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COLUMN ELECTROPHORESIS ON THE APOLLO-SOYUZ TEST PROJECT

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I. INTRODUCTION

Electrokinetic separation techniques have been widely used for the analysis and characterization of charged materials of biological origin.[3,4] Under terrestrial conditions preparative methods based on zone electrophoresis, isotachophoresis, and isoelectric focusing are prominent in the purification of charged macromolecules and small particles but have only been of limited usefulness in the separation of biological cells and larger particles. The major difficulties in the latter applications arise primarily from

In the Apollo 16 experiment, a mixture of polystyrene latices was processed in an improved version of the apparatus used on Apollo 14. Whereas with such a device it is not possible to conduct the processing under terrestrial conditions without the appearance of extensive thermal convection which destroys the separation boundaries, under microgravity conditions flight photographs showed no boundary deterioration, but did indicate that electroosmosis was a major factor in producing overlapping elongated sample zones in the cylindrical tube. Consequently, more subtle perturbations of sample zones were not distinguishable.

The "Electrophoresis Technology Experiment" aboard the Apollo-Soyuz Test Project was the first attempt to process viable biological cells by zone electrophoresis in a microgravity environment and to return the processed samples to earth; and also to demonstrate isotachophoresis of biological cells under near zero gravity conditions. The objectives of the project included the refinement and operational testing of a space-rated static electrokinetic separation apparatus which embodied the satisfactory design features developed in the Apollo 14 and 16 experiments. The refinements included development and testing of column coating procedures devised to eliminate electroosmotic fluid flow; a method for introducing biological samples into the electrophoresis column to form well-defined sample zones; development of multiple sample processing capability; maintenance of sterility in media which contacted cell samples; and facilities for freezing separated samples for return to earth. Equipment was designed and tested

for transporting sample cells to and from space in a frozen state. The electrophoresis unit (EU) was tested with mixtures of native red cells for isotachophoresis and chemically stabilized red blood cell populations with differing electrophoretic mobilities for zone electrophoresis. Viable cell sample types of current interest (human peripheral blood lymphocytes and kidney cells) were processed by zone electrophoresis. This communication presents the results of the hardware development work carried out for the ASTP experiment, the operational tests of the flight apparatus and a discussion of the technical and tactical difficulties which remain to be solved in order to realize the full potential of this type of microgravity processing.

II. PRINCIPLES OF STATIC ZONE ELECTROPHORESIS

Electrophoresis is the movement of a charged liquid or solid phase with respect to a continuous liquid phase under the influence of an applied electrical field. A variety of electrophoretic techniques are used for analytical purposes as well as for separation of materials differing in their electrophoretic mobilities, where the electrophoretic mobility, U_e, is defined as the electrophoretic velocity, v, of a particle per unit field strength, x. Electroosmosis is the flow of a liquid relative to a solid under the influence of an external electrical field. In a closed cylindrical system the observed velocity of a particle, V_o, under the influence of an electrical field can arise from the electrophoretic mobility of the particle, V_e, as well as the electroosmotic fluid velocity:

$$V_o = V_e + V_{os} \left(\frac{2r^2}{R^2} - 1 \right) \quad (1)$$

Where V_{os} is the electroosmotic fluid velocity at the tube wall and r is the distance of the particle from the tube axis with radius, R.

It can be seen that in the presence of electroosmosis there are fluid flow contributions to the velocities of particles at all radial positions in the tube except at r = 0.707R. which is called the stationary level. With biological cells in glass tubes the ratio $\frac{V_{os}}{V_e} \sim 2$ so that sample bands of biological cells assume a pronounced "bullet" shape during electrophoresis which seriously compromises the resolution of particles with different U_e values. In the ASTP zone electrophoresis experiment sample migration behavior was predicted on the basis of analytical particle electrophoresis results collected as described by Seaman [7] and modifications of equation 1 which expressed the migration distance of a sample band in terms of the behavior of individual particles, their electrophoretic mobilities at a standard test temperature, the dimensions of the column and the properties of the particle suspending medium at the temperature of the flight experiment. Accordingly, the band migration expression provided the distance, d_I^T(mm), traversed into the column at temperature, T, by the particles originally located at the longitudinal midpoint of the sample plug and at a distance, r(cm), from the tube axis:

$$d_r^T = \left(\frac{^{25}I \times 6 \times 10^{-2}}{\pi R^2 K^T T} \right) \left(U_e^{25} + U_{os}^{25} \left(\frac{2r^2}{R^2} - 1 \right) \right) t - \frac{d_o}{2} \quad (2)$$

where η^{25} and η^T are the viscosities in poises of the suspending medium at 25°C and at the run temperature, T, respectively; I is the constant current setting in amperes for the operation of the flight column; R is the radius of the electrophoresis column in cm; K^T is the conductivity in mho/cm of the suspending medium at temperature, T; U_e^{25} is the mean electrophoretic mobility (anodic) in μm/sec/volt/cm of the particles at 25°C in the suspending medium; U_{os}^{25} is the electroosmotic mobility (anodic) of the suspending medium in flight type columns at 25°C; t is the electrophoresis run time in minutes; d_o is the sample plug thickness in mm; and 6×10^{-2} is a combined factor for converting μm to mm and minutes to seconds.

III. ISOTACHOPHORESIS

Isotachophoresis (ITP) is a relatively new technique of electrophoretic separation in which a discontinuous electrolyte system is used at the site of sample injection.[8, 9] The term isotachophoresis stems from the Greek and means equal speed. The method consists of placing a mixture of charged particles (sample) of the same sign (co-ions) in a column or on some solid support medium containing in addition two electrolytes one of which has all of its co-ions of greater mobility (leading buffer) than any sample co-ions while the other electrolyte has all of its co-ions of

of lower mobility (terminator buffer) than any sample co-ion, the entire electrolyte having a common counter-ion. The polarity of the electric field must be such that the leading ion migrates toward the electrode that is placed on the same side of the sample as the leading electrolyte.

Upon application of a constant direct current, the sample co-ions initially move at different velocities until a steady state is reached in which the sample ions have separated into contiguous zones with sharp interfaces in order of their mobilities. At steady state the zones migrate at the same velocity, however, the compartments are of various lengths, ion concentrations and conductivities such that the product of the co-ion mobility and the field gradient is the same within every zone.

In contrast to electrophoresis the boundaries between species of different mobility are sharply defined and stabilized by electrical forces and consequently are highly self-recuperative and will reform if stirred or disrupted by other factors, including convection. The concentration of each substance within a compartment is uniform and remains constant throughout the run once the separation has been achieved. Much higher concentrations of components can be handled than in zone electrophoresis with no deterioration of the sharp boundaries. It should be noted, however, that the sample compartments are contiguous to each other and never separate to form an intermediate zone of clean buffer unless a "spacer ion" of suitable intermediate mobility is present.

In ITP the heating of the column is not uniform, i.e., the progressively lower mobility compartments are exposed to proportionally higher potential gradients at steady state. The final temperature is a complicated function of both radial cooling and frontal migration of each compartment. The highest temperature is reached in the terminator buffer.

The potential application of the method in a microgravity environment has been reviewed by Bier and coworkers.[8] The density gradients which arise in isotachophoresis are even more difficult to overcome under terrestrial conditions than in zone electrophoresis. These gradients are produced by (i) the steep concentration differences at the sample band boundaries; and (ii) the non-uniform heating of the column during application of the electrical field. In order to obviate these problems ground-based isotachophoresis of proteins has had to be conducted in gels. As a consequence of these problems, plus sedimentation in the case of biological cells, an ASTP experiment involving cell isotachophoresis was planned and developed.

IV. DESCRIPTION OF THE APPARATUS

The major elements of the flight experimental equipment consisted of an electrophoresis module; a cryogenic (liquid nitrogen) freezer for transport into orbit of slides containing the control samples and the samples for processing in the electrophoresis unit and for transport to earth of processed samples and controls; modular columns (six for electrophoresis and two for isotachophoresis)

photography equipment (70 millimeter electric camera, 80 mm lens and extender tube); and timer.

The characteristics of each major hardware element and its subelements were as follows:

A. Electrophoresis Unit

External dimensions of the unit, Figure 1, (cover closed) were 20.3 and 40.6 cm. Launch weight was 13.6 Kg. Subassemblies of the electrophoresis unit were:

1. Controls and displays.

2. Fluorescent light assembly for illumination of the experiment columns during processing.

3. Thermoelectric (TE) module for rapidly cooling and freezing the electrophoresis columns.

4. Self-contained power supplies that required only an external 208 volt ac (115-volt line-to-neutral), three-phase, 400 Hz connection.

5. Buffer solution circulation system for purging of electrolysis products from column electrode compartments.

6. Phase separator assemblies for removing gases (H_2 and O_2) liberated into the buffer circulation systems.

7. Camera attachment assembly for mounting the camera to the electrophoresis unit cover.

8. Individual storage compartments for eight experiment column assemblies.

The electrophoresis unit control and display panel (Figure 2) is described as follows. In the ON position, the POWER switch

