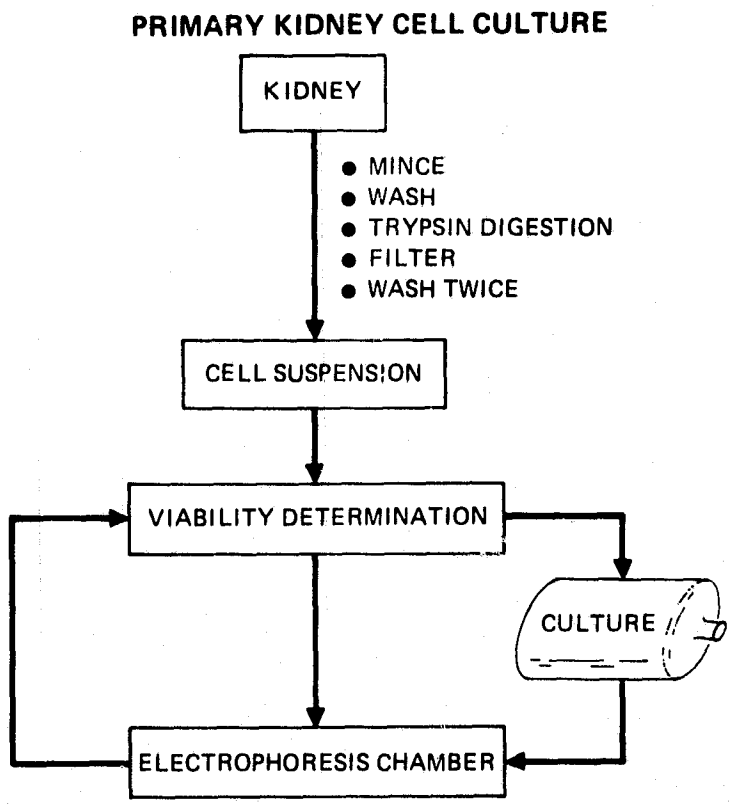
GROWTH HORMONE ASSAY REFERENCES

1. Biochem. J., 89:114, 1963.
2. Metabolism, 13:1135, 1964.
3. J. Lab. Clin. Med., 70:973, 1967.
4. Methods of Hormone Radioimmunoassay, B. M. Jaffe and H. R. Behrman, Academic Press, NY, 1974.

PRIMARY KIDNEY CELL CULTURE

A. Kidney - Minced and Washed (1)
B. Trypsin Digestion
C. Filter Cell Suspension
D. Wash and Centrifuge - twice in a buffer
E. Determine Cell Viability (2)
   a. Culture (1) and
   b. Electrophoresis
F. Determine viability

(Numbers in parenthesis indicate references for each procedure.)



PRIMARY KIDNEY CELL CULTURE REFERENCES

1. Tissue Culture: Methods and Applications, Academic Press, NY, 1973. P. E. Kruse, Jr., and M. K. Patterson, Sr.
2. Experimental Cell Research, 11:297, 1956.

GRANULOCYTE COLONY STIMULATING FACTOR (CSF) SEPARATION

I. Kidney Cells
   A. Cell Suspension (1)
   B. Viability Test (2)
      a. Culture (1) - Electrophoresis and/or CSF Assy (3, 4, 5), or
      b. Electrophoresis - Culture and/or CSF Assay

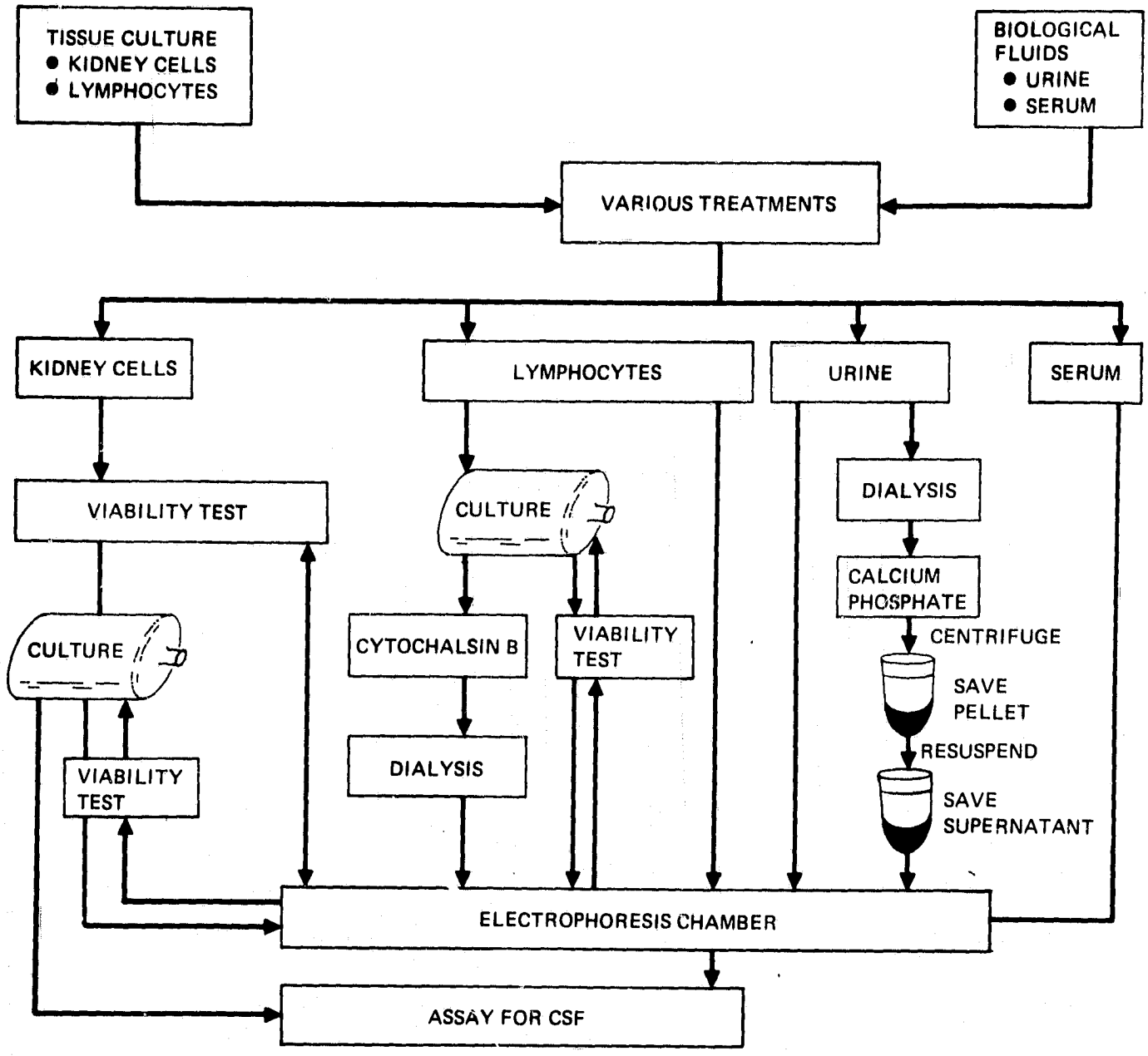
II. Lymphocytes
   A. Electrophoresis - Culture and CSF Assay, or
   Culture (1)
   B. Culture Lymphocytes
   C. Add Cytochalsin B (6)
   D. Dialysis CSF Media
   E. Electrophoresis - Culture and CSF Assay

