Electrophoretic Separation of Cells and Particles from Rat Pituitary (PITUITARY)

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In this report we describe the results of a continuous flow electrophoresis (CFE) experiment done on STS-65 in which we tested the idea that intracellular growth hormone (GH) particles contained in a cell lysate prepared from cultured rat anterior pituitary cells in microgravity might have different electrophoretic mobilities from those in a synchronous ground control cell lysate. Collectively, the results suggested that CFE processing in microgravity was better than on earth; more sample could be processed/time (6x) and more variant forms of GH molecules could be resolved as well. We had also hoped to do a pituitary cell CFE experiment, but failure of the hardware required that the actual cell electrophoresis trials be done on earth shortly after Shuttle landing. Data from these experiments showed that spaceflown cells were more electrophoretically mobile than ground control cells, thereby offering evidence for the idea that exposure of cultured cells to microgravity can change their net surface charge--especially when the cells are fed. Collectively, the results from this pituitary cell experiment document the advantage of using coupled cell culture and CFE techniques in the microgravity environment.

INTRODUCTION

Even though continuous flow electrophoresis (CFE) was first introduced about 35 years ago[1) 2)], it is only quite recently that there has been renewed interest in this technology[3) 4)]. As pointed out by Roman and Brown "...the tremendous growth of biotechnology over the past decade has necessitated the development of purification methods for isolating a single compound from a complex biological matrix"[3)]. The CFE process has attracted interest because it is continuous, does not use organic solvents, and avoids the use of support media (e.g. gels).

A number of studies indicate that the CFE process may also be quite useful for applications requiring cell separation. In fact, a recent book summarizes its theoretical basis in addition to providing a description of results from many laboratories using this technology for the separation of diverse cell types such as subpopulations of mammalian lymphocytes and human cancer cells[5)].

Distortions of the sample stream occur during CFE processing; these are hydrodynamic,

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electrodynamic and electrohydrodynamic in character. Some are limited by gravity. It is therefore not surprising that the theoretical advantages of doing CFE processing in microgravity have been tested in spaceflight experiments dating back to 1982. This history has been reviewed by Morrison[6]. Perhaps the best evidence for enhanced resolution of separated cells by CFE in low gravity was obtained using fixed red blood cells from 3 different species during a 5 minute suborbital rocket flight[7].

Our laboratory has applied electrophoresis technology to the separation of rat pituitary cells and their subcellular constituents[8) 9]. In 1983 we attempted a CFE experiment in microgravity[10]. Increased bandspread of the recovered cells suggested enhanced resolution of the different hormone containing cell types, but poor recoveries and biological contamination did not establish this point definitively. The availability of Japanese cell culture kits (CCK) and the NASDA free flow electrophoresis unit (FFEU) during the 14 day IML-2 mission allowed us to design a CFE trial that was coupled to pituitary cells in the CCK. Although problems with the FFEU were encountered during spaceflight operations (see Discussion) sufficient data were collected which continue to support the idea that CFE processing in microgravity is advantageous. This report describes those data.

MATERIALS AND METHODS

In its original design, two continuous flow electrophoresis (CFE) trials were to have been done in microgravity. One used a freshly prepared rat pituitary cell lysate; the other used enzymatically dispersed rat pituitary cells. Both samples were to have been prepared for CFE processing in microgravity using the cell culture kits (CCK's) described in the companion report[11]; however, because of technical problems with the CFE hardware, only the cell lysate CFE experiment was actually attempted in microgravity. The procedures which were followed in this trial are outlined in Fig. 1. Pituitary cells (4×10^7) in each of 2