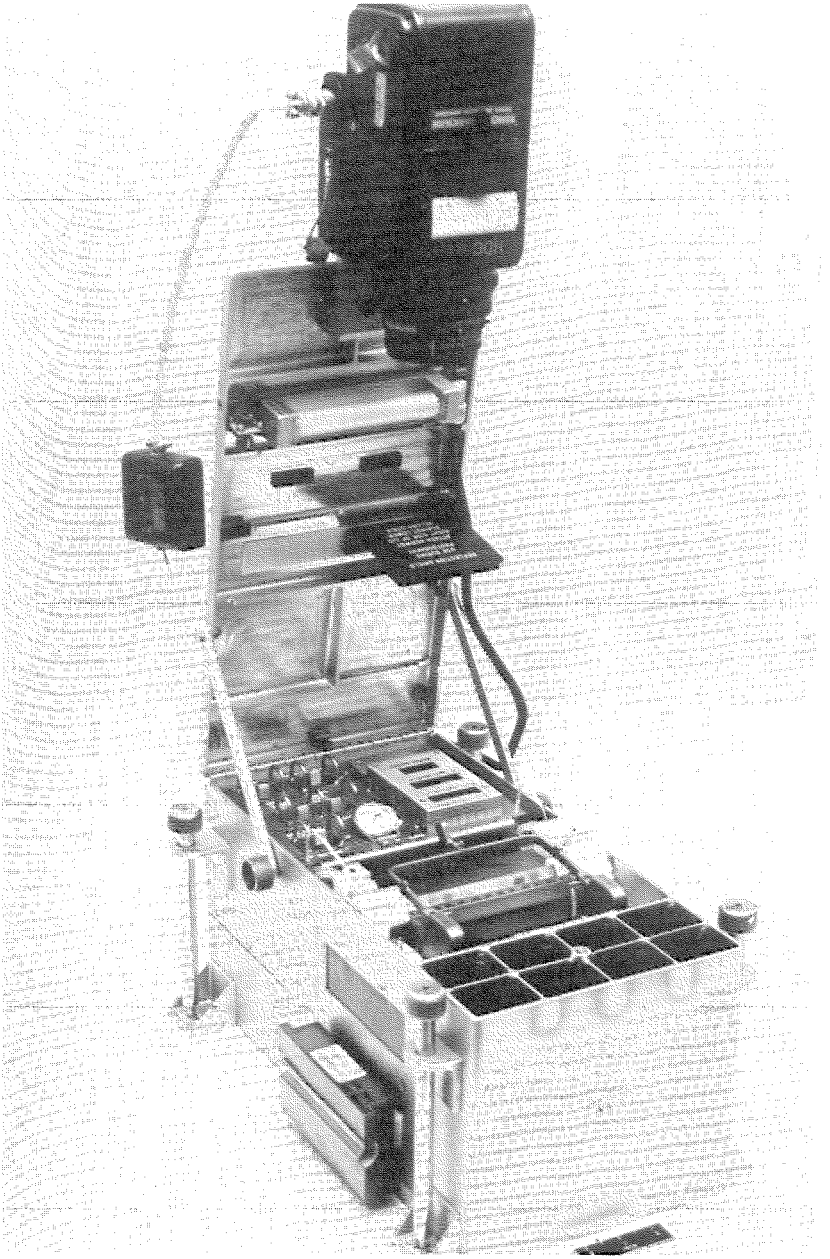

FIGURE 1
Electrophoresis Unit

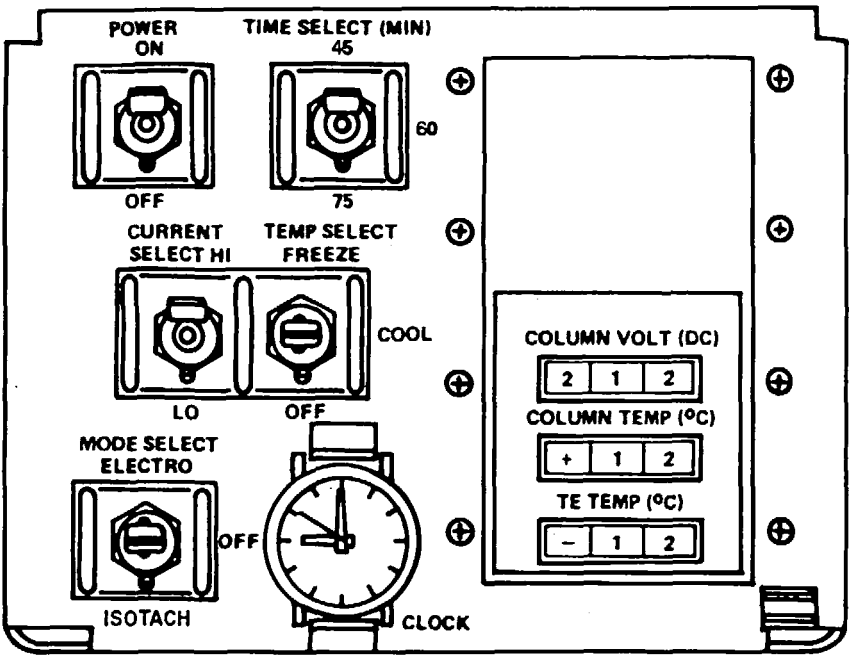

FIGURE 2
Control and Display Panel

energizes the fluouescent light assembly and the digital displays and also activates the TE module cooling blower.

The TEMP SELECT switch has positions FREEZE, COOL, and OFF. The FREEZE position is selected to freeze the electrophoresis column after completion of each electrophoretic separation. A thermoelectric temperature controller allows a minimum temperature of approximately -40°C. When the COOL position of the switch is selected, the temperature controller will control the TE units up to temperatures of 5°C. The switch is placed in the cool position

for the viable cell experiments. Because isotachophoresis required neither cooling nor freezing of the column, the OFF position was used.

The MODE SELECT switch has positions ELECTRO (for electrophoresis), OFF, and ISOTACH (for isotachophoresis).

The TIME SELECT (MIN) switch is used to select a run time of either 45, 60, or 75 minutes.

The CURRENT SELECT switch is used to select a current flow either 4.0 milliamperes (HI) for electrophoresis or 1.3 milliamperes (LO) for isotachophoresis.

The COLUMN VOLT (DC) digitally displays the voltage across the experiment column electrodes. The range of the display is from 0 to 999 volts in increments of 1 volt, with an accuracy of $\pm$ 2 volts.

The TE TEMP (^{o}C) displays the temperature of the thermoelectric module assembly cold plate. The range of this display is from -99^{o} to 99^{o}C in increments of 1^{o}C, with an accuracy of $\pm$ 2^{o}C.

The purpose of the electrophoresis unit clock (a commercial panel-mounted wristwatch) is to allow time correlation of the photographs taken during the experiment. A metric scale (0 to 130 mm in 1 mm graduations) is mounted next to the experiment column cradle. Illumination was provided by a fluorescent light assembly mounted on the electrophoresis unit cover.

The electrophoresis unit contains a TE assembly consisting of four three-stage thermoelectric coolers to remove heat from the electrophoresis columns during the experiment and to freeze

the columns after each separation has been performed. The electrophoresis column cradle is mounted on a cold plate which contains a thermistor device to sense its temperature. A thermal cover joins the column cradle to provide thermal continuity around the periphery of the column. The thermal cover was used for all freezing operations and during electrophoresis of lymphocytes and kidney cells.

A buffer solution reservoir (~58 ml capacity), mounted on a removable tray, contains a cylindrical rubber diaphragm with a hemispherical end. The buffer solution enters the reservoir from a phase separator assembly. The reservoir is covered with a hydrophobic felt cover to ensure containment of the buffer solution if the diaphragm ruptured.

A double peristaltic pump (capacity 35 ml/min) circulates the buffer solution through the fluid system to allow removal by the phase separators of gas bubbles generated at the electrophoresis column electrodes. The pump receives buffer solution from one side of the fluid system (e.g., the column anode side) and circulates it to the other end of the column (Figure 3).

Two identical phase separators are provided, consisting of two cylindrical membranes each. One membrane is hydrophilic (porous polyethylene) and the other is hydrophobic (porous polytetrafluoroethylene). The buffer and gas mixture enters between the membranes. The hydrophobic membrane allows passage of gas and the hydrophilic membrane allows passage of liquid to the storage reservoir. One separator is connected to the column anode side of the

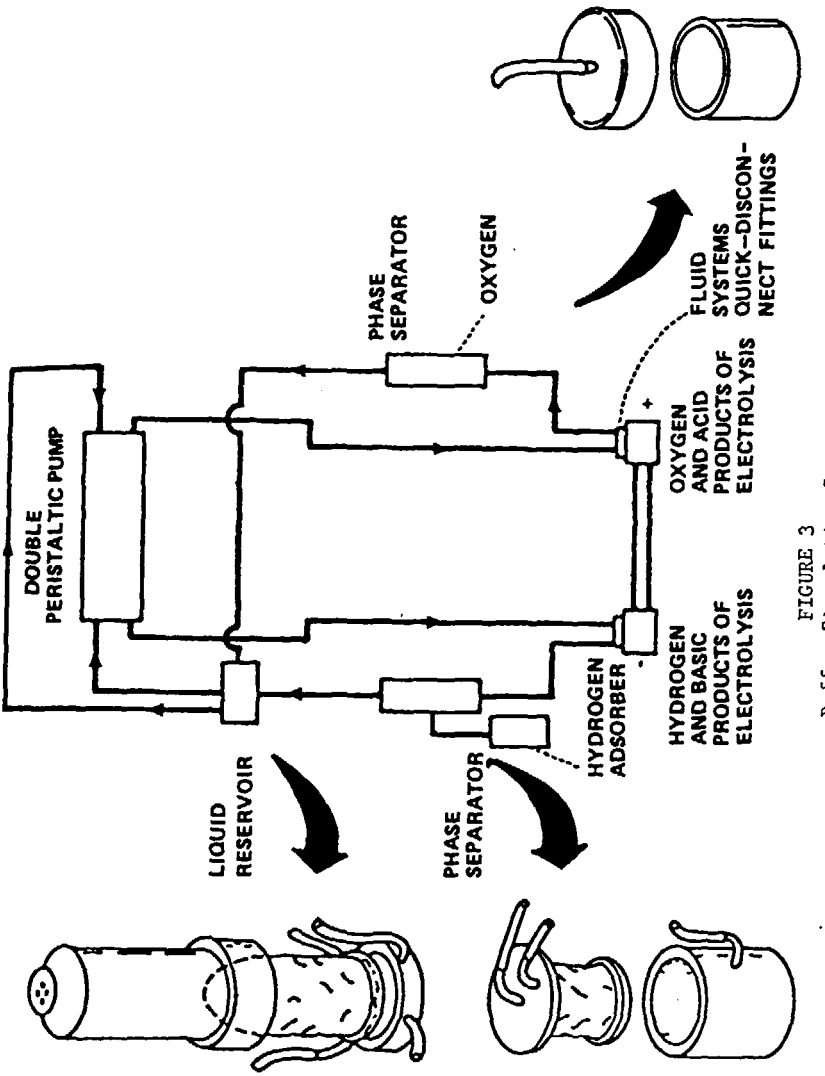

FIGURE 3
Buffer Circulating System

fluid system and removes the oxygen gas liberated in the buffer solution at the anode. The oxygen is vented to cabin air. The other separator is connected to the cathode side of the fluid system and removes hydrogen gas which is routed to a hydrogen adsorber assembly (a cannister containing precipitated palladium (palladium black) and silica gel contained in a fine-filament fiberglass bag).

B. Cryogenic Freezer

The cryogenic freezer, manufactured by Minnesota Valley Engineering (Figure 4), was used to contain the experiment samples before insertion into the experiment columns for processing and to maintain the frozen electrophoresis columns after the separation.

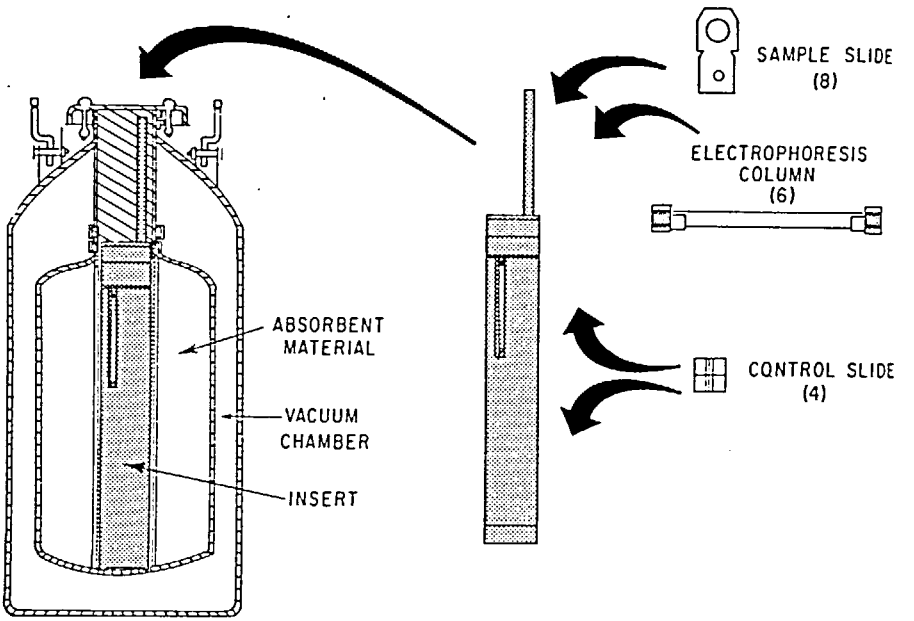

FIGURE 4
Cryogenic Freezer

The cryogenic freezer consists of two concentric flasks. The space between the flasks is evacuated for thermal insulation. The inner flask has an outside diameter of 31 cm and has a cavity to accommodate a cylindrical sample storage canister with dimensions of 5 cm diameter and 48 cm length. The maximum launch weight, when charged with nitrogen, is 10.4 kg.

The liquid nitrogen cooling medium was contained in a saturated adsorbent of the inner flask. The cryogenic freezer maintained the samples at a temperature no higher than -85°C for 20 days.

C. Electrophoresis Columns

Each electrophoresis column (Figure 5) consisted of a transparent Pyrex glass tube 0.95 cm outside diameter by 0.64 cm inside diameter. The tube was 14.94 cm long, split lengthwise and rejoined with a composite RTV 140/RTV 560 silicone seam to allow for expansion of the buffer solution when the electrophoresis columns were frozen. A thermistor bonded to the tube wall at the center of the length of each tube was used to monitor the column temperature.