III. Urine
   A. Electrophoresis, or
   B. Purification of CSF (4)
      a. Dialysis
      b. Add Calcium Phosphate
      c. Centrifuge and Save Pellet
      d. Resuspend Pellet and Centrifuge
      e. Save Supernatant
      f. CSF Assay

IV. Serum - Electrophoresis

(Numbers in parenthesis indicate references for each procedure.)

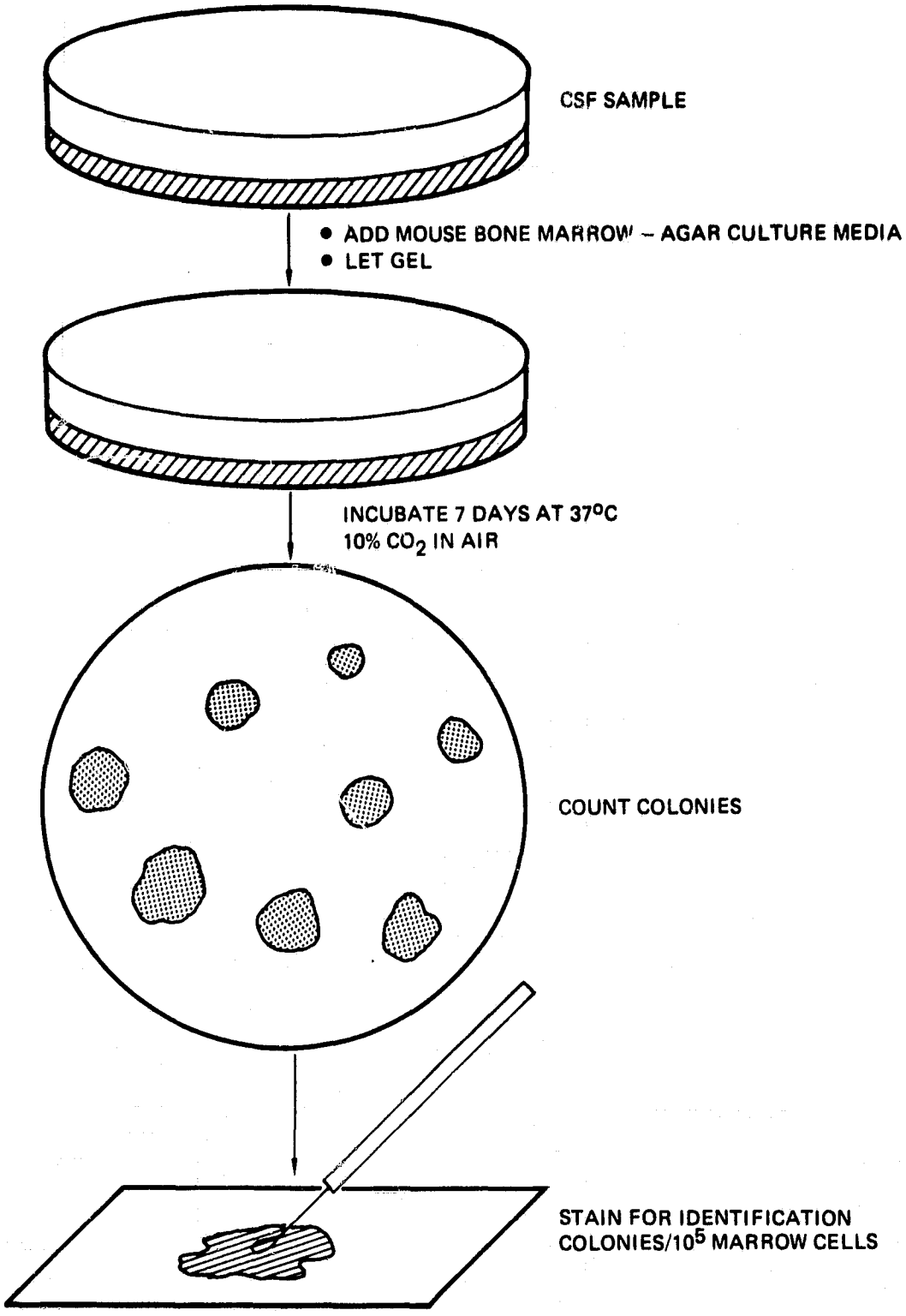
GRANULOCYTE COLONY STIMULATING FACTOR (CSF) SEPARATION



GRANULOCYTE COLONY STIMULATING FACTOR (CSF) SEPARATION REFERENCES

1. Tissue Culture: Methods and Applications, Academic Press, NY, 1973. P. E. Kruse, Jr. and M. K. Patterson, Jr.
2. Experimental Cell Research, 11:297 (1656).
3. J. Cell Phys. 76:89, 1970.
4. J. Lab Clin Med, 79(4):657, 1972.
5. Selected Histochemical and Histopathological Methods, S. W. Thompson (ed.), Charles C. Thomas Company, Illinois, 1966.
6. Cell Immun. 36:388, 1979.

GRANULOCYTE COLONY STIMULATING FACTOR (CSF) ASSAY



GRANULOCYTE COLONY STIMULATING FACTOR ASSAY REFERENCES

1. J. Cell Physiology, 76:89, 1970.
2. J. Lab. Clin. Med., 79(4):657, 1972.
3. Selected Histochemical and Histopathological Methods, S. W. Thompson (ed.), Charles C. Thomas Company, Illinois, 1966.

APPENDIX B

CURRENT GOOD MANUFACTURING PRACTICES FOR FINISHED PHARMACEUTICALS - 1977 CODE OF FEDERAL REGULATIONS

PART 211—CURRENT GOOD MANUFACTURING PRACTICE FOR FINISHED PHARMACEUTICALS

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AUTHORITY: Secs. 501, 701, 52 Stat. 1049-1050 as amended, 1055-1056 as amended (21 U.S.C. 351, 371).