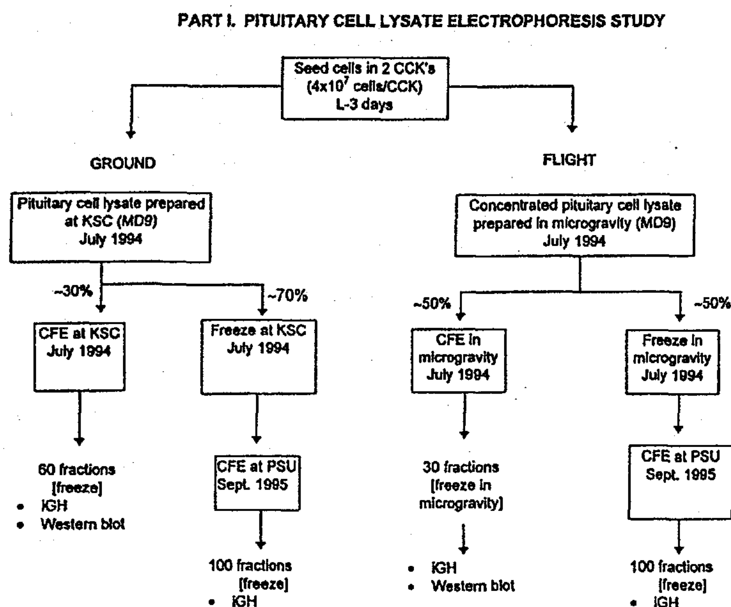

Fig. 1 Experimental design and timing of operations involving CFE of pituitary cell lysate. See Materials and Methods for details.

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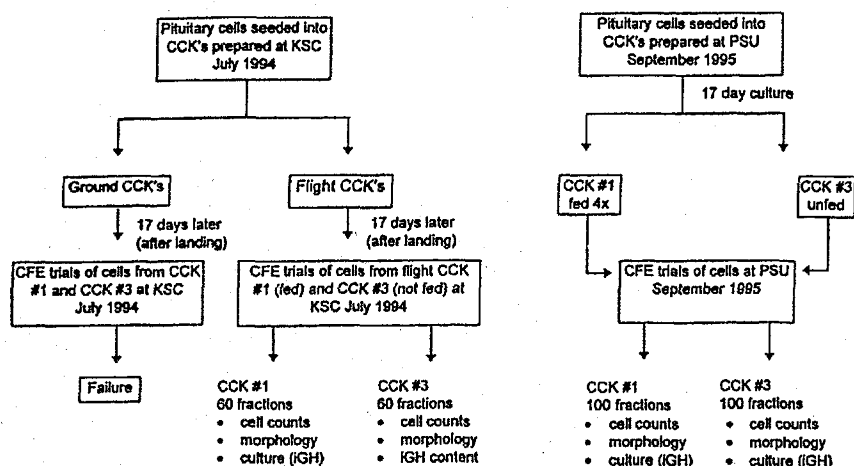

Fig. 2 Experimental design and timing of operations involving CFE of pituitary cells after microgravity exposure. Cells from flight CCK #1 and #3 were processed at Kennedy Space Center within 8 hrs. of Shuttle landing using a device (identified as device #2 in Table 1) made for this experiment by Mitsubishi Heavy Industries, LTD. This device malfunctioned after processing of cells from both flight CCK's; accordingly, an asychronous ground control experiment was done later using another CFE device (identified as device #1 in Table 1).

CCK's were prepared at L-3 days exactly as described for the cell culture portion of this experiment[11]. Cells in these two CCK's were not fed prior to preparation of the cell lysate on mission day 9. As shown in Fig. 1, only (50 % of each ground and flight lysate was actually used for processing in the July, 1994 experiment; the remainder were processed by CFE at Penn State later.

The procedures which were followed in the cell electrophoresis study are outlined in Fig. 2. After Shuttle landing cells were removed from CCK #1 (fed 4x in microgravity) and from CCK #3 (not fed during the 14 days in microgravity)[11] using our routine trypsinization procedures[12]. Cell viabilities at this point averaged 93 and 90 % for CCK #3 and #1 respectively. Sufficient cells were obtained after CFE processing of the cells from CCK #1 to do a 6 day culture with the separated cells; this was not possible for those from CCK #3 because cell numbers were insufficient.

Cell Lysis.

On mission day 9, cell culture medium was removed and the cells in the CCK were washed briefly (~1.5 min) with 15 ml distilled water; this step diluted any residual serum containing medium that had been left behind which would, in turn, discourage cell lysis. Cell lysis was accomplished using H_2O containing 0.2 mM $ZnCl_2$ and soybean trypsin inhibitor (1 μg/ml). The $ZnCl_2$ was included to maintain integrity of nuclei and hormone-containing secretory granules[13][14]. Lysis, monitored microscopically, was complete after ~10 min. This diluted lysate was then concentrated to ~3 ml by (1) drawing the lysate out of the CCK into a syringe containing 20 mg DNAse (Type I), solubilized, and

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then reinjected back into the CCK, and (2) then drawn from the CCK into a concentrating device containing 1 ml of 10x electrophoresis buffer and consisted of a Spectra pore 7 dialysis membrane (10,000 MW) surrounded by polyethylene glycol 60 (60,000 MW).

CFE.

