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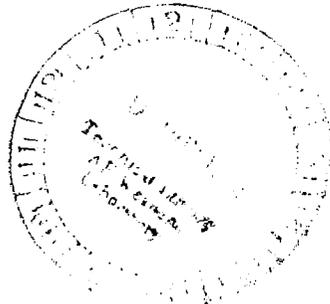


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ELECTROPHORETIC CHARACTERIZATION
OF ALDEHYDE-FIXED RED BLOOD CELLS,
KIDNEY CELLS, LYMPHOCYTES
AND CHAMBER COATINGS

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16. ABSTRACT This study was conducted for additional ground-based electrokinetic data on the Electrophoresis Flight Experiment to be flown on the Apollo-Soyuz Test Project (ASTP) Experiment MA-011. The candidate cells--aldehyde-fixed red blood cells, embryonic kidney cells and lymphocytes--were evaluated by analytical particle electrophoresis. This report documents the results which aided in the interpretation of the final analysis of the MA-011 experiment. The electrophoresis chamber surface modifications, the buffer, and the material used in the column system are also discussed.			
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Scope of Report

During spring 1974, the need was identified for additional ground-based electrokinetic analyses and consultation on the development work for the ASTP electrophoresis technology experiment (MA-011) scheduled for flight in July 1975. This contract which went into effect on July 1, 1974 established the following services to be provided by the contractor:

1. Analyze candidate cell populations (aldehyde-treated red cells, embryonic kidney cells and lymphocytes) by stringently standardized analytical particle electrophoresis techniques in order to determine:
 - a) the expected separability of the candidate populations in the ASTP apparatus; and b) the electrokinetic stability of the populations under conditions expected to be encountered during the ASTP experiment,
2. Conduct postflight analytical electrophoresis of the separated populations of red blood cells to provide additional data for the assessment of the success of the experiment;
3. Develop and employ rapid screening procedures for evaluating the suitability of electrophoresis chamber surface modifications designed to minimize loss of resolving power in the ASTP electrophoresis apparatus due to electroosmotic flow;
4. Act as technical advisors in the area of analytical electrophoresis of biological particles essentially on a standby basis; and
5. Supply ad hoc reports, postflight analysis data, etc. as and when required.

Since the results of much of this work were critical for making decisions on methodology prior to the flight, the data were communicated in monthly progress reports and memos to MSFC as soon as they were obtained. This report documents and summarizes those principles, methodologies and results which bear

most directly on the conduct and interpretation of the electrophoresis technology experiment as it was ultimately carried out.

A. Definition of the Flight Experiment Buffer (A-1)

Composition of A-1 Buffer

In July 1974, the final formulation was established for the buffered liquid medium to be used in the zone electrophoresis flight experiment. This buffer was selected as the candidate which was most compatible with the biological samples to be flown and the flight hardware and operation conditions. In the flight experiment, the buffer reservoir and circulation system, and the electrophoresis columns were filled with this buffer for all zone electrophoresis runs. Buffer formulations which differed from A-1 were used for the preparation of sample suspensions of red blood cells and human lymphocytes for filling the flight sample and control slides.

In order to assure that the A-1 buffer properties would be uniform at the various laboratories involved in the experiment, the directions for preparing the buffer were drafted which included the pH, viscosity, osmolality and density of the buffer so prepared as checks of the preparation (July-August 1974 Monthly Progress Report). The composition of the A-1 buffer was:

<u>Substance</u>	<u>Mol. Wt.</u>	<u>g/liter</u>	<u>g/2 liters</u>	<u>Calculated Millimolarity*</u>
Na ₂ HPO ₄ ·7 H ₂ O	268.07	0.472	0.944	1.76
KH ₂ PO ₄	136.09	0.050	0.100	0.367
NaCl	58.44	0.375	0.750	6.42
Na ₂ EDTA·2 H ₂ O	372.24	0.125	0.250	0.336
D-glucose	180.16	40.0	80.0	222.
Glycerol	92.11	47.3	94.6	514.

* Based on 100% purity assay

All reagents used in making up the buffer were analytical reagent grade with assays of at least 99% purity by weight and were weighed to within 1% of the values given. The buffer was sterilized by filtration through a pre-sterilized (steam sterilized, 15 minutes at 250°C) Millipore filter of 0.22 μ m pore size.

Physical Properties of A-1 Buffer

The electrokinetic behavior of particles is influenced by the physical properties and composition of the suspending medium. The major factors of concern are the dynamic viscosity, ionic strength, and pH. In addition, the concentration of ions and molecules which do not pass freely through the membranes of biological cells must be maintained at levels (isotonic) which will not produce cell damage and leakage of cell contents.

The electrophoresis experiments involved runs at $\sim 15^\circ\text{C}$ for viable cells and at $30\text{--}35^\circ\text{C}$ for aldehyde treated red cells. The columns were operated in a constant current mode (4.0 mA) so that the voltage gradient in the column depended on the conductivity of the A-1 buffer at the operation temperature. Consequently, it was necessary to determine the temperature dependence of the pH, conductivity, and dynamic viscosity so that the behavior of the flight experiment could be predicted from ground-based analytical particle electrophoresis data.

The ionic strength at 25°C was calculated to be 9.7×10^{-3} mole/l (July-August Progress Report). The pH of the buffer at 25°C for several batch preparations ranged from 7.25 to 7.31. The temperature dependence of pH was small: where the pH was 7.30 at 20°C , lowering the temperature to $\sim 1^\circ\text{C}$ produced an increase of 0.08 pH units. The osmolality of the buffer in the absence of glycerol was 248 mOsmoles/kg based on its freezing point depression. In the presence of glycerol, the osmolality was 840 mOsmoles/kg. The former figure represents the effective osmolality or tonicity of the buffer since glycerol

freely permeates biological cells.

The temperature dependence of the conductivity of four batches of A-1 buffer made up in this laboratory were determined over the range 10° to 35°C. The conductivities were measured using a Radiometer CDM2e conductivity meter equipped with a CDM104 conductivity cell, a platinized platinum three electrode cell with a cell constant of 0.94. The measuring accuracy of the instrument is 1% of full scale deflection on the ranges from 0-50 $\mu\text{mho/cm}$ to 0-150 $\mu\text{mho/cm}$. On other ranges it is 2%. The range used in these determinations introduces an uncertainty of 2% or $\pm 30 \mu\text{mho/cm}$. A precisely prepared aqueous solution of 0.0100 M KCl with buoyancy and temperature taken into account was used as a standard to check the operation of the instrument. Care was taken to rinse the conductivity cell several times in distilled water and in an aliquot of the solution to be tested prior to making a measurement. The conductivity measurements were made from 10° to 35°C at 5° intervals with the conductivity cell placed in a water bath controlled to $\pm 0.1^\circ\text{C}$ for each temperature.

The dynamic viscosity, η (poise), was calculated from the kinematic viscosity, ν (Stokes), and the density, ρ (g/cc), of the buffer at 10, 18, 25, 30 and 35°C:

$$\eta = \rho\nu$$

Kinematic viscosities were measured in an Ostwald viscometer (H_2O flow time ~ 80 sec. at 25°C) immersed in a circulating water bath controlled to $\pm 0.1^\circ\text{C}$. Density was determined with a Pyrex pycnometer (nominal 10 cc capacity). The viscometer and pycnometer were calibrated with distilled H_2O at each of the measurement temperatures.

The data are given in Table I.

TABLE I: TEMPERATURE DEPENDENCE OF CONDUCTIVITY, DENSITY AND DYNAMIC VISCOSITY OF A-1 BUFFER.

Temperature (°C)	Conductivity, κ (mmho/cm)	Density, ρ (g/cc)	Dynamic Viscosity η (poise)
10	0.67	1.025	0.0167
15	0.76	-	-
18	-	1.024	0.0133
20	0.86	-	-
25	0.96	1.022	0.0111
30	1.06	1.021	0.00988
35	1.19	1.020	0.00887

B. Evaluation of Electrophoresis Chamber Surface Modifications for the Elimination of Electroosmotic Flow.

Background

Electroosmotic fluid flow arising from the effects of the charged wall of the electrophoresis chamber introduces problems in the determination of electrophoretic mobility of a particle. In analytical particle electrophoresis the measured electrophoretic velocities are in error unless the measurements are obtained for particles located at a position in the chamber where the electroosmotic flow is negligible (stationary level) (1). In preparative or analytical applications where a concentrated suspension (sample) is subjected to electrophoresis in a closed cylindrical tube the sample boundary becomes paraboloidal in contour as a result of electroosmosis of the suspending medium. The non-planar sample distribution introduces difficulties in separating or resolving species differing in electrophoretic mobility. Either the run time or sample size must then be compromised in order to obtain satisfactory separations.

Electroosmotic flow arises from the effect of the applied electrical field on the increased concentrations of ions of opposite charge sign to the

chamber wall near that surface. The increase in concentration of ions is due to the electrostatic potential of the chamber surface which may arise either from:

- a) ionization of surface groups
- b) adsorption or desorption of ions from the suspending medium, or
- c) chemical reaction of surface groups with components of the suspending medium.

Glass which is a hydrophilic surface exemplifies case a) while examples for b) are commonly associated with hydrophobic surfaces.

Rationale

The electrophoretic mobility of a particle and electroosmotic fluid flow in a closed electrophoresis system are both manifestations of surface charge, in the former case that of the particle and in the latter that of the chamber. Each is a function of the electrostatic potential at the plane of fluid shear (hydrodynamic shear plane) between the charged surface and the bulk liquid medium.

There are two general principles of approach to the elimination of surface charge:

- a) covalent bonding to surface groups to eliminate the negative groups by chemical modification (total charge zero) or to introduce positive groups such that the net charge is zero, or a combination of these types of surface modification.
- b) to use substances, usually macromolecules, which physically adsorb to the glass surface, thereby shifting the electrophoretic plane of shear out from the original glass surface to the new macromolecular surface. By this means the charge on the original surface will have a negligible influence on ion distribution at the new plane of slip during electrophoresis.

Tests of the stability properties and effectiveness of different surface coatings should be based on a sensitive measurement of charge at the hydrodynamic shear plane. Electrophoresis constitutes such a measure and may be conducted in two ways: 1) Following coating of the electrophoresis chamber tube, the electroosmotic flow is calculated from measurements of the electrophoretic velocities of a standard particle at various distances from the tube wall; or 2) The electrophoretic mobilities of particles made from the same materials as the electrophoresis chamber tubes are measured by standard particle electrophoresis following coating. The first test approach is the desirable final test of any coating procedure, but is severely handicapped by the time needed for the setup of the apparatus and the modification of individual tubes. The second test approach would significantly reduce the time required in the initial screening or testing of coating procedures, but may be limited by differences between particle and tube surface properties. Consequently the time savings and flexibility of the use of model particles support their use during the screening and initial testing of coating procedures and only those procedures showing promise should then be tested as coatings of chamber tubes.

Criteria for satisfactory surface coatings or modification

The vast number of surface reactions encompassed by the above two approaches can be severely restricted by an examination of the criteria required for a satisfactory surface coating or modification. The following properties of the surface coating should be considered in judging its general suitability and application limitations:

1) Physical properties:

- a) transparent to light, at least over the same range of wavelengths as the original glass.
- b) small coating thickness relative to the radius of the electrophoresis tube.

- c) capable of being applied uniformly.
 - d) low electrical conductivity and surface conductance.
 - e) low fixed or acquired electrostatic charge in buffers. For aqueous sodium chloride solutions with an ionic strength ≥ 0.01 g ions liter⁻¹ the zeta potential at the tube wall should be < 2 practical mV over the range of pH used for experiments.
 - f) poor surface for gas bubble nucleation and formation.
 - g) nonadhesive to particles or biological cells to be examined in the coated tube.
- ii) Chemical and biological properties:
- a) hydrophilic to minimize adsorption of components from samples.
 - b) compatible with system to be examined, e.g., non-toxic towards living cells.
 - c) not subject to attack or degradation by any biological specimens under test.
 - d) will not desorb or change the electrokinetic properties of the particles under examination.
- iii) Electrokinetically stable (2) for duration of studies to:
- a) buffers up to physiological ionic strengths (~ 0.15 g ions liter⁻¹).
 - b) pH range, 2 to 11.
 - c) temperature range $\sim 0-50^{\circ}\text{C}$.
 - d) shear rates encountered when filling or cleaning the electrophoresis tube.

Possible coating materials or surface modifiers

Taking the above criteria into consideration possible coating materials or surface modifiers include:

- i) Polysaccharides [agarose; dextran; ficoll; glycogen; methylcellulose; and starch].

- ii) Methacrylates and acrylamides [2-hydroxyethylmethacrylate (HEMA) (3); 2,3-dihydroxypropylmethacrylate (DHPMA) (3); and polyacrylamide].
- iii) Polymeric alcohols [polyethylene glycol (PEG); and polyvinyl alcohol (PVA)].
- iv) Silane derivatives as either sub-layer agents or primary coatings [γ -glycidoxypropyltrimethoxysilane (Dow Corning, Z6040) (4,5,6); γ -aminopropyltriethoxysilane (Union Carbide, A-1100) (4,6); γ -methacryloxypropyltrimethoxysilane (Dow Corning, Z6030) (4,5); and vinyltrimethoxysilane (4,5)].

To date, polysaccharides have offered the most promising features as coatings. They are hydrophilic, have small numbers of charged groups and are generally biocompatible. As a class they may be crosslinked and their residual charge groups eliminated or chemically reduced by procedures such as those used by Porath et al. (7) on agarose. In addition polysaccharides may be derivatized or oxidized and mixtures used to produce coatings with either a net negative, net positive or net zero charge (8). Table II summarizes certain of the properties of the candidate coating materials.

TABLE II: PROPERTIES OF POSSIBLE COATING AND SURFACE LINKAGE

	Surface Functional Groups	Expected Charge at Neutral pH (H ₂ O)	Crosslink. Functional Groups	Hy Hy
Substrata				
Glass	>SiOH	-ve		
Lexan	COO ⁻	?		
Coatings				
Methyl cellulose	>C-OH, (COO ⁻)*	zero*	>C-OH	
Dextran, starch, glycogen	>C-OH, (COO ⁻)	zero	>C-OH	
Ficoll	>C-OH, (COO ⁻)	zero	>C-OH	
Agarose	>C-OH, (COSO ₃ ⁻ , COO ⁻)	-ve	>C-OH	
HEMA	>C-OH, (COO ⁻)	zero	RC=CR	
DHPMA	>C-OH, (COO ⁻)	zero	RC=CR	
Polyethylene glycol	>C-OH	?	>C-OH	
Polyvinylalcohol	>C-OH	?	>C-OH	
Dow-Corning Z-6040	>C-OH	?	-C-C- 0	
Union Carbide A-1100	>C-NH ₂	+ve		
γ-methacryloxypropyltri-methoxysilane	-C=CH ₂ Me	?	RC=CR	
vinyltrimethoxysilane	>C=CH ₂	?	RC=CR	

* Only zero in the absence of oxidized forms of the material which will possess neg carboxyl. Functional groups in parentheses are those expected at a trace level.

Objectives

The original objectives of this work were to (a) develop techniques employing analytical electrophoresis of model particles for rapid screening of electrophoresis column surface modifications designed to eliminate electro-osmotic flow during electrophoresis, and (b) use these techniques to evaluate cleaning and modification procedures and the quality and stability of the modified surfaces over a range of conditions likely to be encountered during the planned MA-011 space electrophoretic studies.

The model particles used for the studies were borosilicate glass derived from the stock of tubing which was used to manufacture the electrophoresis columns for the flight experiment. The following work was directed at (a) development of rigorous cleaning procedures which would minimize variations in surface properties prior to modifications, and (b) development of surface coating materials which could be easily applied directly to a cleaned borosilicate glass surface under mild conditions to form a very thin stable layer which neutralizes or masks the glass surface charge.

Materials and Methods

Borosilicate particles were prepared at MSFC from Corning #7740 borosilicate tubing used in the fabrication of flight electrophoresis columns. Samples of tubing in distilled water were ground in an Al_2O_3 ball mill for 16 hours.

The sample of #7740 Corning borosilicate particles obtained from Dr. W. J. Patterson (MSFC) was sized and cleaned in preparation for the coating experiments. The very large particles were eliminated by sedimentation for one hour from suspension in a column of distilled H_2O , 7.5 cm deep at room temperature. The procedure was repeated one more time with both the sedimented particles and the supernatant suspension. The majority of the resulting particles had diameters of less than 5 μm and sedimented slowly enough that electrophoretic measurements could be obtained easily.

The particles were suspended in 3 volumes of aqua regia and left overnight. Following centrifugation, the particle pack was resuspended five times in 10 volumes of H₂O followed by centrifugation. Particle aggregation appeared during the procedure which indicated contamination of the suspension. The aqua regia wash was repeated, and was followed by washes with 15 volumes distilled H₂O, 15 volumes of warm alcoholic NaOH, and 10 volumes distilled H₂O. Some aggregation was still present at this step. The washing was continued with 20 volumes warm 6N HCl followed by 20 volumes 0.015 M NaCl. The centrifuged particle pack contained gray discolorations which were not apparent earlier. A repeated wash with 15 volumes aqua regia followed by 6N HCl (60°C), and 2 washes with 15 volumes 0.015 M NaCl did not eliminate the discoloration or the aggregation. The particles were then washed twice with 10 volumes of toluene (ethanol added until residual water dissolved), four times in 15 volumes of isopropyl alcohol, and once with 50-60°C 0.015 M NaCl. The resulting pack had no pronounced gray discolorations and could be resuspended to form a suspension with no aggregation. The particles were then washed five more times with 15 volumes of hot (60-70°C) 0.015 M NaCl. Samples of the particles for electrophoresis were drawn prior to washing with organic solvents, prior to the final 5 saline washes, and from the final preparation.

During pilot studies, samples of methylcellulose (Dow Methocel MC, 8000 cps premium), agarose (BioRad Laboratories, gel electrophoresis grade) and dextran (Pharmacia, $M_w \sim 2 \times 10^6$ daltons) were derivatized in order to introduce positively charged groups. Diethylaminoethyl (DEAE) derivatives were synthesized essentially as described for insoluble cellulose (9) in which the polysaccharide is alkylated with 2-chlorotriethylamine hydrochloride under strongly basic conditions. All of the compounds derivatized were soluble to some extent in water, so the modified method of Peterson and Sober (10) was employed for the derivitization procedure. Subsequently, attempts were made

to produce a triethylaminoethyl derivative (TEAE) from each DEAE derivative by treatment with ethyl bromide as described for the preparation of TEAE-agarose (11).

Following these pilot syntheses, two batches of approximately 45 grams each of DEAE-methylcellulose were prepared from Dow Premium 8000 cps Methocel MC, which was the candidate coating for the flight experiment. As above the DEAE group was added to the methylcellulose by alkylation of the latter with 2-chlorotriethylamine hydrochloride under basic conditions. The degree of addition is roughly proportional to the amount of alkylamine employed. Thus in the first batch termed 1X, 7 grams of the alkylamine were reacted with 60 g Methocel and in the second, 35 grams was used to form the derivative termed 5X. Sodium borohydride was included (0.5 g/100 g methylcellulose) in the sodium hydroxide reaction medium in order to reduce carbonyl groups in the Methocel which would in the original procedure be susceptible to air oxidation at alkaline pH (7,12).

The reaction products were dialyzed extensively against distilled water, precipitated from cold acetone, lyophilized and ground up in a Waring blender. The 5X derivative goes into aqueous solution more easily than the 1X. The pH of a 0.1% to 0.2% w/v solution of each in distilled water is about 10. Preliminary titration data indicated that the 5X product contains roughly 5 times as many DEAE groups as the 1X product and that the pK_a of the DEAE group falls in the range from 9 to 10.

The nitrogen contents of the 1X and 5X derivatives were determined for the lyophilized materials by the micro-Kjeldahl method (13).

Dow-Corning Z-6040 was supplied by W.J. Patterson (MSFC) as a solution of 3.1 g Z-6040 in 100 cc H_2O + 25 cc methanol.

The influence of cleaning procedures and surface modifications on the electrostatic charge of the glass particles was assessed by analytical particle

electrophoresis as described by Seaman (1).

Suspending media viscosities were measured at $25.0 \pm 0.1^\circ\text{C}$ in an Ostwald viscometer with a flow time of ~ 80 sec. for water.

Results

The electrophoretic mobility (μ) data for the glass particles at 25°C at various stages of washing are given in Table III for various suspending media. Washing of the particles in aqua regia to remove residual Al_2O_3 and multivalent ions followed by alcoholic base and 6N HCl washes increased the electrophoretic mobility to a maximum of $-5.8 \mu\text{m/s/volt/cm}$ in 0.015 M NaHCO_3 from an initial value of $-2.5 \mu\text{m/s/volt/cm}$. Further washing with organic solvents and dilute NaCl appeared to remove some discoloration of the sedimented particle packs and diminished the level of contaminants causing aggregation but did not significantly alter the particle electrophoretic mobilities.

TABLE III: ELECTROPHORETIC MOBILITY, μ , OF CORNING 7740 BOROSILICATE PARTICLES DURING CLEANING PROCEDURES.

Sample	Medium	$\mu \pm s$ ($\mu\text{m/s/volt/cm}$)*	(No.)
Unwashed particles	0.015 M NaHCO_3	-2.5 ± 0.3	(60)
Particles before organic washes	0.015 M NaHCO_3	-5.8 ± 0.6	(20)
Particles after organic washes	0.015 M NaHCO_3	-5.8 ± 0.4	(80)
Particles after organic washes	A-1 buffer	-4.8 ± 0.4	(25)
Final particle prep.	0.015 M NaHCO_3	-5.8 ± 0.3	(30)
" " "	0.015 M NaHCO_3	-5.5 ± 0.4	(20)
" " "	0.015 M NaCl pH 7.3	-5.7 ± 0.4	(20)
" " "	0.15 M NaCl pH 7.1	-2.9 ± 0.1	(20)
" " "	A-1 buffer	-4.8 ± 0.3	(40)

* Electrophoretic mobility, μ , at 25°C and standard deviation, s , for the number of particles measured given in parentheses.

These particles were then used in a pilot study to test for the reduction of surface charge by agarose, methylcellulose, dextran and their DEAE and TEAE derivatives (Table IV).

TABLE IV: ELECTROPHORETIC MOBILITIES AT 25°C OF BOROSILICATE GLASS PARTICLES IN 0.015 M NaCl-NaHCO₃ pH 7.2 ± 0.2 CONTAINING COATING MATERIALS AT 0.1% w/v.^a

Treatment	Observed ^c $\bar{u} \pm s$ (n)	Viscosity Corrected $\bar{u} \pm s$
1. None	-5.50 ± 0.27 (20)	
2. Methylcellulose	-3.65 ± 0.22 (30)	-8.40 ± 0.51
" 1x wash	-5.67 ± 0.22 (20)	
3. DEAE-Methylcellulose	+1.47 ± 0.10 (28)	+1.60 ± 0.11
" 3x wash	+1.63 ± 0.10 (20)	
" 3x wash pH 9.0	+1.14 ± 0.07 (20)	
4. TEAE-Methylcellulose	+1.36 ± 0.11 (38)	+1.48 ± 0.12
" 3x wash	+1.61 ± 0.08 (20)	
5. Agarose ^b	-0.33 ± 0.07 (52)	-0.46 ± 0.10
6. DEAE-Agarose	+1.14 ± 0.10 (40)	+1.73 ± 0.15
7. TEAE-Agarose	+0.91 ± 0.06 (30)	+1.30 ± 0.08
" 1x wash	+0.80 ± 0.06 (30)	
" 3x wash	+0.79 ± 0.06 (32)	
8. Dextran 2000	-5.18 ± 0.20 (20)	-5.59 ± 0.10
9. DEAE-Dextran	+1.99 ± 0.09 (20)	+2.13 ± 0.10
10. TEAE-Dextran	+2.05 ± 0.11 (20)	+2.46 ± 0.13
11. Z-6040	-4.86 ± 0.46 (22)	
" 7 days 4° storage	-5.11 ± 0.22 (20)	

^a Washed samples were suspended in > 100 volumes of pH 7.2 saline containing no coating material, centrifuged and resuspended in fresh saline the number of times indicated and then examined in saline.

^b Particles heated to 86°C in Agarose, then cooled to 25°C.

^c Electrophoretic mobility in $\mu\text{m/s/volt/cm} \pm$ sample standard deviation (n = no. of measurements).

The tests were made by measuring the electrophoretic mobilities ($\mu\text{m/s/volt/cm}$) of the particles suspended in saline (0.015 M NaCl-NaHCO₃ pH 7.2 ± 0.2) containing one of the materials at a concentration of 0.1% w/v. The electrophoretic mobilities which were measured in the presence of polymers were then corrected for the viscosity effects of the polymers. Subsequently, the particles suspended in

methylcellulose and its derivatives and in TEAE-agarose were washed one to three times with saline containing no polymer and the mobility measurements were repeated.

The viscosity corrected mobility of glass particles in methylcellulose was increased, but no such increase was observed following one wash of the particles. This result suggests weak adsorption of the methylcellulose to the cleaned glass surface, as had been previously observed for weak and reversible adsorbed neutral polymers (14).

Dextran did not alter the particle mobility significantly. Agarose coated the particles only when the suspension of particles was heated to 86°C and then cooled to 25°C. The majority of the coated particles had very low electrophoretic mobilities and tended to aggregate.

The DEAE and TEAE derivatives all reversed the charge of the particles to mobility values ranging from +1.3 to 2.5. Following one to three saline washes of particles suspended in DEAE-methylcellulose, TEAE-methylcellulose, or TEAE-agarose the mobilities were still positive. Consequently, the adsorption of the positively charged polymers is strong enough that they are not easily removed by dilute saline media. The mobility of 3x washed DEAE-methylcellulose coated particles was lower at pH 9 than at pH 7 as would be expected if the tertiary amino group is less protonated at the higher pH.

A sample of particles was washed once in distilled water and once in methanol and was treated with Z-6040 reagent (supplied by W.J. Patterson) for 15 minutes at room temperature followed by 15 minutes in a 60°C water bath. The particles were centrifuged from the Z-6040 reagent and washed once in saline. The treatment reduced the particle mobility by ~ 10% and following one week storage at 4°C in saline, the mobility had only slightly increased.

The data suggested that a DEAE-derivative of methylcellulose, whose degree

of amination is lower than the material used for these studies, would be readily adsorbed to a cleaned borosilicate glass surface and would be effective in producing flight configured tubes with zero or slightly positive zeta potentials.

At this stage of the studies, the major emphasis of coatings development at MSFC was on methylcellulose and there was too little time left to allow the testing of an alternate material. Consequently, effort was directed toward modifications of methylcellulose which might improve the characteristics of a methylcellulose coating.

The two batches of DEAE-methylcellulose, 1X and 5X, were prepared in sufficient quantity to be used for the flight experiment if they proved successful. Samples of each were sent to MSFC for testing at the same time as studies were being performed in this laboratory.

The nitrogen content of the 1X and 5X derivatives were found to be 0.37 and 1.28 mg/gram, respectively, by micro-Kjeldahl assay.

The effects of each derivative on the surface charge of borosilicate glass were examined using the glass particle electrophoresis model system. In Table V are the electrophoretic mobility data which show that each compound adsorbs to a clean glass particle surface to give a positive surface charge at pH 7.8-8.0. Higher positive mobilities were observed for the 5X than for the 1X derivative as expected. The batches of glass particles exposed to the two derivatives were then recovered from the 0.1% w/v DEAE-methylcellulose-0.015 M NaCl media and were washed with ~ 100 volumes of 0.015 M NaCl-NaHCO₃ (pH 7.2) and were reexamined in the latter medium. In each case one saline wash significantly reduced the positive electrophoretic mobility, while two subsequent washes had little effect. The washed particles still had positive mobilities.

TABLE V: ELECTROPHORETIC MOBILITIES AT 25°C OF BOROSILICATE GLASS PARTICLES TREATED WITH DEAE-METHYLCELLULOSE.

Treatment	Observed ^a $\bar{u} \pm s$ (n)	Viscosity Corrected ^b $\bar{u} \pm s$
1. None	-5.66 ± 0.23 (22)	
2. 1X DEAE-Methylcellulose pH 7.8 ^c	+0.60 ± 0.04 (20)	+1.23 ± 0.08
" " 1x wash	+0.76 ± 0.05 (20)	
" " 3x wash	+0.70 ± 0.05 (20)	
3. 5X DEAE-Methylcellulose pH 8.0 ^c	+1.21 ± 0.04 (20)	+2.65 ± 0.09
" " 1x wash	+0.93 ± 0.05 (20)	
" " 3x wash	+0.96 ± 0.05 (20)	

^a Electrophoretic mobility, \bar{u} , in $\mu\text{m/s/volt/cm} \pm$ standard deviation (n = no. of measurements).