SOURCE: 40 FR 14025, Mar. 27, 1975, unless otherwise noted.

Subpart A—General Provisions

§ 211.1 Finished pharmaceuticals; manufacturing practice.

(a) The criteria in §§ 211.20-211.115, inclusive, shall apply in determining whether the methods used in, or the facilities or controls used for, the manufacture, processing, packing, or holding of a drug conform to or are operated or administered in conformity with current good manufacturing practice

to assure that a drug meets the requirements of the act as to safety and has the identity and strength and meets the quality and purity characteristics which it purports or is represented to possess as required by section 501(a)(2)(B) of the act.

(b) The regulations in this part permit the use of precision automatic, mechanical, or electronic equipment in the production and control of drugs when adequate inspection and checking procedures are used to assure proper performance.

§ 211.10 Personnel.

(a) The personnel responsible for directing the manufacture and control of the drug shall be adequate in number and background of education, training, and experience, or combination thereof, to assure that the drug has the safety, identity, strength, quality, and purity that it purports to possess. All personnel shall have capabilities commensurate with their assigned functions, a thorough understanding of the manufacturing or control operations they perform, the necessary training or experience, and adequate information concerning the reason for application of pertinent provisions of this part to their respective functions.

(b) Any person shown at any time (either by medical examination or supervisory observation) to have an apparent illness or open lesions that may adversely affect the safety or quality of drugs shall be excluded from direct contact with drug products until the condition is corrected. All employees shall be instructed to report to supervisory personnel any conditions that may have such an adverse effect on drug products.

Subpart B—Construction and Maintenance of Facilities and Equipment

§ 211.20 Buildings.

Buildings shall be maintained in a clean and orderly manner and shall be of suitable size, construction, and location to facilitate adequate cleaning, maintenance, and proper operations in the manufacturing, processing, packing, labeling, or holding of a drug. The buildings shall:

(a) Provide adequate space for:

(1) Orderly placement of equipment and materials to minimize any risk of mixups between different drugs, drug components, in-process materials, packaging materials, or labeling, and to minimize the possibility of contamination.

(2) The receipt, storage, and withholding from use of components pending sampling, identification, and testing prior to release by the materials approval unit for manufacturing or packaging.

(3) The holding of rejected components prior to disposition to preclude the possibility of their use in manufacturing or packaging procedures for which they are unsuitable.

(4) The storage of components, containers, packaging materials, and labeling.

(5) Any manufacturing and processing operations performed.

(6) Any packaging or labeling operations.

(7) Storage of finished products.

(8) Control and production-laboratory operations.

(b) Provide adequate lighting, ventilation, and screening and, when necessary for the intended production or control purposes, provide facilities for adequate air-pressure, microbiological, dust, humidity, and temperature controls to:

(1) Minimize contamination of products by extraneous adulterants, including cross-contamination of one product by dust or particles of ingredients arising from the manufacture, storage, or handling of another product.

(2) Minimize dissemination of microorganisms from one area to another.

(3) Provide suitable storage conditions for drug components, in-process materials, and finished drugs in conformance with stability information as derived under § 211.60.

(c) Provide adequate locker facilities and hot and cold water washing facilities, including soap or detergent, air drier or single service towels, and clean toilet facilities near working areas.

(d) Provide an adequate supply of potable water (§ 1250.82 of this chapter) under continuous positive pressure in a plumbing system free of defects that could cause or contribute to

contamination of any drug. Drains shall be of adequate size and, where connected directly to a sewer, shall be equipped with traps to prevent back-siphonage.

(e) Provide suitable housing and space for the care of all laboratory animals.

(f) Provide for safe and sanitary disposal of sewage, trash, and other refuse within and from the buildings and immediate premises.

§ 211.30 Equipment.

Equipment used for the manufacture, processing, packing, labeling, holding, testing, or control of drugs shall be maintained in a clean and orderly manner and shall be of suitable design, size, construction, and location to facilitate cleaning, maintenance, and operation for its intended purpose. The equipment shall:

(a) Be so constructed that all surfaces that come into contact with a drug product shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the drug or its components beyond the official or other established requirements.

(b) Be so constructed that any substances required for operation of the equipment, such as lubricants or coolants, do not contact drug products so as to alter the safety, identity, strength, quality, or purity of the drug or its components beyond the official or other established requirements.

(c) Be constructed and installed to facilitate adjustment, disassembly cleaning and maintenance to assure the reliability of control procedures uniformity of production and exclusion from the drugs of contaminants from previous and current operations that might affect the safety, identity, strength, quality, or purity of the drug or its components beyond the official or other established requirements.

(d) Be of suitable type, size, and accuracy for any testing, measuring, mixing, weighing, or other processing or storage operations.

Subpart C—Product Quality Control

§ 211.40 Production and control procedures.

Production and control procedures shall include all reasonable precautions, including the following, to assure that the drugs produced have the safety, identity, strength, quality, and purity they purport to possess:

(a) Each significant step in the process, such as the selection, weighing, and measuring of components, the addition of ingredients during the process, weighing and measuring during various stages of the processing, and the determination of the finished yield, shall be performed by a competent and responsible individual and checked by a second competent and responsible individual; or if such steps in the processing are controlled by precision automatic, mechanical, or electronic equipment, their proper performance is adequately checked by one or more competent and responsible individuals. The written record of the significant steps in the process shall be identified by the individual performing these tests and by the individual charged with checking these steps. Such identifications shall be recorded immediately following the completion of such steps.

(b) All containers, lines, and equipment used during the production of a batch of a drug shall be properly identified at all times to accurately and completely indicate their contents and, when necessary, the stage of processing of the batch.

(c) To minimize contamination and prevent mixups, equipment, utensils, and containers shall be thoroughly and appropriately cleaned and properly stored and have previous batch identification removed or obliterated between batches or at suitable intervals in continuous production operations.

(d) Appropriate precautions shall be taken to minimize microbiological and other contamination in the production of drugs purporting to be sterile or which by virtue of their intended use should be free from objectionable microorganisms.

(e) Appropriate procedures shall be established to minimize the hazard of

cross-contamination of any drugs while being manufactured or stored.

(f) To assure the uniformity and integrity of products, there shall be adequate in-process controls, such as checking the weights and disintegration times of tablets, the adequacy of mixing, the homogeneity of suspensions, and the clarity of solutions. In-process sampling shall be done at appropriate intervals using suitable equipment.