The carrier buffer consisted of 5 mM HEPES, 30 mM glycine, 0.2 mM potassium acetate, 0.3 mM $MgCl_2$, 0.03 mM $CaCl_2$, 220 mM glycerol, 44 mM sucrose, 0.2 mM $ZnCl_2$. Its conductivity and osmolarity was 260 -μS and 300 mOSM respectively. Results from several preflight CFE trials indicated that this buffer was superior to several others in terms of maintaining cell viability (>80 % over 17 days).

Three different CFE devices were used in carrying out this experiment. Two have been described in previous publications[10) 15)]; the third was designed to serve as the ground control unit when synchronous flight and ground processing was done. See Table 1 for operating details and specific sample applications.

Post CFE Analysis.

Procedures involving cell preparation, GH immunoassay, HPLC gel filtration and ion exchange chromatography, immunocytochemistry and cell culture were as described previously[16) 17)]. Western blotting was done on 5-15 % gradient gels under non-reducing conditions as in previous reports[14)].

Table 1 CFE operating conditions used in this study

Sample	Sample in space or ground	Process in space or ground	FFE Device +	Field strength	Carrier buffer rate ++	Sample rate	% recovery *	Fig #
Lysate	ground	ground	2	25 v/cm	5 ml / min	0.2 ml / hr	228	4
Lysate	ground	ground	2	25 v/cm	3 ml/min	0.15 ml/hr	73	6B
Lysate	flight	flight	3	25 v/cm	7 ml/min	2 ml/hr	17	5,6A
Lysate	ground	ground	1	15 v/cm	18 ml/min	2.7 ml/hr	34	6C
Lysate	flight	ground	1	15 v/cm	18 ml/min	2.7 ml/hr	32	6C
Cells	ground (no media change)	ground	2	25 v/cm	5 ml/min	0.15 ml/hr	54	11 (top)
Cells	ground (no media change)	ground	2	25 v/cm	5 ml/min	0.15 ml/hr	34	11 (middle)
Cells	ground (no media change)	ground	2	25 v/cm	5 ml/min	0.15 ml/hr	34	11 (bottom)
Cells	flight (4x media change)	ground	2	25 v/cm	3 ml/min	0.15 ml/hr	18	12
Cells	flight (no media change)	ground	2	25 v/cm	3 ml/min	0.15 ml/hr	37	12
Cells	ground (4x media change)	ground	1	15 v/cm	18 ml/min	2.7 ml/hr	11	12
Cells	ground (no media change)	ground	1	15 v/cm	18 ml/min	2.7 ml/hr	16	12
								Chamber dimensions
+ FFE Device:		#1 1.5 mm Continuous Flow Electrophoresis Device (Hymer et al. 1987)						120 cm x 8.2 cm x 1.8 mm
		#2 Japanese 1.0 mm ground Free Flow Electrophoresis Unit						10 cm x 6 cm x 1 mm
		#3 Japanese 4 mm flight Free Flow Electrophoresis Unit (Akiba et al. 1994)						10 cm x 6 cm x 4 mm
++ Carrier Buffer Formulation:		5 mM HEPES, 30 mM Glycine, 0.2 mM Potassium Acetate, 0.3 mM MgCl2, 0.03 mM CaCl2, 220 mM Glycerol, 44 mM Sucrose, 0.2 mM ZnCl2						
* either GH or total cells								

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RESULTS

The ultrastructure of a rat pituitary cell lysate as well as enzymatically dispersed rat pituitary cells after a 9 day culture is shown in Fig. 3. These micrographs represent the type of samples that were used in this microgravity continuous flow electrophoresis (CFE) experiment.

Lysate Processing (Earth).

A typical CFE distribution profile of GH particles contained in a freshly prepared lysate from an eight day pituitary cell culture shows a dominant anodal GH peak; a small cathodal GH peak and immunopositive material which did not migrate in the field (Fig. 4). This pattern is similar to others using freshly prepared rat anterior pituitary homogenates[9].