The inside surfaces of the glass columns were cleaned, coated with Z-6040 (γ-glycidoxypropyltrimethoxysilane, Dow Corning) and then coated with methyl-cellulose in order to obtain a stable surface with close to zero zeta potential,[10] thereby eliminating electroosmotic fluid flow. Confirmation of the adequacy of the coating was obtained by experimentally determining the electrophoretic velocity of fixed red blood cells at various levels in the coated columns. The columns were filled before flight with a sterile

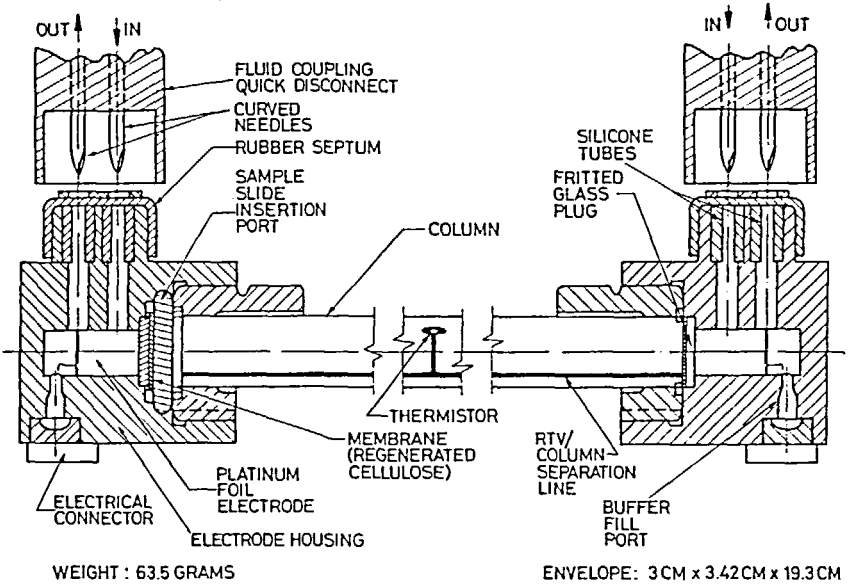

FIGURE 5
Electrophoresis Column

buffer solution. Each column was sealed in a laminated bag that was then placed in a fine-filament fiberglas bag. Each end of the electrophoresis column has an electrode chamber assembly containing a platinum electrode, a printed c rcuit electrical connector, and a matching electrophoresis unit. The electrode chamber assembly containing the column cathode had a cavity into which the sample slide was inserted for experiment operation. A blank slide was contained in the cavity before experiment operation.

The buffer, designated A-1, used in the zonal electrophoresis columns consisted of a mixture of 1.76 mM Na_2HPO_4, 0.367 mM KH_2PO_4,

6.42 mM NaCl, 01336 mM Na_2EDTA, 222 mM glucose, and 514 mM glycerol in water. It has a pH of 7.30 $\pm$ 0.10 at 20°C and a calculated ionic strength of 0.0097 mol/liter. At 25°C, the conductivity was 0.96 mmho/cm; the density, 1.022; and the dynamic viscosity, 0.0111 poise. The buffer was demonstrated to be compatible with the column coating, all components of the electrophoresis system, and the cells to be flown on the mission. The average mobilities of fixed rabbit, human and horse red blood cells in this buffer as determined by analytical particle electrophoresis were 1.56, 1.94 and 2.38 μm/sec/V/cm, respectively. The buffer had adequate cryoprotectant properties for the freezing procedure used for all cell-types under study.

D. Isotachophoresis Columns

Each isotachophoresis column consisted of a glass tube having dimensions of 0.97 cm outside diameter, 0.64 cm inside diameter, and 14.94 cm length. Each end of the tube had an assembly containing a flat, disk-shaped, three-lobed electrode (one electrode assembly with a silver anode and the other with a palladium cathode) a rubber diaphragm and a printed circuit electrical connector. The design of the columns had to be slightly modified from those used for zonal electrophoresis. Some of the features of the Skylab 4 isotachophoresis module[8] were also incorporated.

The isotachophoresis assembly containing the palladium cathode had a cavity into which the sample slide was inserted for the experiment. A blank slide was again contained in the cavity before processing the columns.

The isotachophoresis column contained a leader buffer made as follows: 0.62 ml of 85% phosphoric acid in 500 ml water, with 42 gm of dextrose and 276 gm of glycerol, adjusted to a pH of 7.4 in one liter of distilled water. The terminator buffer contained 2 gm of serine, 42 gm of dextrose, and 276 gm of glycerol, adjusted to pH 8.2 in one liter of distilled water.

E. Sample Slides

Each experimental sample was mounted in a slide assembly (Figure 6). Before processing, the slides were stowed in the cryogenic freezer sample canister. Each slide assembly was contained in a tetrafluoroethylene cover 0.13 mm thick to avoid contamination during handling. Every two slides removed from the canister ex-

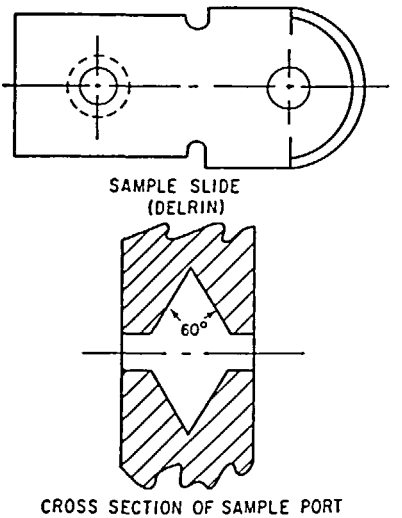

FIGURE 6
Slide Assembly

posed a return storage space in the canister for two electrophoresis columns. In addition to the slides to be processed, two "control" slides containing lymphocytes and two control slides containing kidney cells were stowed in spaces on the circumference of the sample canister. These "control" slides were designed to assess the effects of the cryogenic freezer environment on the viable sample materials; they were returned to Earth without processing.

Each sample slide was numbered to correspond to the appropriate column. The slides were removed from the canister and inserted into the experiment column electrode (cathode) chamber assembly for processing.

F. Slicing Device

After the electrophoretic separation was completed, the frozen columns were returned to Earth in the cryogenic freezer and were subsequently sectioned for analysis. In slicing the columns, several requirements had to be satisfied, as noted: (1) the frozen columns sliced at -40°C or colder; (2) contamination from the slicing device minimized; (3) each slice contained asceptically. A slicing device was designed, constructed and tested for this specific purpose.[11] As shown in Figure 7, the slicing assembly consisted of an extrusion assembly where the frozen column was placed. The extrusion rod was used to push the ice column into a teflon tube contained in the slicing assembly. After proper alignment, a razor blade was used to slice the frozen column. The 5 mm slices were stored at -196°C. The slicing assembly was operated in

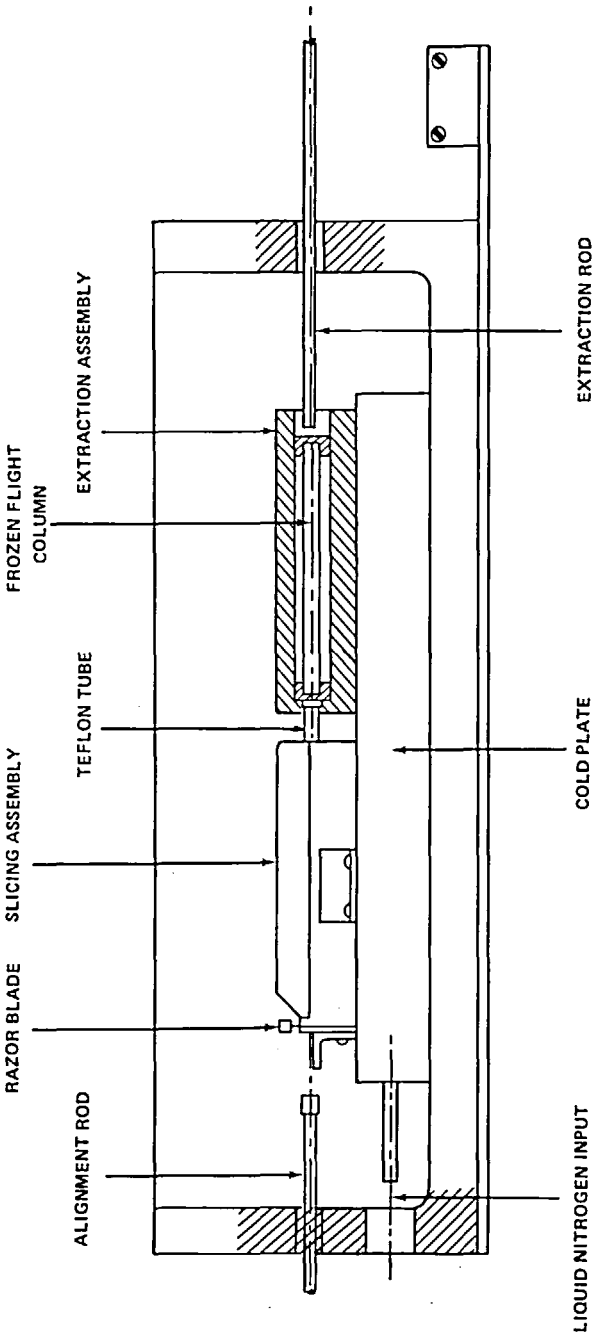

FIGURE 7
Slicing Assembly

a laminar flow hood and operated under sterile conditions. Subsequent analyses of the cultured kidney cells proved that no bacterial contamination resulted from the operation of this device.

V. SELECTION OF EXPERIMENTAL SAMPLES

The samples used in the MA-011, Electrophoresis Technology Experiment, were fixed red blood cells (rabbit, human and horse), human lymphocytes, cultured human kidney cells, fresh human and rabbit red blood cells. There were eight columns processed during the experiment including two for isotachophoresis. The order in which the samples were processed and the processing technique utilized are shown in Table 1. The sample selected and their importance is as follows:

A. Fixed Red Blood Cells

In order to assess the resolution and sharpness of the bands formed by zone electrophoresis, well-characterized particle species

TABLE 1. SAMPLE PROCESSING SCHEME FOR FLIGHT EXPERIMENT

Column Number[a]	Operation Mode	Sample Processed
1	Electrophoresis	Rabbit + Human + Horse Erythrocytes[b]
5	"	"
2	"	Human Lymphocytes
6	"	"
3	"	Human Kidney Cells
7	"	"
4	Isotachophoresis	Rabbit + Human Erythrocytes[b]
8	"	Rabbit + Human Erythrocytes

a. Column number gives order of processing sequence, i.e. 1,2,3.
b. Formaldehyde-treated.

are required. Although polystyrene latices were the model particles for the Apollo 16 experiment, the red blood cells used on the ASTP flight (erythrocytes fixed in formaldehyde fixative) provide an almost equally indestructible sample material, resistant to mechanical stress and hemolytic agents. These fixed red blood cells are stable for months under varying temperature conditions, and extensive electrophoretic mobility measurements of the cells using a variety of buffers have been published.[7] The types of cell were selected to be morphologically distinguishable under microscopic examination in order to aid discrimination of different cell-types in the samples returned to Earth. The brown color of the cells makes them clearly distinguished in photographs of the experiment while in progress.

B. *Lymphocytes*

Interest in the separation of lymphocytes stems originally from the recognition of a role for lymphocytes in the immune response to foreign antigens and to foreign tissue. With the increasing emphasis on cellular interactions and the cellular basis of immunity, simple methods have been sought for obtaining pure fractions of lymphocytes from heterogeneous cell populations.

