Electrophoretic Separation of Human Kidney Cells at Zero Gravity

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

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

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

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

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

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

Methods

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

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

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

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

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

Results

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

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

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

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

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

Discussion

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

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

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

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

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

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

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TABLE 1

Tube Number	Wt in grams Tube, ring, sample	Wt in grams tube	Wt in grams teflon ring	Wt in grams frozen sample	Buffer pH
R	1.4292	1.1061	None	[illegible]	7.85
L	1.3623	1.08425	None	0.27835	7.85
1	1.3483	1.11375	0.0885	0.15430	7.8
2	1.3381	1.0926	0.0944	0.15[illegible]	7.9
3	1.3150	1.0861	0.08695	0.14[illegible]	7.8
4	1.3612	1.1047	0.08955	0.16695	7.8
5	1.3521	1.09775	0.09115	0.16329	7.85
6	1.3350	1.0905	0.08625	0.15825	7.6
7	1.3290	1.09205	0.08850	0.14845	7.85
8	1.3568	1.104	0.09030	0.16250	7.75
9	1.3573	1.0943	0.09105	0.17195	7.75
10	1.3350	1.0869	0.08905	0.15805	7.85
11	1.3581	1.11405	0.08970	0.15435	----
12	1.3749	1.10465	0.09260	0.17765	7.65
13	1.3301	1.08585	0.08805	0.15620	7.85
14	1.3156	1.10695	0.01765	0.10103	7.45
15	1.2970	1.08865	0.07495	0.13349	7.5
16	1.3154	1.08785	0.08830	0.13525	7.65
17	1.4090	1.10985	0.09510	0.20[illegible]	7.25
18	1.3296	1.0915	0.08805	0.14975	----
19	1.2915	1.0854	0.08595	0.12[illegible]	7.45
20	1.3240	1.08648	0.08840	0.14515	----
21	1.3443	1.0908	0.09235	0.16175	----
22	1.3714	1.10855	0.09590	0.16695	7.45
23	1.3494	1.09885	0.08825	0.16250	----
24	1.3408	1.09785	0.09[illegible]	0.15[illegible]	----
25	1.3829	1.09895	0.09850	0.18545	----
26	1.2727	1.1061	0.08[illegible]	0.11975	7.4

TABLE 2

SEQUENCE OF EVENTS

PRIMARY CULTURE
(35 mm) Petri Dishes

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

TABLE 3

SEQUENCE OF EVENTS

SUBCULTURE 1

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

TABLE 4

SEQUENCE OF EVENTS

SUBCULTURE 2

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

TABLE 5

RESULTS
PRIMARY CULTURE

FRACTION	UK ASSAY units/dish	VIABLE CELLS $X10^5$	UK UNITS 100 cells
11	45	.07	.64
13	535	.696	.77
15	240	.12	2.0
17	225	.744	.3
B CONTROL	61	.068	.9
D CONTROL	81	.288	.3
	SUBCULTURE 1		
14-1	85	.60	.14
16-1	124	.132	.94
17-1	205	.9	.23
19-1	359	.222	1.62