^b Corrected for the bulk medium viscosity effects of the methylcellulose derivatives.

^c Particles suspended in 0.015 M NaCl + 0.1% w/v of the methylcellulose derivative.

An attempt was made to chemically crosslink adsorbed DEAE-methylcellulose on the glass surface with epichlorohydrin, an agent commonly used for cross-linking of dextran and agarose (7,12). A modification of Flodin's procedure (12) was used in which the glass particles were suspended to produce a milky white suspension in a 0.2% w/v solution of 5X DEAE-methylcellulose. The particles were centrifuged from suspension and were resuspended in 1.4 M NaOH to which NaBH_4 (1 mg/100 cc) had been added to prevent air oxidation of the polymer. Redistilled epichlorohydrin was added to a concentration of 0.2% w/v. The suspension was incubated for 4 hours at 70°C. Subsequently the particles were centrifuged, then washed three times in 0.015 M NaCl- NaHCO_3 , pH 7.2.

The electrophoretic mobilities of uncoated borosilicate particles were compared over the range of pH from 2 to 12 with those of particles treated with DEAE-methylcellulose and epichlorohydrin (Figure 1). The suspending medium was 0.015 M NaCl (ionic strength, $\Gamma/2 = 0.015$) to which 0.015 M NaOH

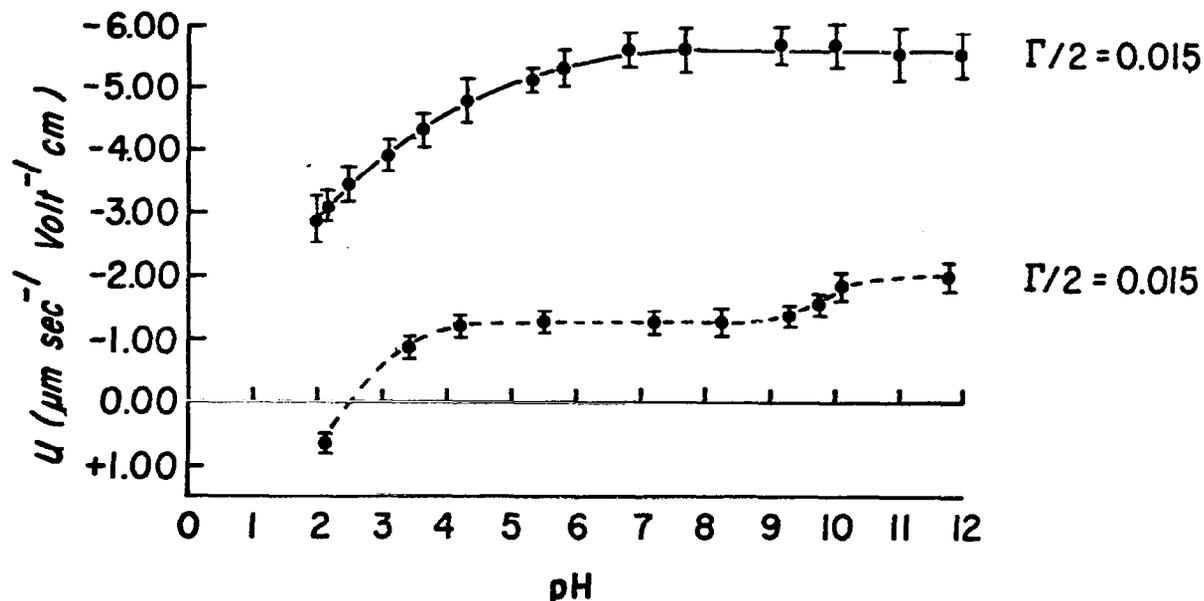


Figure 1. The electrophoretic mobility of bare borosilicate particles (—) and crosslinked DEAE-methylcellulose coated particles (---) as a function of pH of the suspending medium. Error bars indicate the standard deviation.

or 0.015 M HCl were added to give the indicated pH. The pH vs. mobility curve for bare glass suggests the presence of 2 or more surface ionogenic groups. The coated particles exhibited a reduced electrophoretic mobility at about pH 7 but the mobility was still negative, in contrast to particles simply coated with DEAE-methylcellulose. The inflection of the pH-mobility curve in the region of pH 9 for the treated particles is consistent with the presence of a positive group of pK ca. 9.5 which is characteristic of DEAE derivatives of cellulose (15). At pH 2, the particles exhibit a positive mobility consistent with the expression of positively charged DEAE groups. The lack of dependence on pH of the mobility in the region from 4 to ~ 8 indicates that the particles were covered by the polymer.

Samples of the 1X and 5X DEAE-methylcellulose were tested as coatings in borosilicate capillaries and in capillaries pretreated with Z-6040 according to

the procedures used for methylcellulose (16). It was found that prolonged rinsing of the capillaries with distilled water removed the bulk of the polymer and resulted in significant electroosmotic flow.

Discussion

The primary aim of this work was the development of a rapid technique which could be used to screen potential cleaning and modification procedures for the MA-011 electrophoresis columns. Originally efforts were to be directed at lexan surfaces. Subsequently, borosilicate glass was chosen when it was found that columns could be fabricated from this material which eliminated fluid permeation through the column walls which was observed with lexan columns. Consequently, efforts were directed toward the borosilicate system.

It was found that gram quantities of particles could be prepared for study by the milling procedure described. Particles in the size range of $< 5 \mu\text{m}$ could be separated from the crude mixture by a simple sedimentation procedure if contaminants were not present which produced particle aggregation.

One of the primary issues was the method by which the glass surfaces should be cleaned prior to any coating procedures. The cleaning procedure used in the preparation of the model particles was designed with the intent of removing inorganic surface contaminants, especially those containing multivalent ions, leachable alkali, and organic matter. The suitability of the cleaning procedures was tested by examining the electrophoretic mobility of the particles which increased significantly following the cleaning procedures. The final electrophoretic mobility values obtained were comparable to those expected based on streaming potential measurements of the electrokinetic potential of 7740 borosilicate glass (17).

A recommended cleaning procedure for glass (Appendix I) was submitted to

MSFC and its major features were incorporated into the final flight experiment procedure.

The screening of coating procedures was directed toward development of a procedure employing a polysaccharide coating agent which could be applied directly to a cleaned glass surface and then crosslinked in situ. The preliminary screening experiments supported the notion that addition of positively charged groups into nearly neutral polysaccharide molecules (dextran, agarose and methylcellulose) markedly enhanced their adsorption to the negative glass surface. However, the adsorption was reversible and in the case of DEAE-methylcellulose the polymer was slowly desorbed by prolonged rinsing of treated borosilicate capillaries with distilled water (16). When DEAE-methylcellulose was used in coating treatments of Z-6040 treated capillaries similar results were obtained where the reduction in electroosmotic flow was not as large as that obtained with methylcellulose. This result was unexpected. At that time methylcellulose treatment of a Z-6040 coated glass surface had been shown to reduce electroosmotic flow in borosilicate capillaries and larger cylindrical tubes to nearly zero (16). It was postulated that methylcellulose was bound covalently through reaction with the epoxide groups of the Z-6040 sublayer (6). Consequently, it appeared probable that inclusion of a small amount of the positively charged DEAE derivative in the unmodified methylcellulose treatment would further reduce the electroosmotic flow to zero. The lack of effective coating by the DEAE derivative on Z-6040 coated glass indicated that the unmodified methylcellulose was physisorbed rather than covalently bound as postulated. Water was a much better solvent for the DEAE derivatives than for the unmodified methylcellulose.

The attempts to crosslink a DEAE-methylcellulose coating on the glass surface were not pursued beyond the pilot experiments reported. Thus, while the approach appeared fruitful, it was discontinued since such a procedure could not be fully tested by flight time.

C. Analytical Particle Electrophoresis of Candidate Cell Populations

Background

The flight experiment plan for the zone electrophoresis portion of the MA-011 electrophoresis technology experiment provided for two runs of each of four types of samples. One type of sample was to be used to test the performance of flight hardware and two experimental biological sample types were to be flown in order to achieve electrophoretic separations at zero g which are either difficult or not possible under terrestrial conditions.

In electrokinetic studies on "living" biological material the population is usually heterogeneous with a short period of viability. In addition the electrokinetic stability (time-wise reproducibility of the electrophoretic mobility) is very dependent on the pH, ionic strength, composition and temperature of the suspending medium (2). Electrokinetic instability may arise from adsorption of cellular leakage products to the peripheral zone of the cell, auto-enzymatic degradation or from effects associated with the applied electrical field such as joule heating.

In order to check the operation and performance of any preparative or analytical electrophoresis apparatus particles which possess stable and standardized electrokinetic properties are necessary. The use of well characterized standard particles would make it possible to detect limitations of the equipment as regards the effects of joule heating, electroosmotic problems or instrumental artifacts.

For the evaluation of performance criteria in electrophoretic separation of biological cells the use of aldehyde-treated red blood cells was recommended. Such cells possess a greatly increased electrokinetic stability so that they may be used over a wide range of suspending media pH and ionic strength (18,19). In addition the relative (in some cases the absolute) distribution in mobilities of cells in the population (biological variability) is often retained thus

simulating the original biological distribution of the parameter more effectively than would be the case if a totally non-biological particle such as a polystyrene latex were to be selected.

The resolving power of the ASTP electrophoresis apparatus was to be evaluated by performing separations on mixtures of aldehyde-fixed red blood cell populations which differ in their mean electrophoretic mobilities. These populations could be obtained from either a) different mammalian species whose red blood cell populations display different electrophoretic mobilities (20,21), or b) from a single species through chemical or enzymatic modification designed to produce a series of subpopulations differing in mean electrophoretic mobility by standard amounts. This modification for example could be achieved by stepwise treatment with neuraminidase, an enzyme which removes sialic acid from the surface of most species of mammalian red blood cell (20,22). The differences in mobility between enzyme treated human red cell populations could be made as small or as large as one desired thereby affording full opportunity to test the sensitivity of the apparatus to differences in electrophoretic mobility. Such an approach would eliminate species differences including size, shape and membrane composition which perhaps play a role in the electrophoretic behavior of cells in the equipment to be tested.

Precise values for the mean electrophoretic mobility including statistical measures of variation in the sample population are available for red blood cells from a variety of mammalian species (20,21). The mobility of cells varies markedly in various buffered media (2) although the mobilities of different types of cell relative to one another remain approximately the same (21).

The viable biological samples proposed for the flight experiment were human peripheral blood B and T lymphocytes (23,24) and human embryonic kidney

cells (25). Electrokinetic data on lymphocytes was being collected (23,24), however in contrast to lymphocytes and red blood cells, there was little published electrokinetic data on kidney cells.

Hamster kidney fibroblasts have been found to have an electrophoretic mobility of -1.30 ± 0.06 S.D. $\mu\text{m/s/volt/cm}$ in a high ionic strength medium at pH 7.0 with no evidence of more than one population of cells (26). Urokinase, an enzyme used therapeutically for the treatment of pulmonary embolism has been found to be produced in about 5 percent of cultured cells from human embryo kidney cells (27). At present the demand for urokinase greatly exceeds the capability to produce it and a possible way to increase production would be to isolate these producing cells. The kidney cells had been separated into a number of fractions by Mr. Grant Barlow of Abbott Laboratories (25) using an endless belt electrophoresis apparatus (Kolin) (28). However, this procedure does not yield quantitative mobility data.

Objectives

The precise determination of the mean electrophoretic mobility of the cell populations or subpopulations thereof permitted prediction of the degree of separation to be expected in the ASTP experiment for given electrical field strengths, experimental run times and varying low values of chamber wall charge. In addition, these data provided control data for postflight electrophoretic measurements for the purpose of establishing whether the samples returned to earth have the same electrokinetic properties as they originally had prior to being introduced into the preparative electrophoresis test sample.

The major objectives of these studies were:

- a. To obtain mean electrophoretic mobility values and the distribution of mobility values for candidate aldehyde-treated red blood cells, human embryonic kidney cells, and lymphocytes in A-1 buffer.
- b. To determine the electrokinetic stability of the candidate cell populations

in A-1 buffer.

- c. To work with MSFC personnel on the processing and experimentation for aldehyde-treated red blood cells.

Methods

Analytical particle electrophoresis was carried out as described by Seaman (1). All measurements were obtained with dilute suspensions ($\sim 0.1\%$ by volume) of the cells at 25.0°C . The details of the various preparation procedures are given under results.

Results and Discussion

1. Aldehyde-treated red blood cells.

Commencing in July 1974, samples of aldehyde-treated red blood cells from various species were obtained from MSFC for electrophoretic analysis. Initially, two questions were posed: which type of aldehyde treatment should be used, and what red cell types could be obtained which could be mixed to produce the test sample for the flight experiment. It was decided that three types of red cells from different species would be sought which differed significantly in their electrophoretic mobilities so that a mixture of the three would be resolved into three bands during the flight experiment.

A second consideration in the selection of the red cell types for the flight experiment was that the red cells differ morphologically (shape, size, etc.) so that a postflight analytical method other than electrophoresis could be used to determine the degree of separation of the different cell types.

Initially dog, human and chicken red cells were treated at MSFC with 1.6% glutaraldehyde in phosphate buffered saline and samples were provided for electrophoretic analysis. Each sample was washed three times in 100 volumes of 0.15 M NaCl-NaHCO₃ pH 7.2 (standard saline) followed by suspension in either standard saline or one additional wash with A-1 buffer.

The electrophoretic mobilities of the samples were measured in standard saline (0.15 M NaCl adjusted to pH 7.2 with 0.15 M NaHCO₃) and in A-1 buffer. The objectives were to obtain mobility information for glutaraldehyde-treated red blood cells in A-1 buffer and to determine whether substantial mobility variations occur between batches of treated red cells from a single species. The following results were obtained. The mobility values tabulated represent the mean electrophoretic mobilities, \bar{u} , at 25°C ± standard deviations in units of $\mu\text{m/s/volt/cm}$.

<u>Species</u>	<u>Fixation Date</u>	<u>A-1 Buffer¹</u>	<u>Standard Saline²</u>
Chicken	3/08/74	-1.69 ± 0.10	-0.91 ± 0.06
"	5/23/74	-1.72 ± 0.11	-0.91 ± 0.07
Human	8/22/74	-2.18 ± 0.10	-1.23 ± 0.03
"	5/17/74	-2.08 ± 0.08	-1.24 ± 0.04
Canine	6/31/74	-2.36 ± 0.10	-1.32 ± 0.05
"	5/16/74	-2.31 ± 0.08	-1.32 ± 0.07

¹ 30 cells each sample

² 20 cells each sample

No significant variations in electrophoretic mobility were observed between batches of fixed cells from any one specie.

A series of questions were raised concerning the procedure for preparing the aldehyde-treated red cells which lead to the drafting and submission of a protocol covering the collection, washing and aldehyde treatment of red cells (Appendix II). The procedure proposed an aldehyde treatment at 37°C in order to maintain cell morphology. This procedure was then checked in an experiment on human and chicken red blood cells.

Three blood samples were collected in trisodium citrate from three, 1 year old White Rock chickens by cardiac puncture following anesthetization with Nembutal. Two of the samples were fixed with glutaraldehyde and one with formaldehyde at 37°C as described in the fixation protocol (Appendix II). Two units of human red blood cells were fixed with glutaraldehyde at 37°C. Each unit was divided in half and one half of each unit was fixed with Ladd

Industries purified glutaraldehyde and the remaining halves were fixed with Matheson Coleman and Bell 50% glutaraldehyde (lower purity).

The mean electrophoretic mobility, $\bar{\mu}$, of each sample of fixed cells was determined at 25°C, in pH 7.2 0.15 M NaCl-NaHCO₃ and were found to be:

<u>Sample</u>	<u>Fixation</u>	<u>$\bar{\mu} \pm$ stand. deviation ($\mu\text{m/s/volt/cm}$)</u>	<u>No. of cells</u>
Chicken-3	None	-0.84 \pm 0.04	50
Chicken-1	Glutaraldehyde (Ladd)	-0.98 \pm 0.09	30
Chicken-2	Glutaraldehyde (Ladd)	-1.12 \pm 0.07	30
Chicken-3	Formaldehyde	-0.97 \pm 0.05	30
Human-1	Glutaraldehyde (Ladd)	-1.08 \pm 0.03	30
Human-1	Glutaraldehyde (MCB)	-1.08 \pm 0.03	30
Human-2	Glutaraldehyde (Ladd)	-1.09 \pm 0.03	30
Human-2	Glutaraldehyde (MCB)	-1.09 \pm 0.03	30

Several features of the fixation were notable. The electrophoretic mobilities of aldehyde treated chicken cells were variable and were higher than those of the unfixed cells. The basis of these increases and the variability was not clear. Formaldehyde fixation at 37°C led to partial hemolysis of chicken red cells.

The properties of human red cells fixed at 37°C in glutaraldehyde of two degrees of purity differ from those of human red cells fixed under similar conditions in glutaraldehyde but at 25°C. The electrophoretic mobilities of the former are the same at pH 7.2 as those of the unfixed cells, unlike the electrophoretic mobilities of cells fixed at 25°C which are elevated by about 10% over those of unfixed cells (19). Also, the red cells fixed at 37°C showed more of a tendency to aggregate during and after the fixation than those at 25°C.

These observations pointed out a need for further examination of the influence of such variables as fixation temperature on the electrophoretic and aggregation properties of red cell populations from humans and chickens so that model cell populations with well-defined and acceptable electrophoretic and aggregative properties could be consistently produced.

In September 1974, the principal investigator set up an analytical particle electrophoresis apparatus (on loan from University of Oregon Health Sciences Center) at MSFC and instructed personnel on its use. Subsequently, the electrophoretic screening of candidate aldehyde-treated red cells was conducted at MSFC.

Analysis of the preliminary electrokinetic data on aldehyde-treated chicken, human and dog red cells indicated that their electrophoretic mobilities were not sufficiently different to afford separation in the flight experiment (29). In April 1975, the principal investigator conducted an electrophoretic screening of aldehyde-treated red cells at MSFC (Appendix III). These data supported the selection of formaldehyde-treated rabbit red cells as a slow migrating species and formaldehyde-treated human red cells as an intermediate migrating species.

Subsequently, horse red cells treated with formaldehyde were found to be suitably faster than human red cells and were chosen as the fast migrating species for the flight experiment.

The electrophoretic mobilities in A-1 buffer at 25°C of the formaldehyde-treated preparations of rabbit, human and horse red cells were reported from MSFC to be 1.75 ± 0.08 $\mu\text{m/s/volt/cm}$, 2.08 ± 0.08 and 2.90 ± 0.10 respectively. These data were employed in the predictions of sample band behavior discussed later. Electrophoretic mobility histograms were constructed for the red cells used for the flight experiment and are reported in the postflight analysis section.

No long term stability studies were conducted on aldehyde-treated red cells stored in A-1 buffer. Short term exposures of the order of a day had no effect on the electrophoretic mobility of the treated cells.

2. Human embryonic kidney cells.

Samples of human embryonic kidney cells (#995-1) were received from Abbott

Laboratories in August, 1974.

The electrophoresis experiment consisted of two parts: one to determine the distribution of electrophoretic mobilities of the kidney cells in A-1 buffer; and a second to determine whether any changes in electrophoretic mobility arise due to short term exposure of the kidney cells to the A-1 buffer.

The kidney cells in monolayer culture (\sim 1 week old, confluent) in Falcon flasks were removed from the flask surface with rubber policeman. The cells were washed and suspended in pH 7.2 phosphate-buffered saline (0.145 M NaCl-0.00772 M Na_2HPO_4 -0.0023 M NaH_2PO_4) and the electrophoretic mobilities of 40 cells averaged $-0.91 \mu\text{m/s/volt/cm}$ with a standard deviation of 0.05. Following transfer to the A-1 buffer, a series of 200 measurements were obtained and then after 4-1/2 hours the cells were transferred back into phosphate-buffered saline. Examination of 40 cells gave an average mobility of $-0.92 \pm 0.06 \mu\text{m/s/volt/cm}$. Thus no significant changes in electrophoretic behavior were produced by short term exposure to the low ionic strength A-1 buffer at 22-24°C.

The results of the 200 mobility measurements of the kidney cells in the A-1 buffer are presented in Figure 2. The observed range of mobilities was -1.32 to $-2.12 \mu\text{m/s/volt/cm}$ with an average of $-1.65 \mu\text{m/s/volt/cm}$ and a standard deviation of 0.16.

In September, 1974, a second batch of cells (#995-2) was received which represented the second culturing of a primary line of kidney cells.

An experiment was conducted on this sample of kidney cells to determine whether reculturing cells from a primary culture altered the electrokinetic properties of the cell population. The electrophoretic mobility histogram obtained for these cells (Figure 3) did not differ significantly from that of the cells from the first culture (Figure 2).

August 22, 1974

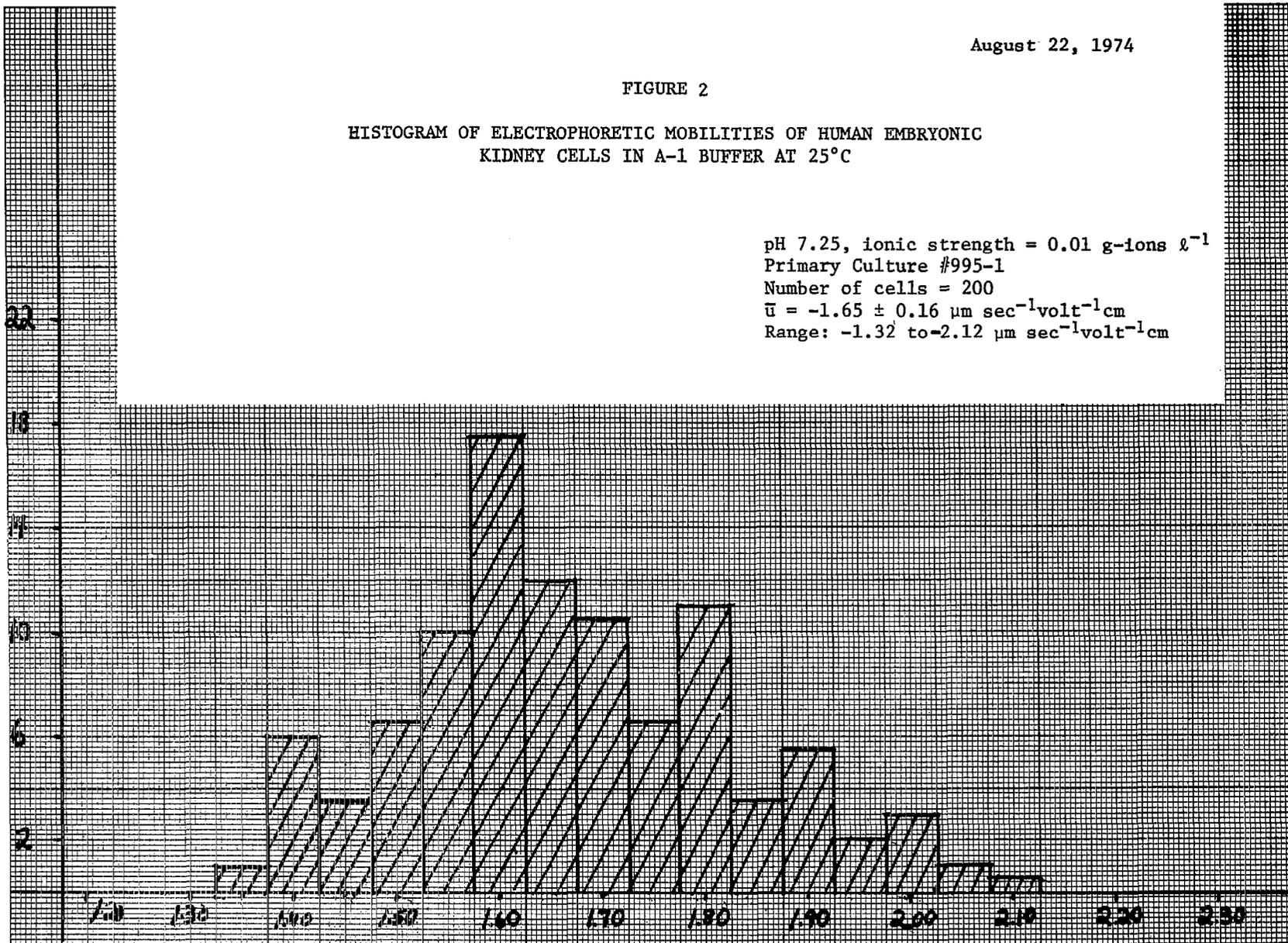
FIGURE 2

HISTOGRAM OF ELECTROPHORETIC MOBILITIES OF HUMAN EMBRYONIC
KIDNEY CELLS IN A-1 BUFFER AT 25°C

pH 7.25, ionic strength = 0.01 g-ions ℓ^{-1}
Primary Culture #995-1
Number of cells = 200
 $\bar{u} = -1.65 \pm 0.16 \mu\text{m sec}^{-1}\text{volt}^{-1}\text{cm}$
Range: -1.32 to -2.12 $\mu\text{m sec}^{-1}\text{volt}^{-1}\text{cm}$

30

PERCENTAGE OF CELLS



MOBILITY, u , ($\mu\text{m sec}^{-1}\text{volt}^{-1}\text{cm}$)

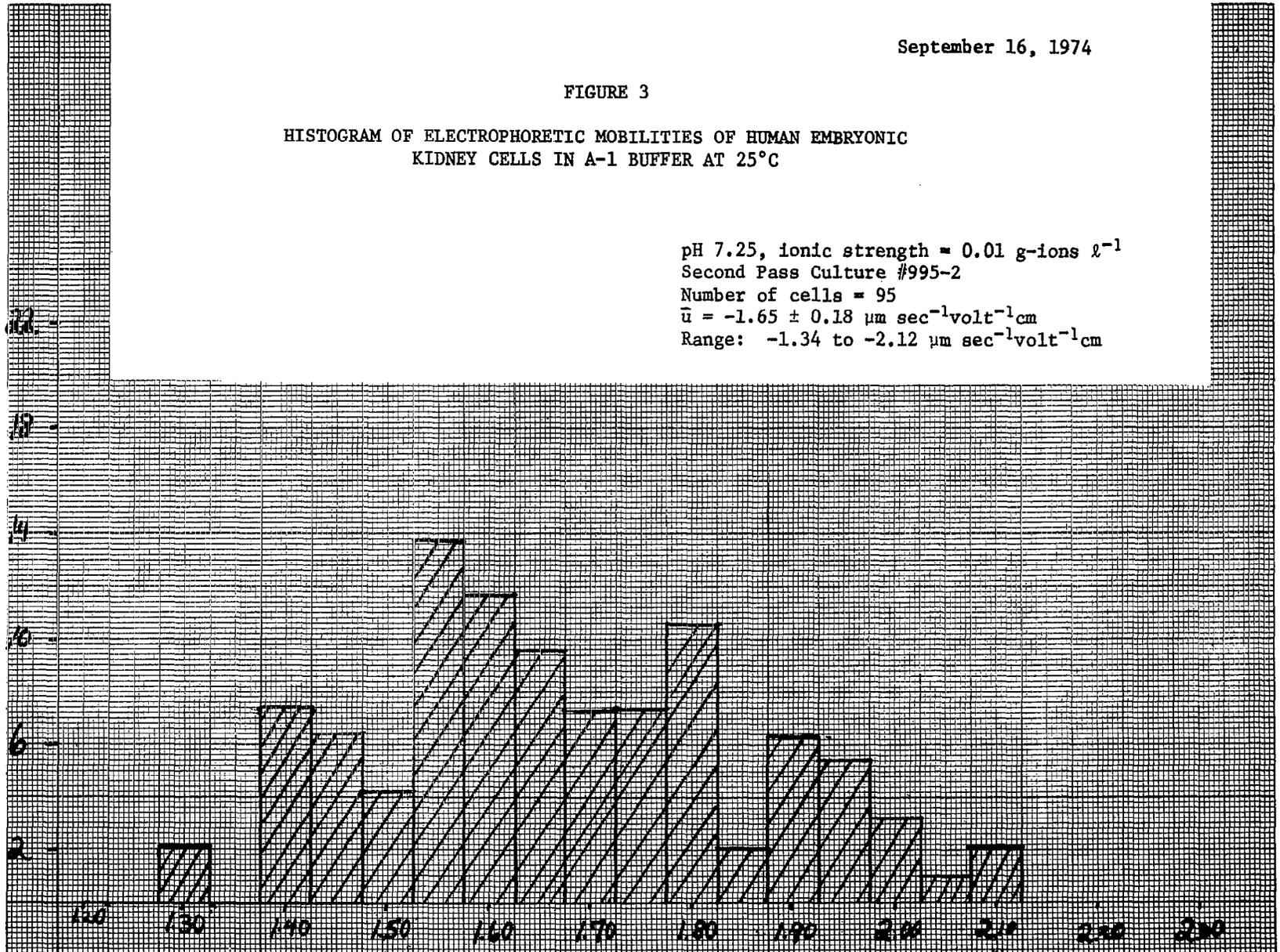
September 16, 1974

FIGURE 3

HISTOGRAM OF ELECTROPHORETIC MOBILITIES OF HUMAN EMBRYONIC
KIDNEY CELLS IN A-1 BUFFER AT 25°C

pH 7.25, ionic strength = 0.01 g-ions ℓ^{-1}
Second Pass Culture #995-2
Number of cells = 95
 $\bar{u} = -1.65 \pm 0.18 \mu\text{m sec}^{-1}\text{volt}^{-1}\text{cm}$
Range: -1.34 to -2.12 $\mu\text{m sec}^{-1}\text{volt}^{-1}\text{cm}$

PERCENTAGE OF CELLS



MOBILITY, u, ($\mu\text{m sec}^{-1}\text{volt}^{-1}\text{cm}$)

These data showed that a. the whole kidney cell population displayed a broad range of electrophoretic mobilities in A-1 buffer; b. short term exposure of the cells to A-1 buffer did not produce alterations in their averaged electrokinetic properties; and c. two successive cultures of cells from a primary cell line displayed no significant differences in their electrophoretic mobilities in A-1 buffer.