Lymphocyte separation is of medical and scientific importance. Many unresolved questions in immunology depend on the availability of pure lymphocyte subpopulations, which are needed for clarifying the nature of the interaction between B and T cells in antibody formation and the nature of the interactions between the classes of T cells in cell-mediated immunity. Also, interest has

developed in the clinical use of various soluble lymphocyte products (lymphokines) in a manner analogous to the use of antisera. The fractionation of the different lymphokines is aided by prior separation of the specific lymphocyte populations responsible for their production. To date, none have been fractionated to an acceptable degree of purity.

C. Kidney Cells

The isolation and production of the enzyme urokinase (UK) has interested biomedical laboratories for many years.[12] This enzyme converts plasminogen to plasmin. This conversion is necessary to accomplish blood clot lysis and the human enzyme has been shown to be effective in treatment of thromboembolic disease.[13, 14] Originally, UK was extracted from urine; however, at least 1500 liters of urine were required to produce a single UK unit dose consisting of 4 million CTA (Committee on Thrombolytic Agents) units. Subsequently, UK has been isolated from cultures of cells obtained from the cortex of the kidney. Currently, the demand for UK greatly exceeds the capability for its production. Bernik and Kwann[15] observed that only a fraction of the cells in the cortex of the kidney produce UK. If the "producing cells" could be isolated and subjected to subculturing techniques, the yield would be increased with a corresponding reduction in the cost of this drug.

VI. MA-011 EXPERIMENT OPERATIONS

During the flight experiment two isotachophoresis and six zone electrophoresis columns were run on the electrophoresis unit

(duplicate runs for zone electrophoresis and single runs for isotachophoresis samples). An electrophoresis or isotachophoresis column was removed from its storage location and installed on the column cradle. Fluid couplings were secured to each electrode chamber of the electrophoresis columns. Then, the sample slide with a frozen sample was removed from the cryogenic freezer, inserted into the column and allowed to thaw. The 70 mm camera, mounted on the open cover, photographed the (erythrocyte) experiment columns during the runs. After each electrophoretic separation was completed, the column was frozen while still in place by the TE module. The frozen column was removed from the column cradle, the electrode chambers were removed and discarded, and the column was placed in the cryogenic freezer for return to Earth. Isotachophoresis specimens were neither frozen nor returned but only photograghed during processing.

VII. RESULTS

The flight equipment and the data collection assemblies in general functioned satisfactorily. The fluid connect lines (located in the electrode housings) of three of the columns were clogged during assembly. Without a fluid purge of the electrode housing, chemical and gas products of electrolysis accumulated in the electrode region and produced column voltage fluctuations and pH changes in the column fluid. These anomalies were seen in the zone electrophoresis columns 2 (lymphocytes), 5 (RBC), and 7 (kidney cell). Operations of the other columns appeared normal.

A. Zone Electrophoresis

Fixed Red Blood Cells

Most of the operational features of the experiment were assessed by analyses of the fixed red blood cell separations. The flight photograph record along with the availability of the recovered samples provided data for assessing the separation while in progress and for determining the efficacy of the recovery and sectioning procedures. The maintenance of viability of living cells and modifications of these cells by the experimental manipulations (e.g. freeze-thaw cycles) was not a problem with fixed cells.

Prior to the flight experiment electrophoretic mobilities were measured by analytical particle electrophoresis for the aldehyde-treated red cells from rabbit, human, and horse suspended in the column buffer (A-1) at 25°C. The mean values $\pm$ standard deviation for one hundred cells of each type were: rabbit, 1.56 $\pm$ 0.10 μm/sec/volt/cm; human, 1.94 $\pm$ 0.10 and horse 2.38 $\pm$ 0.13. The electrophoretic mobility distribution was essentially Gaussian for each of the populations. Thus, the test mixture of the three species covered the mobility range from 1.6-2.4 μm/sec/volt/cm which encompasses most of the known biological cell mobilities in this medium and provided a nontrivial separation problem. These mobility values were used with equation (2) to predict the appropriate run time (60 min.) for the flight experiment which would give sample band migration through 80-90% of the available column

length, maximal resolution, and signs of problems such as electro-osmosis which would compromise the effectiveness of the separation.

During the flight experiment direct observations and the flight photographs recorded that the red cell sample in Column 1 moved into the field of view as a uniform band with a planar front. The band split and moved regularly as the run progressed. In contrast, in the second red cell run (Column 5) the band was distorted with a nonplanar front by the time it had emerged from the sample slide into the observable part of the column (~20 mm) and subsequently migrated sporadically during the run.

Migration distance data for visually detectable sample band boundaries was collected by microscopic examination of the negatives of the flight photographs which were taken at three-minute intervals throughout the RBC runs. Figures 8 and 9 illustrate the migration behavior for Columns 1 and 5, respectively, as a function of run time.

Examination of the flight photo negatives for Column 1 showed the presence of two bands with five boundaries generally being detectable to the extent that their position could be followed from frame to frame. The fifth boundary was located approximately midway inside the second band. The linear displacement for all the boundaries indicates that the particles were traveling at a constant electrophoretic velocity during the course of the experiment. The velocity of the slowest boundary was 1.3 and the fastest 1.9 mm/min., equivalent to electrophoretic mobilities of 1.5 to

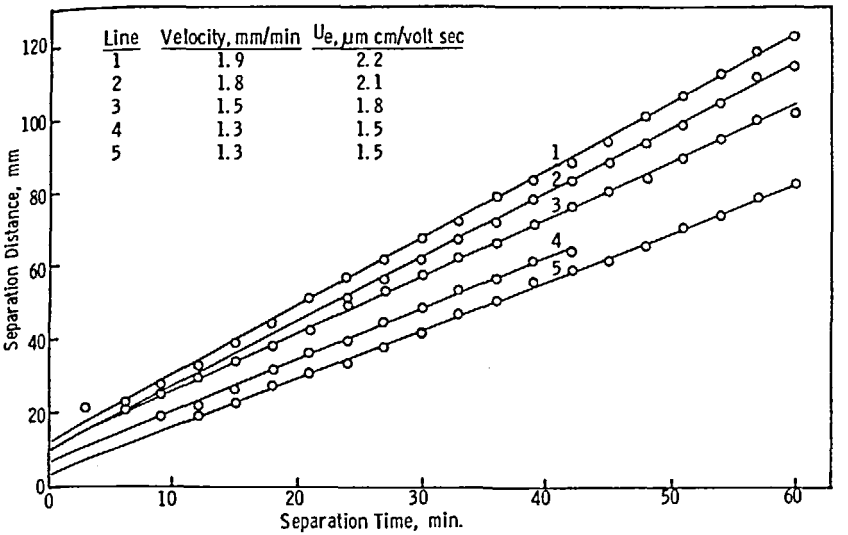

FIGURE 8
Migration Behavior for Column 1

2.2 μm/sec/volt/cm which agreed well with the analytical values for rabbit RBC ($U_e^{25} = 1.56$) as the slow band and horse RBC ($U_e^{25} = 2.38$) as the fast band.

Three boundaries were visible in Column 5. The extensive flat regions in Figure 9 indicate a breakdown in the electrical circuit and, hence, the potential gradient. The velocities which could be measured during the periods of current flow varied from 2.2 to 2.6 mm/min., i.e., higher than those of Column 1. The higher velocities in Column 5 are probably due to increased flow of suspending medium brought about by blockage of the electrode buffer circulation system.

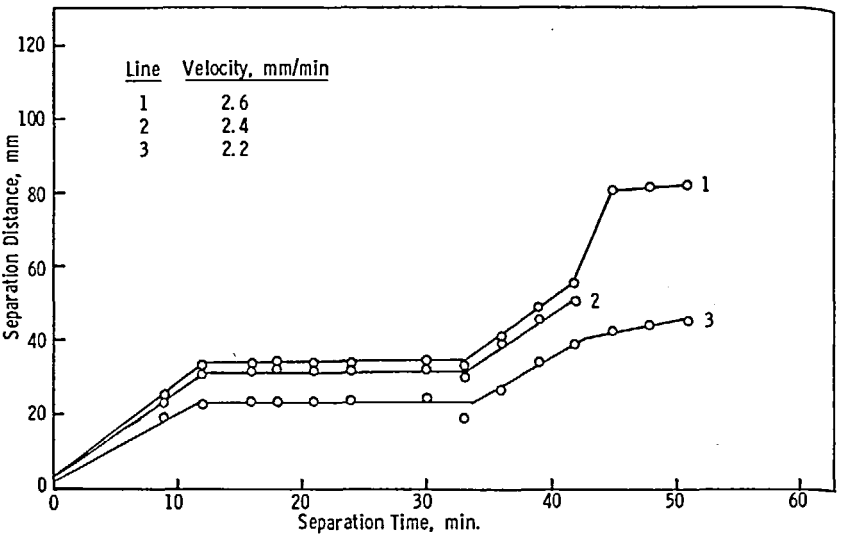

FIGURE 9
Migration Behavior for Column 5

The migration rates for both columns did not extrapolate to zero distance at zero minutes. This was expected since the voltage gradient through the sample slide was about 75% greater than that in the column because of the sample slide aperture diameter being less than that of the column with the apparatus operated in a constant current mode.

The migration rate data for Column 1 obtained from the flight photographs indicated that the sample bands migrated in agreement with their predicted behavior and suggested that fluid flow perturbations due to electroosmosis had been minimal. Elimination of electroosmosis was a major problem which had to be solved during the preparations for the flight experiment. If present, in a

closed system electroosmosis produces fluid flow in the direction of cell migration near the axis of the column and in the opposite direction near the wall. Thus for a band of sample particles with identical electrophoretic mobilities, significant electroosmotic fluid flow causes the cells in the center of the column to migrate faster than those near the wall to give an increased longitudinal spread of the sample band and a crescent shape. The methylcellulose coating technique was used to decrease the electrostatic charge on the inside walls of the columns and hence the electroosmotic fluid flow. Prior to treatment, the electroosmotic mobilities for A-1 buffer in the borosilicate glass columns were approximately -4.5 μm/sec/volt/cm (anodic), which is about twice the magnitude of the electrophoretic mobilities of the sample cells. Following coating, the electroosmotic mobilities were less than -0.3 μm/sec/volt/cm for all of the flight columns[10]. Even with this low level of electroosmotic flow (-0.3 μm/sec/volt/cm) the bands were predicted to spread to about 2.5 times the length computed for zero electroosmotic fluid flow after 60 minutes of run time. However, not all of this spread would be visually observable since at the boundaries the cell concentrations would be lower than the threshold concentration for visualization ($\sim 10^6$ cells/cc).