TABLE 6

RESULTS

Human Granulocyte Conditioning Factor

Subculture 1

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

*Colonies formed corrected for control plate

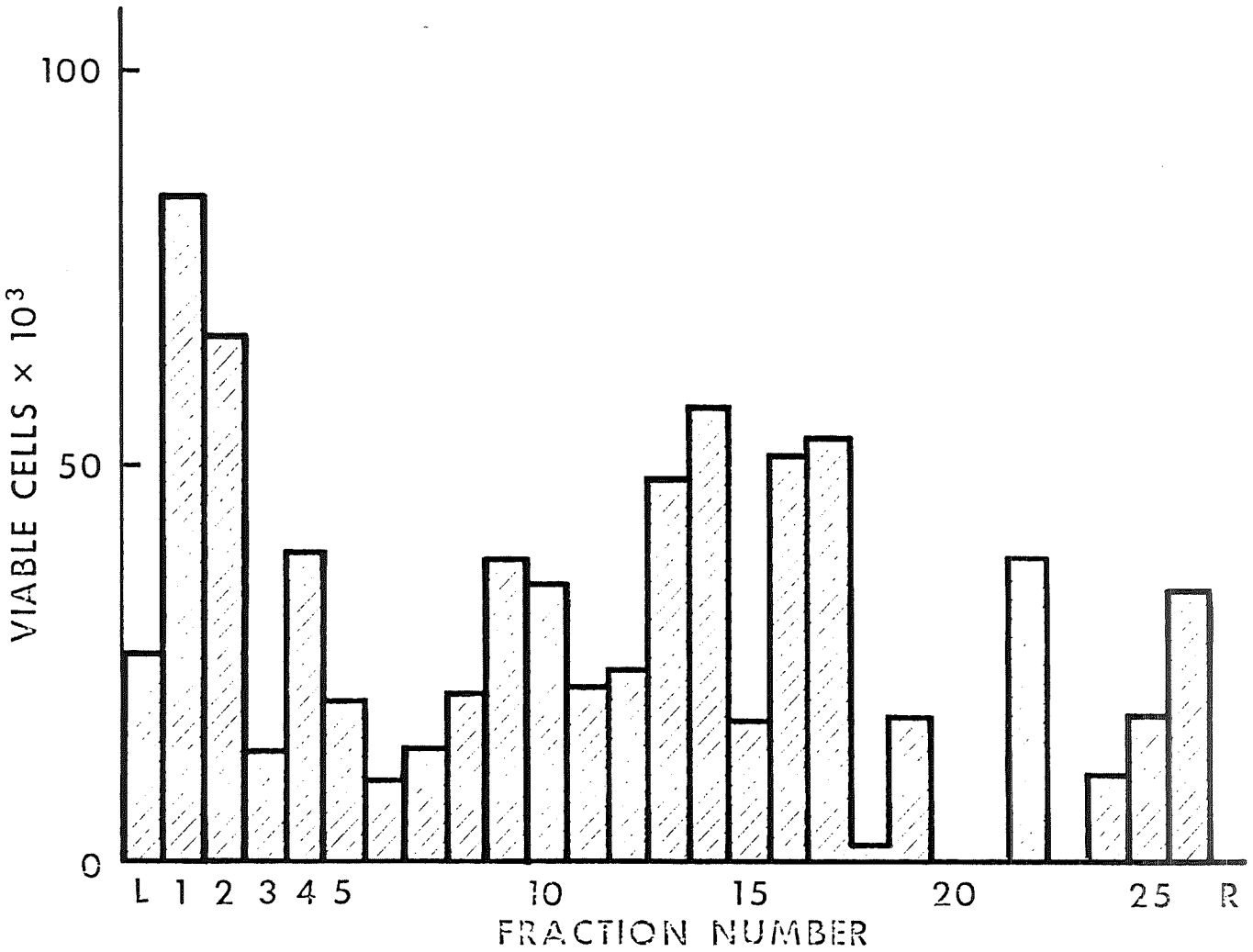

Figure 1.- Viable cell distribution in fractions from sliced gel.