Experiments were not conducted on kidney cells from different donors so that it was not established whether cell lines from different donors displayed the same properties. Also no work was completed on the effects of freezing and thawing on the electrokinetic properties of the cells in A-1 buffer since equipment was not available which would properly control freezing and thawing rates at the University of Oregon Health Sciences Center.

3. Human B and T lymphocytes.

One sample of human B and T lymphocytes was received from Dr. P. Bigazzi (SUNY) in October 1974. Due to mechanical difficulties these were not examined until about three weeks later and had been exposed to -20°C storage temperatures during that interval. Electrophoresis of these lymphocytes in A-1 buffer at 25°C gave values ranging from -1.49 to -1.89 $\mu\text{m/s/volt/cm}$. Due to the small sample size and the prolonged storage, values for only 20 cells were obtained, which is too small a sampling to establish a reliable histogram. No further lymphocyte studies were conducted since electrophoresis equipment had been set up at SUNY.

D. Theoretical Prediction of the Behavior of Candidate Cell Populations in the Flight Experiment

Mathematical expressions describing sample migration behavior

A mathematical treatment of the migration behavior of samples in the cylindrical electrophoresis chamber of the MA-011 flight apparatus was

drafted and submitted to MSFC in January 1975 (Appendix IV). This treatment described the particle migration in terms of the electrophoretic mobility and dispersity of the particle populations, electroosmotic fluid flow, the voltage gradient; run time and the electrophoresis tube dimensions. Also equations were derived for calculating the spread of a single population of particles in terms of its electrophoretic mobility distribution.

The basic band displacement equation (equation 6, Appendix IV) was modified to provide for temperature differences between the anticipated flight experiment temperature and the standard 25°C conditions employed for ground-based measurements:

$$d_r^T = \left[\frac{\kappa^{25}}{\kappa^T} \right] \left[\frac{\eta^{25}}{\eta^T} \right] E^{25} t \left[U_e^{25} + U_{OSM}^{25} \left(\frac{2r^2}{R^2} - 1 \right) \right]$$

in which d_r^T is the distance (in μm) traversed by a particle in t seconds at temperature, T , at a point $r(\text{cm})$ from the axis of the tube with radius, $R(\text{cm})$. E^{25} is the voltage gradient in practical volts/cm at 25°C through the tube filled with A-1 buffer, U_e^{25} and U_{OSM}^{25} are the mean particle electrophoretic and the fluid electroosmotic mobilities at 25°C in $\mu\text{m/s/volt/cm}$. η^T and η^{25} are the dynamic viscosities in Poises and κ^T and κ^{25} are the specific conductances in mho/cm of A-1 buffer at temperature, T , and at 25°C, respectively.

In April, 1975, it was found that the measurement of the voltage gradient, E , in the electrophoresis column would interfere with the particle migration since the voltage probes were producing gas bubbles. The question was raised as to whether elimination of the voltage probes would compromise the post-flight calculation of the electrophoretic mobilities of samples in the flight experiment. The principal investigator, then at MSFC, submitted a memo which included the computation of the voltage gradient, E , in the absence of voltage probes. Since the electrophoresis column was supplied by a constant current power supply, the voltage gradient, E^T , may be calculated for a given column

temperature, T, with the expression:

$$E^T = \frac{V^T}{l} = \frac{I}{\kappa^T \pi R^2}$$

in which V^T is the voltage drop in volts through a length of column l (cm), I is the current through the column in amperes (set at 4.0×10^{-3} amp for the flight experiment) and the other terms are as defined earlier.

The voltage gradient calculated with this expression was in agreement with ground based voltage measurements made prior to the elimination of the voltage probes.

This expression was incorporated into the band displacement equation to give the displacement in mm in terms of known or measurable parameter values:

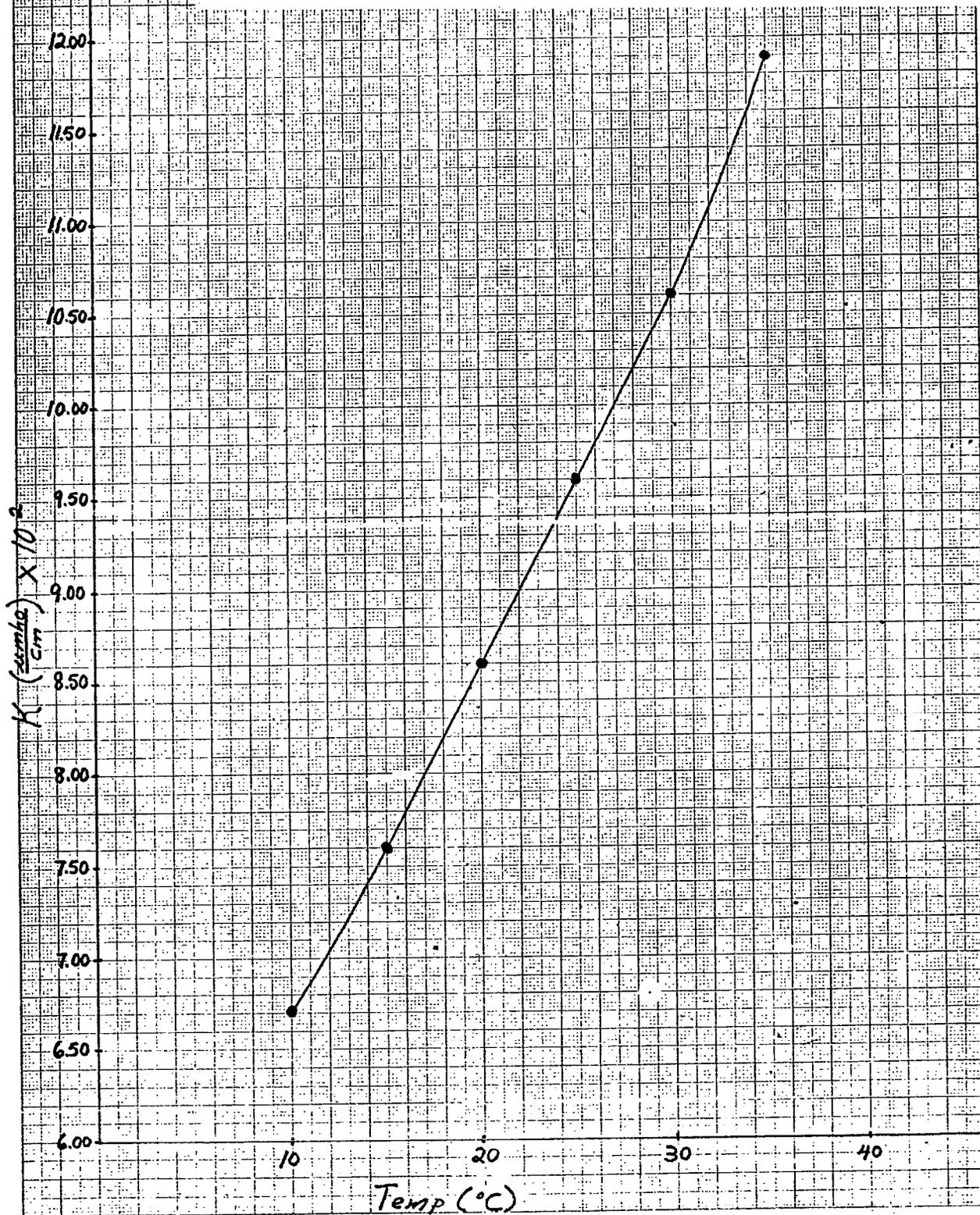
$$d_r^T = \left[\frac{\eta^{25} I \times 6 \times 10^{-2}}{\pi R^2 \kappa^T \eta^T} \right] \left[U_e^{25} + U_{Osm}^{25} \left(\frac{2r^2}{R^2} - 1 \right) \right] t$$

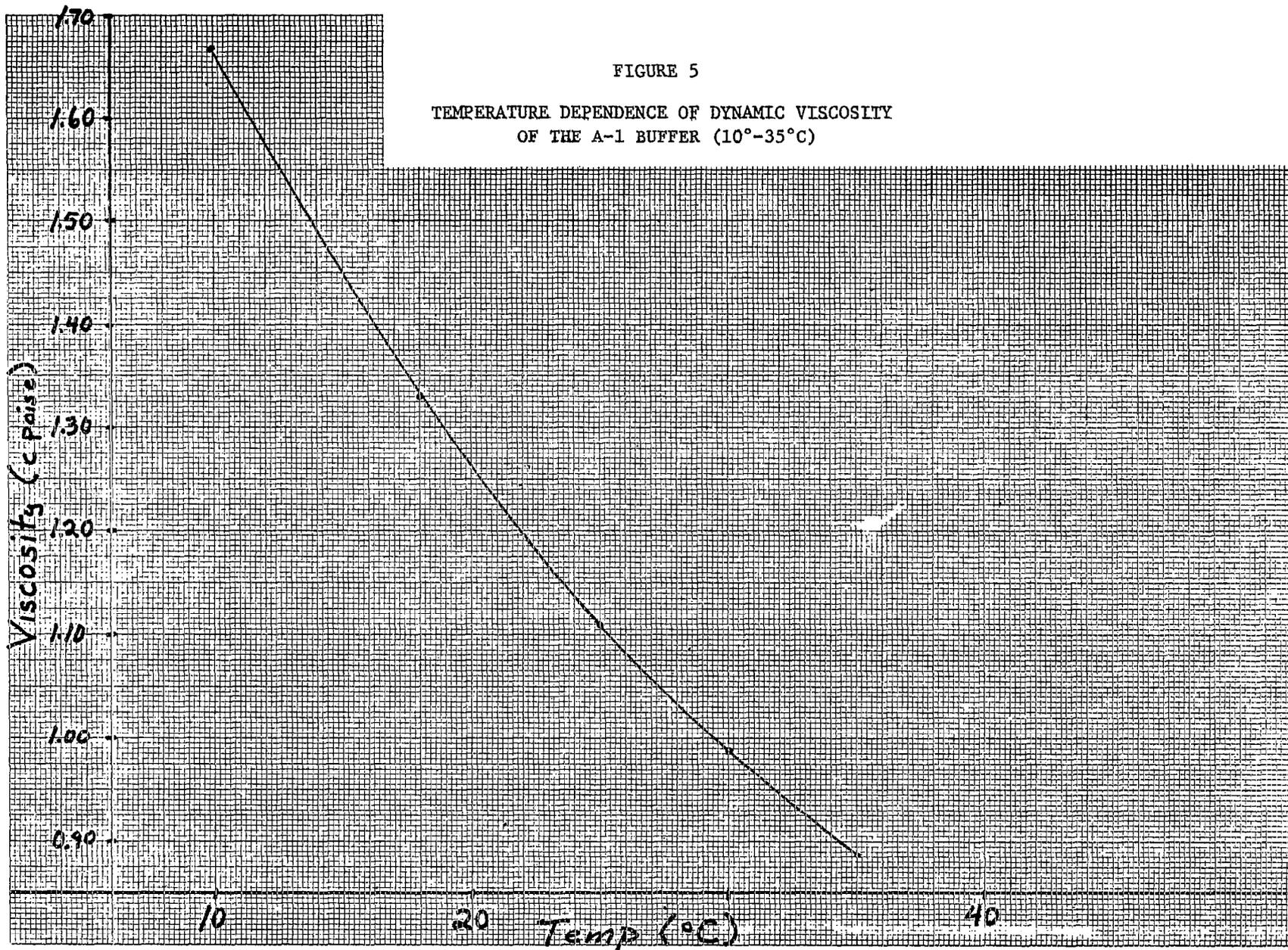
where the units of d_r^T are in mm, t in minutes, and 6×10^{-2} is a combined factor for converting μm to mm and minutes to seconds. This basic equation was employed in the prediction of band migration behavior and in the calculation of equivalent electrophoretic mobilities at 25°C from flight photograph data if the flight column temperature is known and substantial temperature gradients do not exist.

It is clear from the above expressions that the voltage gradient and consequently the sample migration in the electrophoresis column depend on the conductivity of the A-1 buffer. In the temperature region from 10 to 35°C , the conductivity of the A-1 buffer increases by 78% (Figure 4). However, the other temperature dependent variable in the band displacement equation, namely the A-1 viscosity, η , decreases by 47% over this temperature range (Figure 5). Since there is an inverse relationship between the temperature dependencies of κ and η , the change in the product $\frac{1}{\kappa^T \eta^T}$ is relatively small even for variations

FIGURE 4

TEMPERATURE DEPENDENCE OF CONDUCTIVITY
OF THE A-1 BUFFER (10°-35°C)





in average temperature up to 20-25°C. Thus, the value of $\frac{1}{\kappa T \eta T}$ is 8.95×10^4 ohm-cm/Poise at 10°C and 9.47×10^4 at 35°C.

Predictions of sample migration behavior

The first prediction was submitted in February 1975 for glutaraldehyde treated chicken, dog and human red cells, and human embryonic kidney cells (Appendix V). For the flight operation conditions envisioned at that time, these three populations of red cells were not predicted to be separated into visually distinguishable bands in the flight experiment. The kidney cells were predicted to be spread through about one third of the column length so that fractions could easily be collected by slicing the column into sections during the postflight analyses.

In May 1975 the predictions were updated to provide for the modification of the constant current settings of the electrophoresis column power supply and the more recently proposed candidate formaldehyde-treated red blood cells from horse, human and rabbit (Appendix VI). The red cells from rabbit and human were predicted to migrate closely together with marginal resolution in the absence of electroosmotic flow while horse red cells were predicted to be clearly resolved. The predictions for kidney cells were modified to be based on the full distribution of mobilities (section C) rather than on mean mobility and standard deviation. Calculations of band spread and resolution showed that even low levels of electroosmotic flow would seriously impair the resolution of electrophoretically different sample populations.

F Postflight Analyses Background

and selected properties of the cells contained in each section, and (b) analyses of the flight photographic record of the progress of each run. The analyses of the runs of aldehyde-treated red cells were intended to establish whether the electrophoresis unit operated as specified and whether unexpected problems attended the sample handling and sectioning. The emphasis of the analyses of the viable flight samples (kidney cells and lymphocytes) was slightly different and focused on the general issue of maintenance of viable cells and on the properties of the different fractions of the whole populations resolved by electrophoresis.

The principal investigator was involved primarily in the postflight analyses of the red blood test sample runs.

Objectives

The major objectives of the postflight analyses of the zone electrophoresis of the mixture of aldehyde-treated red blood cells from horse, human and rabbit were to answer the following questions:

1. Did the samples migrate at the rates predicted from the ground-based experiments?
2. Did the total migration of the samples agree with the predictions?
3. Was electroosmotic fluid flow effectively eliminated by the methyl-cellulose coatings so that the resolution capability of the apparatus was not reduced due to electroosmotic flow?
4. Were the test red cells quantitatively recovered following the column sectioning procedure?
5. Were the electrokinetic properties of the reclaimed cells in agreement with their position in the flight column?

Results and Discussion

The principal investigator travelled to MSFC to assist in the postflight analysis of the sectioned red cell columns. Subsequently, selected samples

were carried back to the University of Oregon Health Sciences Center where electrophoretic analyses were completed. The results of these analyses were drafted and submitted to MSFC in October and November 1975 in the form appended (Appendicies VII and VIII).

The following data and information are included:

<u>Item</u>	<u>Appendix</u>
a. Flight sample analysis log	VII
b. Red cell count data for sample slides	"
c. Red cell count data for electrophoresis column sections	"
d. Fluid recovery from electrophoresis column sections	"
e. Electrophoretic mobility data for control red blood cells and cells recovered from column sections	"
f. Histograms of electrophoretic mobility data and cell count data	"
g. Typing of recovered red cells by electrophoretic mobility	VIII
h. Updated prediction of sample band behavior for actual flight conditions	"
i. Plot of red cell band migration as a function of run time from flight photograph measurements	"

These data were summarized in a report presented at MSFC on October 28, 1975 by the principal investigator (Appendix IX). The major conclusions of these analyses were:

- a. There was good general agreement between the predicted and observed migration behavior for the red cell migration behavior in the first test run (column #1 S/N 072). Small deviations from the expected behavior remain unexplained.
- b. No signs of electroosmotic flow was apparent from visual examination of the flight photographs. However, the photographs were of poor quality and the effects of low levels of electroosmotic flow ($< 0.1 \mu\text{m/s/volt/cm}$) would be difficult to detect.
- c. It was not possible to correlate the electrophoretic properties of the reclaimed cells with their positions in the flight column since the first column broke during the sectioning procedure and the original position of the collected samples is uncertain. In the second column, the

flight experiment did not proceed as planned.

- d. The red cell test runs indicated that the electrophoresis unit is capable of resolving small samples of cells on the basis of their electrophoretic mobility and that reasonable estimates of the electrophoretic mobility of the sample cells may be obtained from photographic records of the progressing electrophoretic separation.

F. General Comments and Recommendations

The results to date indicate that the ASTP MA-011 electrophoresis unit may be used with some refinement for separating small quantities of biological cells and will provide data on the electrophoretic mobilities of these materials. For such an instrument to be of optimal use in later zero g applications, its design and applicability to separation problems should be critically evaluated. Such a review would provide guidelines for improvements in this generation of instrumentation and as well as for its judicious use. The review of applicability should include resolution capability, sample capacity, compatible media, etc.

The procedure for introducing samples into the electrophoresis columns as frozen discs in a sample slide appeared to work satisfactorily. One potential difficulty should be recognized and that is that there is no provision for dispersing weakly aggregated sample particles in this procedure. Some aggregation of aldehyde-treated red cells was observed in backup flight sample slides which were examined during the counting of the numbers of cells loaded per slide. Aggregates of particles where the particles differ in their electrophoretic mobilities, are expected to migrate with an averaged mobility during electrophoresis so that incomplete separation will result. It is not known to what extent the red cell test runs were influenced by such aggregation.

The interpretation of the migration behavior of the human embryonic kidney cells cannot be completed until additional electrokinetic data are obtained. Ground-based electrophoretic mobility measurements were obtained for washed confluent kidney cells. The cells used in the flight experiment were treated with trypsin prior to the washing procedure. The histograms of kidney cell count versus position in the flight column indicated that the cells migrated slower than expected.

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CLEANING OF GLASS SURFACES FOR COATING EXPERIMENTS

I. Glass Cleaning

A. General Considerations

The nature of the glass surface is known to vary considerably, being influenced not only by the bulk composition or type of glass, but also by the history of the sample during the manufacturing and subsequent processes. Thus, in order to ensure successful application of coating methods to be developed, the glass tubes used during the developmental work should be of the same type of glass expected to be used for any possible flight applications.

The cleaning method outlined has the following intents:

- a. To remove grease, debris, and gross surface contaminants;
- b. To remove the surface layer of the glass whose nature is particularly dependent on the history of the surface; and
- c. To leach the newly formed surface in order to remove alkaline substances and polyvalent ions which will decrease the stability of the surface coating to be applied.

B. Water Quality (See Appendix)

Pure water is a necessity in the reproducible conditioning of the glass surface for subsequent coating experiments. While the question of the degree of water purity necessary may be arguable, the question of whether the water should be of comparable purity during a series of experiments is not.

The contaminants in water may be categorized, somewhat arbitrarily, as soluble constituents, both ionic and nonionic, with and without surfactant properties and insolubles such as silica particles, dust, cellulose fibers, bacteria, etc.

C. Cleaning Procedure for Glass Tubes

To clean glass tubes carry them through the following series of steps. Put them in a screw cap pyrex container and carry all steps out in the same container in order to avoid contamination arising from unclean vessels or contact with hands, etc. The persons carrying out the procedure should use rubber or plastic gloves in order to avoid burns or absorption of toxic materials such as hydrofluoric acid.

1. Sonicate the tubes in alcoholic NaOH for 15 minutes followed by 10 to 15 rinses with distilled water. Alcoholic NaOH: dissolve 120 gm NaOH in 120 ml H₂O and bring to 1 liter with 95% ethanol. This solution will turn to a tan color overnight unless the alcohol is free of aldehydes.
2. Immerse the tubes in 1 M HF (2% w/v in H₂O) for 15 min. Rinse 10-15 times with distilled H₂O.
3. Immerse the tubes in warm aqua regia (18 ml conc. HNO₃ + 82 ml conc. HCl) overnight. Rinse the tubes 20-30 times in distilled H₂O and follow the pH and conductivity of samples of the rinses to plateau values. Store the tubes in distilled water in the same vessel used during the cleaning operations

sealed from the atmosphere. Rinse each tube with fresh distilled water upon removal from storage for use.

Contact of any part of the clean tubes with contaminated surfaces should be avoided prior to surface coating since surfactants may rapidly spread over the surface to the insides of the tubes.

These tubes should be wetttable and should possess constant electroosmotic mobilities when tested under standard conditions. Once the electroosmotic standard test values are established they will provide a baseline for assessing modifications in the cleaning procedure.

The manner in which any flight configured tubes are to be prepared should be established as early as possible to avoid problems arising from incompatibilities of voltage probe materials, exterior coatings, RTV, etc. with the cleaning and surface modification procedures.

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APPENDIX
ULTRA-PURE WATER

Ultra-pure water may be prepared by either a) distillation, b) ion-exchange treatment, c) freezing, d) electrophoresis or by a combination of these methods (1). The final quality of the purified water obtained will depend on the resistance of the still and storage container materials to dissolution in the water and on the amount of contamination carried over during the purification procedures. An additional source of water contamination arises from the use of plastic tubing of various types to connect storage vessels to one another or to the purification equipment (2).

The two methods normally used either separately or in combination for water purification are distillation and ion exchange. Water which is free of volatile impurities can be effectively purified by distillation. Problems may arise from the presence of a continuous film of water in the condenser which may permit non-volatile impurities to enter the distillate and also from any spray of liquid droplets which may be carried over without distillation. If the water to be purified contains volatile organic impurities addition of alkaline potassium permanganate prior to distillation is effective in destroying the majority of such contaminants. However organic amines and ammonia, if present will be carried through the distillation process under alkaline conditions, thus, if these are anticipated to pose problems in the planned experiments, the final distillation should be made on acidified water.

Ion exchangers effectively remove ionized impurities but suspended and unionized dissolved substances may remain in the effluent. Ion exchangers often introduce organic and surface active contaminants into the solution (3) unless extensively preconditioned in the manner described by Vanderhoff et al. (4). The ion exchange purification procedure should never be used as the final purification stage when the water is to be used for 'surface research'.

Materials recommended for the fabrication of storage vessels for distilled water include borosilicate glass, quartz, polyethylene and teflon (1).

The quality testing of water purity has been discussed by Hickman et al. (5) who recommends measurements of electrical conductivity, assessment of gas content, bacteriological tests and standard assays for pyrogens. Care should be exercised to determine that the method used for assay of the water does not of itself produce significant contamination of the water! The quality of ultra-pure water may be assessed by measurement of the following characteristics:

- a) Specific conductivity at an established temperature.

The intrinsic specific conductivity for pure water has been calculated to be $0.0548 \times 10^{-6} \text{ mho cm}^{-1}$ at 25°C . This value is not altered significantly by ionized impurities at the fractional parts per billion level nor unfortunately by significant levels of unionized impurities or insoluble particles (1). Although specific conductivities as low as 0.06 to $0.07 \times 10^{-6} \text{ mho cm}^{-1}$ have been obtained for water distilled from borosilicate glass or quartz the product water will be contaminated by the dissolution of materials from the still itself and gradually from the containers used for storage of the distilled water. A realistic figure for distilled water from a two-stage borosilicate glass still is in the range 0.5 to $1.0 \times 10^{-6} \text{ mho cm}^{-1}$. Measurements should be made on a good quality conductance

bridge capable of accurate measurements over the range 1.0×10^{-4} mho cm^{-1} to 1.0×10^{-8} mho cm^{-1} .

b) Foam shake test.

At a clean air-water surface a bubble bursts within about 0.01 second from the time its upper side touches the water surface. The quantity of a surfactant impurity necessary to produce a transient foam is usually extremely small, e.g., a concentration of 0.0005% w/v saponin is capable of producing a transient foam (3). A small volume of water shaken in a clean test tube should exhibit a plane air-water interface within one second after cessation of vigorous shaking of the contents. The method is simpler and more sensitive than a surface tension measurement.

c) pH.

The pH of CO_2 free ultra-pure water should be 7.0 ± 0.2 at 25°C .

d) Particulate content.

The content of particles may be evaluated by either optical or electronic means. The number of particles greater than $0.5 \mu\text{m}$ in diameter per ml of water may be determined using a Coulter counter equipped with a $30 \mu\text{m}$ diameter orifice.

e) Bacteriological tests.

Sterility may be checked by taking twenty 1 ml samples of the product water and placing each sample in a sterile test tube containing 10 ml of trypticase soy broth (Baltimore Biological Laboratory). If any samples develop turbidity after incubation at 37° for 3 days they should be streaked over trypticase soy agar in plates and then incubated at 37°C for one week. Colonies may be inspected by light microscopy. Filtration through a bacterial millipore filter should yield sterile water.

f) Assays for pyrogens.

Upon request, certified commercial laboratories carry out standard assays for pyrogens in distilled water using dogs or rabbits.

g) Electrophoretic mobility of dilute suspensions of polystyrene particles in 0.001 M aqueous NaCl with pH adjusted to 7.0 ± 0.2 with 0.001 M aqueous NaHCO_3 using the ultra-pure water to be tested. A useful criterion for water quality is whether the values of experimental parameters being measured are independent of the source of water. The electrophoretic mobility of polystyrene particles depends sensitively on the ionic and surfactant content of the suspending medium. An absence of change in the mean electrophoretic mobility of the polystyrene particles from one water batch to another would indicate adequate quality for surface studies. It should be noted that scrupulous cleanliness of all vessels and equipment plus careful handling procedures is required in order to avoid significant contamination of the ultra-pure water during the course of the procedures being used for its quality evaluation.

References

See page 2 of main text entitled 'Cleaning of glass surfaces for coating experiments.'

PROCEDURE FOR WASHING OF HUMAN RED BLOOD CELLS
AND SUBSEQUENT FIXATION WITH ALDEHYDES

Scope of Instructions

This protocol has been drawn up to cover the washing and fixation of human erythrocytes for cell/enzyme modification procedures, electrokinetic measurements and rheological experiments. The washing procedure to be described is routinely employed and has been tailored primarily for experiments on the electrokinetic properties of erythrocytes and on the flow behavior of their suspensions. Consequently the procedure may require modification in instances where abnormal cells or red blood cells from different species are to be used, or in cases where the nature of the studies is different from those normally conducted in this laboratory.