Figure 10 compares the progress of band migration in the flight experiment (Column 1) with the predicted behavior. In the predictive model an electroosmotic mobility of -0.2 μm/sec/volt/cm and a radial temperature gradient of 2°C were assumed based on the

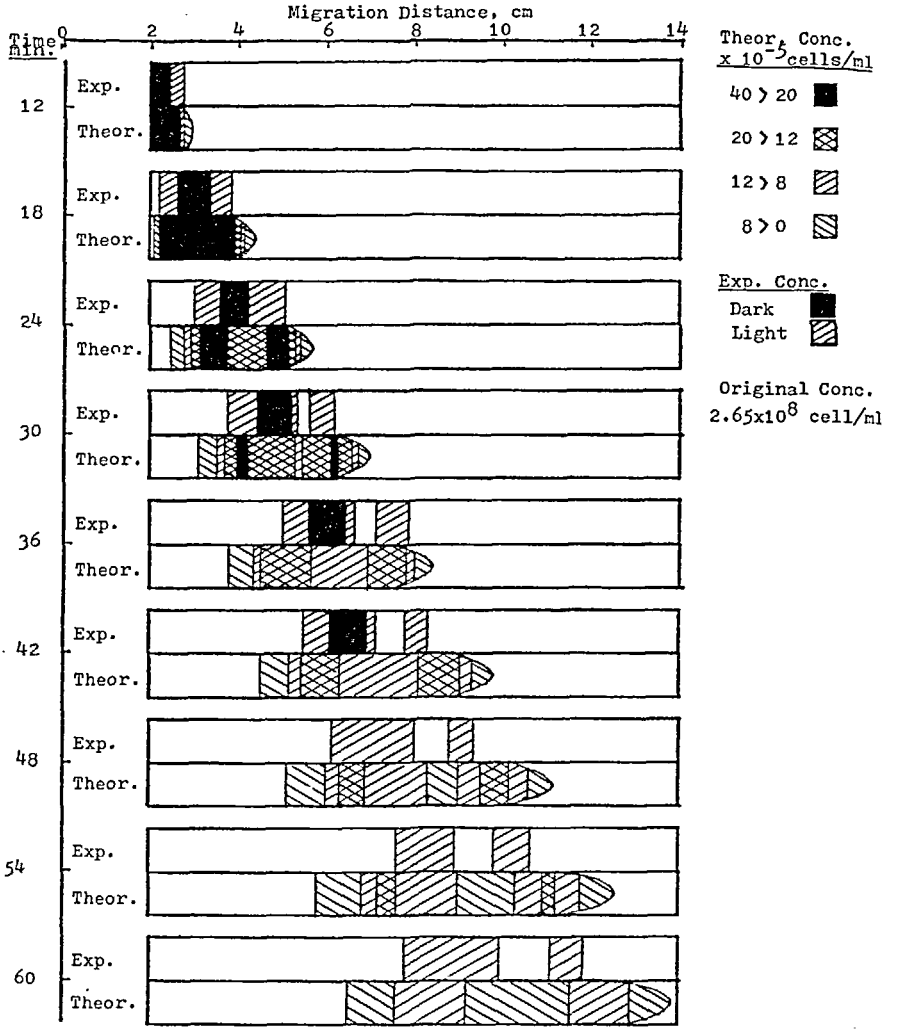

FIGURE 10
Comparison of Band Migration With Predicted Behavior

best available ground-based tests. Cell concentrations were predicted with a computerized model at all migration distances in the column as a function of elapsed run time. The influence of these low levels of electroosmosis and temperature gradient is readily seen as a paraboloidal advancing boundary which is pronounced even during the earlier stages of the 60-minute run. No sign of such contouring was visible in the flight photographs nor were there indications of cells between the separated bands. The predicted cell concentrations for these regions was in the region of the threshold concentration for direct visualization. Preliminary high precision densitometer scan measurements were made on Column 1 for four frames of the original flight transparencies. One of the frames analyzed was at zero time, i.e. before appearance of the particles, in order to obtain a background density scan of the column which could be subtracted from the subsequent density scans which contained particles. The results of this analysis are presented in Figures 11, 12 and 13 for separation times of 24, 42 and 60 minutes, respectively. These results show that cells, debris or leakage products were present at a very low level over a wider region than was detectable by visual observation of the flight film. Overall, the flight migration behavior of the red cells agreed well with the predicted behavior and indicated that electroosmosis had been largely eliminated as a problem in the separation.

The second phase of the flight experiment was the sample recovery operations and the analysis of the sections of the frozen

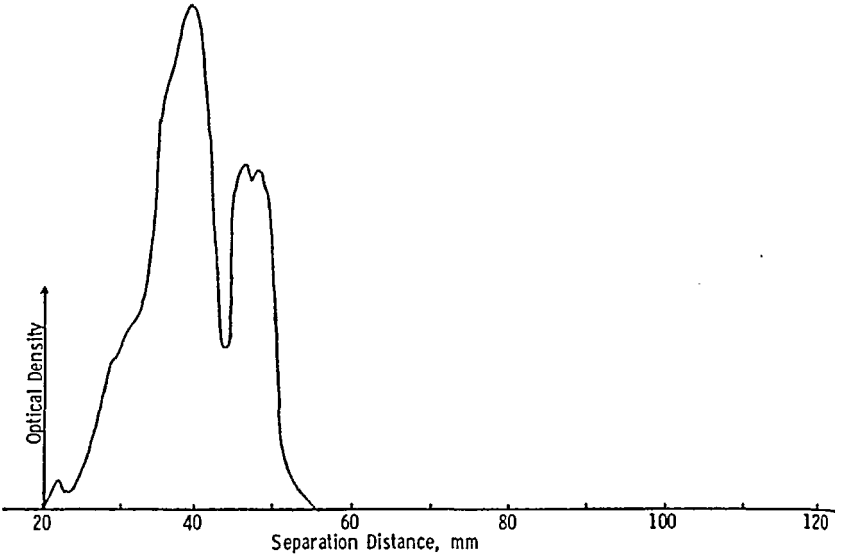

FIGURE 11
Microdensitometer Analysis of Column 1 After 24 Minutes

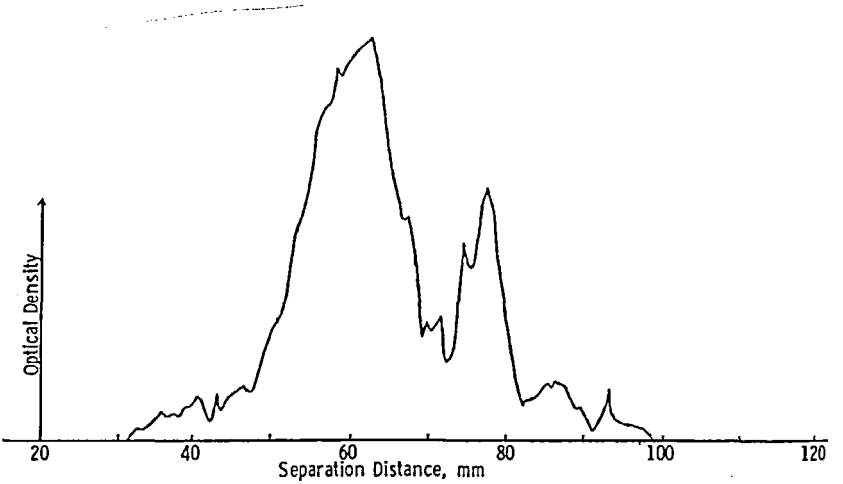

FIGURE 12
Microdensitometer Analysis of Column 1 After 42 Minutes

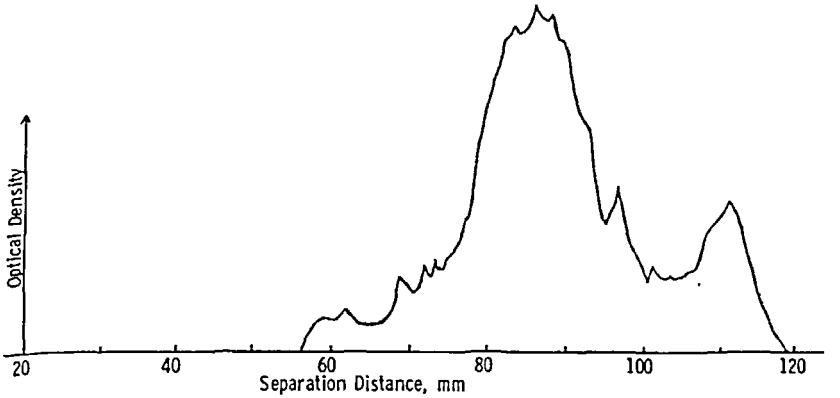

FIGURE 13
Microdensitometer Analysis of Column 1 After 60 Minutes

column core. The frozen column core containing the distributed sample was sliced at 5 mm intervals, the individual sample slices were weighed, then thawed and the number of red cells in each were enumerated and typed. During the recovery of the frozen sample suspension plug from Column 1, the glass column collapsed and the regions of the sample plug containing the sample bands were crushed so that the bands were mixed. Column 5 slicing proceeded smoothly giving 93% of the theoretical sample volume and 90% of the original number of sample cells. Hemacytometer cell counts were obtained for each sample slice and the distribution of cells in the column was constructed on the basis of the volumes of the sample slices (Figure 14).

In order to establish that the different cell types were being resolved by electrophoresis, a nonelectrophoretic method was

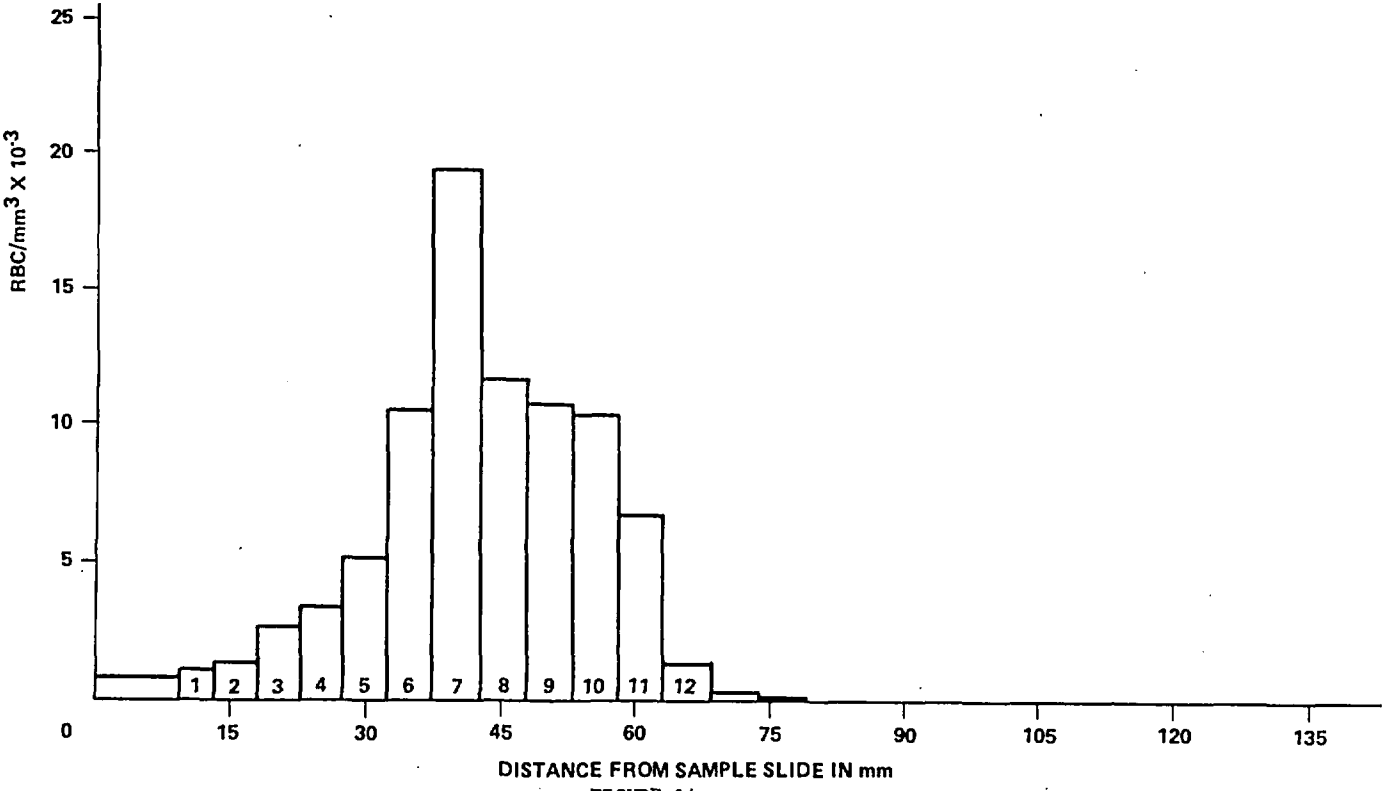

FIGURE 14
Cell Count Distribution in Sample Slices - Column 5

used to determine the relative quantities of rabbit, human and horse red cells in each slice. Control studies with mixtures of the three cell types established that classifications to within ~10% accuracy could be made on the basis of the major diameters of the cells in photographs. This noninvasive technique provided the data in Table 2 for Column 5 sample slices and is plotted in Figure 14. This data established that although Column 5 operation was impeded by the blockage of the electrode-compartment buffer circulation system, the electrophoretic separation of the three species of red cells was progressing as predicted.