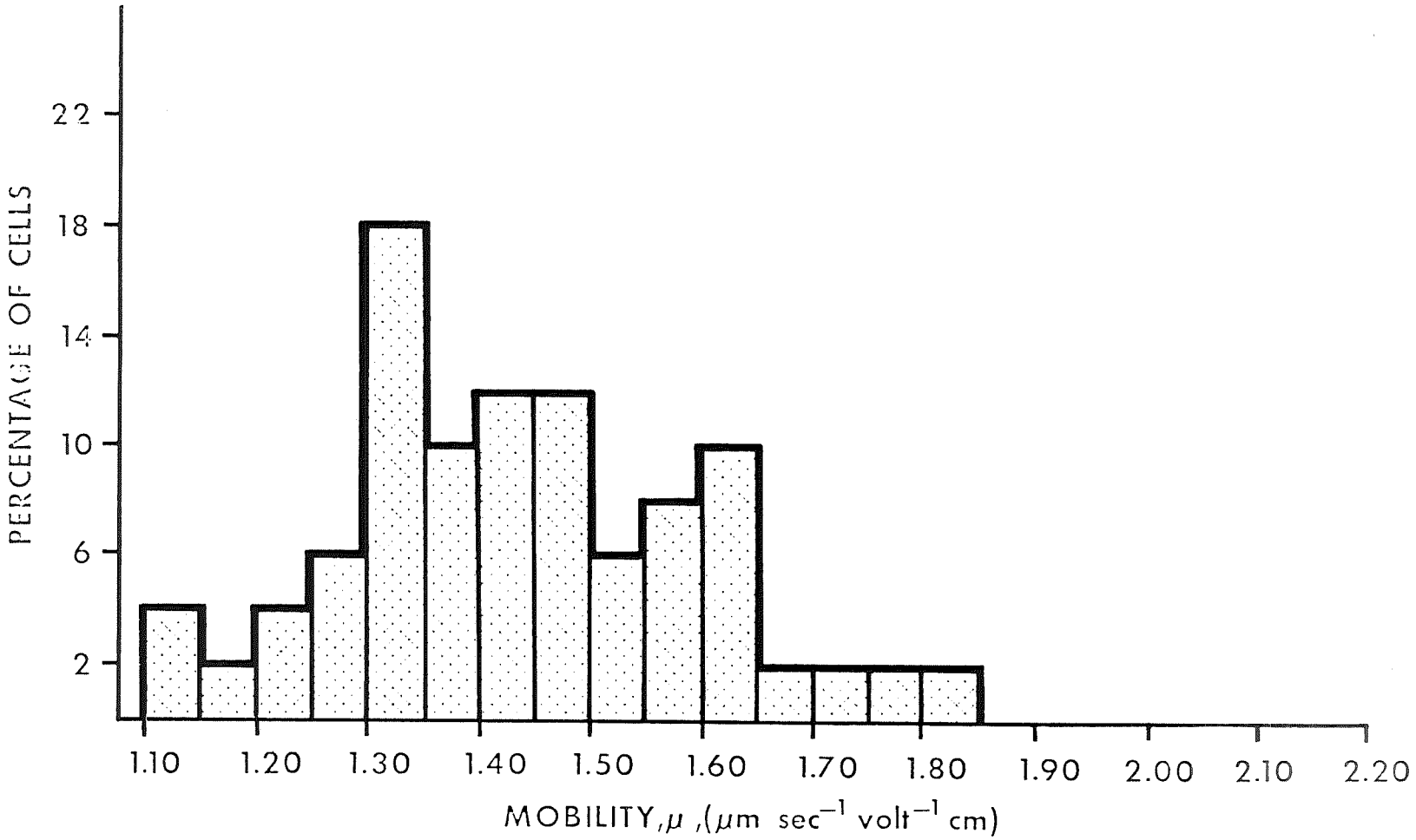

Figure 2.- Analytical electrophoretic mobility distribution on cell subculture 2 of fraction 14.