General Considerations

The general purpose of cell washing procedures is to isolate the red blood cells from other blood elements, chylomicra and soluble components in the serum or plasma. During such procedures the composition of the wash medium (buffer) must be designed or selected such that the population of cells is not appreciably altered as a result of hemolysis or differential centrifugation. Irreversible changes in cell parameters such as deformability, geometry, or electrokinetic charge should be avoided by appropriate choice of buffer osmolarity, ionic strength, pH and temperature. Conditions for centrifugation of the red cells should be precisely specified in terms of temperature, g force, and duration. Temperature shocks to the red blood cells should be avoided by ensuring that the blood or red cell suspension is always at the same temperature as the wash medium or fixative solution prior to addition or mixing.

Specific Considerations

Erythrocytes undergo hemolysis, changes in geometry and a number of other changes when exposed to suspending media of nonphysiological pH, ionic strength

and osmolarity. These suspending media parameters should be specified and rigidly controlled. Physiological osmolarities are about 300 milliosmolar - lower values produce cell swelling and leakage while higher values result in cell shrinkage. Physiological pH is 7.4 and this is the value for maximum erythrocyte stability in media of different ionic strengths. In 0.150 M aqueous NaCl red cells are relatively stable from about pH 6 to 8 while in 0.007 M aqueous NaCl they are stable only in the range from about pH 6.8 to 7.5.

Source of Blood

i) Blood of established phenotype may be obtained by venipuncture from healthy fasting adults and either defibrinated or taken into sterile 3.8% w/w trisodium citrate (1 volume of the citrate to 9 volumes of whole blood) at room temperature. Other anticoagulants including ACD, CPD, EDTA or oxalate may be used. It is not advisable to use heparin since it is usually ill-defined and also is metabolized under routine blood transfusion service storage conditions. Consideration should be given to the choice of vein (antecubital), method of cleaning arm and the gauge and length of needle in relation to size of syringe. The material used in construction of the syringe (plastics, glass) may also be relevant in certain studies.

ii) Units of whole blood or packed cells may be obtained from the Regional Center of the American Red Cross. Such blood should not be more than four days old at the time of washing and fixation and should have been stored at +4°C. The development of aggregated or adhesive material in the blood or packed cells may be checked microscopically or by subjecting samples of the blood to a Screen Filtration Pressure measurement. Such microaggregates may be removed by filtration through a blood clot filter prior to washing the red blood cells.

Washing Procedure

Centrifuge the blood sample at about 2500 xg for 20 minutes at room temperature. Use a temperature controlled centrifuge and select centrifuge tubes or buckets of

such a size that the whole procedure can be carried out in one centrifuge. The choice of material for the centrifuge tubes or buckets may be of relevance in certain studies. Aspirate off the serum or plasma and then the buffy coat (thin yellowish-white layer of white cells and platelets). Avoid removing more than the absolute minimum number of red blood cells. Suspend the packed red blood cells in phosphate buffered saline (PBS)* using a minimum of 5 volumes of PBS to one volume of packed red blood cells. Centrifuge the suspension for 15 minutes at 2500 xg at about 20°C. Repeat the washing procedure four times. Remove the supernatant PBS.

Fixation Procedure

Resuspend the washed human red blood cells to about 50% v/v concentration in the PBS at about 20°C. Raise the temperature of the system gradually to 37°C in a 37° water bath or incubator and then add dropwise from a separatory funnel or buret with stirring a volume of freshly prepared aldehyde fixative solution* (37°C) equal to the red cell suspension volume. Maintain the system for two hours at 37°C with stirring, then centrifuge the suspension for 15 minutes at 2500 xg and remove the supernatant aldehyde. Resuspend the red blood cells to about 50% v/v concentration in PBS at 37° and add an equal volume of fresh aldehyde to the partially fixed RBC and transfer the 'fixed' red blood cell suspension into an airtight glass vessel in order to minimize absorption of CO₂ and O₂. Note if any visible leakage of colored material appears in the supernatant fluid during or post fixation. It is advisable also to periodically monitor the pH of the supernatant fixative solution.

* See Appendix

APPENDIX

I. Solutions

For each 100 ml of packed red blood cells fixed according to the procedure ^{litus} 2.5 of 1X PBS and 500 mls of aldehyde fixative (the aldehyde of choice in PBS) are required.

1. Phosphate Buffered Saline (PBS)

<u>Constituent</u>	<u>Formula Wt.</u>	<u>1X Molarity</u>	<u>1X g/l</u>	<u>2X g/l</u>
NaCl	58.44	0.075	4.383	8.766
NaH ₂ PO ₄ ·H ₂ O	138.01	0.0134	1.849	3.698
Na ₂ HPO ₄ ·7 H ₂ O	268.09	0.0537	14.396	28.792

The final pH of the 1X PBS is 7.3 and the osmolality is 282 mosmoles/kg. It should be stored at 4°C to retard bacterial growth.

2. 4% w/v Formaldehyde Fixative Solution

For 1 liter, mix 40 grams of paraformaldehyde with 500 ml of 2X PBS and about 400 ml distilled H₂O in a 2 liter Erlenmeyer flask. Insert a magnetic stir bar, and warm the stirring suspension to 60°C and maintain it at 60°C for 30 min, during which time the paraformaldehyde will dissolve. Cool the solution to room temperature and filter it through Whatman No. 1 filter paper into a 1 liter volumetric flask. Wash held up solution through the filter with a couple of distilled H₂O rinses of less than 50 ml and then bring the total volume up to 1 liter with distilled water. The pH of the solution is usually not more than 0.1 pH unit lower than that of PBS.

3. 3.3% w/v Glutaraldehyde Fixative Solution

For one liter, dissolve 33 grams of pure glutaraldehyde or 47 ml of 70% w/v Polysciences glutaraldehyde in 500 ml of 2X PBS in a one liter volumetric flask and bring the volume to one liter with distilled H₂O.

R.J. Knox
G.V.F. Seaman

APPENDIX III

SUMMARY OF ANALYTICAL ELECTROPHORESIS DATA ON ALDEHYDE-TREATED
RED BLOOD CELLS FROM DIFFERENT SPECIES
(Collected at MSFC April 21-30, 1975)



NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

GEORGE C. MARSHALL SPACE FLIGHT CENTER

MARSHALL SPACE FLIGHT CENTER, ALABAMA 35812



REPLY TO: EH35(75-194)
ATTN OF:

May 2, 1975

TO: EH35/Dr. Allen

FROM: EH35/Dr. Knox

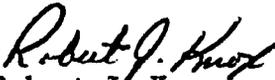
SUBJECT: Summary of Analytical Electrophoresis Data on Aldehyde-Treated Red Blood Cells Conducted at MSFC April 21-30, 1975.

The analytical electrophoresis apparatus was reassembled, checked out, and found to be operational. Samples of aldehyde-treated red blood cells were obtained from Frances Scott. These were drawn from batches of fixed cells from human, chicken, dog, turkey, cow, horse, and rabbit. The samples were washed 4-5 times in 50-100 volumes of A-1 buffer and were electrophoresed in same at 25°C. The electrophoretic data and the sample identifications are compiled in Table 1.

The ranges for the electrophoretic mobilities of samples from the different batches roughly in order of decreasing mobility were:

<u>Species</u>	<u>Fixative</u>	<u>U_e Range</u> ²⁵	<u>No. of Samples</u>
Chicken	Autoclaved formaldehyde	-2.49	1
Dog	Glutaraldehyde	-1.37 -2.36	3
	Formaldehyde	-2.01 -2.25	5
Horse	Formaldehyde	-2.18	1
Human	Glutaraldehyde	-1.69 -2.18	3
Turkey	Formaldehyde	-2.00 -2.02	2
Chicken	Formaldehyde	-1.91 -1.99	4
Human	Formaldehyde	-1.88 -1.95	5
Pig	Formaldehyde	-1.77 -1.88	2
Chicken	Glutaraldehyde	-1.69 -1.72	2
Cow	Formaldehyde	-1.52 -1.54	2
Rabbit	Formaldehyde	-1.36 -1.40	2

The large mobility ranges listed for glutaraldehyde treated dog and human red cells are due to one sample of each which was drawn from samples fixed in 1974 by people from Brown. In both cases the mobilities were much lower than the mobilities of two samples each of glutaraldehyde treated dog and human red cells which were sent to us by Frances Scott in August 1974.


Robert J. Knox
University of Oregon Health Sciences Center

Enclosure

<u>Sample</u>	<u>Date</u>	<u>Anticoagulant</u>	<u>Fixative</u>	U_e^{25} ($\mu\text{m}/\text{s/volt/cm}$)	<u>Comments</u>
	3/7/75	Defib.	Formald.	-1.92 -2.25	Bad fixation - Broad U_e range.
	3/17/75	ACD	Formald.	-2.06	
	6/31/74	?	Glutarald.	-2.36	Run in Aug. 1974
	5/16/74	?	Glutarald.	-2.31	Run in Aug. 1974
	6/31/74	?	Glutarald.	-1.37	Fixed by Brown people. Questionable procedure
Turkey	10/24/75	EDTA	Formald. (37°)	-2.02	Some slow cells
	10/24/75	EDTA	Formald. (Room T.)	-2.00	
Cow	2/25/75	EDTA	Formald.	-1.54	B1 Angus
	2/26/75	EDTA	Formald.	-1.52	Jersey-Supposedly sent to Micale for -2.9 mobility.
Rabbit	4/23/75	EDTA	Formald.	-1.40	
	4/26/75	EDTA	Formald.	-1.36	Untreated $U_e = 0.99$
Horse	4/24/75	ACD	Formald.	-2.18	
Pig	#1 4/28/75	EDTA	Formald.	-1.77	
	4/28/75	EDTA	Formald.	-1.88	Less stirring than #1 during aldehyde treatment.

Table I

Electrophoretic Mobilities of Aldehyde-Treated Red Cells in A-1 Buffer at 25°C

<u>Sample</u>	<u>Date</u>	<u>Anticoagulant</u>	<u>Fixative</u>	U_e^{25} ($\mu\text{m}/\text{s/volt/cm}$)	<u>Comments</u>
Human	11/1/74	EDTA	Formald.	-1.92	Unfixed $\bar{U} = -2.36$
	10/18/74	EDTA	Formald.	-1.88	
	3/12/75	Defib.	Formald.	-1.94	
	3/19/75	EDTA	Formald.	-1.90	
	4/25/75	Defib.	Formald.	-1.95	
	8/22/75	----	Glutarald.	-2.18	Meas. in Aug. 1974
	5/17/74	----	Glutarald.	-2.08	
	8/19/74	?	Glutarald.	-1.69	
Chicken	10/10/74	EDTA	Formald.	-1.91	
	3/14/75	Defib.	Formald.	-1.92	
	3/14/75	EDTA	Formald.	-1.97	
	4/4/75	Defib.	Formald.	-1.99	White Rock
	3/8/74	Defib.	Glutarald.	-1.69	Meas. in Aug. 1974
	5/23/74	?	Glutarald.	-1.72	Meas. in Aug. 1974
	3/5/75	EDTA	Formald.	-1.78	#2 Sample to Beckman - not chicken
	?	?	Formald.	-2.49	Autoclaved 2 hours A-11 buffer without glycerol, glucose or dextran
Dog	2/22/75	EDTA	Formald.	-2.01	
	3/5/75	ACD	Formald.	-2.23	Sample #2 sent to Beckman - sample returned had lower mobility - probably bacterial contaminator
	3/7/75	ACD	Formald.	-2.14	

APPENDIX IV

BAND MIGRATION BEHAVIOR FOR ELECTROPHORESIS OF CANDIDATE
CELL POPULATIONS IN CLOSED CYLINDRICAL SYSTEMSA. Single Particle Behavior

A charged particle when exposed to a voltage gradient will migrate toward the electrode of opposite charge. The electrophoretic mobility of the particle, U_e , is defined as:

$$U_e = \frac{V_e}{E} \quad (1)$$

where U_e = electrophoretic mobility in $\mu\text{m}/\text{sec}/\text{volt}/\text{cm}$

V_e = electrophoretic velocity in $\mu\text{m}/\text{sec}$

E = voltage gradient in volts/cm

In this treatment cathodic mobility will be positive, i.e., a negatively charged particle which migrates to the anode would have a negative mobility.

When a suspension of charged particles is contained in a closed cylindrical tube whose walls carry a static charge, an applied voltage causes the particles to migrate and will produce circulation of the suspension, or electroosmosis. The observed velocity, V_o , of the charged particle will be the sum of the electrophoretic velocity, V_e , and the fluid velocity, V_w :

$$V_o = V_e + V_w \quad \text{in } \mu\text{m}/\text{sec} \quad (2)$$

The magnitude of V_w may be expressed as a function of the radial distance from the axis of the tube and of the fluid velocity at the tube wall:

$$V_w = V_s \left[\frac{2r^2}{R^2} - 1 \right] \quad (3)$$

where V_s = fluid velocity at the tube wall in $\mu\text{m}/\text{sec}$

r = radial distance from the tube axis in cm

R = radius of the tube in cm

The magnitude of V_s is a function of the charge on the tube wall and is proportional to the applied voltage. Thus an electroosmotic mobility, or osmobility, U_{osm} , is defined as:

$$U_{osm} = \frac{V_s}{E} \quad (4)$$

where U_{osm} = osmobility in $\mu\text{m}/\text{sec}/\text{volt}/\text{cm}$.

A negative tube wall charge produces a flow of the adjacent fluid toward the cathode, hence a positive osmobility.

The distance, $d(\mu\text{m})$, traversed by a particle in time, $t(\text{sec})$, at a point r cm from the tube axis will be:

$$d = V_o t = V_e t + V_s t \left[\frac{2r^2}{R^2} - 1 \right] \quad (5)$$

or in mobility terms,

$$d = Et \left[U_e + U_{osm} \left(\frac{2r^2}{R^2} - 1 \right) \right] \quad (6)$$

From equation (6) it is seen that at $r = 207R$ is an envelope, termed the stationary level, where the contribution of U_{osm} vanishes.

If a sample population of electrophoretically monodisperse particles is introduced into the chamber as a disc one particle in thickness and of radius r_D (cm), the final displacement and profile of the sample particles as a function of radial distance may be computed with equation (6) if it is assumed that:

- a. The system is closed, i.e., only internal circulation occurs in the cylindrical electrophoresis tube;
- b. Fluid flow is laminar in the tube (no convective disturbances);
- c. The particle population behaves as the sum of the behaviors of individual particles which remain at a fixed radial distance throughout the run (absence of particle-particle interaction);
- d. The voltage gradient and the temperature are constant.

B. Sample Plug Behavior and Resolution

Analysis of a single population of particles:

In a more general treatment of the behavior of a sample plug during electrophoresis, additional terms must be added to the single particle treatment in order to account for the effects of sample plug thickness and biological cell population variation.

When the sample plug has a thickness, x_0 , greater than one particle, sample particles will commence electrophoresis from different starting points. The edge of the sample plug adjacent to the ^{cathode} Λ will be used as a reference point such that particles at this edge commence migration at distance of 0 mm while those at the leading edge of the plug commence migration at x_0 mm.

Biological variation in any single population of cells will produce band broadening and will adversely influence the resolution of two or more cell populations. The influence of population variation (in electrophoretic mobility) on the band width may be expressed in terms of the sample standard deviation of the electrophoretic mobilities of the population. If we assume a normally distributed population, as observed for human red blood cells, 68.3% of the mobilities lie within ± 1 standard deviation unit (s) of the mean, 95.4% within ± 2 units and 99.7% within 3 standard deviation units. If we consider the sample disc to be made up of a large but finite number of layers one particle thick, and that after electrophoresis, each layer will be distributed normally, then the sample disc will be broadened to the limits of the particle mobility distribution. Greater than 99% of the layer of particles at the band front will be found in the region:

$$d_f = Et \left[U_e \pm 3s + U_{osm} \left(\frac{2r^2}{R^2} - 1 \right) \right] + x_0 \quad (7)$$

and those at the trailing edge of the band at:

$$d_t = Et \left[U_e \pm 3s + U_{osm} \left(\frac{2r^2}{R^2} - 1 \right) \right] \quad (8)$$

At $t = 0$, $d_f - d_t = \text{disc thickness} = x_0$. The thickness of the band, x_t , then at time, t , will be the difference between the extremes of d_f and d_t , i.e.

$$x_t = d_f - d_t = Et \left[\left[U_e + 3s + U_{\text{osm}} \left(\frac{2r^2}{R^2} - 1 \right) \right] - \left[U_e - 3s + U_{\text{osm}} \left(\frac{2r^2}{R^2} - 1 \right) \right] \right] + x_0 \quad (9)$$

At one value of r this equation simplifies to:

$$x_t(r) = Et (6s) + x_0 \quad (\text{Band spread equation}) \quad (10)$$

i.e. 99.7% of the particles will be found within this region. Note that this equation gives the thickness of the band at a given value of radius r . In the presence of electroosmotic flow the overall distribution of the band would be between the limits $r = 0$ and $r = \text{plug radius}$, i.e., for the ASTP apparatus the leading edge of the band will be located at $r = 0$ and the trailing edge of the band at $r = 0.752R$.

Inserting these values of r into equation (9) we obtain:

$$\begin{aligned} x_t &= Et \left[\left[U_e + 3s + U_{\text{osm}} \left(\frac{0}{R^2} - 1 \right) \right] - \left[U_e - 3s + U_{\text{osm}} \left(\frac{2(0.752R)^2}{R^2} - 1 \right) \right] \right] + x_0 \\ &= Et \left[6s - U_{\text{osm}} - U_{\text{osm}} \left(\frac{2(0.752R)^2}{R^2} - 1 \right) \right] + x_0 \\ &= Et \left[6s - U_{\text{osm}} \left(\frac{2(0.752R)^2}{R^2} \right) \right] + x_0 \end{aligned} \quad (11)$$

This equation describes the band spreading behavior of a single normally distributed population of particles as a function of population dispersity and electroosmotic flow.

Analysis of a mixed population of particles:

The basic criterion for resolution of two particle populations is that the front of the band of slower particles be separated from the trailing edges of the band of faster moving particles. When particle and wall charge have the same sign, U_e and U_{osm} are of opposite sign. For U_e negative and U_{osm} positive, the leading edge of a sample band is at $r = 0$ and the trailing edge is at $r = 0.752R$. For U_{osm} negative (positive wall charge) the converse is true.

For the former case, where $U_{e_1} > U_{e_2}$, the trailing edge of the faster population will be located at d_{t_1} :

$$d_{t_1} = Et \left[U_{e_1} - 3s_1 + U_{osm} \left(\frac{2(0.752R)^2}{R^2} - 1 \right) \right] \quad (12)$$

while the forward edge of the slower population will be located at d_{f_2} :

$$d_{f_2} = Et [U_{e_2} + 3s_2 + U_{osm}(-1)] + x_0 \quad (13)$$

The separation distance between faster and slower bands is:

$$\begin{aligned} \Delta d &= d_{t_1} - d_{f_2} = Et \left[U_{e_1} - 3s_1 + U_{osm} \left(\frac{2(0.752R)^2}{R^2} - 1 \right) \right] - Et \left[U_{e_2} + 3s_2 - U_{osm} \right] - x_0 \\ &= Et \left[U_{e_1} - 3s_1 - U_{e_2} - 3s_2 + U_{osm} \left(\frac{2(0.752R)^2}{R^2} \right) \right] - x_0 \end{aligned} \quad (14)$$

C. Values of ASTP MA-011 Parameters

Electrophoresis tube length	14.94 cm
Electrophoresis tube radius	0.318 cm
Sample plug radius	0.239 cm
Sample plug thickness	0.312 cm
Voltage probe distance	14.7 cm
Current setting	4.8 mA
Voltage (A-1 buffer) ¹	220 volts
Optional run times	45, 60 and 75 min.

Run temperatures²:

Fixed red cells	30°C
Viable cells (kidney and lymphocytes)	15°C
Radial temperature gradient	To be established
Vertical temperature gradient	" " "

Electrophoretic mobility values \pm
sample standard deviation (25°C)³

Glutaraldehyde treated red cells:

Chicken	1.69 \pm 0.10 $\mu\text{m/s/volt/cm}$
Human	2.08 \pm 0.08
Dog	2.36 \pm 0.10
Embryonic kidney cells	1.65 \pm 0.18
Human lymphocytes	To be established

¹ This value is an estimate based on agarose-filled column measurements.

² Estimates of average run temperatures.

³ Mobility values at $T \neq 25^\circ\text{C}$ may be calculated to compensate for viscosity differences in A-1 buffer at different temperatures from 10-35°C:

$$U_e^{25} \eta^{25} = U_e^T \eta^T$$

where U_e^T and η^T are the electrophoretic mobility and viscosity, respectively, at the given temperature.

PREDICTION OF THE SEPARABILITY OF THE CANDIDATE BIOLOGICAL
CELL POPULATIONS IN THE ASTP ELECTROPHORESIS APPARATUS

I. Fixed Red Cells

Band displacements and contours were computed for glutaraldehyde treated chicken, human and dog red blood cells for electrophoresis chamber wall ζ potentials which give U_{OSM}^{25} values of 0.3, 0.2, 0.1, 0 and -0.1 $\mu\text{m}/\text{sec}/\text{volt}/\text{cm}$. The theoretical treatment of these computations was appended to our December progress report. In order to simplify computations A-1 buffer viscosity and conductivity corrections were incorporated into the basic equations so that mobility data and voltage values at 25°C could be used directly in the computation of band electrophoretic behavior at temperatures other than at 25°C. Equation (6) from the December report then takes the form:

$$d_r^T = \left(\frac{\kappa^{25}}{\kappa^T} \right) \left(\frac{\eta^{25}}{\eta^T} \right) E^{25} t \left[U_e^{25} + U_{\text{OSM}}^{25} \left(\frac{2r^2}{R^2} - 1 \right) \right]$$

in which d_r^T is the distance (μm) traversed by a particle in t seconds at temperature, T , at a point r (mm) from the axis of the tube with radius, R mm. E^{25} is the voltage gradient in volts/cm at 25°C through the tube filled with A-1 buffer, U_e^{25} and U_{OSM}^{25} are the particle electrophoretic and the fluid electroosmotic mobilities at 25°C in $\mu\text{m}/\text{sec}/\text{volt}/\text{cm}$. η^T and η^{25} are the dynamic viscosities in Poises of A-1 buffer at temperature, T , and at 25°C, respectively, and κ^T and κ^{25} are the specific conductances of A-1 buffer in $\mu\text{mho}/\text{cm}$ at T and 25°C, respectively. Viscosity, specific conductance, and electrophoretic mobility data were drawn from those reported in our earlier progress reports. The voltage gradient at 25°C was fixed at 220 volts/14.7 cm or 15.0 volts/cm based on reports from MSFC that the voltage ranged from 220 to 230 volts at about 25°C.

In Figure 1, the red cell migration data are plotted as cross sectional views for bands one cell thick at $T = 30^\circ\text{C}$ and $t = 60$ minutes. The shaded areas on each end of the depicted electrophoresis columns represent the portion of the tubes

obscured by Delrin collars in the flight configured columns. The slowest band is composed of chicken red cells, the intermediate of human red cells, and the fastest band of dog red blood cells. The dog red cell band is either obscured by the collar at U_{OSM}^{25} values of 0.3 to 0.1 or is very close to the collar for lower values. In Figure 2, is a similar plot except for a run time of 45 minutes. The bands are well centered in the tube and are not broadened as much at any given value of U_{OSM}^{25} as in the 60 minute run.

The spread of a band 3.12 mm thick was next calculated based on the dispersity of the red cell populations and the operation parameters used in computing the data plotted in Figure 2 for $U_{OSM}^{25} = 0$. The equation used was:

$$\chi_t = \left(\frac{\kappa^{25}}{\kappa T} \right) \left(\frac{\eta^{25}}{\eta T} \right) E^{25} t (ns) + \chi_0$$

where χ_t is the broadness of the sample band at time, t , n is 2 or 4 and s is the sample standard deviation of the mean electrophoretic mobility for a given red blood cell population, χ_0 is the thickness of the sample plug (3.12 mm).

These data are plotted in Figure 3. In 3a are the band distributions for chicken and dog red cells. The blackened areas depict the zones where 68% of the cells should be located and the adjacent unshaded areas enclose the regions where an additional 28% of the cells will be located. The latter zones are only separated by 8 mm for chicken and dog red cells. In 3b human red cells are included. All three populations overlap: the human and dog red cells bands overlap being the most pronounced. In 3c are plotted the cross sectional distributions of dog and chicken red cells where $U_{OSM}^{25} = 0.1 \mu\text{m}/\text{sec}/\text{volt}/\text{cm}$.

The cases plotted represent optimistic estimations. A number of detrimental factors have not been included since either reliable experimental data are not available or the appropriate theoretical framework is not developed. These include the following:

- a. Temperature gradients. In the absence of cooling a radial temperature gradient is anticipated where the center of the column is warmer than the periphery. Ground based estimates suggest that the magnitude of such gradients is less than 1°C in agar filled columns. In the region of 30°C , a 0.5°C gradient will enable particles in the center of the column to migrate approximately 1% faster than those in the periphery. This would correspond roughly to the influence of an additional electroosmotic flow of about $0.02 \mu\text{m}/\text{sec}/\text{volt}/\text{cm}$.
- b. Cell-cell interaction. At a volume concentration of 20%, the viscosity of the red cell sample plug will be approximately twice that of the suspending medium. Red cell concentrations of 10-20% are anticipated to give significant cell-cell interaction resulting in decreased total migration and a further loss of resolution.

II. Viable Cell Populations

A. Human Embryonic Kidney Cells

Band migration and broadness were calculated for run times of 45 min. (Fig. 3d) and 60 min. (Fig. 3e) at 15°C and $U_{\text{osm}}^{25} = 0$ from electrophoretic mobility range data which we have previously reported. The computed values include no provision for cell-cell interaction, sample plug viscosity or electrophoretic effects due to dextran, or thermal gradients. Either of the 45 or 60 min. run times gives satisfactory displacement of the sample band. Our electrophoretic mobility distribution data provide no evidence for discrete subpopulations of cells which would separate into discrete bands under the conditions included in the computation. The greatest concentration of cells is expected in the blacked out areas of the bands.

B. Human Peripheral Lymphocytes

Our electrophoretic data are sketchy for lymphocytes. From one experiment we obtained $U_e^{25} = 1.61 \pm 0.13 \mu\text{m}/\text{sec}/\text{volt}/\text{cm}$ for a mixed B and T lymphocyte population. Thus the lymphocyte band should migrate to approximately the same

position as the kidney cells in Fig. 3d and e. We have no evidence at present that B and T subpopulations are present whose mean mobilities differ by more than 0.2 to 0.3 $\mu\text{m}/\text{sec}/\text{volt}/\text{cm}$ which is about the minimum required for separation if $U_{\text{osm}} \approx 0$ and the dispersity of the populations is small.

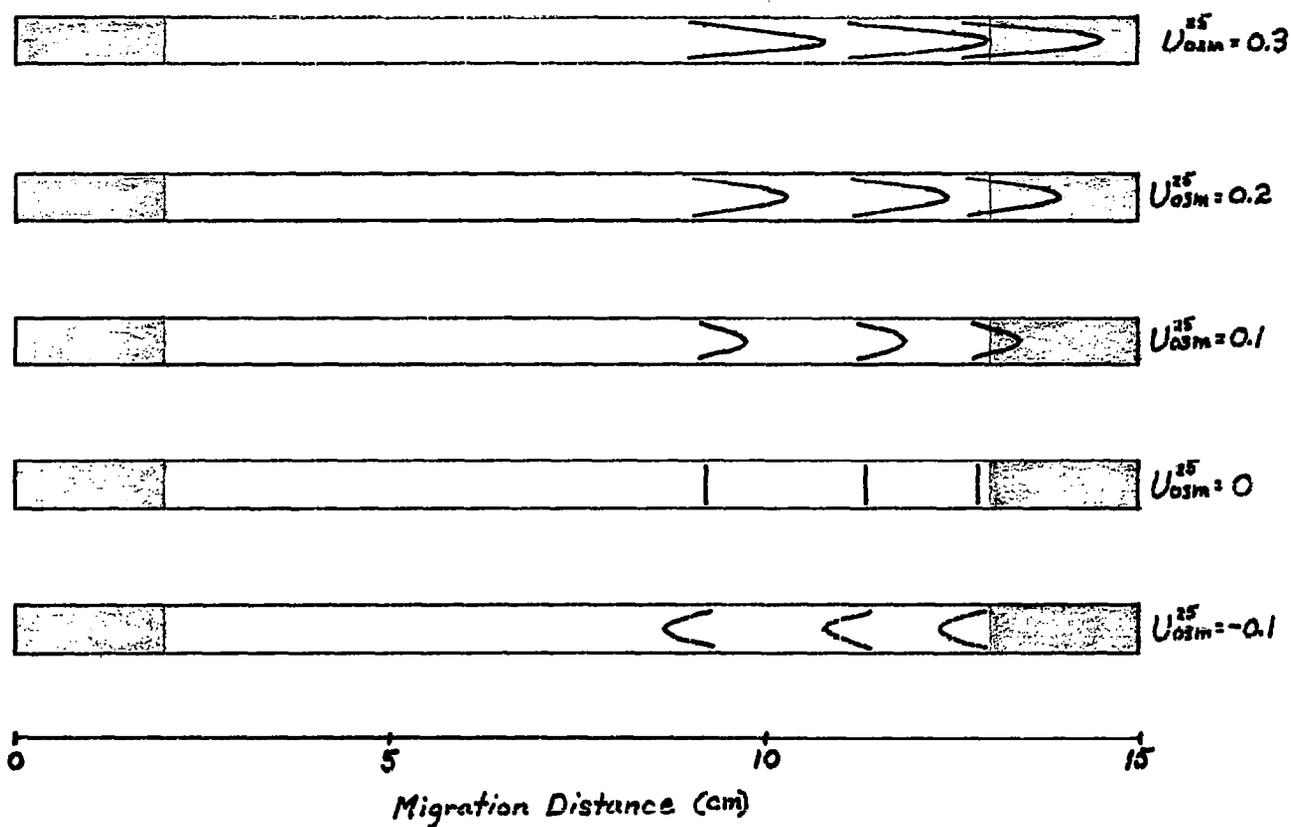


Fig. 1. Predicted Migration For Single Layers Of Glutaraldehyde-Treated Red Blood Cells.