B. Human Lymphocytes

Approximately 1.5 x 10^7 human lymphocytes were loaded into the 0.06 milliliter volume of each sample slide. The control cells

TABLE 2. DIFFERENTIAL COUNT DATA FOR COLUMN 5 SAMPLE SLICES

	Percentage of Erythrocytes		
Slice No.	Horse	Rabbit	Human
1	6	56	38
2	3	66	31
3	--	60	40
4	--	64	36
5	7	36	57
6	12	47	41
7	37	31	32
8	41	17	41
9	61	12	27
10	66	11	23
11	56	10	34
12	69	4	27
13	78	--	22

that were taken to orbit but not thawed, showed an unusually low viability, 14% and 13%. It is deduced that because of the small size of the control slides these lymphocytes froze and thawed faster than the prescribed rate. In pre-flight tests, the cells showed routinely a recovery of 55 to 63% viability when frozen and thawed using the sample keys. These sample keys are much larger than the control slides, although their cell-compartments are the same size.

Migration of the cells was not detected in either column. In Column 2 current was established for two very brief periods (0 to less than 3 minutes) because of fluid line blockage, which resulted in the formation of gaseous products around the electrodes. These gas bubbles blocked the flow of current and prevented operation of the experiment. In addition, the cell viability was only 6%. No explanation can be found for this observation, as the pH of the column fluid varied from 6.69 to 6.85.

In Column 6 the current was on for approximately 30 minutes. In this case, the electrode housings were returned, and an investigation confirmed that the right electrode chamber fluid lines were blocked. This condition resulted in an acid pH throughout the column. The pH of the fluid in Column 6 was 2.54 on the cathode end, 2.28 in the center and 2.21 on the anode end. As would be expected, the viability of the lymphocytes was only 1%.

C. Human Kidney Cells

In the human kidney cell experiments, approximately 2.0 x 10^6 cells were loaded into each sample slide. Only Column 3 was

subjected to electrophoresis in orbit. The processing of Column 7 was discontinued when a fluid leak developed.

The frozen slices obtained from Column 3 were thawed rapidly at 37°C, centrifuged and resuspended in growth media. These fractions were weighed, tared and the pH determined. An aliquot was taken for viable cell count and based on this information, the cells were cultured. Cultured cells were examined for production of urokinase, human granulocyte conditioning factor and erythropoietin as indicators of separation of functionally different cell species. Limited analytical electrophoretic mobility data was also collected on selected sub-cultured sample-slice cells.

The distribution of viable cells recovered from Column 3 based on erythrosin B dye exclusion is shown in Figure 15. The pH of the sample slices ranged from 7.0-7.9 and provided no indication of significant contamination of the column contents by electrolysis products from the electrode compartment.

Attempts to culture the recovered viable cells produced cell adhesion to the glass surfaces, but only fractions between 11 and 20 grew. After 28 days, only fractions 11 to 19 had reached confluency. The other fractions were removed from the culture plates and tested for urokinase activity by the fibrin plate method[16] and showed no fibrinolytic activity. The disposition of the primary culture plates and subcultures is shown in Table 3. Those cells that were put on production media were tested for urokinase activity at various times. The cells that were subcultured were removed from the dishes with EDTA and then recultured.

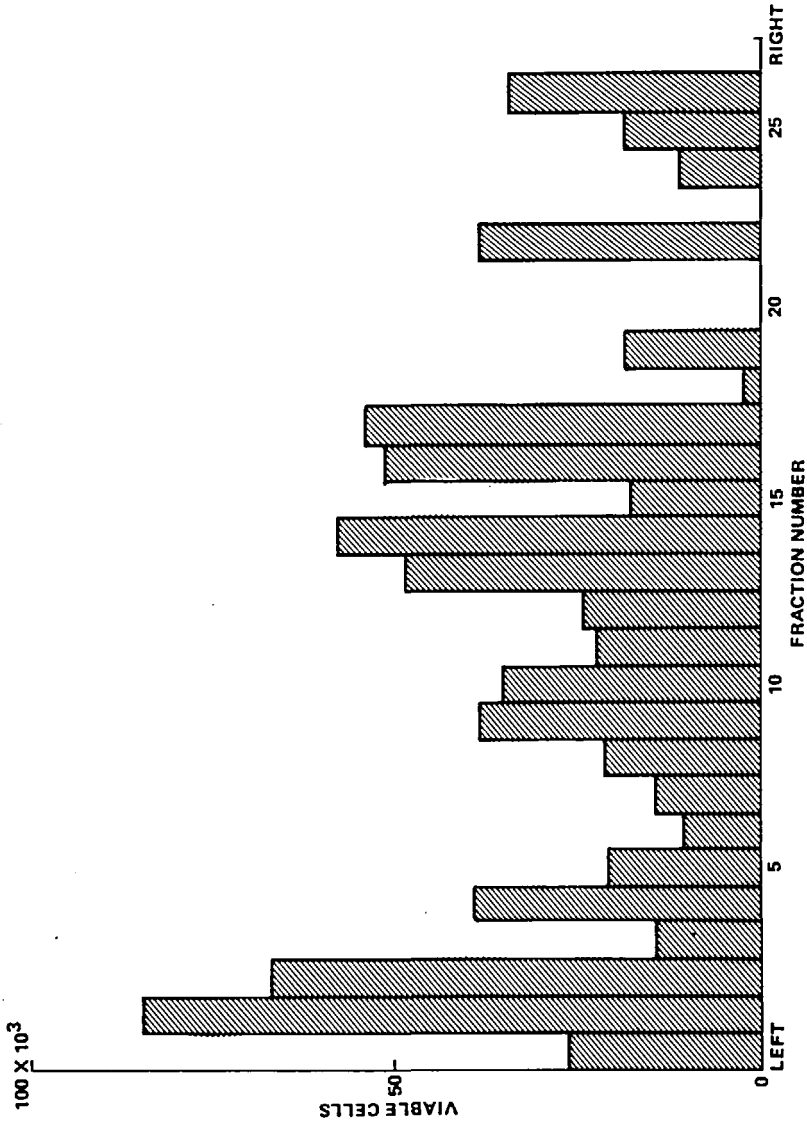

FIGURE 15
Distribution of Viable Cells in Column 3

TABLE 3. DISPOSITION OF KIDNEY CELL CULTURES AND SUBCULTURES FROM COLUMN 3

Slice No.	Number of Dishes	Condition	Disposition		
			Prod.[a]	Subcult.[b]	Other
Subculture					
11	1	Confluent	1		
13	1	"	1		
14	1	"		1	
15	1	"	1		
16	1	"		1	
17	2	"	1	1	
19	1	"		1	
B Control	1	"	1		
D Control	1	"	1		
Subculture 1					
14-1	2	Confluent	1	1	
16-1	2	50% Confluent	1		1[c]
17-1	2	Confluent	1	1	
19-1	2	"	1	1	
Subculture 2					
14-2	2	Confluent	1		1[d]
17-2	2	"	1		1[d]
19-2	2	"	1		1[d]

a. Incubation in production medium for assay of urokinase production
b. Recultured in growth medium
c. No growth resulted from this subculture
d. Harvested for analytical electrophoretic mobility measurements

Table 4 shows the results of the urokinase production obtained with the primary and subculture 1 cells after 35 days on production. There is an obvious enrichment of urokinase activity in fraction 15. An increased production is also seen in several other fractions. Control experiments with the same cells cultured using

TABLE 4. ASSAY DATA FOR UROKINASE, ERYTHROPOIETIN AND HGCF PRODUCTION BY KIDNEY CELLS CULTURED FROM COLUMN 3 FRACTIONS

Fract. No.	Viable Cells x10^5	UK Assay Units/ dish	UK Assay Units/ 100 cells	Erythropoietin Units/ml	HGCF Colonies[a] Formed
Primary Culture					
11	0.07	45	0.64		
13	0.70	535	0.77	Inhib.	
15	0.12	240	2.0	"	
17	0.74	225	0.3	"	
B Control	0.068	61	0.9	0.8	
D Control	0.29	81	0.3	0	
Subculture 1					
14-1	0.60	85	0.14	1.1	40
16-1	0.13	124	0.94	--	65
17-1	0.90	205	0.23	0.9	123
19-1	0.22	359	1.62	1.65	0
Subculture 2					
14-2		0		5.0	
17-2		0		2.0	
19-2		0		1.0	

a. Colonies formed corrected for control plate

ground-base conditions gave the value of 0.28 UK units per 100 cells. The subculture 2 cells did not produce urokinase when placed on production media.

The cells of subculture 1 were also tested for the presence of human granulocyte conditioning factor (HGCF) and the results are shown in Table 4. It is evident that the highest concentration of these HGCF producing cells were in a separate band (fraction 17).

During the final phase of this program, analyses were made for erythropoietin activity. All assays were performed using Ery-

thropoietin Standard Step 3 (CMRL), Lot #3005-1 from Connaught Labs. This standard had a potency of 2.2 units/mg. The harvest from the primary, subculture 1 and from subculture 2 were assayed for erythropoietin activity. The results reported in Table 4 are the average of two sets of assays. The results are most encouraging as the peak activity for erythropoietin is found in faction 14. This is a different fraction than for either urokinase (fraction 15) or human granulocyte conditioning factor peak (fraction 17). These data indicate that a different subpopulation of cells are responsible for each activity assayed. Part of the cells from subculture 2 were analyzed for electrophoretic mobility distribution and the results of three such fractions is shown in Figure 16.

Preflight electrophoretic mobility data for kidney cells in A-1 buffer at 25°C gave a mean mobility of 1.66 μm/sec/volt/cm with a standard deviation of 0.19 with a range of 1.3 to 2.1 μm/sec/volt/cm. Postflight data was collected for 50 cells from each of three subcultured sample slices. The theoretical mobilities of these cells assuming no change during culturing was calculated with equation 2 for the final location of the cells when returned from the flight column:

	U_e^{25} in A-1 Buffer	
Fract. No.	Obs. Mean	Predicted Range
14	1.44	1.30-1.36
17	1.56	1.50-1.61
19	1.58	1.68-1.76

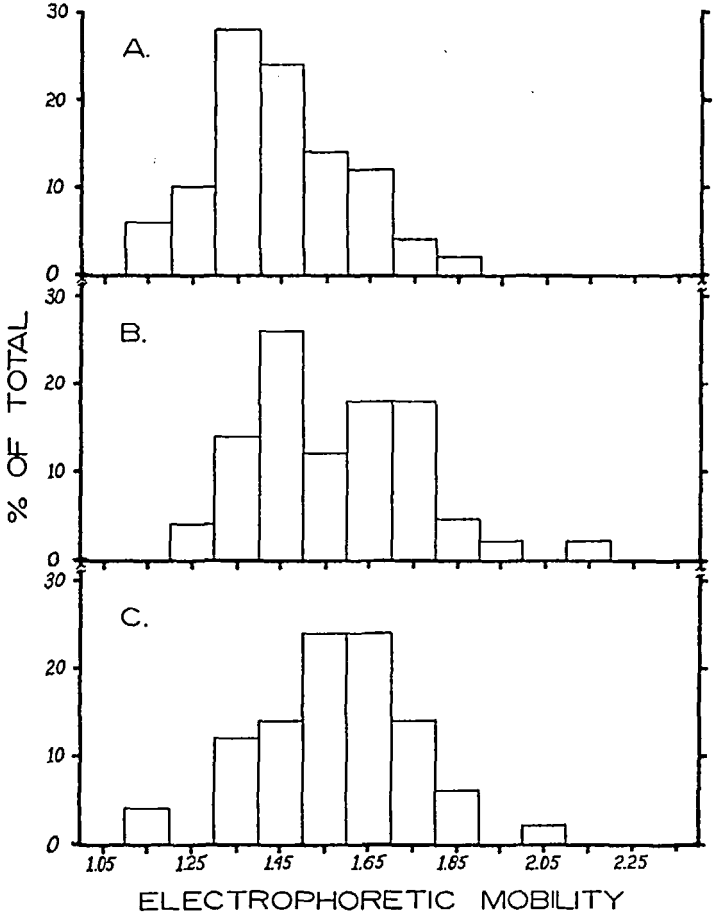

FIGURE 16

Electrophoretic mobility distributions for kidney cells grown from flight sample slices. The bar graphs give the percentage of the total cells examined which were observed in each electrophoretic mobility interval (μm/sec/volt/cm). Fifty cells were measured in A-1 buffer at 25°C for the second culture of kidney cells from the flight sample slices: A. slice 14; B. slice 17; and C. slice 19.