(g) Representative samples of all dosage form drugs shall be tested to determine their conformance with the specifications for the product before distribution.

(h) Procedures shall be instituted whereby review and approval of all production and control records, including packaging and labeling, shall be made prior to the release or distribution of a batch. A thorough investigation of any unexplained discrepancy or the failure of a batch to meet any of its specifications shall be undertaken whether or not the batch has already been distributed. This investigation shall be undertaken by a competent and responsible individual and shall extend to other batches of the same drug and other drugs that may have been associated with the specific failure. A written record of the investigation shall be made and shall include the conclusions and followup.

(i) Returned goods shall be identified as such and held. If the conditions under which returned goods have been held stored, or shipped prior to or during their return, or the condition of the product, its container, carton, or labeling as a result of storage or shipping, cast doubt on the safety, identity, strength, quality, or purity of the drug, the returned goods shall be destroyed or subjected to adequate examination or testing to assure that the material meets all appropriate standards or specifications before being returned to stock for warehouse distribution or repacking. If the product is neither destroyed nor returned to stock, it may be reprocessed provided the final product meets all its standards and specifications. Records of returned goods shall be maintained and shall indicate the quantity returned, date, and actual disposition of the product. If the reason for returned goods implicates associated batches, an appropriate investigation shall be made in accordance with the requirements of paragraph (h) of this section.

(j) Use of asbestos-containing or other fiber-releasing filters: (1) Filters used in the manufacture, processing or packaging of components of drug products for parenteral injection in humans shall not release fibers into such products. No asbestos-containing or other fiber-releasing filter may be used in the manufacture, processing, or packaging of such products unless it is not possible to manufacture that drug product or component without the use of such a filter. Filtration, as needed, shall be through a non-fiber-releasing filter. For the purposes of this regulation a non-fiber-releasing filter is defined as a non-asbestos filter that, after any appropriate pretreatment such as washing or flushing, will not continue to release fibers into the drug product or component that is being filtered. A fiber is defined as any particle with length at least three times greater than its width.

(2) If use of a fiber-releasing filter is required, an additional non-fiber-releasing filter of maximum pore size of 0.22 microns (0.45 microns if the manufacturing conditions so dictate) shall subsequently be used to reduce the content of any asbestos-form particles in the drug product or component. Use of an asbestos-containing filter with or without subsequent use of a specific non-fiber-releasing filter is permissible only upon submission of proof to the appropriate bureau of the Food and Drug Administration that use of a non-fiber-releasing filter will, or is likely to, compromise the safety or effectiveness of the drug.

(3) Substitution for a fiber-releasing filter shall be achieved on or before September 14, 1976. If such substitution is not achieved on or before March 14, 1976, the manufacturer of the drug product for parenteral injection who requires the additional 6 months to develop new manufacturing procedures so as to utilize non-fiber-releasing filters in place of fiber-releasing filters shall submit monthly reports to the appropriate bureau of the Food and Drug Administration indi-

cating progress in substituting the new filters. Such a substitution shall be shown to have been effected without loss of the safety or effectiveness of the drug.

[40 FR 14025, Mar. 27, 1975, as amended at 41 FR 16933, Apr. 23, 1976]

§ 211.42 Components.

All components and other materials used in the manufacture, processing, and packaging of drug products, and materials necessary for building and equipment maintenance, upon receipt shall be stored and handled in a safe, sanitary, and orderly manner. Adequate measures shall be taken to prevent mixups and cross-contamination affecting drugs and drug products. Components shall be withheld from use until they have been identified, sampled, and tested for conformance with established specifications and are released by a materials approval unit. Control of components shall include the following:

(a) Each container of component shall be examined visually for damage or contamination prior to use, including examination for breakage of seals when indicated.

(b) An adequate number of samples shall be taken from a representative number of component containers from each lot and shall be subjected to one or more tests to establish the specific identity.

(c) Representative samples of components liable to contamination with filth, insect infestation, or other extraneous contaminants shall be appropriately examined.

(d) Representative samples of all components intended for use as active ingredients shall be tested to determine their strength in order to assure conformance with appropriate specifications.

(e) Representative samples of components liable to microbiological contamination shall be subjected to microbiological tests prior to use. Such components shall not contain microorganisms that are objectionable in view of their intended use.

(f) Approved components shall be appropriately identified and retested as necessary to assure that they conform to appropriate specifications of identity, strength, quality, and purity at time of use. This requires the following:

(1) Approved components shall be handled and stored to guard against contaminating or being contaminated by other drugs or components.

(2) Approved components shall be rotated in such a manner that the oldest stock is used first.

(3) Rejected components shall be identified and held to preclude their use in manufacturing or processing procedures for which they are unsuitable.

(g) Appropriate records shall be maintained, including the following:

(1) The identity and quantity of the component, the name of the supplier, the supplier's lot number, and the date of receipt.

(2) Examinations and tests performed and rejected components and their disposition.

(3) An individual inventory and record for each component used in each batch of drug manufactured or processed.

(h) An appropriately identified reserve sample of all active ingredients consisting of at least twice the quantity necessary for all required tests, except those for sterility and determination of the presence of pyrogens, shall be retained for at least 2 years after distribution of the last drug lot incorporating the component has been completed or 1 year after the expiration date of this last drug lot, whichever is longer.

§ 211.55 Product containers and their components.

Suitable specifications, test methods, cleaning procedures, and when indicated, sterilization procedures shall be used to assure that containers, closures, and other component parts of drug packages are suitable for their intended use. Containers for parenteral drugs, drug products or drug components shall be cleansed with water which has been filtered through a non-fiber-releasing filter equivalent to that indicated in § 211.40(j) (2). Product containers and their components shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the

drug or its components beyond the official or established requirements and shall provide adequate protection against external factors that can cause deterioration or contamination of the drug.

(Secs. 501, 502, 701, 52 Stat. 1049-1051, 1055-1056, as amended; (21 U.S.C. 351, 352, 371))

§ 211.58 Laboratory controls.

Laboratory controls shall include the establishment of scientifically sound and appropriate specifications, standards, and test procedures to assure that components, in-processed drugs, and finished products conform to appropriate standards of identity, strength, quality, and purity. Laboratory controls shall include:

(a) The establishment of master records containing appropriate specifications for the acceptance of each lot of drug components, product containers, and their components used in drug production and packaging and a description of the sampling and testing procedures used for them. Said samples shall be representative and adequately identified. Such records shall also provide for appropriate retesting of drug components, product containers, and their components subject to deterioration.

(b) A reserve sample of all active ingredients as required by § 211.42(h).