$t = 60 \text{ min.}$
 $T = 30^\circ \text{C}$

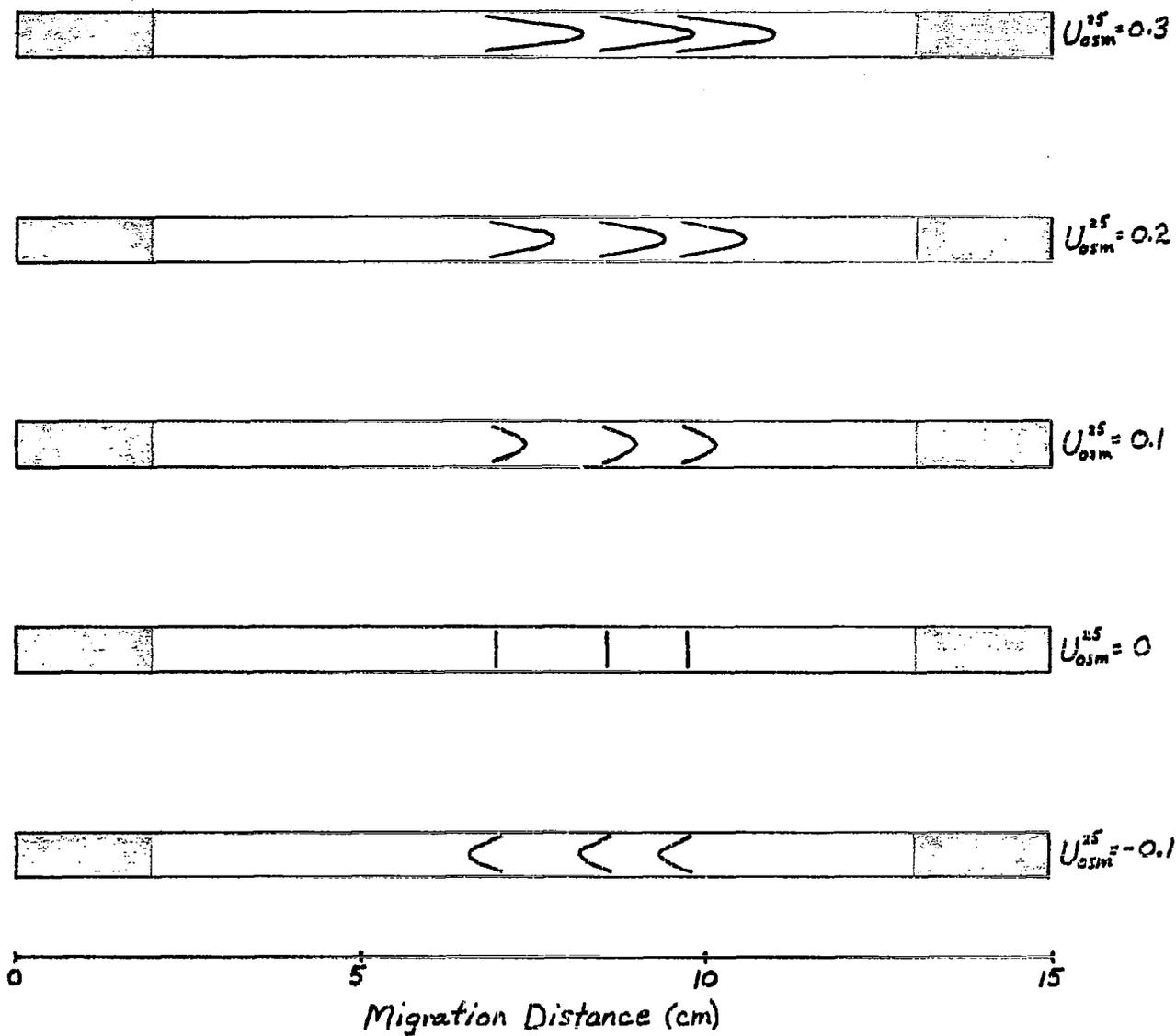


Fig. 2. Predicted Migration For Single Layers Of Glutaraldehyde-Treated Red Blood Cells.

$t = 45 \text{ min.}$

$T = 30^\circ \text{C}$

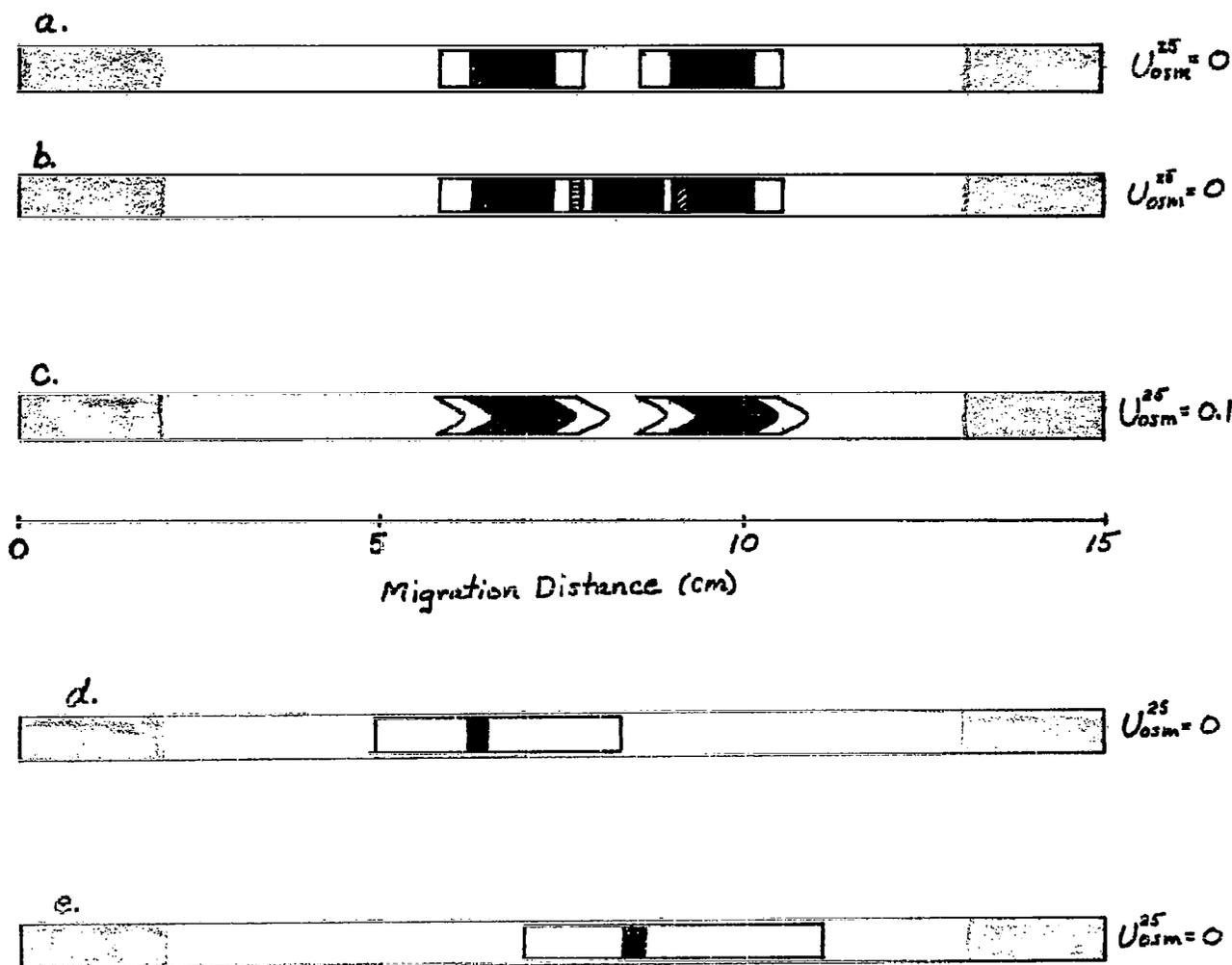


Fig. 3. Predicted Band Migration For Red Cells And Embryonic Kidney Cells.

- a. Chicken and Dog RBC $t = 45 \text{ min}$ $T = 30^\circ \text{C}$
- b. Chicken, Human; Dog RBC $t = 45 \text{ min}$ $T = 30^\circ \text{C}$
- c. Chicken and Dog RBC $t = 45 \text{ min}$ $T = 30^\circ \text{C}$
- d. Kidney Cells $t = 45 \text{ min}$, $T = 15^\circ \text{C}$
- e. Kidney Cells $t = 60 \text{ min}$, $T = 15^\circ \text{C}$

APPENDIX VI

UPDATED PREDICTION OF THE BAND MIGRATION BEHAVIOR OF THE CANDIDATE CELL POPULATIONS IN THE ASTP ELECTROPHORESIS APPARATUS (MA-011)

a. Migration Data

The particle migration predictions were updated to take into account the modification of the constant current setting and the more recently proposed candidate red cell populations.

The following equation was used to calculate the distance, d_r^T (mm), traversed into the electrophoresis column by particles originally located at the longitudinal midpoint of the sample plug and a distance, r (cm) from the tube axis:

$$d_r^T = \left[\frac{\eta^{25} I \times 6 \times 10^{-2}}{\pi R^2 \kappa^T \eta^T} \right] \left[U_e^{25} + U_{osm}^{25} \left(\frac{2r^2}{R} - 1 \right) \right] t - \frac{\chi_0}{2} \quad (1)$$

where η^{25} and η^T are the viscosities in poise of A-1 buffer at 25°C and the run temperature, T , respectively; I is the constant current setting in amperes, R is the radius of the electrophoresis tube in cm., κ^T is the conductivity in mho/cm of A-1 buffer at temperature, T ; U_e^{25} is the mean electrophoretic mobility (anodic) in $\mu\text{m}/\text{sec}/\text{volt}/\text{cm}$ of the particles at 25°C in A-1 buffer; U_{osm}^{25} is the electroosmotic mobility (anodic) of A-1 buffer at 25°C; t is the electrophoresis run time in minutes; χ_0 is the sample plug thickness; and 6×10^{-2} is a combined factor for converting μm to mm and minutes to seconds.

The following parameter values were employed in the analysis. It is assumed that the average column temperature is 30°C.

R	0.3175 cm
I	4.0×10^{-3} amp
χ_0	0.312 cm
r	up to 0.239 cm, the sample plug radius
η^{25}	0.0111 poise
η^{30}	0.00987 poise
κ^{30}	1.06×10^{-3} mho/cm
t	45, 60 or 75 min.
$\frac{\eta^{25} I \times 6 \times 10^{-2}}{\pi R^2 \kappa^{30} \eta^{30}}$	$0.8040 \left(\frac{\text{volts}}{\text{cm}} \right) \left(\frac{\text{sec-mm}}{\text{min-}\mu\text{m}} \right)$

The following electrokinetic data for red blood cells was drawn from memo 5 EH35 (75-239) May 5, 1975:

	$U_e \pm s$ (anodic)
Rabbit	1.75 ± 0.08
Human	2.08 ± 0.08
Horse	2.90 ± 0.10

The $U_e^{25} \pm s$ values for kidney cells was the same as previously used, i.e. 1.65 ± 0.18 $\mu\text{m/s/volt/cm}$ with an observed range of U_e^{25} values from 1.33 to 2.12 $\mu\text{m/s/volt/cm}$.

Figure 1 (a & b) shows the cross sectional profiles and migration distances for the layer of particles at the mid point of each sample band for the mixed red cell sample after 45 minutes and 60 minutes, respectively. The solid lines represent the profiles in the absence of electroosmotic flow and the dotted curves are the profiles for the same samples. After a run time of 45 minutes all the bands should be in the visible portion of the electrophoresis tube. In contrast, after 60 or 75 minutes, the horse red cell band will have passed out of the field of view unless particle-particle interaction or some other effect has slowed the migration significantly.

In Figure 1 (c-e) are the predicted regions where the kidney cells should be found following 45, 60 or 75 minute runs. These band lengths were calculated from the extreme mobilities observed for kidney cells in A-1 buffer. After 75 minutes the leading edge of the kidney cell band, which is expected to contain the urokinase producing cells, is approaching the end of the electrophoresis column (13 cm out of 15 cm total). Similar behavior is expected for lymphocytes.

b. Band Spreading and Resolution

The resolution of the sample red cell populations is very sensitive to electroosmotic flow. The distance, Δd (mm), between the leading edge of a slower band and the trailing edge of a faster band may be computed with the equation (derived in December progress report):

$$\Delta d = \left(\frac{\eta^{25} I x 6 x 10^{-2}}{\pi R^2 \kappa T \eta T} \right) \left[|\Delta U_e^{25}| - (s_1 + s_2) + U_{osm}^{25} \left(\frac{2(0.753R)^2}{R^2} - 1 \right) \right] t - \chi_0 \quad (2)$$

where s_1 and s_2 are the standard deviations of the mobility averages U_{e1}^{25} and U_{e2}^{25} , respectively; and other terms are as defined for equation (1). Substitution of the appropriate constant values gives:

$$\Delta d = 0.8040 \left[|\Delta U_e^{25}| - (s_1 + s_2) + 1.133 U_{osm}^{25} \right] t - \chi_0 \quad (3)$$

The computed separations for the rabbit and human cell bands and the human and horse cell bands are given in Table 1 for run times of 45 to 75 minutes and U_{osm}^{25} values from 0 to -0.3 $\mu\text{m/sec/volt/cm}$. Negative values indicate overlap. Human and horse cell bands are predicted to be resolved under all of these conditions. Rabbit and human cell bands are only marginally resolved: at U_{osm}^{25} values greater than 0 to -0.1 the bands overlap. If the standard deviations of the mobility values were zero (Δd column three) the bands will overlap when U_{osm}^{25} is greater than -0.2 to -0.3 $\mu\text{m/sec/volt/cm}$.

Note that the resolution is predicted to improve when longer run times are employed. However, the large mobility of the horse cells precludes use of these times unless factors which have not been taken into account (such as particle-particle interactions) significantly slow the bands.

The longitudinal spread of the bands is a major factor in the loss of resolution and can be calculated as a function of particle mobility standard deviation and electroosmotic flow. In the presence of electroosmotic flow, a bullet shaped band is produced and the distance, χ_t (mm), from the tip to the tail of the band may be estimated with the relationship (December progress report):

$$\chi_t = \left[\frac{\eta^{2.5} I \times 6 \times 10^{-2}}{\pi R^2 \kappa T \eta T} \right] \left[2s - U_{osm}^{2.5} \left(\frac{2r^2}{R^2} - 1 \right) \right] t + \chi_0 \quad (4)$$

where χ_t is the longitudinal distance in the electrophoresis tube in which 68% of the cells should be located.

The values of χ_t are tabulated in Table 1 for 5 values of 0.08 and 0.10 $\mu\text{m}/\text{sec}/\text{volt}/\text{cm}$, which were reported for human, rabbit and horse red cells. The concentrations of cells in these bands may be estimated, assuming only longitudinal migration:

$$\text{Mean cell concentration (cells/cc)} = \frac{0.68N_i}{\pi r^2 \chi_t} \quad (5)$$

in which N_i = the initial total number of cells of a given type loaded in the sample slide.

r = radius of the sample plug in cm = 0.239 cm.

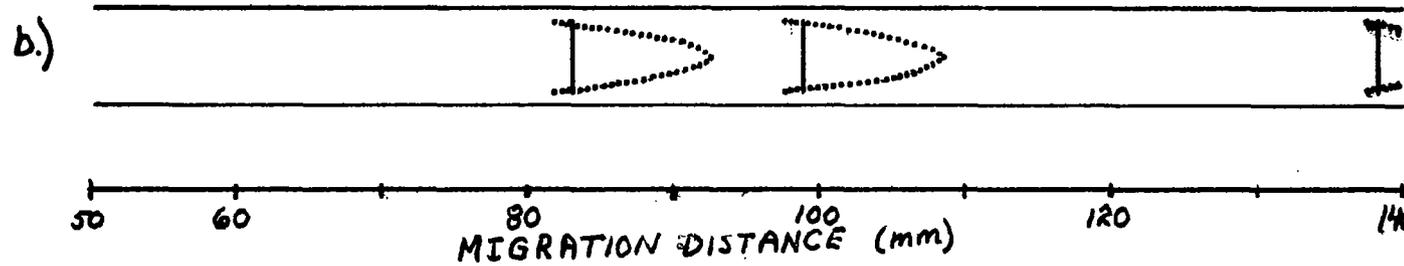
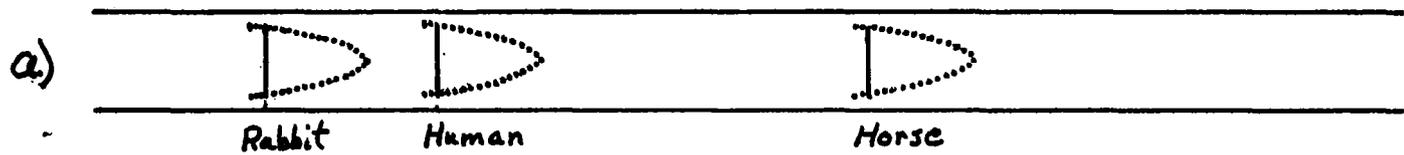
χ_t = length of sample band in cm.

Substitution of the parameter values gives:

$$\text{Mean cell concentration (cells/cc)} = \frac{3.79 N_i}{\chi_t} \quad (6)$$

As the electroosmotic flow increases, χ_t increases leading to a smaller final mean cell concentration.

Figure 1 : Cross-sectional Views of Mixed Red Cell Bands and



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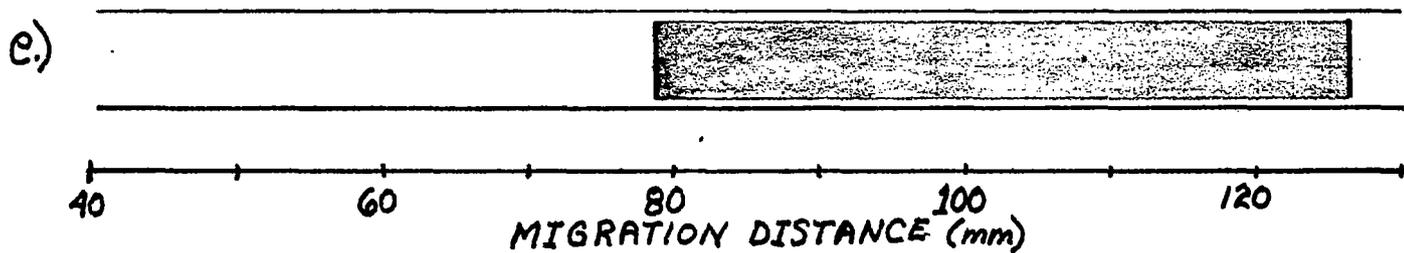
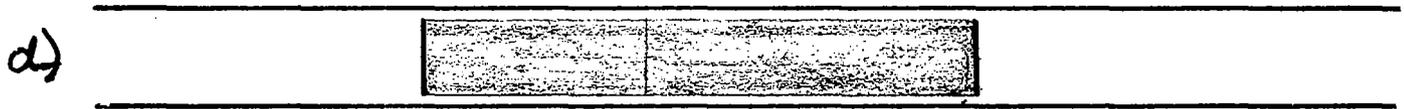
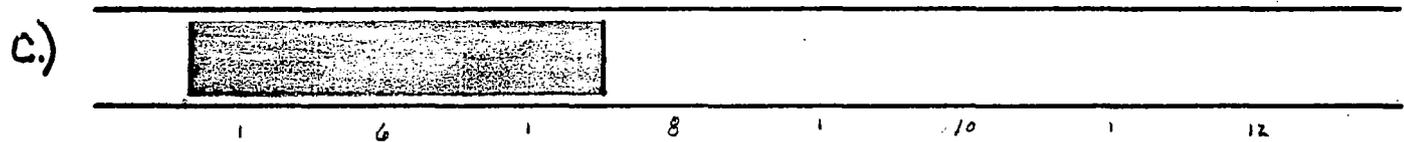


TABLE 1

CALCULATED BAND SEPARATIONS AND SPREAD OF BANDS FOR
RABBIT, HUMAN AND HORSE RED CELLS (ALDEHYDE-TREATED)

U _{0sm}	t(min)	Band Separation, Δd (mm)			Band Spread, χ _t (mm)	
		Rabbit-Human	Human-Horse	*Rabbit-Human	S = 0.08	S = 0.10
0	45	3.0	20.0	8.8	8.9	10.4
-0.1	45	-1.1	15.9	4.7	13.0	14.4
-0.2	45	-5.2	11.8	0.6	17.1	18.6
-0.3	45	-9.3	7.7	-3.5	21.2	22.6
0	60	5.1	27.8	12.8	10.8	12.8
-0.1	60	-0.4	22.3	7.3	16.3	18.2
-0.2	60	-5.8	16.8	1.9	21.8	23.7
-0.3	60	-11.3	11.4	-3.6	27.2	29.2
0	75	7.1	35.5	16.8	12.8	15.2
-0.1	75	0.3	28.6	10.0	19.6	22.0
-0.2	75	-6.5	21.8	3.1	26.4	28.8
-0.3	75	-13.4	15.0	-3.7	33.3	35.7

*. For $S_1 + S_2 = 0$

APPENDIX VII

POSTFLIGHT ANALYSES OF ASTP ELECTROPHORESIS TECHNOLOGY
EXPERIMENT (MA-011) RED BLOOD CELL RUNS

I. Ground Control Data

A. Electrophoretic Mobilities of Candidate Red Blood Cells

Ground control electrophoretic mobilities were measured in A-1 buffer at 25°C for samples of aldehyde-treated rabbit, human and horse red blood cells (RBC). These samples were drawn from the same red cell batches in formaldehyde fixative as were used in the preparation of the sample slides for the flight experiment.

The cells were washed four times in 50-100 volumes of A-1 buffer. The mobilities of 100 cells from each preparation were measured employing the microinjection technique used for the flight samples.

Normally a sample volume of 1 to 2 cc of sample suspension containing 10^6 to 10^7 RBC/cc is required to fill the analytical electrophoresis chamber. However, only a small part of this volume is examined through the microscope. The microinjection technique consisted of injecting 10-30 μ l of cell suspension into the observation region of the chamber. In this way, measurements could be obtained on samples containing 10^4 to 10^5 cells.

Histograms (Figure 1, 2 and 3) were constructed for each species of red cell. This mobility data provides the basis for the prediction of the behavior of the cells in the flight experiment.

B. Sample Slide Cell Counts

The flight sample slides were prepared at MSFC by adding to the sample slide 20 μ l from each of three stock red cell suspensions. The reported composition was:

<u>Red Cell</u>	<u>Stock RBC/ml</u>	<u>RBC/20 μl</u>	<u>% of Total</u>
Rabbit	2.61×10^8	5.22×10^6	32.8
Human	1.72×10^8	3.44×10^6	21.6
Horse	3.63×10^8	7.26×10^6	45.6
	Total	1.59×10^7	100

The cell count was checked for in two sample slides which were prepared at the same time as those which were flown. Each slide was thawed. The cells were observed to be concentrated on one side of the sample plug leaving almost clear A-1 buffer on the other side. The sample from each slide was drawn into a plastic pipet, transferred to a glass test tube, followed by 2-3 rinses of the slide and pipet with A-1 buffer. The last traces of cells could not be washed from the sample slide groove.

Each sample was mixed on a vortex mixer and then drawn into a 2.0 ml volumetric pipet followed by A-1 buffer to give a 2.0 ml volume. Each suspension was thoroughly mixed and was transferred back to the glass tubes. Aliquots of each suspension were counted in an AO Spencer Hemacytometer:

<u>Slide No.</u>	<u>Counts/80 small divisions</u>	<u>Mean \pm SD</u>	<u>RBC/mm³</u>	<u>Total RBC</u>
032	240;227;253	240 \pm 13	(1.20 \pm 0.06) $\times 10^4$	2.40 $\times 10^7$
035	240;260;293;264	264 \pm 22	(1.32 \pm 0.11) $\times 10^4$	2.64 $\times 10^7$

The average total RBC per sample slide was 2.52×10^7 . This value is significantly larger than the theoretical 1.59×10^7 cells per slide.

II. Flight Sample Analysis Log

Sept. 3, 1975. Samples L 72.7—L 72.29 (column 1) were removed from cryogenic freezer and thawed at room temperature. Each sample tube was centrifuged briefly in order to separate the thawed sample from the teflon sleeve. The teflon sleeves were removed with tweezers from the tubes. Negligible losses of sample fluid occurred during the procedure. The sample tubes were then weighed.

Each sample was agitated with a vortex mixer to achieve suspension of cells. An aliquot of $\sim 10 \mu\text{l}$ was withdrawn and transferred to a hemacytometer and counted. Following counting, the samples were transferred back to the sample containers which were then stored at 4°C .

Sept. 4, 1975. Samples L 72.1—L 72.6, L 72.R, L 72.L, L 52.1—L 52.24, L 52.R and L 52.L were removed from the cryogenic freezer, thawed at room temperature and treated the same as samples L 72.7—L 72.29.

Sept. 5, 1975. Samples L 72.1, L 72.3, L 72.4, and L 52.5 through L 52.11 were stored in ice and carried by R.J. Knox to the University of Oregon Health Sciences Center for electrophoretic mobility studies. Samples from each of the stocks of aldehyde-treated red cells (rabbit, horse, human) which had been used to make up the flight sample slides were also obtained for electrophoretic analysis.

Sept. 10-13, 1975. Following storage at 4°C , aliquots of samples L 72.1, L 72.3, L 72.4, L 52.5, L 52.8 and L 52.11 were examined by electrophoresis in order to obtain appropriate analytical data. All of the flight samples brought to Oregon, with the exception of L 72.3, were centrifuged and the supernatant A-1 buffer was carefully aspirated for pH measurements. The cells were suspended in 100-200 μl of fresh sterile A-1 buffer.

Sept. 13, 1975. All flight samples were turned over to Frances Scott for transport back to Marshall Space Flight Center.

III. Flight Sample Analysis Data

The sample slice data collected at Marshall Space Flight Center for column 1 and column 5 sample slice series are given in Tables I and II, respectively. The computed volume, V , of each slice was obtained from the measured sample mass, m , and the density of A-1 buffer, ρ :

$$V = \frac{m}{\rho} \quad (1)$$

The density of A-1 buffer at 25°C (1.022 g/cc) was used.

The computed length, ℓ , in mm represents the length of the electrophoresis column which would be filled by the given sample slice. This length, ℓ , was calculated from the sample volume, V in mm^3 and the radius of the electrophoresis column, R in mm:

$$\ell = \frac{V}{\pi R^2} = \frac{V}{\pi (3.18 \text{ mm})^2} \quad (2)$$

The computed lengths of the sample slices should approximate to the preset sample slice thicknesses (2 mm for column 1 and 5 mm for column 5). For column 5 where no problems were encountered during the slicing of the column, the average computed slice thickness was 5.07 mm with a standard deviation of 0.38 mm. This agrees well with the preset value of 5.0 mm. For column 1, the average slice thickness for slices 7 to 29 (excluding 26) was 1.36 mm with a standard deviation of 0.26 mm. This represents only 68% of the theoretical 2.0 mm value. Personnel involved in the slicing of columns 1 and 5 noted that some of the sample slices from column 1 had an aerated appearance. It is probable that column 1 was cracked at the time of removal of the frozen sample column and that desiccation of the column occurred during the storage of the column in the cryogenic freezer. The significant amounts of debris and small glass particles in slices 7-29 support this notion. These slices were located in the part of the frozen column which had been pushed out of the glass column prior to the collapse of the glass column.