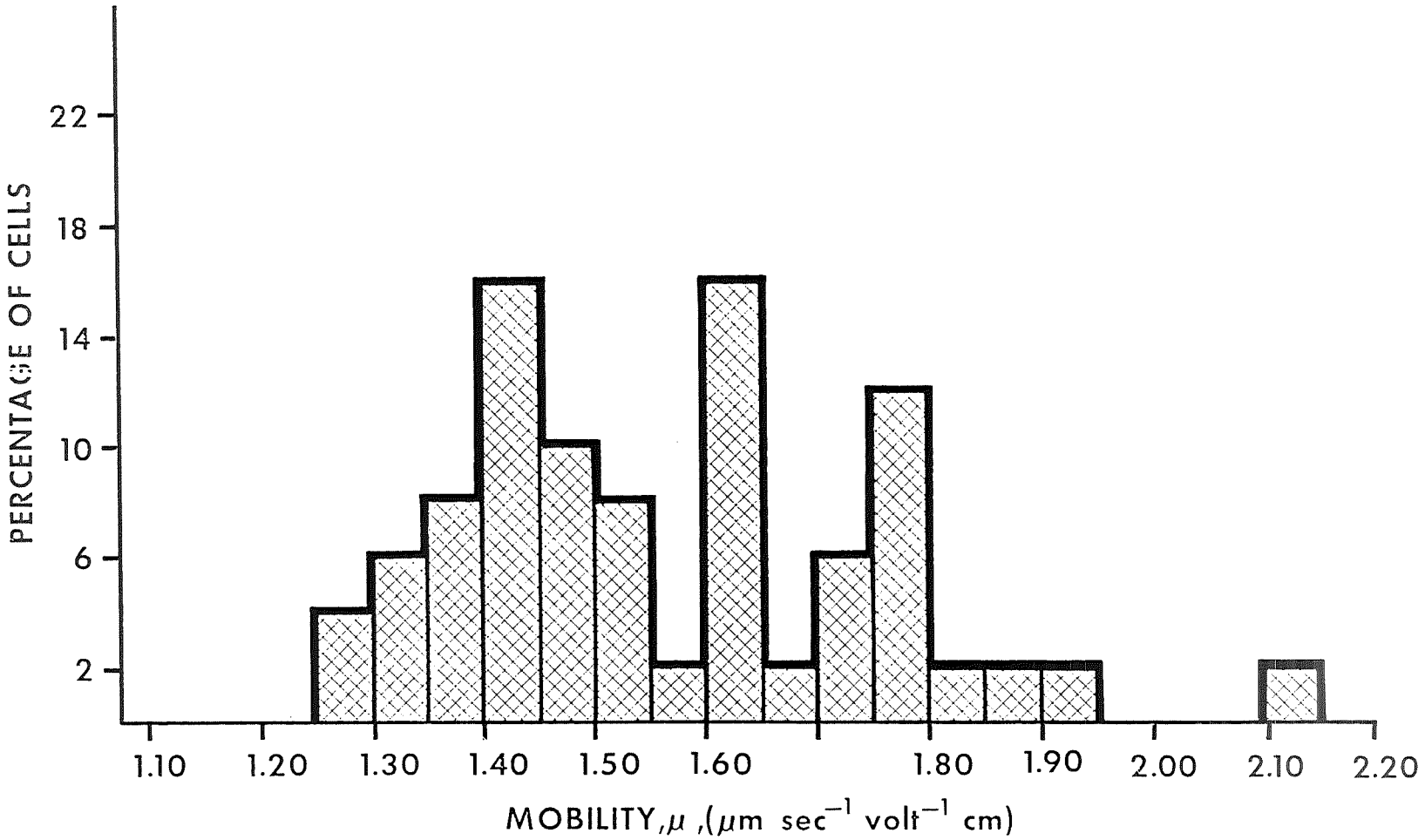

Figure 3.- Analytic electrophoretic mobility distribution on cell subculture 2 of fraction 17.

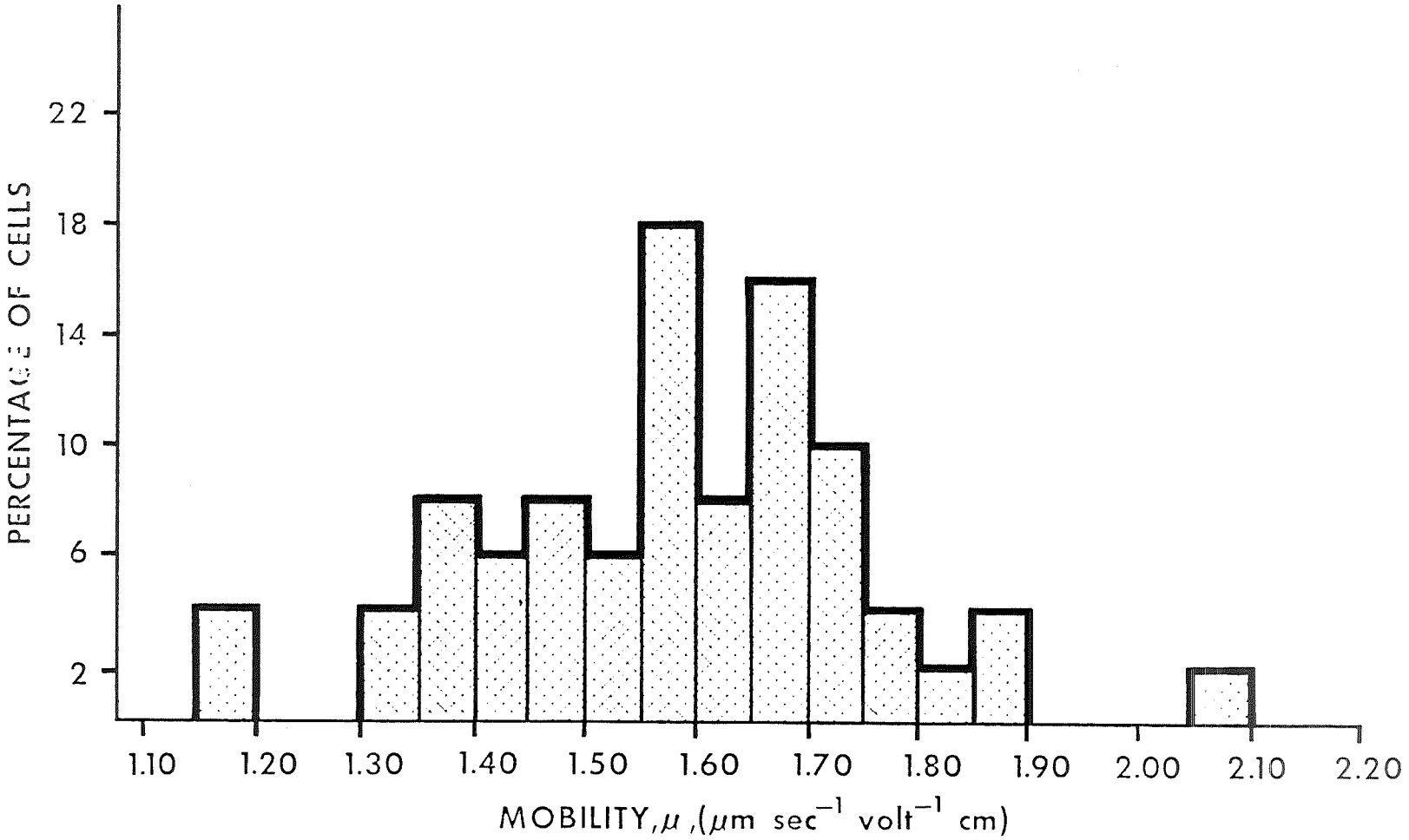

Figure 4.- Analytic electrophoretic mobility distribution on cell subculture 2 of fraction 19.