(c) The establishment of master records, when needed, containing specifications and a description of sampling and testing procedures for in-process drug preparations. Such samples shall be adequately representative and properly identified.

(d) The establishment of master records containing a description of sampling procedures and appropriate specifications for finished drug products. Such samples shall be adequately representative and properly identified.

(e) Adequate provisions for checking the identity and strength of drug products for all active ingredients and for assuring:

(1) Sterility of drugs purported to be sterile and freedom from objectionable microorganisms for those drugs which should be so by virtue of their intended use.

(2) The absence of pyrogens for those drugs purporting to be pyrogen-free.

(3) Minimal contamination of ophthalmic ointments by foreign particles and harsh or abrasive substances.

(4) That the drug release pattern of sustained release products is tested by laboratory methods to assure conformance to the release specifications.

(f) Adequate provision for auditing the reliability, accuracy, precision, and performance of laboratory test procedures and laboratory instruments used.

(g) A properly identified reserve sample of the finished product (stored in the same immediate container-closure system in which the drug is marketed) consisting of at least twice the quantity necessary to perform all the required tests, except those for sterility and determination of the absence of pyrogens, and stored under conditions consistent with product labeling shall be retained for at least 2 years after the drug distribution has been completed or at least 1 year after the drug's expiration date, whichever is longer.

(h) Provision for retaining complete records of all laboratory data relating to each batch or lot of drug to which they apply. Such records shall be retained for at least 2 years after distribution has been completed or 1 year after the drug's expiration date, whichever is longer.

(i) Provision that animals shall be maintained and controlled in a manner that assures suitability for their intended use. They shall be identified and appropriate records maintained to determine the history of use.

(j) Provision that firms which manufacture nonpenicillin products (including certifiable antibiotic products) on the same premises or use the same equipment as that used for manufacturing penicillin products, or that operate under any circumstances that may reasonably be regarded as conducive to contamination of other drugs by penicillin, shall test such nonpenicillin products to determine whether any have become cross-contaminated by penicillin. Such products shall not be marketed if intended for use in man and the product is con-

taminated with an amount of penicillin equivalent to 0.05 unit or more of penicillin G per maximum single dose recommended in the labeling of a drug intended for parenteral administration, or an amount of penicillin equivalent to 0.5 unit or more of penicillin G per maximum single dose recommended in the labeling of a drug intended for oral use.

§ 211.60 Stability.

There shall be assurance of the stability of finished drug products. This stability shall be:

(a) Determined by reliable, meaningful, and specific test methods.

(b) Determined on products in the same container-closure systems in which they are marketed.

(c) Determined on any dry drug product that is to be reconstituted at the time of dispensing (as directed in its labeling), as well as on the reconstituted product.

(d) Recorded and maintained in such manner that the stability data may be utilized in establishing product expiration dates.

§ 211.62 Expiration dating.

To assure that drug products liable to deterioration meet appropriate standards of identity, strength, quality, and purity at the time of use, the label of all such drugs shall have suitable expiration dates which relate to stability tests performed on the product.

(a) Expiration dates appearing on the drug labeling shall be justified by readily available data from stability studies such as described in § 211.60.

(b) Expiration dates shall be related to appropriate storage conditions stated on the labeling wherever the expiration date appears.

(c) When the drug is marketed in the dry state for use in preparing a liquid product, the labeling shall bear expiration information for the reconstituted product as well as an expiration date for the dry product.

Subpart D—Packaging and Labeling

§ 211.80 Packaging and labeling.

Packaging and labeling operations shall be adequately controlled: To assure that only those drug products that have met the standards and specifications established in their master production and control records shall be distributed; to prevent mixups between drugs during filling, packaging, and labeling operations; to assure that correct labels and labeling are employed for the drug; and to identify the finished product with a lot or control number that permits determination of the history of the manufacture and control of the batch. An hour, day, or shift code is appropriate as a lot or control number for drug products manufactured or processed in continuous production equipment. Packaging and labeling operations shall:

(a) Be separated (physically or spatially) from operations on other drugs in a manner adequate to avoid mixups and minimize cross-contamination. Two or more packaging or labeling operations having drugs, containers, or labeling similar in appearance shall not be in process simultaneously on adjacent or nearby lines unless these operations are separated either physically or spatially.

(b) Provide for an inspection of the facilities prior to use to assure that all drugs and previously used packaging and labeling materials have been removed.

(c) Include the following labeling controls:

(1) The holding of labels and package labeling upon receipt pending review and proofing against an approved final copy by a competent and responsible individual to assure that they are accurate regarding identity, content, and conformity with the approved copy before release to inventory.

(2) The maintenance and storage of each type of label and package labeling representing different products, strength, dosage forms, or quantity of contents in such a manner as to prevent mixups and provide proper identification.

(3) A suitable system for assuring that only current labels and package labeling are retained and that stocks of obsolete labels and package labeling are destroyed.

(4) Restriction of access to labels and package labeling to authorized personnel.

(5) Avoidance of gang printing of cut labels, cartons, or inserts when the labels, cartons, or inserts are for different products or different strengths of the same products or are of the same size and have identical or similar format and/or color schemes. If gang printing is employed, packaging and labeling operations shall provide for added control procedures. These added controls should consider sheet layout, stacking, cutting, and handling during and after printing.

(d) Provide strict control of the package labeling issued for use with the drug. Such issue shall be carefully checked by a competent and responsible person for identity and conformity to the labeling specified in the batch production record. Said record shall identify the labeling and the quantities issued and used and shall reasonably reconcile any discrepancy between the quantity of drug finished and the quantities of labeling issued. All excess package labeling bearing lot or control numbers shall be destroyed. In event of any significant unexplained discrepancy, an investigation should be carried out according to § 211.40(h).

(e) Provide for adequate examination or laboratory testing of representative samples of finished products after packaging and labeling to safeguard against any errors in the finishing operations and to prevent distribution of any batch until all specified tests have been met.

Subpart E—Records and Reports

§ 211.101 Master production and control records; batch production and control records.

(a) To assure uniformity from batch to batch, a master production and control record for each drug product and each batch size of drug product shall be prepared, dated, and signed or initialed by a competent and responsible individual and shall be independently checked, reconciled, dated, and signed or initialed by a second competent and responsible individual. The master production and control record shall include:

(1) The name of the product, description of the dosage form, and a specimen or copy of each label and all other labeling associated with the retail or bulk unit, including copies of such labeling signed or initialed and dated by the person or persons responsible for approval of such labeling.

(2) The name and weight or measure of each active ingredient per dosage unit or per unit of weight or measure of the finished drug, and a statement of the total weight or measure of any dosage unit.