The total computed volume of each flight column was 4.73 cc. The total recovered volumes from columns 1 and 5 were 3.57 cc and 4.39 cc, respectively, which represented 76% and 93% of the theoretical. Thus a substantial volume of fluid was lost from the column 1 sample.

The red blood cell concentration was obtained from hemacytometer counts of the undiluted sample slices. The sample counts ranged from 0 to about 400. Typically two or more aliquots were counted for samples in which the cells were concentrated.

The pH measurements were those obtained by direct measurement with a combination glass electrode at $\sim 23^\circ\text{C}$. Samples with volumes less than 150 μl could not be measured by this technique.

The histograms containing the cell count versus position in electrophoresis columns 1 and 5 are given in Figures 4 and 5, respectively. The histogram in Figure 4 represents an attempt to place the collected samples commencing with the end of the column away from the sample slide. There is some uncertainty in this placement due to unaccounted losses in the recovered fluid volume and possible mixing of the samples when the column collapsed during the recovery operation.

The electrophoresis data for the six sample slices examined are shown in Figures 6-11, and are summarized in Table III.

Table I. Sample Slice Data for Column 1, Series S/N 072

Sample No.	Mass (g)	Computed Volume (cc)	Computed Length (mm)	RBC/mm ³ x10 ²	Total RBC x 10 ⁵	pH (23°C)
L-72-R	0.3165	0.3096	9.74	0.50	0.16	
" L	0.2782	0.2721	8.56	0.35	0.95	
1	0.7405	0.7243	22.8	71.5	51.8	7.07
2	0.2781	0.2720	8.56	17.5	4.76	
3	0.1507	0.1474	4.64	90.0	13.3	
4	0.6390	0.6251	19.7	36.0	22.5	7.09
5	0.0900	0.0880	2.77	27.5	2.42	
6	0.0544	0.0532	1.67	23.5	1.25	
7	0.0452	0.0442	1.39	0.85	0.38	
8	0.0329	0.0322	1.01	0.85	0.27	
9	0.0368	0.0360	1.13	0.95	0.34	
10	0.0513	0.0502	1.58	0.95	0.48	
11	0.0488	0.0477	1.50	0.50	0.24	
12	0.0279	0.0273	0.86	0.30	0.082	
13	0.0409	0.0400	1.26	0.25	0.10	
14	0.0483	0.0472	1.48	0.40	0.19	
15	0.0372	0.0364	1.14	0.95	0.35	
16	0.0423	0.0414	1.30	0.95	0.39	
17	0.0493	0.0482	1.52	1.05	0.51	
18	0.0458	0.0448	1.41	0.35	0.16	
19	0.0374	0.0366	1.14	1.3	0.48	
20	0.0410	0.0401	1.26	0.85	0.34	
21	0.0403	0.0394	1.24	0.95	0.37	
22	0.0455	0.0445	1.40	0.95	0.42	
23	0.0481	0.0470	1.48	0.75	0.35	
24	0.0476	0.0466	1.47	1.4	0.65	
25	0.0484	0.0473	1.49	1.6	0.73	
26	0.1345	0.1316	4.14	1.5	0.70	
27	0.0561	0.0549	1.73	1.6	0.85	
28	0.0660	0.0646	2.03	2.0	1.3	
29	0.0348	0.0340	1.07	2.6	0.88	

Table II. Sample Slice Data for Column 5, Series S/N 052

Sample No.	Mass (g)	Computed Volume (cc)	Computed Length (mm)	RBC/mm ³ x 10 ²	Total RBC x 10 ⁵	pH (23°C)
L-52-R	0.2379	0.233	7.49	0	0	
" L	0.2942	0.288	9.06	6.00	1.72	
1	0.1308	0.128	4.02	9.00	1.15	
2	0.1546	0.151	4.76	12.5	1.89	
3	0.1550	0.152	4.77	24.5	3.72	
4	0.1611	0.158	4.96	32.7	5.15	
5	0.1530	0.150	4.71	51.0	7.63	9.33
6	0.1632	0.160	5.02	105	16.8	9.57
7	0.1748	0.171	5.38	184	31.5	7.90
8	0.1633	0.160	5.03	118	18.8	7.38
9	0.1699	0.166	5.23	108	18.0	6.97
10	0.1615	0.158	4.97	104	16.4	6.56
11	0.1682	0.164	5.18	68.0	11.2	
12	0.1734	0.170	5.37	12.5	2.12	
13	0.1552	0.152	4.78	1.80	0.273	
14	0.1598	0.156	4.92	0.30	0.047	
15	0.1676	0.164	5.16	0	-	
16	0.1825	0.178	5.62	0	-	
17	0.1488	0.146	4.58	0	-	
18	0.1666	0.163	5.13	0	-	
19	0.1675	0.164	5.16	0	-	
20	0.1759	0.172	5.42	0	-	
21	0.1783	0.174	5.49	0	-	
22	0.1642	0.160	5.06	0	-	
23	0.1916	0.187	5.90	0	-	
24	0.1660	0.162	5.11	0	-	

Table III. Summary of Postflight Electrophoretic Mobility Measurements
in A-1 Buffer at 25°C†

Sample	$\bar{U}_e \pm \text{SD}$	n	Median	Mode
Fresh Human RBC*	2.319 ± 0.102	160		
HCHO-Rabbit RBC	1.564 ± 0.096	100	1.57	1.62 ± 0.02
HCHO-Human RBC	1.937 ± 0.103	100	1.94	1.92 ± 0.02
HCHO-Horse RBC	2.377 ± 0.130	100	2.39	2.37 ± 0.02
L-72-1	2.049 ± 0.297	100	2.16	2.32 ± 0.02
L-72-3	2.167 ± 0.285	100	2.29	2.32 ± 0.02
L-72-4	2.073 ± 0.304	100	2.10	2.27 ± 0.02
L-52-5	1.907 ± 0.225	100	1.91	1.82 ± 0.02
L-52-8	1.901 ± 0.279	100	1.95	2.12 ± 0.02
L-52-11	1.950 ± 0.309	100	1.98	2.12 ± 0.02

† Mean electrophoretic mobility, \bar{U}_e , and standard deviation, SD, given for number of cells measured, n.

* Earlier measurement series gave 2.36 ± 0.07 (n = 100)

FIG. 1

Red Cell Histogram: HCHO-Rabbit Red Cells Used in Flight Samples

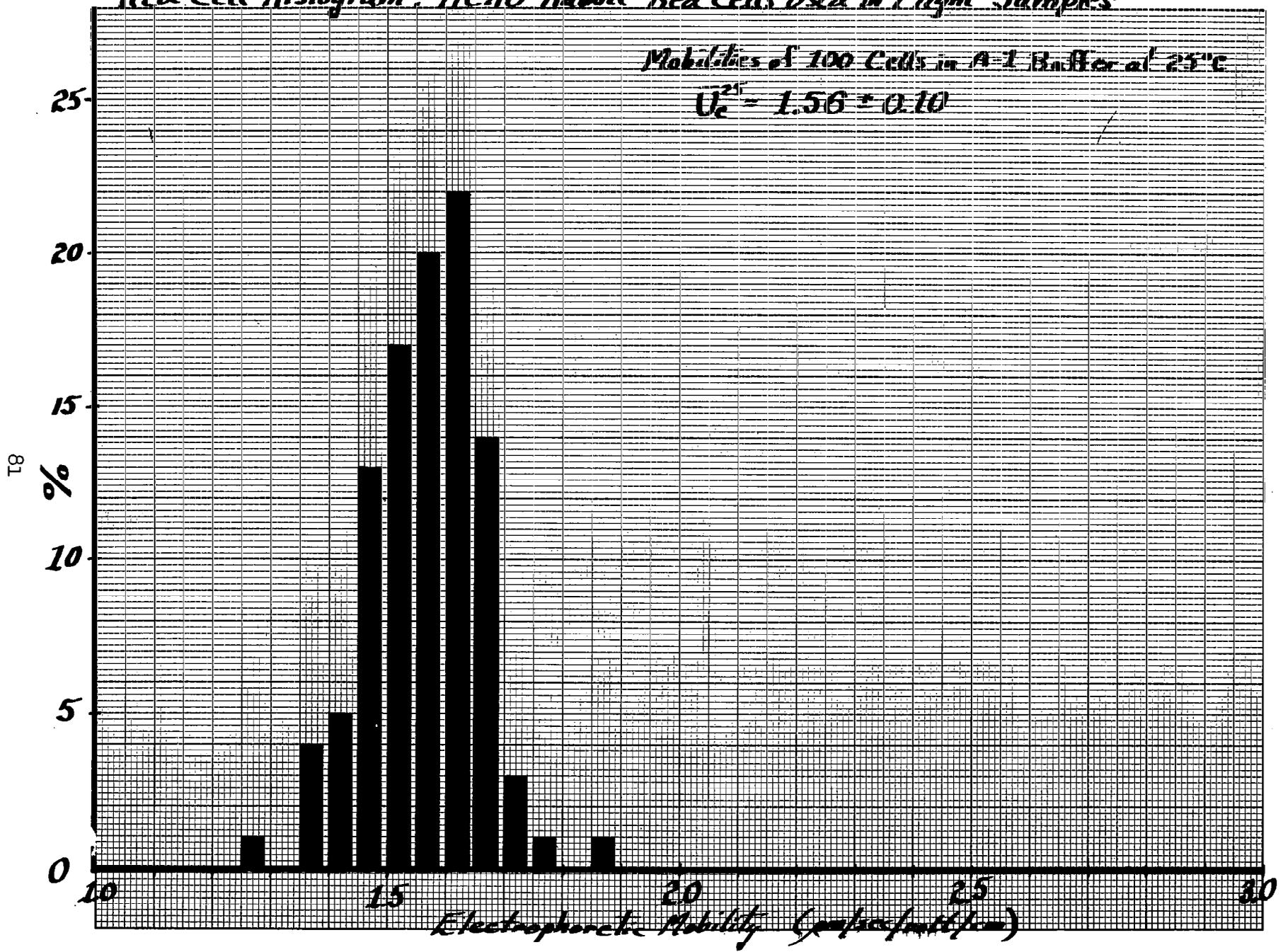


FIG. 2

Red Cell Histogram: HCHO - Human Red Cells Used in Flight Samples

Mobilities of 100 Cells in A-T Buffer at 25°C

$$U_c = 1.94 \pm 0.10 \mu\text{m/sec/volt/cm}$$

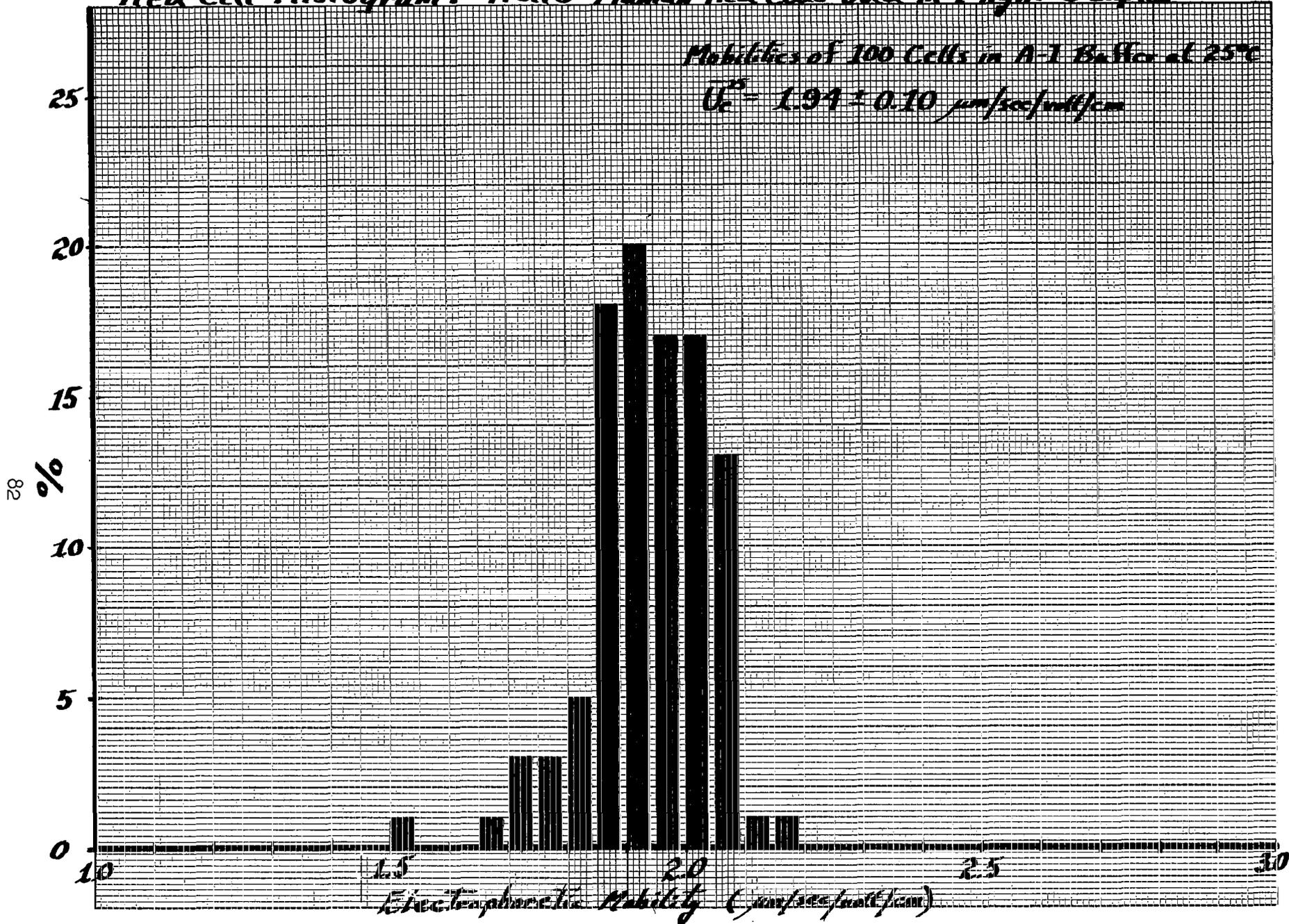


FIG.3

Red Cell Histogram: HCHO-Horse Red Cells Used in Flight Samples

Mobilities of 100 Cells in A-1 Buffer at 25°C

$$\bar{U}_E = 2.38 \pm 0.13 \mu\text{m/sec/volt/cm}$$

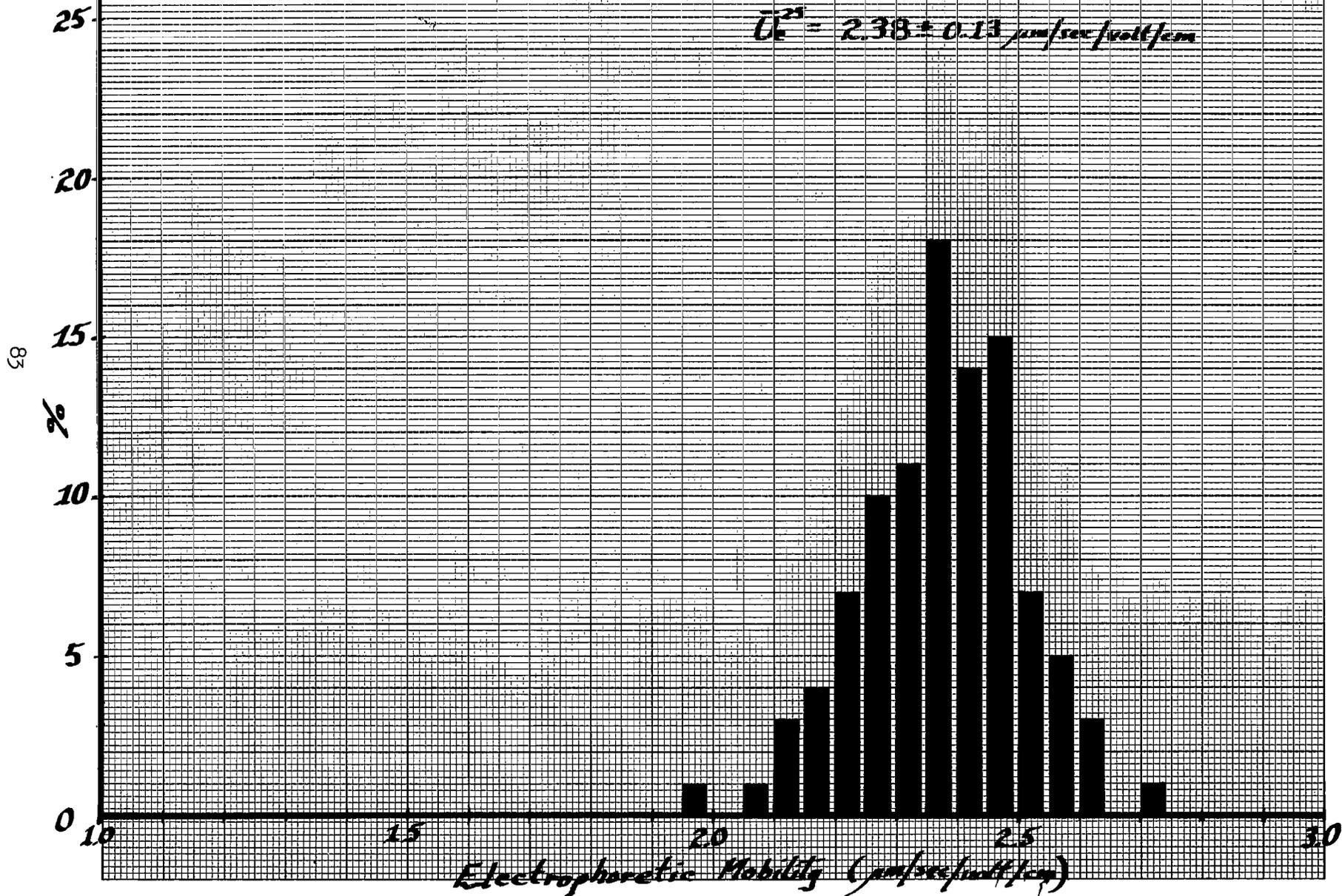


FIG. 4 Cell Count Histogram: Column 1, Sample s/n 072

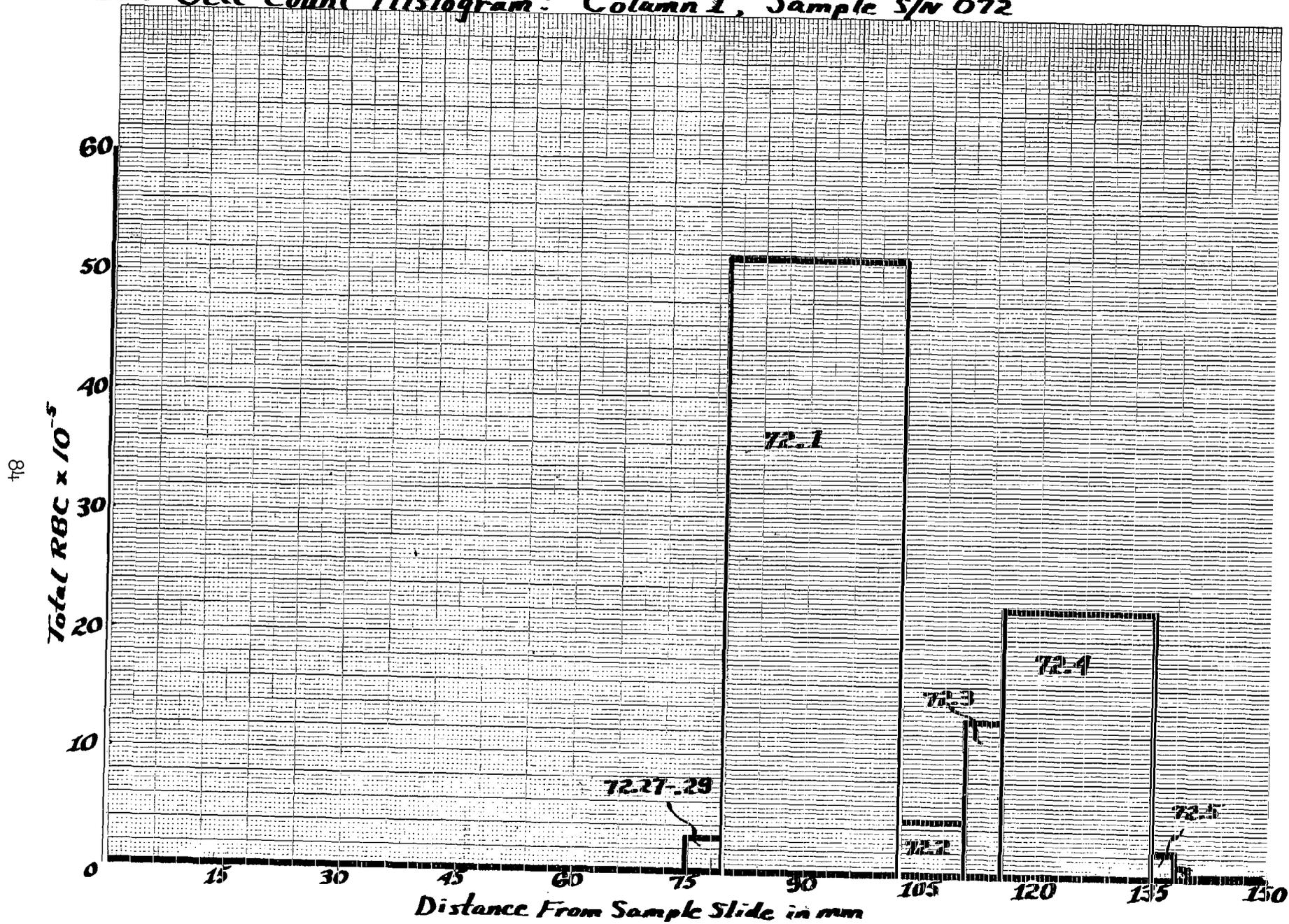
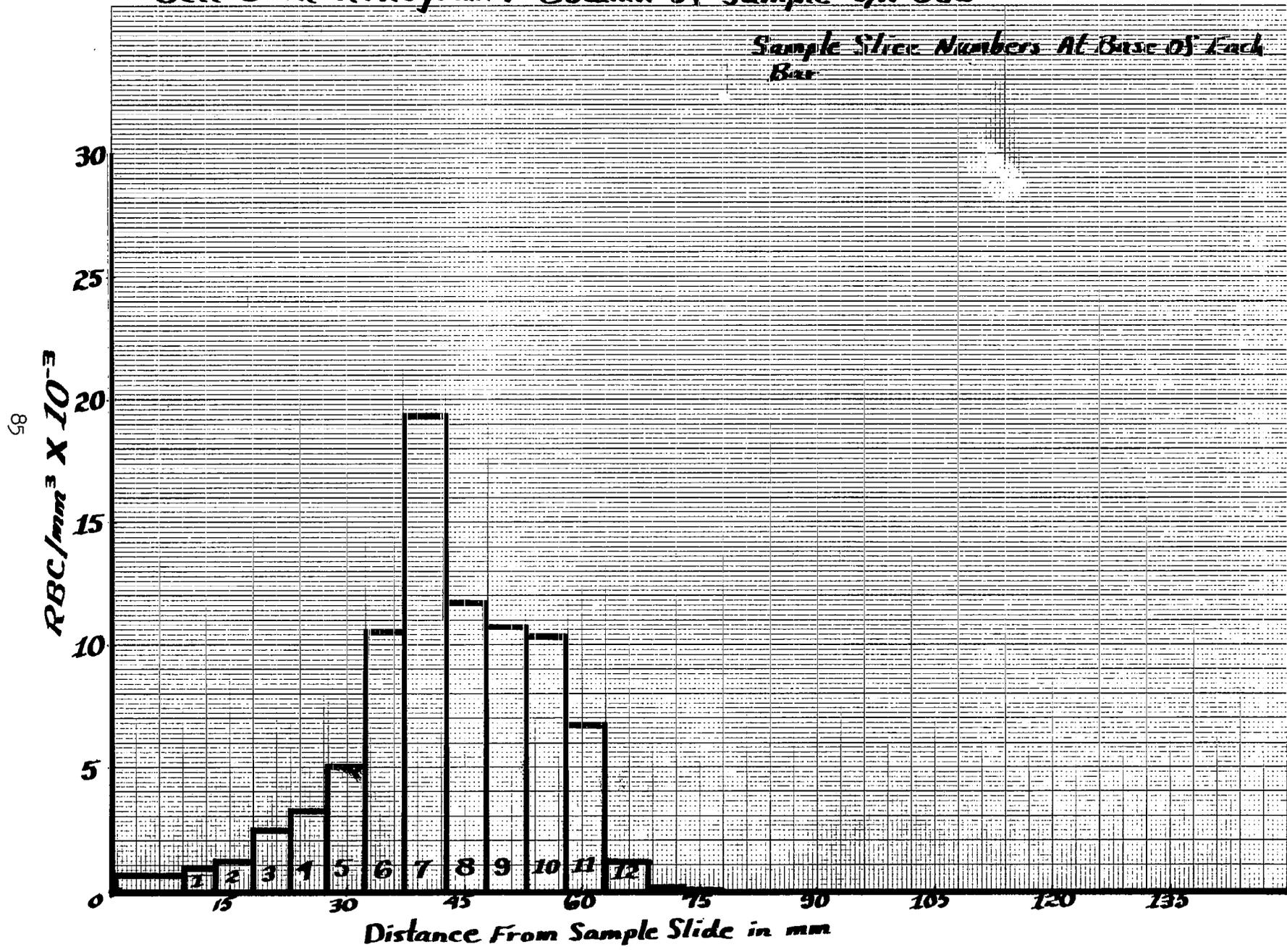