D. Isotachophoresis

Ground-based studies on the isotachophoresis (ITP) of cells has led to the development of a new small capacity apparatus (Rotofor)[17] which uses boundary stabilization through rotation of a horizontal tube. The apparatus is equipped with a rotating seal fraction collector thus permitting ready elution of the migrating cells from the column. The development of the apparatus was critical for purposes of testing cell separation under terrestrial conditions and for defining suitable buffers, candidate test cells and handling procedures for the flight experiment.

The requirements of ITP flight experiment called for freezing of the red cell samples in a medium containing suitable buffer ions for ITP and yet with low overall electrolyte concentration. However, many of the usual leader/terminator buffers, while compatible with the freezing of the red cells, caused cell aggregation during an actual ITP run. Using the Rotofor, non-aggregating buffers were eventually found. Sixty per cent recovery of fresh red cells could be obtained using a cryogenic mixture containing 4.2% dextrose and 2.8 to 3.5 M glycerol. Using non-frozen red blood cells in phosphate-serine buffer systems, ITP separation of cells from hemoglobin (or various added dyes) was easily achieved but fractionation of mixtures of sheep and rabbit on human and rabbit red cells was not obtained.

With frozen red cell mixtures, however, ready separation by ITP was achieved. As freezing results in partial hemolysis of the red cell population it appeared that hemolysis liberated mat-

erial of suitable mobility to act as a spacer ion. Attempts to find suitable spacers (ampholines and amino acids) for fresh or fixed red blood cell mixtures were unsuccessful.

It was decided for the ASTP experiment to fly one sample of fresh-frozen red blood cells and one sample of fixed red cells. In Column 4 isotachophoresis of a mixture of formaldehyde-fixed human and rabbit red cells was carried out and in Column 8 the isotachophoresis of fresh human and rabbit red cells. The concentration of fixed cells for Column 4 was approximately the same as that for Columns 1 and 5. The sample slide in Column 8 contained 4.5×10^8 cells in a cavity volume of 0.098 ml which was achieved by loading equal volumes each of 40% hematocrit human and rabbit red cells into the sample slide cavity.

During the isotachophoresis run in Column 4 photographs were taken at three-minute intervals, examples of which are shown in Figures 17-18. The first frame showed the current off; the following frames, covering a time-span of 40 minutes, showed the current on; and the last three frames indicated that the current was off again. Thus there is an uncertainty of up to 6 minutes in the total run time. The estimated initial migration velocity of about 1.1 mm/min is close to the predicted value of approximately 1 mm/min. However, if the whole time-span is used, i.e., the last photograph was taken at a minimum of 40 minutes (Figure 18) and a maximum of 43 minutes migration, the overall rate of migration is somewhat below or a little above 0.7 mm/min depending upon the actual time the current was on. The first visible boundary

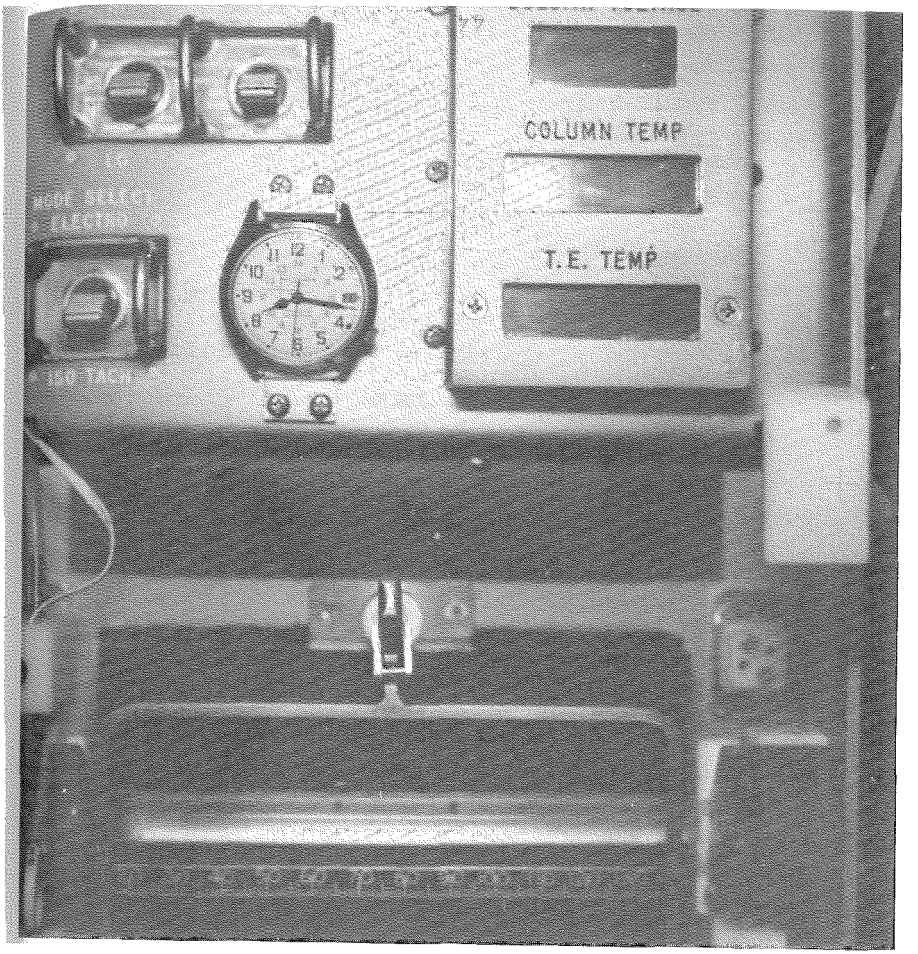

FIGURE 17
Photo of Isotachophoresis in Column 4 After 31 Minutes

(Figure 17) appears to be slightly fuzzy, but the last boundary, four frames later, is characteristically sharp and flat. The initial voltage was 113 volts, final voltage 133, this increase being typical of ITP.

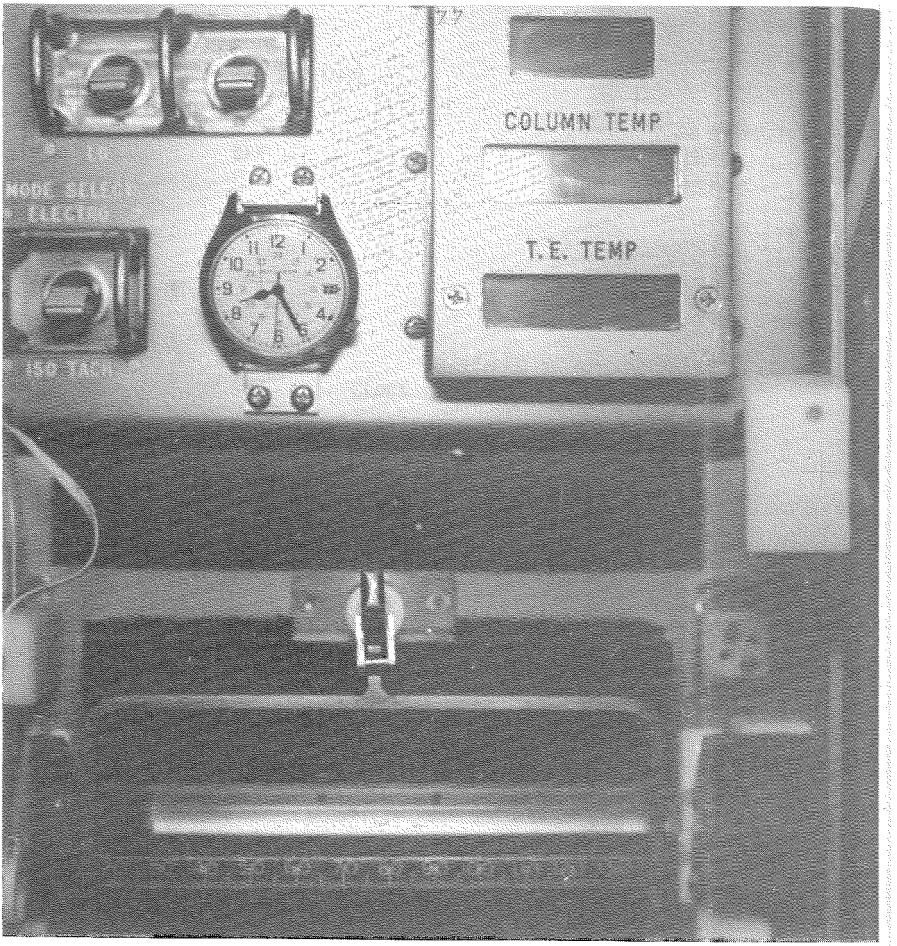

FIGURE 18
Photo of Isotachophoresis in Column 4 After 40 Minutes

The ITP of fresh-frozen red cells was carried out in Column 8 (Figures 19-20). The experimental run was carried out twice by the astronauts since the first run showed no current applied. The

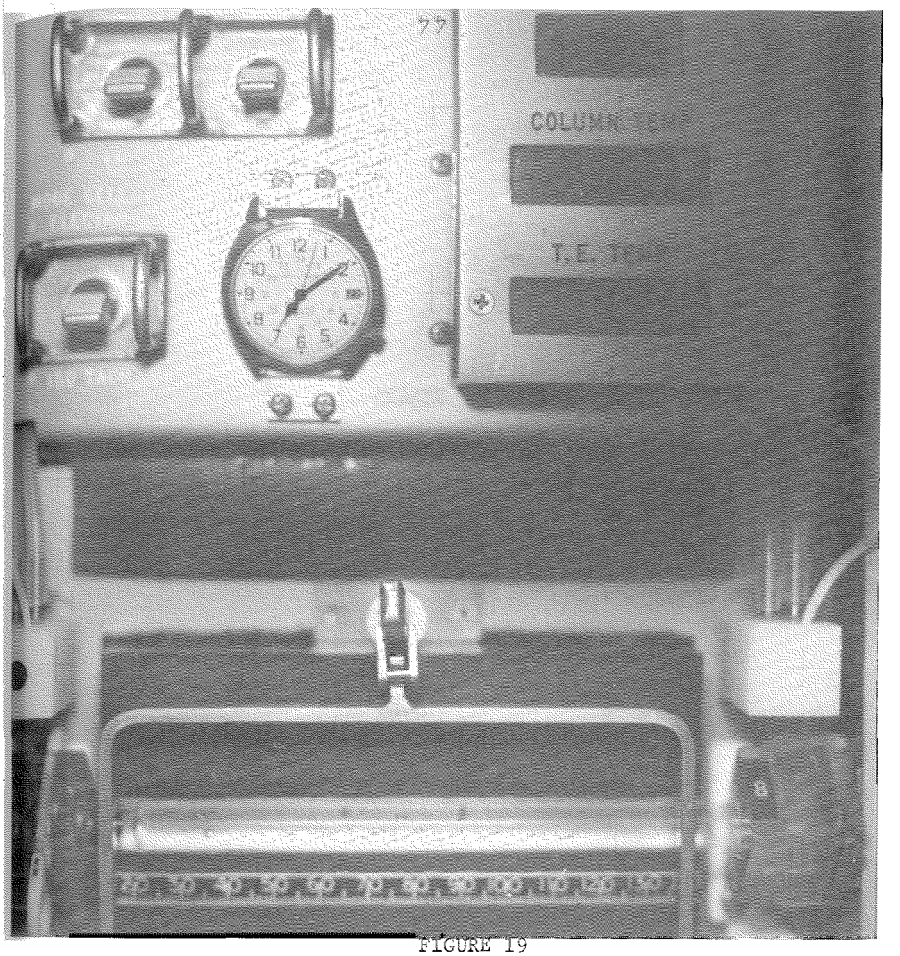

FIGURE 19
Photo of Isotachophoresis in Column 8 After 30 Minutes

run was repeated with the current on for 39 minutes. However, as the second run was initiated at least 70 minutes after insertion of the sample there is considerable uncertainty as to the location of