(3) A complete list of ingredients designated by names or codes sufficiently specific to indicate any special quality characteristic; an accurate statement of the weight or measure of each ingredient regardless of whether it appears in the finished product, except that reasonable variations may be permitted in the amount of components necessary in the preparation in dosage form provided that provisions for such variations are included in the master production and control record; an appropriate statement concerning any calculated excess of an ingredient; an appropriate statement of theoretical weight or measure at various stages of processing; and a statement of the theoretical yield.

(4) A description of the containers, closures, and packaging and finishing materials.

(5) Manufacturing and control instructions, procedures, specifications, special notations, and precautions to be followed.

(b) The batch production and control record shall be prepared for each batch of drug produced and shall include complete information relating to the production and control of each batch. These records shall be retained for at least 2 years after the batch distribution is complete or at least 1 year after the batch expiration date, whichever is longer. These records shall identify the specific labeling and lot or control numbers used on the batch and shall be readily available during

such retention period. The batch record shall include:

(1) An accurate reproduction of the appropriate master formula record checked, dated, and signed or initialed by a competent and responsible individual.

(2) A record of each significant step in the manufacturing, processing, packaging, labeling, testing, and controlling of the batch, including: Dates; individual major equipment and lines employed; specific identification of each batch of components used; weights and measures of components and products used in the course of processing; in-process and laboratory control results; and identifications of the individual(s) actively performing and the individual(s) directly supervising or checking each significant step in the operation.

(3) A batch number that identifies all the production and control documents relating to the history of the batch and all lot or control numbers associated with the batch.

(4) A record of any investigation made according to § 211.40(h).

§ 211.110 Distribution records.

(a) Finished goods warehouse control and distribution procedures shall include a system by which the distribution of each lot of drug can be readily determined to facilitate its recall if necessary. Records within the system shall contain the name and address of the consignee, date and quantity shipped, and lot or control number of the drug. Records shall be retained for at least 2 years after the distribution of the drug has been completed or 1 year after the expiration date of the drug, whichever is longer.

(b) To assure the quality of the product, finished goods warehouse control shall also include a system whereby the oldest approved stock is distributed first whenever possible. (See 21 CFR 1304 for regulations relating to manufacturing and distribution records of drugs subject to the Drug Abuse Control Amendments of 1965; Public Law 89-74.)

§ 211.115 Complaint files.

Records shall be maintained of all written and oral complaints regarding each product. An investigation of each complaint shall be made in accordance with § 211.40(h). The record of each investigation shall be maintained for at least 2 years after distribution of the drug has been completed or 1 year after the expiration date of the drug, whichever is longer.

APPENDIX C

PROCESS PROTOCOL - ERYTHROPOIETIN

PROTOCOL
ERYTHROPOIETIN PRODUCTION IN SPACE

A. PREFLIGHT OPERATIONS

1. CELL SOURCE: Kidney cortex can be either minced and partially purified to separate glomeruli using a sieving technique or the needed (not purified strain) cells can be purchased from tissue culture supply houses if available.
2. FROZEN-STORAGE: Immediately after obtaining cells by the sieving method above, the cells must be frozen in a medium that will protect them from internal chemical distortion and possible cell rupture during the freeze/thaw cycle as well as providing a nutrient source for the very low cellular metabolism while in the stored state. Additives such as glycerol, dimethyl sulphoxide, sucrose and phosphates are typical preservatives. The cells should be rate frozen to -196°C for storage up to one year; -96°C for storage up to six months; -56°C for storage up to two months and -20°C for storage up to two weeks.
3. SYSTEM STERILIZATION: The system must be sterilized prior to flight and remain in that sterile condition during storage and flight.

B. FLIGHT OPERATIONS

1. THAWING CELL SAMPLE: Cells must be quick-thawed for best survival. They should be brought from the frozen storage temperature to 4°C at a rate of 2 ml of sample per minute.
2. BUFFER EXCHANGE: Immediately after thawing, the cell suspension must be washed of the cryoprotectant substances, and a buffered nutrient media added to allow the cells to survive for several days if not directly followed by the electrophoretic procedure. Immediately prior to processing the cell sample through the electrophoresis system, the sample must be "cleansed" of any constituents that would interfere with the electric field and separation capabilities of the unit. At the same time as these constituents are removed, additional nonreactive constituents must be added to the sample to restore and maintain osmotic equilibrium within the cell. During this exchange process all operations must be conducted in a 4°C $\pm$2°C environment. The sample volume should be adjusted to 85 milliliters containing 1×10^8 cells/ml.

3. CELL ELECTROPHORETIC SEPARATION: Once these cells have gone through the buffer exchange procedure they must be processed through the electrophoretic system as soon as possible to maintain their viability; preferably entering the separation chamber within several minutes and no longer than 20 minutes after completion of the fluid replacement. The exchanged buffer must have some but only minimal,protective ability. It cannot contain nutrients to maintain cell viability.

Residence time of each cell in the electrophoresis chamber will be approximately 50 minutes. As long as the electrophoretic separation process is conducted at 4-8°C, a cell viability loss of less than 10% is anticipated. The total separation time for the full sample is expected to be 85 hours.

3.1 BUFFER RECLAMATION: Each electrophoretic chamber outlet tube must be monitored to detect the presence of the cellular material of interest. The central 50% of this bandspread of each outlet tube must be collected. The effluent of the remaining outlet ports will be collected and subjected to a "cleansing" process to remove particulates, organics, and ionic elements. The resultant water must then be held until a preselected volume is obtained. Preweighed and packaged chemicals must then be mixed into this volume of water in order to produce additional buffer material for continuous operation of the electrophoresis chamber.

4. COLLECTED SAMPLE MEDIA EXCHANGE: As the separated cells emerge from the electrophoresis chamber outlet tubes they must be transfered into a more protective nutrient media which will allow cell growth. This media must contain all essential growth factors (solids and gases) and be of sufficient quantity to keep the collected cells viable until the complete sample has been collected and transfered to the erythropoietin-producing cell culturing vessel.

5. CELL CULTURE INOCULATION: The collected cells are transfered to a small culture vessel of 500 ml volume. This initial inoculation must have a minimum concentration of 4.4×10^5 cells/ml. Nutrient media must be continuously cycled through the vessel while temperature, pH and oxygen concentrations are maintained within controlled intervals. There is a growth lag phase of about three days as the cells acclimatize to their new environment. Once growth begins, the cells will double approximately every 35 hours.