FIG.5 Cell Count Histogram: Column 5, Sample S/N 052



85

FIG 6

Red Cell Histogram: L-72-1, Col. 1, MA-011

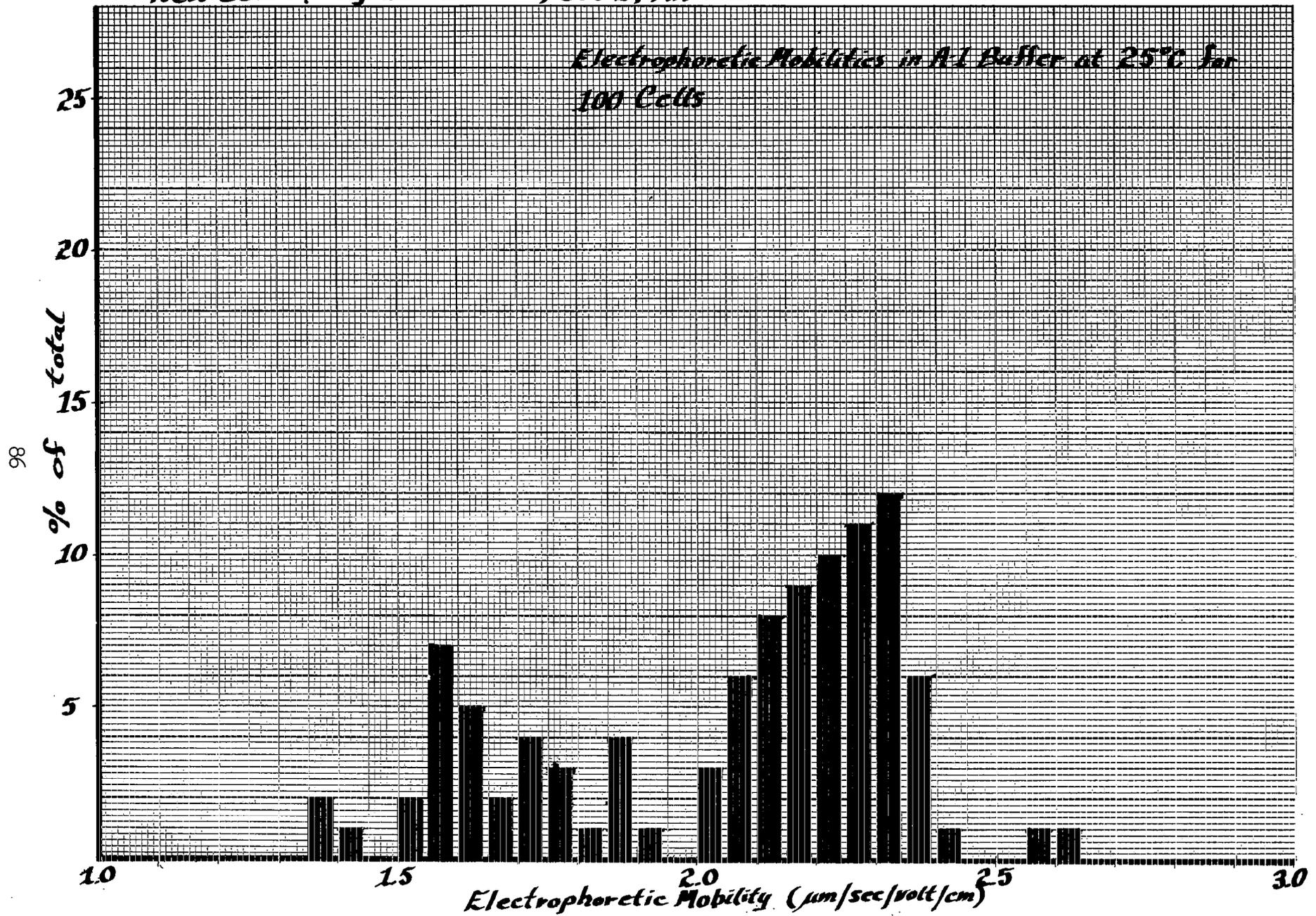


FIG. 7

Rad Cell Histogram: L-72-3, Column #1 MA-011

Electrophoretic Mobilities in A-1 Buffer: 100 cells

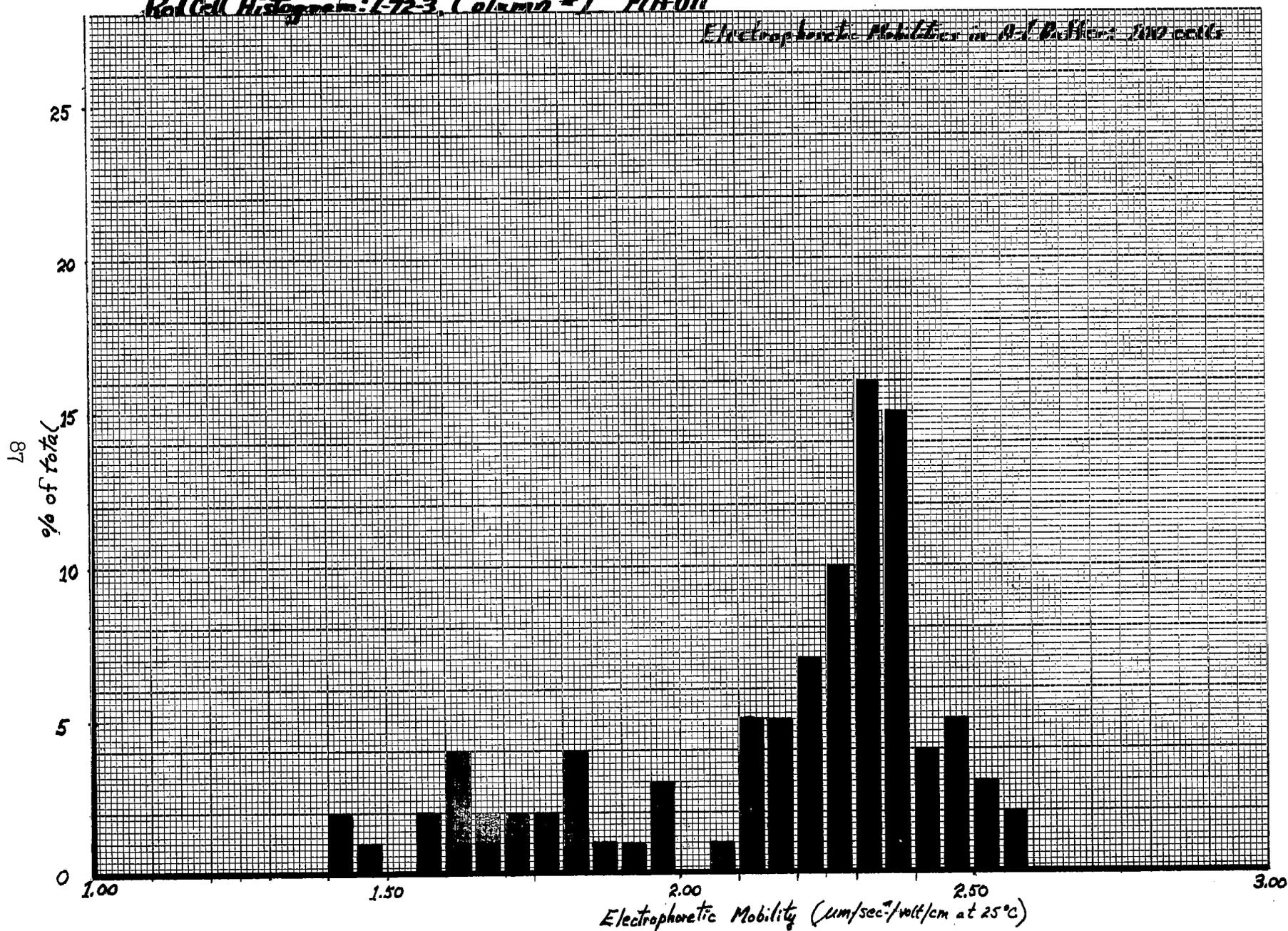


FIG 8

Red Blood Cell Histogram: L-72-4, Col 1, MA-011

Electrophoretic Mobilities in A-1 Buffer at 25°C for
100 cells

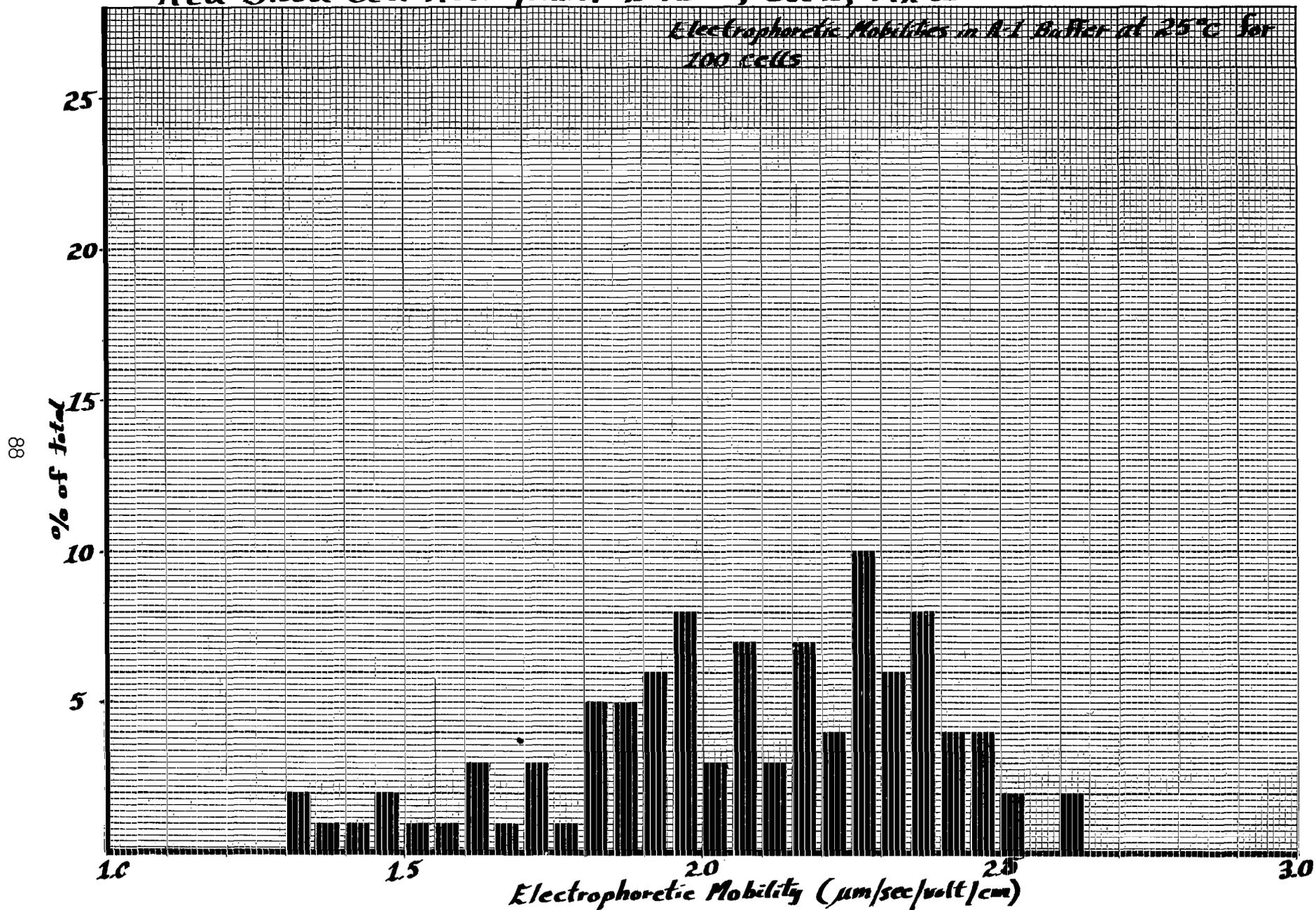


FIG. 9

Red Cell Histogram: L-52-5, Column #5, MA-011

68

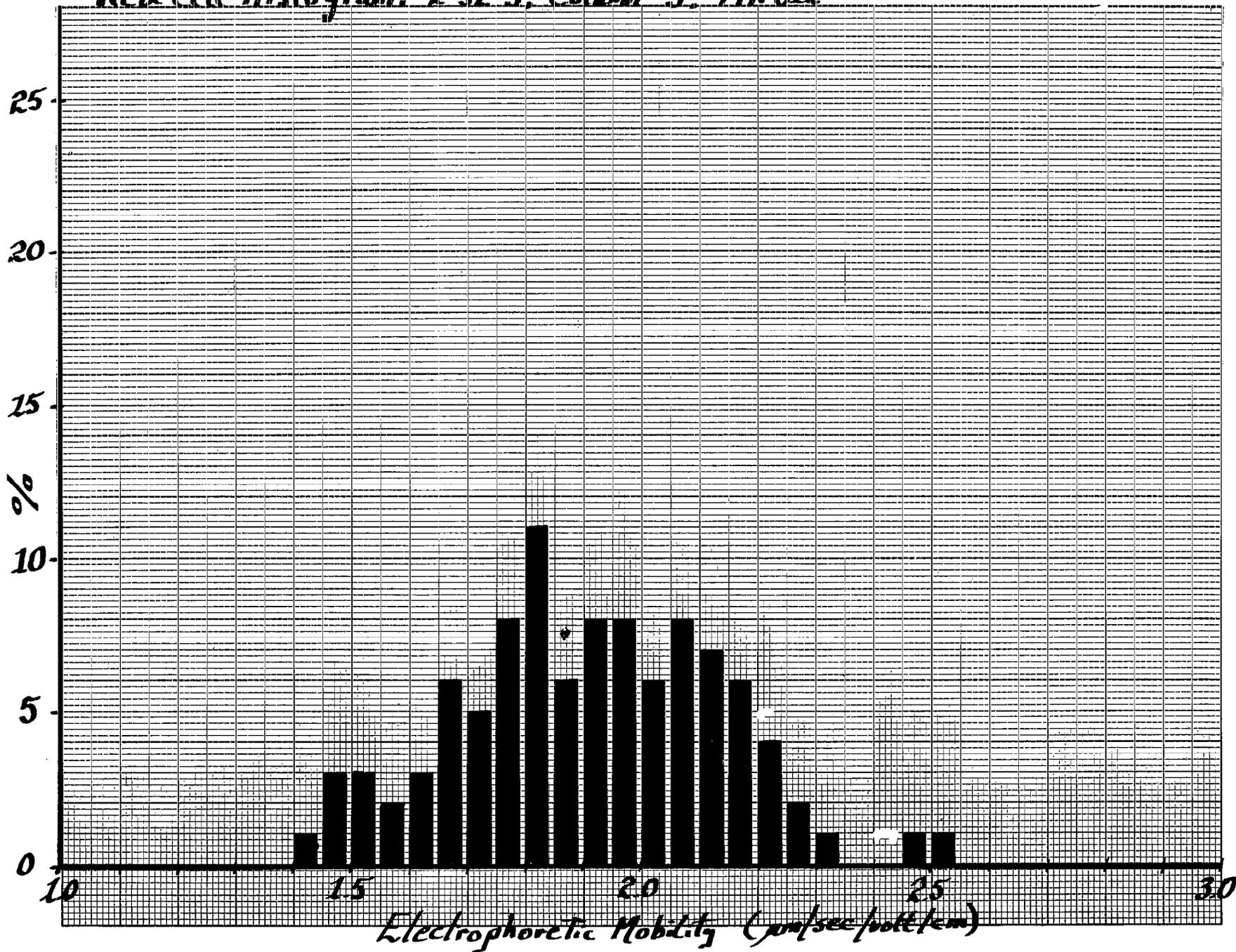


FIG. 10

Red Cell Histogram: 152-8, Column #5, MA-011

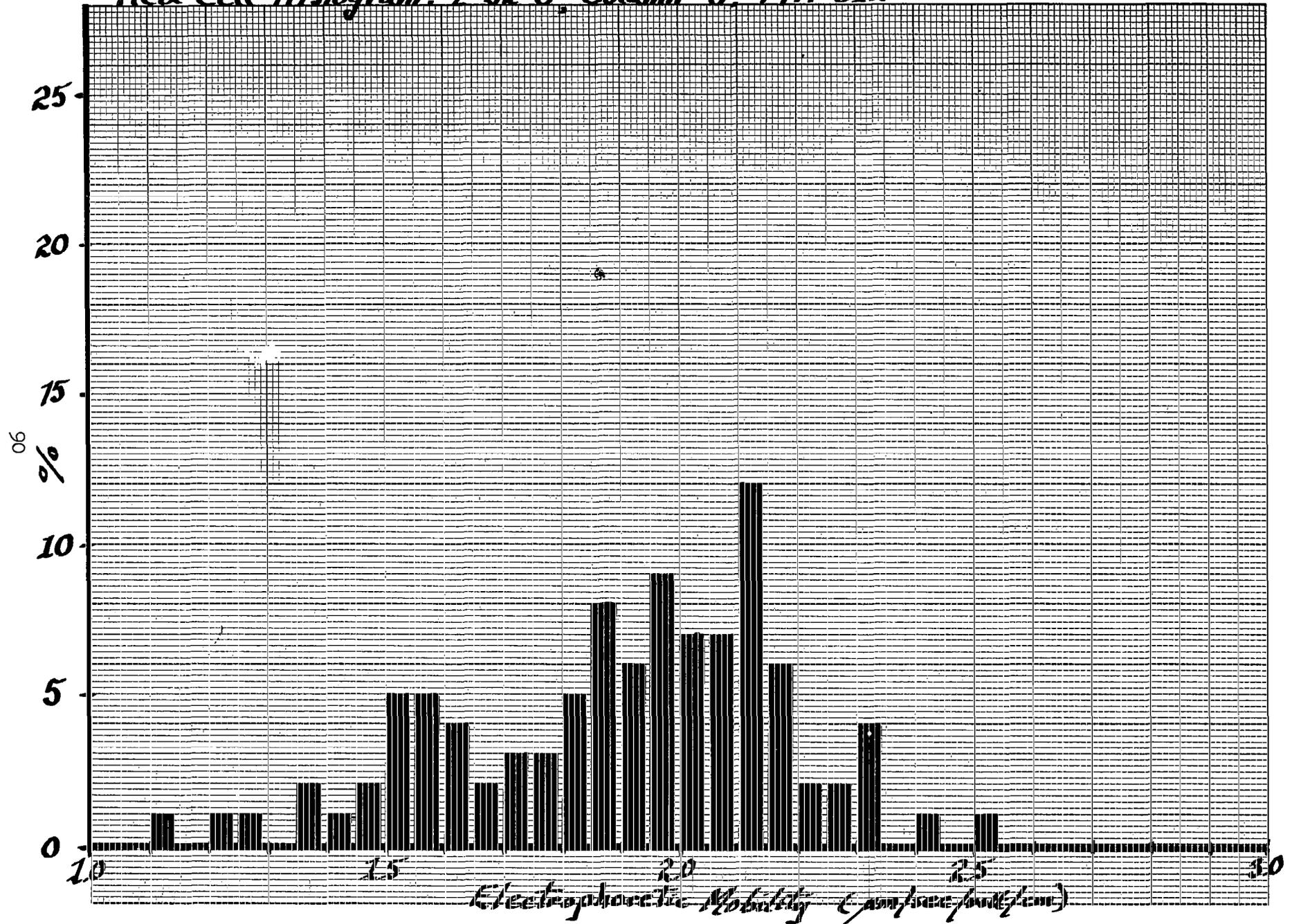
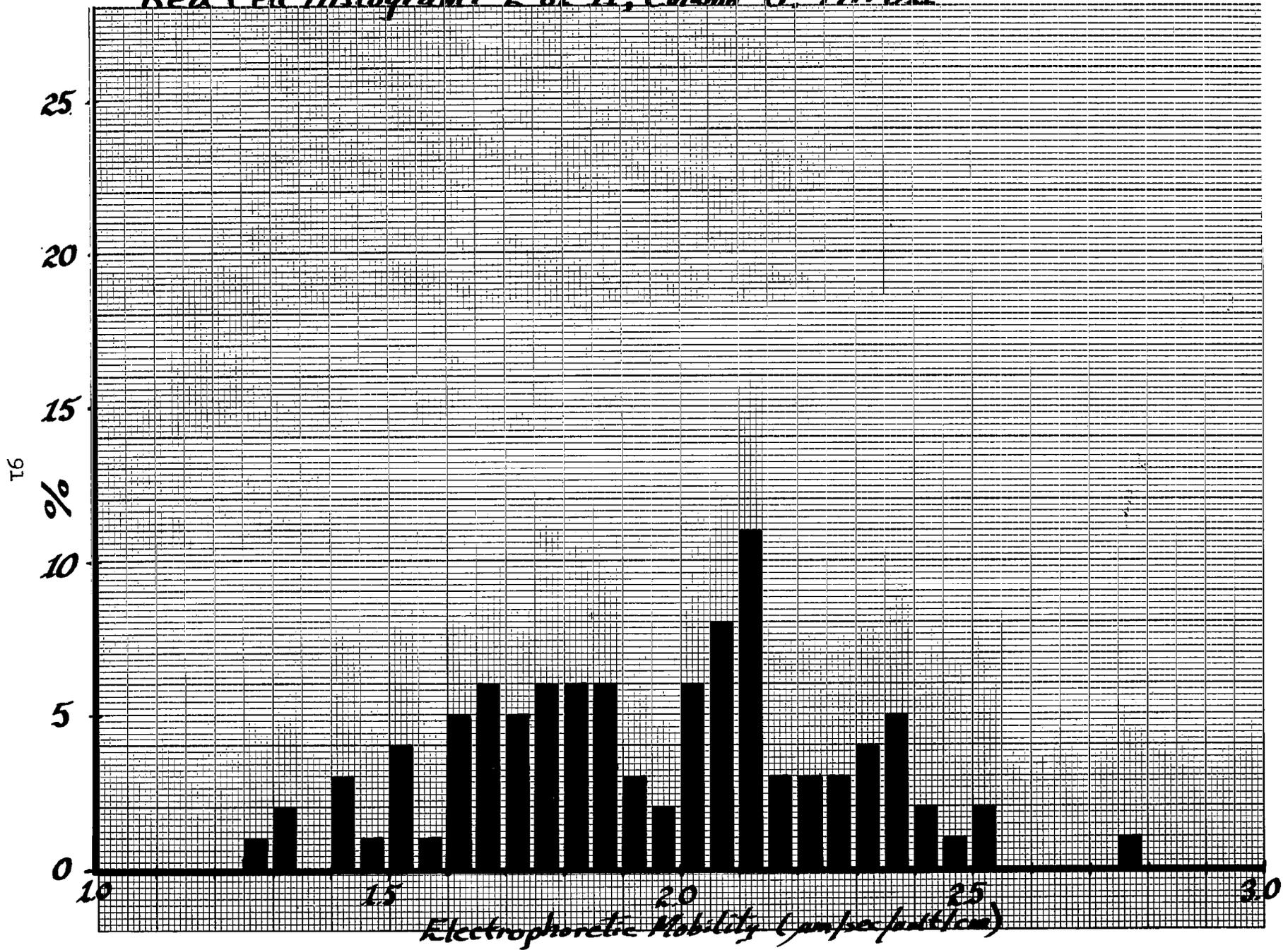


FIG 11

Red Cell Histogram: L 52-11, Column #5, MA-011



APPENDIX VIII

Postflight Analyses of ASTP Electrophoresis
Technology Experiment (MA-011) Red Blood Cell Runs

I. Flight Sample Analysis Data

The aldehyde treated red blood cells which were recovered from the flight samples and examined by analytical particle electrophoresis were typed according to their electrophoretic mobilities. The electrophoretic mobility, U_e^{25} , intervals used for this typing were obtained from the electrophoretic mobility distributions in A-1 buffer at 25°C of samples of aldehyde-treated rabbit, human and horse red blood cells taken from the batches used in the preparation of the flight sample slides. The intervals were as follows:

<u>Cell Type</u>	<u>*U_e^{25} Interval</u>	<u>% in Interval</u>	<u>†% Overlap (Overlapped Type)</u>
Rabbit	≤ 1.69	96	4 (Human)
Human	1.70-2.09	96	2 (Rabbit) 2 (Horse)
Horse	≥ 2.10	99	1 (Human)

* Electrophoretic mobility in $\mu\text{m}/\text{sec}/\text{volt}/\text{cm}$.

† The percent overlap is the percentage of the given type of cells which is located in the adjacent mobility interval.

The typing results are presented along with the morphological typing data reported on September 22 by Dr. Cate at MSFC. The electrophoretic typing data are shown in parenthesis:

<u>Sample Slice No.</u>	<u>% of Total Cells</u>		
	<u>Rabbit</u>	<u>Human</u>	<u>Horse</u>
L-72-1	17 (19)	26 (22)	57 (59)
L-72-3	19 (11)	48 (17)	33 (72)
L-72-4	15 (12)	31 (38)	54 (50)
L-52-5	36 (18)	57 (60)	7 (22)
L-52-8	17 (24)	41 (48)	41 (28)
L-52-11	10 (23)	34 (42)	56 (35)

In general, the agreement is reasonable considering the possible errors in each technique. The electrophoretic typing method is subject to a greater compounding of experimental errors than the morphological technique and should be regarded as a rough estimate.

I. Flight Photograph Analyses

A. Updated Prediction of Sample Band Behavior.

In Figure 1 is the calculated electrophoretic mobility histogram for the flight sample slides containing aldehyde-treated rabbit, human and horse red blood cells. The histogram was constructed based on the slide composition data, i.e., the percentage of each cell type reported by MSFC, and the measured electrophoretic mobility distributions in A-1 buffer at 25°C for each type. The left ordinate of Fig. 1 is the % of the total number of cells for the bar graph and the right ordinate is the accumulated percent of the total for the integral curve. The mobility distribution is clearly trimodal.

This mobility distribution was used along with the conditions of the flight experiment to predict the migration behavior of the red cell bands in the flight experiment. For flight column 1 (S/N 072) the temperature throughout the course of the 60 minute experiment ranged from 32°C to 36°C, averaging 35.1°C. The current through the column was set at 4.0 mA with a maximum power supply voltage of ~ 400 volts. Since the dimensions of the column are known along with the conductive properties of the A-1 buffer, the voltage gradient and migration distances may be calculated for the individual red cell bands using equation 1 (May Progress Report):

$$d_r^T = \left[\frac{\eta^{25} I \times 6 \times 10^{-2}}{\pi R^2 \kappa_T \eta^T} \right] \left[U_e^{25} + U_{OSM}^{25} \left(\frac{2r^2}{R^2} - 1 \right) \right] t - \frac{\chi_0}{2} \quad (1)$$

in which d_r^T (mm) is the distance traversed into the electrophoresis column by particles originally located at the longitudinal midpoint of the sample plug and at a distance r (cm) from the tube axis for a column temperature T ; η^{25} and η^T are the viscosities in poise of A-1 buffer at 25° and T° C, respectively; I is the constant current setting in amperes, R is the radius of the electrophoresis tube in cm; κ^T is the conductivity in mho/cm of A-1 buffer at temperature, T ; U_e^{25} is the mean electrophoretic mobility (anodic) in $\mu\text{m}/\text{sec}/\text{volt}/\text{cm}$ of the particles at 25°C in A-1 buffer; U_{OSM}^{25} is the electroosmotic mobility (anodic) of A-1 buffer at 25°C; t is the run time in minutes; χ_0 is the sample plug thickness in mm; and 6×10^{-2} is a combined factor for converting μm to mm and minutes to seconds.

Equation 1 was simplified by assuming $U_{OSM}^{25} \approx 0$ and by eliminating the $\chi_0/2 = 1.6$ mm term since the starting point of the cells was not certain. The final expression for 35°C was:

$$d^{35} = \left[\frac{\eta^{25} I \times 6 \times 10^{-2}}{\pi R^2 \kappa^{35} \eta^{35}} \right] t U_e^{25} = 47.75 U_e^{25} \quad (2)$$

The calculated average voltage gradient at 35°C was 10.6 volts/cm and 18.7 volts/cm in the sample slide.

In Figure 2 is the plot of U_e^{25} versus band displacement, d^T , as calculated from equation 2. With this plot, the mobility distribution data for the sample slide cells (Fig. 1) was translated into a bar graph showing the predicted % of the total cell load (right hand ordinate) at various positions in the flight column following a 60 minute run at an average temperature of 35°C.

This graph may also be used to predict what the expected mobility of cells taken from various locations in the flight column.

B. Flight Photograph Data.

Twenty flight photographs which were taken at three minute intervals through the sixty minute run of column 1 were examined. The band boundary locations were established visually and were plotted as a function of total elapsed run time (Figure 3). The open circles refer to the fast band boundaries, the closed circles to the slower band complex. The dashed line refers to what appeared to be a boundary in the slow band complex. The mobility values given were calculated from the slopes of the plots and are expressed in terms of the electrophoretic mobility at 25°C. The plots do not extrapolate to zero which may reflect in part the effects of the elevated voltage gradient in the sample slide aperture.

The computed mobilities for the fast band and the front boundary of the slower band agree well with the ground-based mobility values for horse and human RBC, respectively. The computed mobility for the slower band tail was larger than the ground-based value for rabbits.