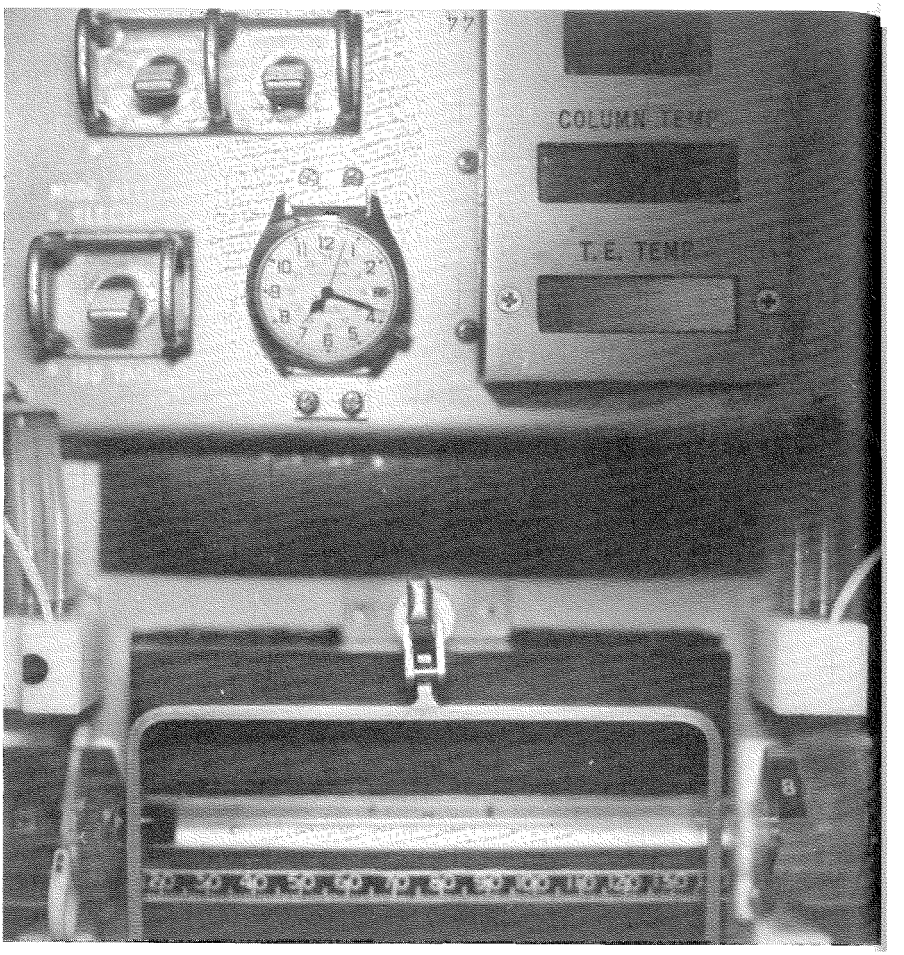

FIGURE 20
Photo of Isotachophoresis in Column 8 After 39 Minutes

the sample at the beginning of the run. Figures 19-20 show the presence of two small air bubbles in the column located at approximately the 20 mm and 36 mm marks. In Figure 19 a typically sharp

front red cell boundary is visible at the 20 mm position. However, the boundary is not flat and is tilted towards the air bubble. In Figure 20 the boundary is straighter, though slightly rounded and is located at the 25 mm position. The overall rate of migration from the beginning of the run was estimated to be about 0.6 mm/min. The initial voltage was 121 decreasing to 109 and then increasing to a maximum of 138 volts. The transient drop in voltage was unexpected, but the overall voltage was within the expected range.

Ground-based examination on the day of the space experiment of two sample slides containing duplicate samples of the fresh-frozen flight cells which had been thawed showed about 52% hemolysis of the red cell population, considerably higher than the usual hemolysis of 20%, routinely observed in prior experiments. However, isotachophoresis performed in the Rotofor using aliquot portions of the flight buffer showed that the remaining red cells were clearly visible as also confirmed for the sample actually used on the ASTP experiment.

VIII. DISCUSSION AND CONCLUSIONS

It is generally recognized that elimination of the force of gravity provides significant qualitative and quantitative advantages in a variety of materials processing techniques which range from controlled crystal growth to separation processes such as electrophoresis.[18] In these processing applications a major issue is what degree of improvement can be expected from minimizing gravitational influences and will realization of the potential provide unique opportunities or economically significant advantages over

ground-based applications. While the overall value of a given product may be difficult if indeed possible to assess, it should be possible to test the basic principles of the applications and assess the magnitude of the advantages that elimination of gravity can offer. On the surface this appears to pose no difficulties, but more detailed examination makes it apparent that portions of the underlying theory and much of the practical experimental work is limited under terrestrial conditions so that the data base is insufficient for making such assessments without the aid of pilot studies in a microgravity environment. The ASTP Electrophoresis Technology Experiment constituted one step in a program designed to provide the appropriate data from terrestrial and microgravity studies for projecting the longer range potential of this type of microgravity processing as well as the circumstances which would warrant its use.

During the Apollo 14 and 16, and Skylab missions basic demonstrations were performed with simplified zone electrophoretic and isotachophoretic instruments which indicated that gravity-driven convective disturbances were markedly reduced in a microgravity environment. The corresponding ground-based experiments could not be performed without extreme perturbation of the separated zones resulting. However, a number of technical difficulties relating to design features and materials selections arose during these experiments. Major emphasis in the ASTP experiment was placed on optimizing the design features of the zone electrophoresis apparatus which was flown aboard Apollo 16. The compact

multiple-use electrophoresis unit was designed to accept columns suitable for performance of both zone electrophoresis and isotachophoresis experiments. Thermoelectric temperature control and freezing capability provided appropriate temperature control during the runs and enabled the samples separated by zone electrophoresis to be preserved at their final migration locations so that post-flight analyses were possible.

The two-week period from launch date to the time when the experiment would be performed posed the problems of maintaining sterility in the systems which contacted the test samples as well as of storing the samples under conditions which would not adversely affect their electrophoretic or functional properties. Sterile packaging techniques and the cryogenic storage facility were developed for these purposes.

The experimental hardware generally functioned as planned. However, the experiment was not totally successful because the fluid lines in some of the columns were blocked. Although each column was thoroughly tested before flight, the fluid lines could not be checked without contaminating the enclosed sterile buffer. Electroosmosis which has been a major obstacle to successful electrophoretic separations in a closed cylindrical tube apparatus, was eliminated in the ASTP electrophoresis experiment. The coating techniques developed for this purpose significantly increased the resolution capability of the electrophoresis unit and will be valuable for ground-based and future space electrophoretic applications.

The red blood cell separations served as the first controls ever successfully run on the same space missions with unknown samples. The red cells were readily visible from the flight photographs and demonstrated the operation of the flight unit in orbit. However, in both zonal electrophoresis columns, sharpness of the boundaries deteriorated with time, as is evident from the photographic record, but the bands were planar and showed no unexpected longitudinal spreading. Post-flight sample slice analysis showed partial separation of cells of the three species in one column according to their electrophoretic mobilities. A breakage of the other column precluded any reliable post-flight analysis of the system.

Equipment problems during the attempted runs of the lymphocytes precluded the acquisition of adequate data and as a consequence no conclusions could be reached regarding the feasibility of separating subpopulations of human lymphocytes in a microgravity environment. As a spin-off from this work, however, the freezing of human granulocytes, by the same method developed for the freezing of human lymphocytes for this mission, appears to be most promising and is being pursued further. The viability of human granulocytes using the ASTP method after freezing and thawing is in the range 82-86%. In addition their phagocytic activity appears undiminished. Also during simulation of and in preparation for the flight experiments, a 1G method for preparative cell electrophoresis was developed, permitting the separation of the fastest-migrating cells (the T-lymphocytes) in a pure state.[19]

The kidney cell separation was most encouraging not only because viable cells were carried into orbit, processed, returned and cultured, but also because the cells which were separated appear to be product specific. The electrophoresis in space separated the kidney cells into at least three and perhaps four subpopulations. These results are in agreement with the best ground-based data obtained by use of the endless belt electrophoresis apparatus, although difficulty in maintaining sterile conditions precluded collection of subpopulations from the endless belt apparatus. Even though each kidney cell fraction from the ASTP flight showed viable cells on the basis of dye exclusion[20] and they all attached to the glass surface only the few fractions between 11 and 20 multiplied. The reason for this is not known.

The results indicated an enrichment but incomplete resolution of urokinase producing cells in the region centering around Fraction 15. The incomplete resolution between the "producing" and "non-producing" cell populations is not surprising in that the experimental conditions (buffer, run time, etc.) were not optimized for kidney cells but generalized for the three different cell samples to be separated. In addition the fractions which produced Human Granulocyte Conditioning Factor and erythropoietin did not coincide with the urokinase producing fraction. This finding indicates that these products are most probably not produced by the same strain of cell. The analytical mobility data obtained from cells from subculture 2 show each fraction to have a rather broad mobility distribution at this stage. This is rather unexpected in

view of the narrow mobility distribution which should have existed in the original fraction. Unfortunately there were insufficient numbers of cells to obtain mobility data on the original cells separated during the ASTP flight and the studies had to be performed on subculture 2 cells instead.

The isotachophoresis experiments demonstrated the remarkable sharpness of isotachophoresis boundaries. With further work on the definition of suitable spacers that can be used with cells, the ITP procedure may permit separations that cannot be accomplished on the ground. Full expectations were not realized as there was only evidence of the frontal boundaries, but not of any possible separation, or of the rear boundaries. An overall migration rate of 1 mm/min was predicted for both samples. The last four frames of the fixed cells run confirm this rate of migration but the overall migration rate was estimated to be substantially slower, 0.7 mm/min. With the fresh red cell experiment, the rate of migration was still slower being about 0.6 mm/min which may have been caused by the unusually large degree of hemolysis.

The cryogenic freezer used to transport the samples to orbit and to bring back the frozen columns performed as designed. While it is planned for future flights to carry viable samples to orbit in a fluid state, nevertheless the method of freezing the cells used on the MA-011 experiment proved to be a satisfactory means of transporting, handling, inserting and collecting the samples.

This experiment provided a significant step forward in the development of a biological processing facility in space. For the first time, all aspects of the technology necessary to transport candidate biological materials for separation to orbit, conduct separations and return the viable separated products to earth were addressed.

IX. ACKNOWLEDGMENTS

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X. REFERENCES AND NOTES

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