Cells must be cultured until they reach a population of 3×10^6/ml. This will require about 7 days. When this population density is reached the entire contents of the vessel is used to inoculate a second culture vessel of 3.5 liter volume. Again, after a 3 day lag and 4 day cell growth period (with care given to controlling cell environment described above) the vessel contents are transfered into a 25 liter vessel for the third expansion of culture volume. When the three day lag and 4 day growth period are completed in this vessel, the contents are transfered to a final production tank of 125 liters. Each transfer and dilution results in an initial inoculation of 4.4×10^5 cell/ml.

6. PRODUCTION CELL CULTURING AND HARVESTING: In the production tank there will be a seven day period of cell acclimalization and growth until the cell concentration reaches 3.6×10^6 cells/ml. When this population density has been achieved, the nutrient media will be changed to that more desirable for erythropoietin production and cell reproduction will rapidly diminish. The culture vessel is continuously drained at the rate of 8.5% of volume per day through a 0.2 micron filter and the effluent collected. The chamber culture volume is maintained by the simultaneous admission of fresh replacement media. The culture vessel is maintained at the optimum temperature, pH and chemical composition required for maximum cell production.

   The production cycle will continue for 14 days. At that point in time, the cell population in the culture vessel will be cut back to 10% (the remainder of the cells disrupted with the membranes and organelles discarded) and the growth phase restarted. This cut back in density will allow removal of any metabolic wastes that may not have been removed with continuous media replacement. The 7 day growth, 14 day production cycle will be repeated three additional times. At the end of the fourth cycle, the tank is drained, cleaned, sterilized and a new cycle with new cultures begun.

7. PROTEIN PRODUCT CONCENTRATION: The resultant harvested media contains the erythropoietin product in very dilute form along with proteins from the growth media. The protein must be concentrated for further purification by another electrophoretic separation. The fluid must be concentrated approximately 15 times to provide a new electrophoresis sample flow rate of 36 ml/hours. The sample must contain about 10% protein per milliliter.

7.1 CULTURE MEDIA RECLAMATION: The excess production culture media available from the concentration procedure will be collected and cleansed of particulates, organics, and ionic elements. This polished water must be mixed with preweighed and packaged chemicals to form additional nutrient media for continuous operation of the culture vessel. Because water is lost each day for the electrophoretic sample, make up water must be added to this additional nutrient media to restore volume and chemical concentration.

8. ERYTHROPOIETIN ELECTROPHORETIC SEPARATION: At 4°C the collected and concentrated media must be processed through the continuous flow electrophoresis chamber at the rate of 36 ml/hour.

9. FRACTION COLLECTION AND CONCENTRATION: As the separated proteins emerge from the electrophoresis chamber outlet tubes, they must be continuously analyzed to determine their identity. Only the middle 90% of each fraction bandwidth must be collected. All collected fractions are subjected to a protein concentration procedure in order to reduce the volume by a ratio of 100:1.

9.1 BUFFER RECLAMATION: The effluent of the remaining chamber outlet ports and the fluid from the concentration fraction must be subjected to a cleansing process to remove particles, organics and ionic elements. The resultant water must be held until a preselected volume is obtained. Make up water will be required since a certain amount is lost for concentration in step 9. Preweighed and packaged chemicals must then be mixed into this volume of water in order to produce additional buffer material for continuous operation of the electrophoresis chamber.

10. PRODUCT LYOPHILIZATION: The erythropoietin must be bulk lyophilized to its powder form. The fluid removed in this process is dumped overboard.

11. BULK STORAGE: The lyophilized material must be held in bulk sterile storage to be brought back to earth for further processing, packaging and sale.

12. SYSTEM STERILIZATION: Since the system remains closed after ground sterilization, there should be no contamination problems. Final product samples must be collected every other day to verify freedom from microbiological contamination and pyrogenicity.

APPENDIX C

PROCESS PROTOCOL-ANTIHEMOPHILIC FACTOR

PROTOCOL
FOR
ANTIHEMOPHILIC FACTOR PRODUCTION IN SPACE

A. PRE-FLIGHT OPERATIONS

1. ANTIHEMOPHILIC FACTOR SOURCE: Cryoprecipitate (the source of AHF) is obtained in its bulk frozen form from the supplier.
2. STORAGE: AHF must be stored at -30°C or below to prevent deterioration.

B. FLIGHT OPERATIONS

1. THAWING AND DILUTION OF MATERIAL: The material is allowed to thaw at 10°C and diluted in a 1:1 ratio with the proper buffer to be used for electrophoretic separation.
2. ELECTROPHORETIC SEPARATION: At 4°C, the diluted cryoprecipitate is processed through the continuous flow electrophoresis chamber at the rate of 36 ml/hour.
3. FRACTION COLLECTION AND CONCENTRATION: As the separated proteins emerge from the electrophoretic chamber outlet tubes they must be analyzed continuously to determine their identity. Only the middle 90% of each fraction bandwidth must be collected. All collected fractions are subjected to a protein concentration procedure in order to reduce the volume by a ratio of 100:1.

3.1 BUFFER RECLAMATION: The effluent of the remaining chamber outlet ports and the fluid from the concentration fractions are subjected to a cleansing process to remove particulates, organics and ionic elements. The resultant water must then be held until a preselected volume is obtained. Makeup water will be required to compensate for that lost during reclamation and lyophilization. Preweighed and packaged chemicals must then be mixed into this volume of water in order to produce additional buffer material for continuous operation of the electrophoresis chamber.

4.0 PRODUCT LYOPHILIZATION: Each concentrated protein fraction is separately bulk lyophilized to its powder form. The fluid removed in this process is dumped overboard.

5.0 BULK STORAGE: The lyophilized material is held in bulk sterile storage to be brought back to earth for further processing, packaging and sale.

6.0 SYSTEM STERILIZATION: Since the system remains closed after ground sterilization, there should be no contamination problems. Final product samples must be collected every other day to verify freedom from microbiological contamination and pyrogenicity.

APPENDIX C.2

PROCESS PROTOCOL-BETA CELLS

PROTOCOL
FOR
BETA CELL PRODUCTION IN SPACE

A. PREFLIGHT OPERATIONS

1. CELL SOURCE: The human pancreas must be minced and partially purified to separate the Islets of Langerhans or Beta cells from the surrounding acinar and connective tissue. This can be accomplished using enzymatic disruption and washed sedimentation techniques. Approximately 7.0×10^9 cells in 68 ml should be collected for this activity.
2. FROZEN STORAGE: Immediately after obtaining the partially purified cells, they must be frozen in a medium that will protect them from internal chemical distortion and possible cell rupture during the freeze/thaw cycle as well as providing a nutrient source for their very low cellular metabolism while in the stored state. Additives such as glyceral, dimethyl sulphoxide, sucrose and phosphates are typical preservatives. The cells should be rate frozen for storage. The depth of freezing and the length of storage viability must still be determined.
3. SYSTEM STERILIZATION: The system must be sterilized prior to flight and remain in that sterile condition during storage and flight.