Calculated Histogram For RBC Flight Slides

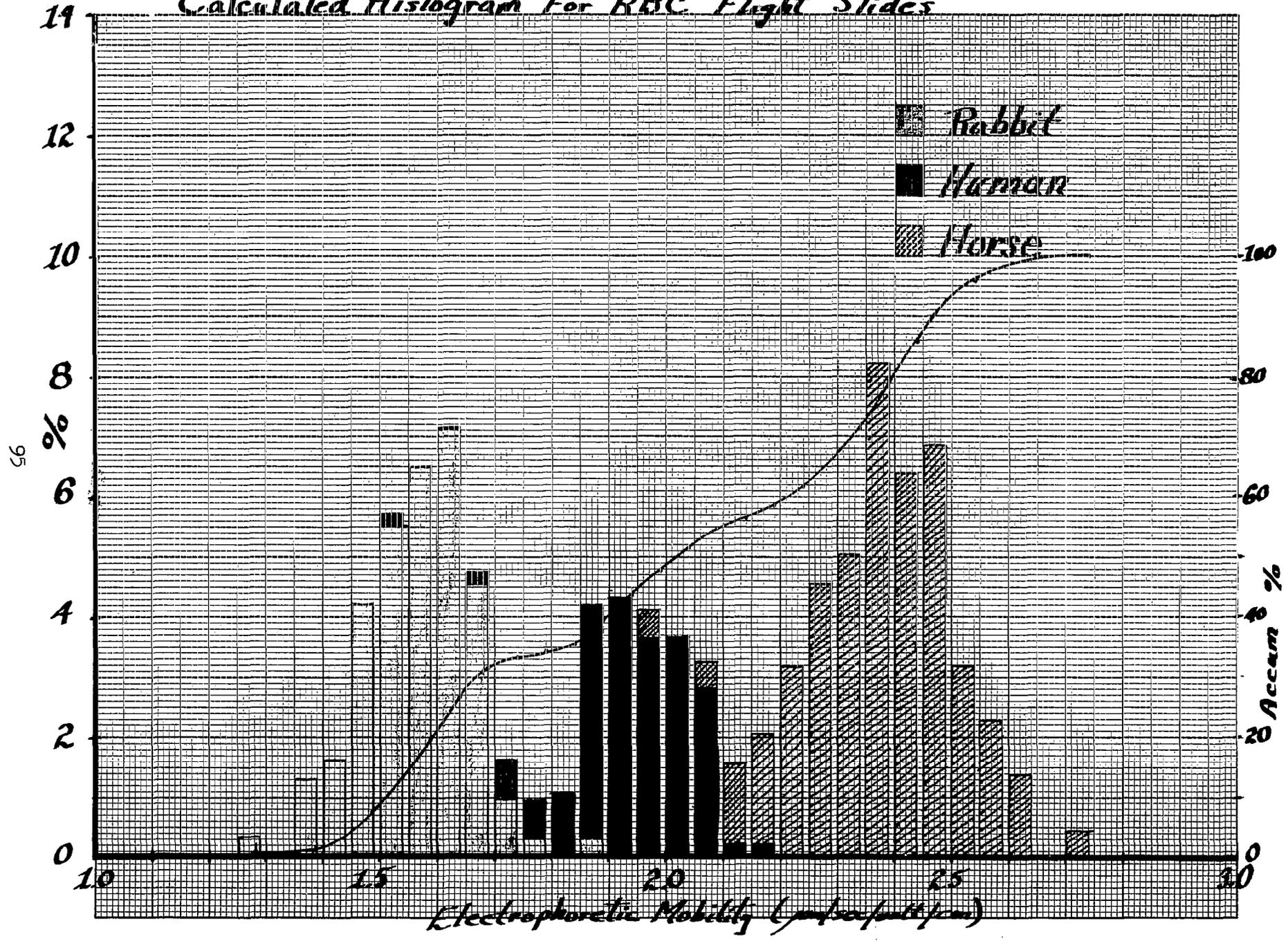


FIG 2

Predicted Band Intensities At Various Migration Distances

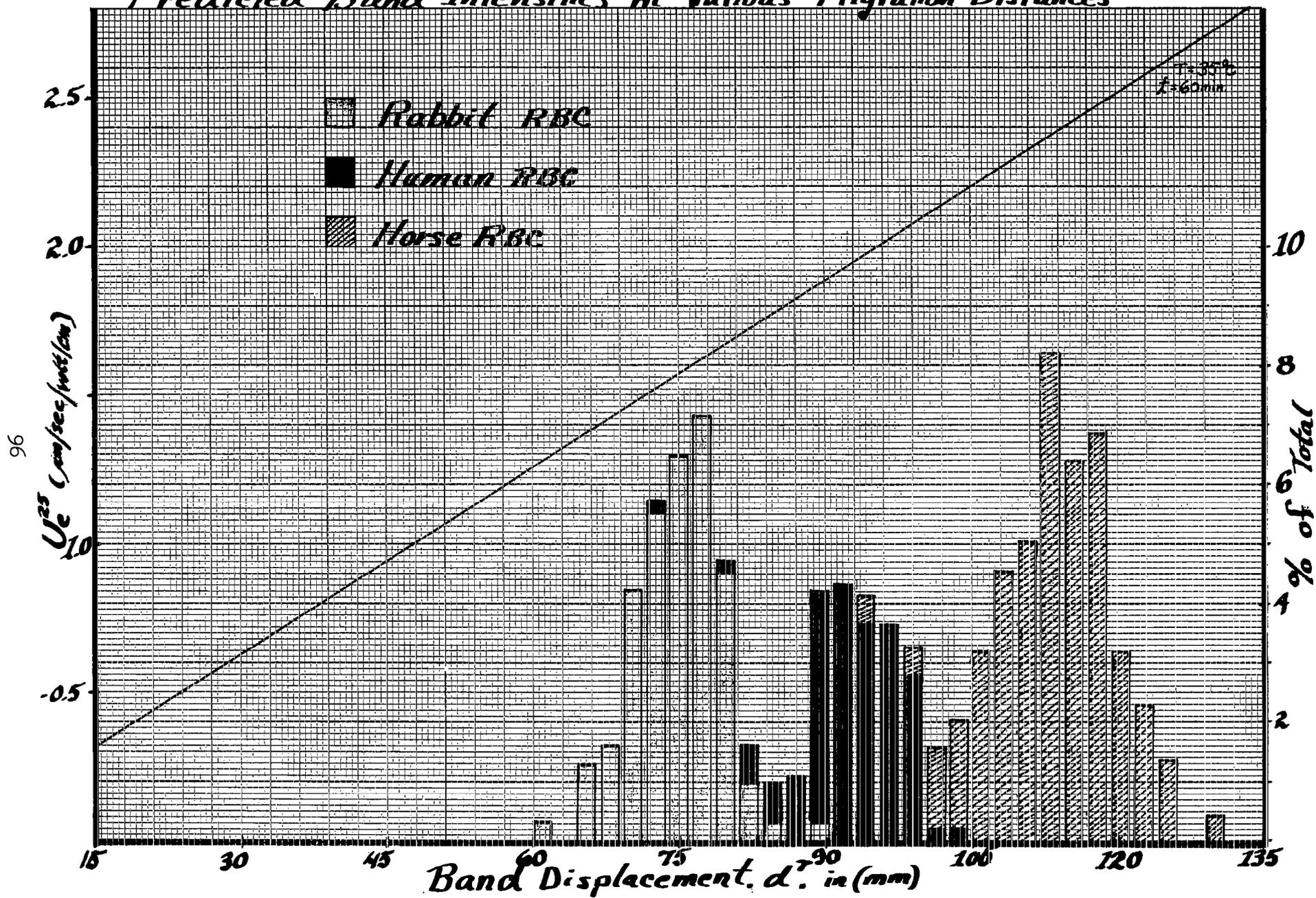
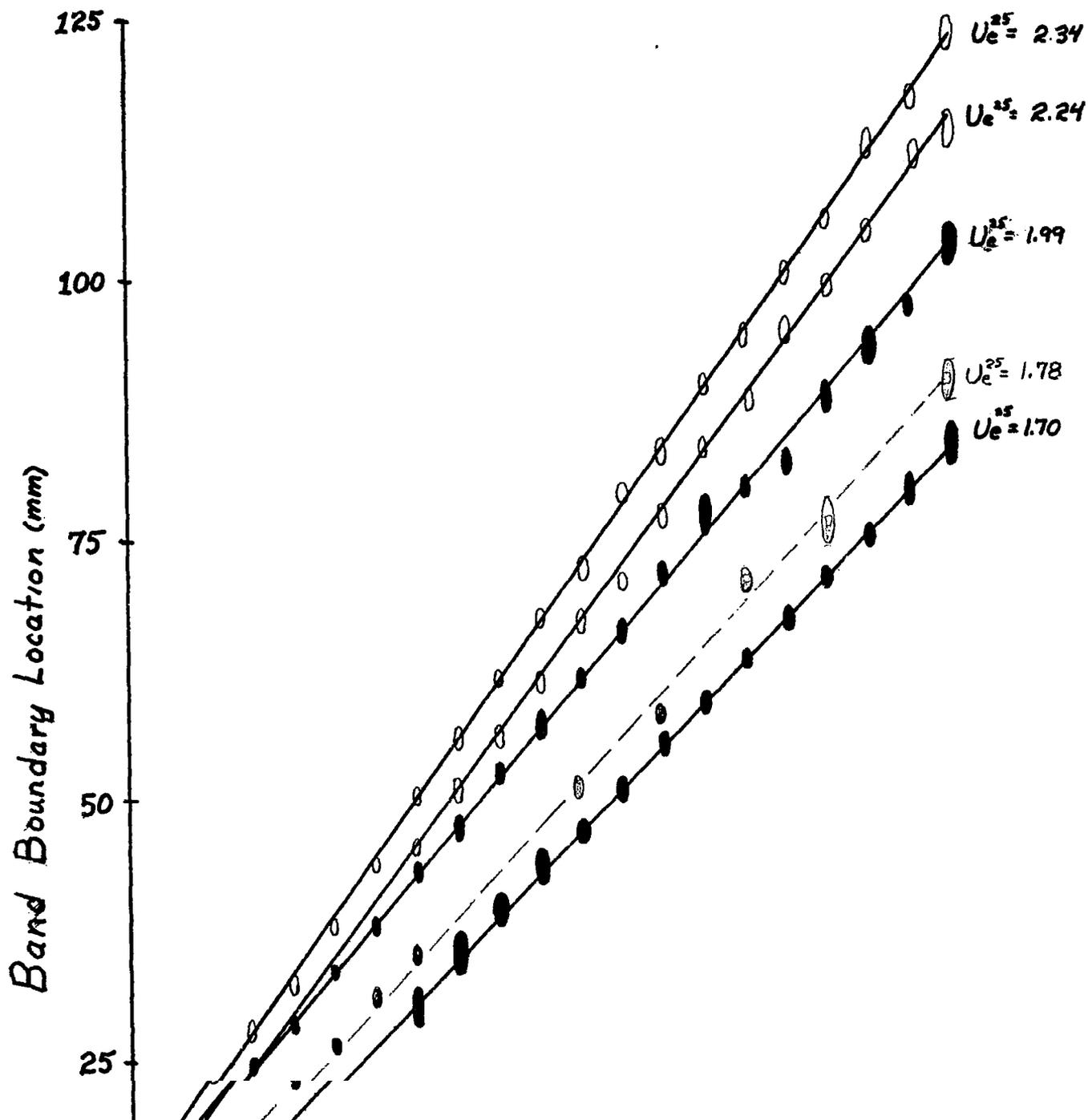


FIG. 3 BAND MIGRATION VERSUS RUN TIME FOR COLUMN 1 (S/N 072)



APPENDIX IX

ELECTROPHORESIS TECHNOLOGY EXPERIMENT

MA-011: Zone Electrophoresis of
Aldehyde-Treated Red Blood Cells

Presented at:

George C. Marshall Space Flight Center
October 28, 1975

By:

Robert J. Knox
Principal Investigator
Contract NAS-30887
University of Oregon Health Sciences Center
Portland, Oregon 97201

INTRODUCTION

Aldehyde-treated red blood cells were used as test particles in the MA-011 zone electrophoresis experiment in order to demonstrate the separation capability of the electrophoresis unit as well as to examine the effectiveness of the postflight sample slicing operation. Red cells were chosen for the following reasons:

- a. They are used as standard particles in analytical particle electrophoresis of biological cells;
- b. Populations of red cells obtained from a given animal display a narrow range of electrophoretic mobilities;
- c. The surfaces of red cells or aldehyde-treated red cells have similar physico-chemical properties to those of human lymphocytes and embryonic kidney cells;
- d. Red cells are visible to the naked eye at the cell concentrations used in the experiment so that their progress could be photographed during the flight experiment.

The red cells were treated with 2% formaldehyde in order to make them insensitive to the shocks of freezing and thawing or long term exposure to the low ionic strength of the flight column fluid (A-1 buffer).

It was expected that if the electrophoresis unit functioned properly, the test mixture of aldehyde-treated red cells from three different species would be resolved into three planar bands.

PREFLIGHT ACTIVITIES

During the preparations for the flight experiment we identified the parameters which had to be measured in order to predict the outcome of the flight experiment. The density, viscosity and conductivity of the flight buffer (A-1) were measured over the temperature range from 10 to 35°C. The electrophoretic mobilities of aldehyde-treated red cells from a variety of species were measured by analytical particle electrophoresis in order to screen three types of red cells for the flight test sample. Rabbit, human and horse red cells were selected for the flight experiment. The mean mobilities of the nearest neighbor populations were separated by about 0.4 $\mu\text{m/s/volt/cm}$ so that a successful separation would not be trivial.

The A-1 buffer properties together with the analytical electrophoretic mobility data were used to predict the band migration and separation for the conditions anticipated for the flight experiment.

Analytical electrophoretic mobility data was also accumulated for human embryonic kidney cells in A-1 buffer so that the run time of the flight experiment could be adjusted to give optimal migration of the kidney cells.

POSTFLIGHT ACTIVITIES

Following the ASTP mission, the prediction of the red cell migration behavior was updated to cover the actual flight conditions. The predicted red cell band intensities as a function of migration distance from the sample slide are shown in Figure 1. This figure was constructed from observed electrophoretic mobility distributions for each of the red cell populations and the number of each cell type loaded in the sample slide at MSFC. The straight curve establishes the predicted band displacement following a 60 minute run at 35°C for cells with mobilities, U_e^{25} , measured at 25°C in A-1 buffer during ground based experiments. The right hand ordinate gives the percentage of the total number of cells in the sample slide found in each bar of the histogram. From the figure it can be seen that the location of visibly dense bands should be: rabbit, ~ 70-81 mm; human ~ 88-100 mm; and horse ~ 105-123 mm.

Photographs of the band migration during the flight experiment were compared with the predictions. Subsequently, the slices of the flight columns were analyzed for a. the number of cells in each sample slice, and b. the electrophoretic mobility of the cells recovered from selected sample slices.

Flight Photograph Analysis

Three major questions were posed for the flight photograph analysis:

1. Was electroosmosis eliminated?
2. Did the bands migrate at rates predicted from the ground based experiments?
3. Did the total migration of the bands agree with the predictions?

A major problem in the preparations for the flight experiment was the elimination of electroosmosis. If present, the fluid flow due to electroosmosis would cause the cells in the center of the flight column to travel faster than those near the column wall. This effect produces "bullet" shaped bands with a cross sectional appearance similar to a crescent and reduces the resolution capability of the apparatus. A methylcellulose coating technique developed at Lehigh University and at MSFC was used to coat the inside walls of the electrophoresis column in order to eliminate electroosmotic flow. Following coating, electroosmotic flow was reduced from about 4.5 $\mu\text{m}/\text{sec}/\text{volt}/\text{cm}$ to less than 0.3 $\mu\text{m}/\text{sec}/\text{volt}/\text{cm}$. With an electroosmotic flow of 0.3 $\mu\text{m}/\text{sec}/\text{volt}/\text{cm}$, the bands should be spread after 60 minutes to about 2-1/2 times the length computed for no electroosmotic flow. Not all of this spread would be observable to the naked eye since at the boundaries the concentrations of cells would be lower than the threshold for direct visualization.

In our examination of the flight photos, we looked for two indications of electroosmosis: a. a curvature of band front boundaries which became more pronounced as the run time increased; and b. a longitudinal spread of the bands in excess of that predicted in the absence of electroosmotic flow.

The band fronts were planar in appearance with no signs of pronounced curvature for column 1. Problems which interfered with the detection of small

curvatures: a. the optical aberration in viewing bands through a cylindrical tube wall; b. the haziness of the flight photos; and c. the definition of the band boundaries with the naked eye.

The spreading behavior of the bands was analyzed by plotting the displacement of the band boundaries from the sample slide as a function of run time. The data were collected by visual examination of twenty flight photographs taken at three minute intervals during the 60 minute run of column 1. The band widths at 60 minutes were taken from the straight curve fits to the boundary migration data. The results are shown in Table I. The bands were slightly more compressed than expected rather than longer. Thus, no band spreading due to electroosmosis was detectable through visual examination.

The observed band locations for column 1 at 60 minutes are compared with the predicted locations in Figure 2. The observed band locations were taken from the plot of band migration versus run time since the visualization of the bands was difficult at the end of the run. The fast band corresponded well with the predicted location for horse red cells and the front of the slower band complex corresponded with the predicted human red cell location. The rear boundary of the slower band complex was 1 to 1.5 cm further into the tube than predicted for rabbit cells. Overall, the bands were slightly more compressed than expected and the separation between horse and human cells was greater than predicted.

TABLE I

Band	Band Width (mm)		Electrophoretic Mobility (25°C) ($\mu\text{m}/\text{sec}/\text{v}/\text{cm}$)	
	Observed	Predicted	Observed	Predicted
Fast (Horse)	8-10	15-18	2.21-2.36	2.38
Slower Band Complex (Human + Rabbit)	20	30	1.70-1.96	1.56-1.94

The electrophoretic mobility of the particles in the band boundaries was computed from the plot of band boundary migration versus run time. The data in Table I show that the calculated mobility at 25°C for the fast band was 2.36 for the band front and 2.21 $\mu\text{m}/\text{sec}/\text{v}/\text{cm}$ for the rear boundary which are slightly lower than the predicted average of 2.38 $\mu\text{m}/\text{sec}/\text{v}/\text{cm}$ for horse cells as observed in ground based analytical particle electrophoresis. The front of the slower band complex corresponded to the mean value for human cells of 1.94 $\mu\text{m}/\text{sec}/\text{v}/\text{cm}$, but the rear boundary had a mobility of 1.70 which is higher than the 1.56 average value observed for rabbit red cells in the ground based experiments.

The analysis of the flight photographs showed good general agreement between the observed migration behavior of the red cell bands and the predicted behavior. Small discrepancies were found which have not yet been explained. Some of these may relate to factors not included in the predictive model of the experiment such as: radial and longitudinal thermal gradients in the flight column, the compression of the voltage field in the sample disc aperture and red cell concentration effects.

Postflight Sample Analysis

During the sample slicing operation at MSFC for column 1, the column walls fractured and the sample plug was crushed. The samples were collected in the hope that minimal mixing and contamination had occurred. In the case of column 5, the flight experiment had not functioned properly. The sample slicing was carried out as planned for column 5.

We proceeded with the postflight analysis of samples from both columns as originally planned. Initially each sample slice was weighed and then a cell count was performed on each. The calculated recovery of fluid volume was 93% for column 5 and 75% for column 1. The histograms of cell count versus the computed distance from the column sample slide are shown for columns 1 and 5 in Figures 3 and 4.

In Figure 3, only 6 fractions were obtained from the region of the column containing the red cell bands. These were placed in the sample histogram starting from the distal end of the column from the sample slide since twenty five percent of the column fluid had not been recovered and the earlier sample slices were shown to contain consistently less volume than calculated. The histogram agreed well with the locations of cells in the flight photographs but this agreement may be fortuitous since cell typing and electrophoretic data indicated considerable mixing of cell types. The recovery of cells was 70% of the theoretical value.

In Figure 4, no discrete bands were shown in the histogram for column 5 which agrees with the flight photographs. The recovery of cells was 90% of the theoretical yield. The front of the continuous band was located at ~ 80 mm in the flight photos in contrast to the location of the cells in the sample slices where the front was at ~ 60 mm. In sample slices 7-10 the pH decreased from 7.9 to 6.5 and reached values of 3 to 4 in slices at larger migration distances.

The cells in three sample slices from each column were examined by analytical particle electrophoresis in order to find whether they displayed the electrophoretic mobility distributions expected from their locations in the flight column. Data was collected for 100 cells from each sample.

The results for column 1 sample slices 1, 3 and 4 were disappointing since all had similar electrophoretic mobilities and did not show the expected trends. Their mean mobilities were all in the range from 2.05 to 2.17. This suggested that the cells had been mixed. The cells in sample slices 5, 8, and 11 from column 5 showed a trend from lower to higher electrophoretic mobilities, respectively, but the separation was not large.

We made estimations of the numbers of each cell type from the electrophoretic mobility data. These were in general agreement with the morphological typing estimations made at MSFC. Both techniques are nonspecies specific. For column 1 the typing data showed similar distributions of the three cell types in each of the sample slices in contrast to the expected behavior. This supported the notion that the samples had been mixed. In column 5, there were enrichments of rabbit cells in the slow region of the band, and horse cells in the fast region.

The results of the postflight sample slice analyses indicate that the samples from column 1 will be of little use in interpreting the behavior of the flight experiment. The column 5 data show partial separation of cells according to their electrophoretic mobilities.

Electroosmosis, which is 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 useful for ground-based and future space electrophoretic applications.

The red blood cell test samples behaved essentially as predicted from ground-based studies. The bands were planar and showed no signs of unexpected longitudinal spreading.

The electrophoresis unit is capable of separating small volumes of sample cells on the basis of their electrophoretic mobilities.

FIG 1

Predicted Band Intensities At Various Migration Distances

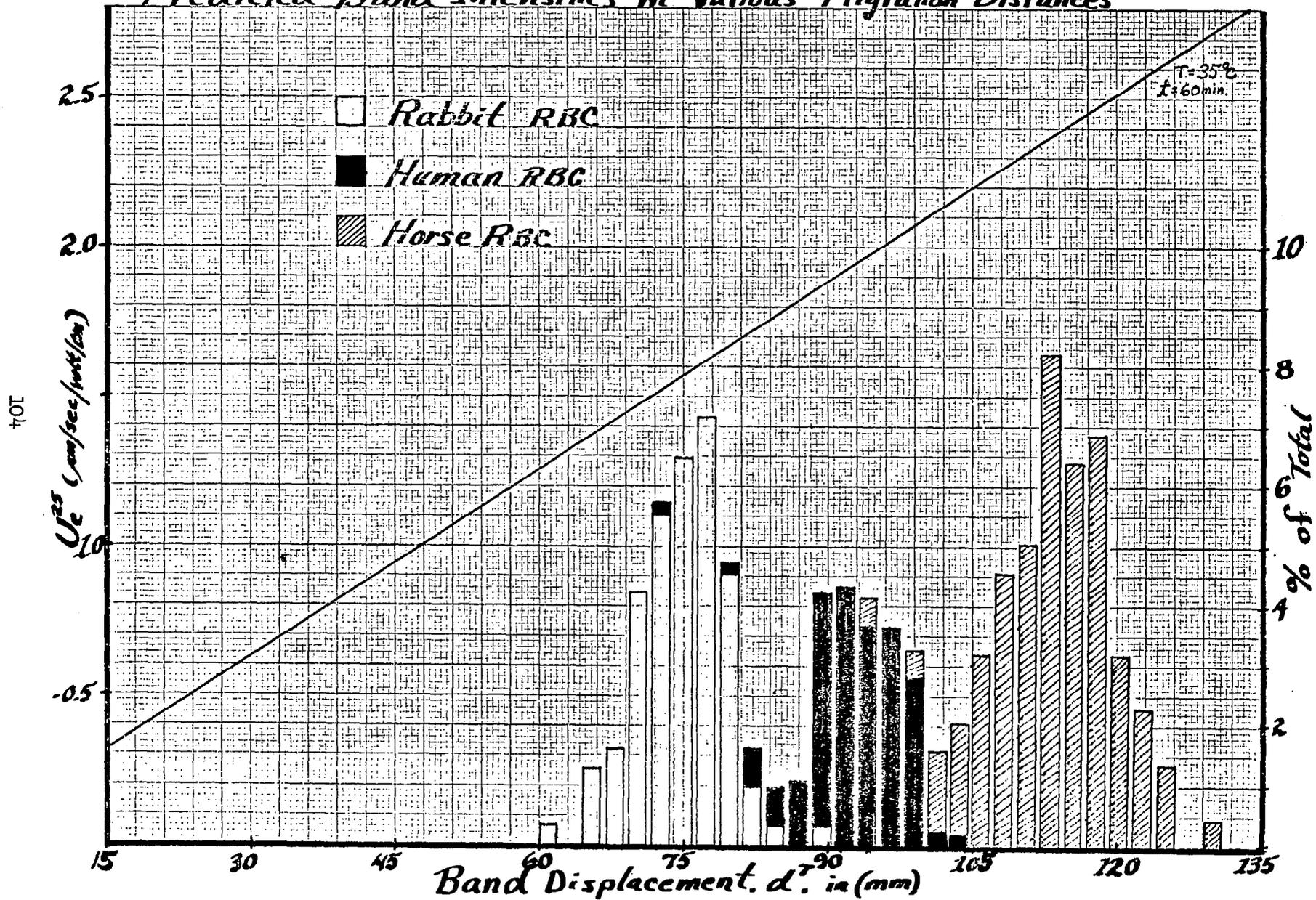


FIG. 2

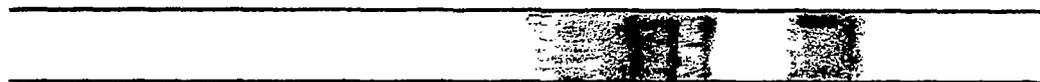
RED BLOOD CELL MIGRATION

PREDICTED



RABBIT HUMAN HORSE

OBSERVED



25

50

75

100

125

150

MIGRATION DISTANCE (mm)

FIG.3 Cell Count Histogram: Column 1, Sample s/n 072

106

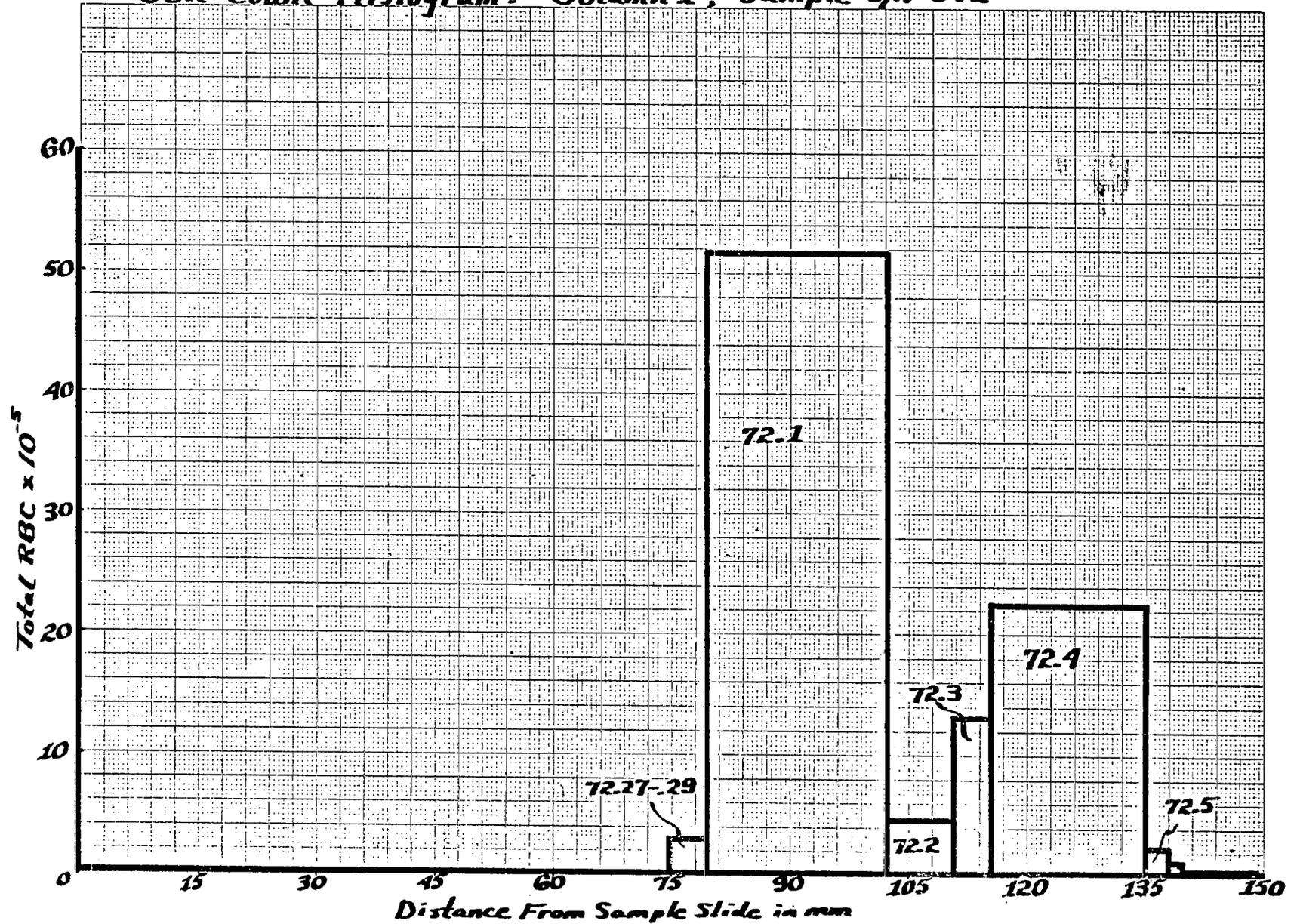


FIG.4 Cell Count Histogram: Column 5, Sample S/N 052