B. FLIGHT OPERATIONS

1. THAWING CELL SAMPLE: Cells must be quick-thawed for best survival. They should be brought from the frozen storage temperature to 4°C at a rate of 2 ml of sample per minute.
2. BUFFER EXCHANGE: Immediately after thawing, the cell suspension must be washed of the cryoprotectant substances and a buffered nutrient media added to allow the cells to survive for several days if not directly followed by the electrophoretic procedure.

Immediately prior to processing the cell sample through the electrophoresis system, that sample must be "cleansed" of any constituents that would interfere with the electrical field and separation capabilities of the unit. At the same time as these constituents are removed, additional nonreactive constituents must be added to the sample to restore and maintain osmotic

equilibrium within the cell. During this exchange process, all operations must be conducted in a 4°C ±2°C environment. The sample volume should be adjusted to 68 milliliters.

3. CELL ELECTROPHORETIC SEPARATION: Once these cells have gone through the buffer exchange procedure, they must be processed through the electrophoretic system as soom as possible to maintain their viability; preferably entering the separation chamber within several minutes and no longer than 20 minutes after completion of the fluid replacement. The exchanged buffer must have some, but only minimal, protective ability. It can not contain nutrients to maintain cell viability.

Residence time of each cell in the electrophoretic chamber will be approximately 50 minutes. As long as the electrophoretic separation process is conducted at 4-8°C, a cell viability loss of less than 10% is anticipated. Total separation time for the full sample is expected to be 68-70 hours.

3.1 BUFFER RECLAMATION: Each electrophoretic chamber outlet tube must be monitored to detect the presence of the cellular material of interest. The central 50% of this band spread of outlet tubes is collected. The effluent of the remaining outlet ports will be collected and subjected to a "cleaning" process to remove particulates, organics and ionic elements. The resultant water must then be held until a preselected volume is obtained. Preweighed and packaged chemicals must then be mixed into this volume of water in order to produce additional buffer material for continuous operation of the electrophoresis chamber.

4. COLLECTED SAMPLE MEDIA EXCHANGE: As the separated cells emerge from the electrophoresis chamber outlet tubes, they are transfered into a more protective nutrient media which will allow cell growth. This media contains all essential growth factors (solids and gases) and is of sufficient quantity to keep the collected cells viable until the complete sample has been collected and transfered to the Beta Cell culturing vessel.

5. CELL CULTURE INOCULATION: The collected cells are transfered to a small culture vessel of 235 ml volume. This initial inoculation has a minimum concentration of 4.4×10^5 cells/ml. Nutrient media must be continuously cycled through the vessel while temperature, pH and oxygen concentrations are maintained within controlled intervals. There is a growth lag

phase of about three days as the cells acclimatize to their new environment. Once growth begins the cells will double approximately every 35 hours. Three reproductive cycles (about 105 hours) will result in a total production of 8.4×10^8 cells in this culture vessel.

When this cell concentration is reached, the culture vessel volume is increased to 1900 ml while new culture media is added until the vessel is full. This dilution results in an inoculation of 4.4×10^5 Beta cells per millimeter for the larger production vessel.

6. PRODUCTION CELL CULTURING AND HARVESTING: After another three day cell acclimatization phase, the cells will again reproduce at a doubling rate of 35 hours. When the culture has reached a concentration of 3.6×10^6 cells per milliliter (approximately 4 days), the culture vessel is continuously drained at the rate of 50% of volume each 35 hours. At the end of each 15th cell culture, the culture is reduced to one-tenth of its original volume to preclude buildup of toxic products. The chamber culture volume is maintained by simultaneous admission of fresh replacement media. The culture vessel will be maintained at the optimum temperature, pH and chemical composition required for maximum cell production.
7. HARVESTED CELL CONCENTRATION: The resultant harvested cells must be concentrated for further purification by another electrophoretic separation. The 651 ml of tissue culture is concentrated approximately 30 times to provide an electrophoresis sample rate of 1 ml/hr. This sample concentration is approximately 1×10^8 cells/ml.

7.1 CULTURE MEDIA RECLAMATION: The excess culture media available from the concentration procedure is collected and "cleansed" of particulates, organic and ionic elements. This "polished" water must be mixed with pre-weighed and packaged chemicals to form additional nutrient media for continuous operation of the culture vessel. Because about 20 ml of water are lost each day for the electrophoretic sample, make-up water is added to this additional nutrient media to restore volume and chemical concentration.

8. BUFFER EXCHANGE: Immediately prior to processing the cell sample through the electrophoresis system, the sample is "cleansed" of any constituents that would interfere with the electrical field and separation capabilities

of the unit. At the same time as these constituents are removed, additional nonreactive constituents must be added to the sample to restore and maintain osmotic equilibrium within the cell. During this exchange process all operations must be conducted in a 4°C ±2°C environment.

9. CELL ELECTROPHORETIC SEPARATION: Once these cells have gone through the buffer exchange procedure,they must be processed through the electrophoresis chamber as soon as possible to maintain their viability, preferably entering the separation chamber within several minutes and no longer than 20 minutes after completior of the fluid replacement. The exchanged buffer must have some, but only minimal, protective ability. Residence time of each cell in the electrophoresis chamber will be approximately 50 minutes. As long as this separation is conducted at 4°-8°C a cell viability loss of less than 10% is anticipated.

10. FINAL PRODUCT CONCENTRATION AND MEDIA EXCHANGE: As the separated cells emerge from the electrophoretic chamber tubes, they are transfered immediately into a more protective nutrient media and cryoprotectant that will allow cell nutrition and preserve viability during frozen storage. During this phase of the process the central 90% of the band spread of the outlet tubes of interest will be collected for final product. All other tubes will be subject to reclamation. Those tubes containing final product will be mixed together and subjected to concentration procedures of Step 7.1 until the sample contains 1×10^8 cells/ml.

10.1 BUFFER RECLAMATION: Identical to step 3.1.

11. FINAL PRODUCT FREEZING AND STORAGE: The final product is rate frozen to prevent cell damage while in the stored state.

12. SYSTEM STERILIZATION: As the system remains closed after ground sterilization, there should be no contamination problems. Final product samples must be collected every other day to verify freedom from microbiological contamination and pyrogenicity.