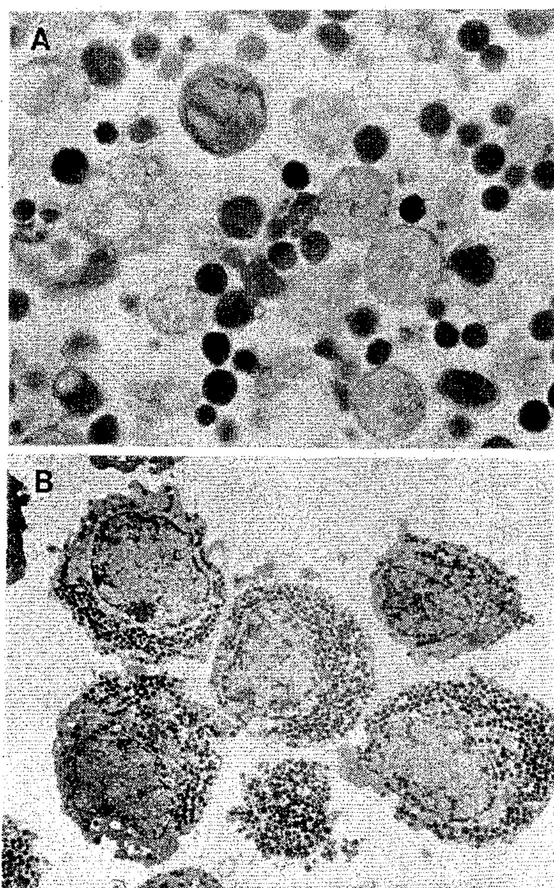

Fig. 3 Electron micrographs of a rat anterior pituitary cell lysate (top) and enzymatically dispersed rat pituitary cells that had been in culture for 9 days (bottom). The culture medium was identical to that used in microgravity[20] and was not changed during the 9 days. Hormone containing secretory granules are prominent. These samples are representative of the types of samples that were subjected to CFE in microgravity (lysate) or after microgravity exposure (cells).

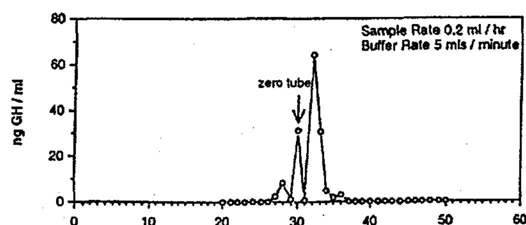

Fig. 4 Representative ground-based CFE distribution profile of GH particles prepared from rat pituitary cells in culture (CCK) for 8 days prior lysis and CFE. See also Table 1. The zero tube identified in this and subsequent figures represents the elution position of the material when no electric field is applied.

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Lysate Processing (Microgravity).

A concentrated pituitary cell lysate was prepared in space from unfed cells on mission day 9 following the procedure described in Methods. CFE processing of this lysate was done according to the experimental design shown in Fig. 1. The down-linked OD 280 profile collected midway through the 21 minute processing trial (Fig. 5A) showed a complex structure which suggested widespread distribution of protein throughout the separation chamber. This pattern was stable during the entire run. Only 30 of 60 possible fractions could be collected at the end of this trial; those selected are indicated in Fig. 5B. On return to earth, only a few of these 30 fractions contained the expected volume (i.e. 2.5 ml), a fact which implies that air bubbles in the separation chamber may have blocked some of the outlets (see Discussion). The concentration of GH in the flight lysate was ~20% greater than that in the synchronous ground control preparation (cf. Fig. 6 A,B), a result which is entirely consistent with the finding that there were greater amounts of GH released into the 9 day culture medium prior to lysate preparation (see companion report, Fig. 4B). After CFE processing in microgravity, 9 of 30 fractions contained detectable GH; after synchronous

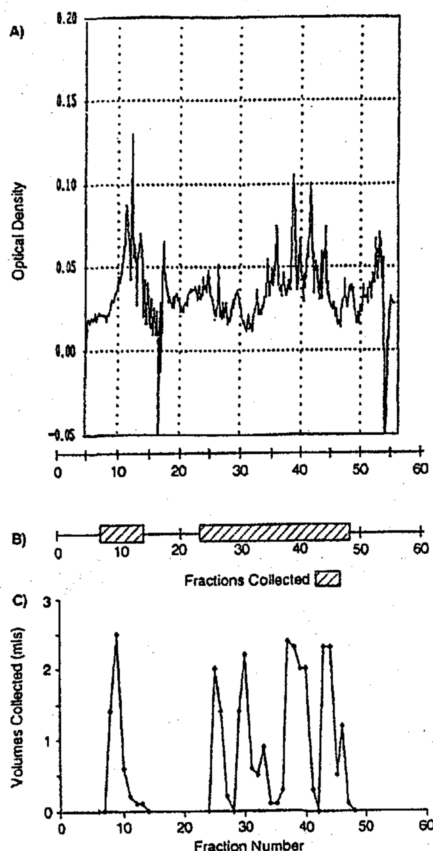

Fig. 5 (A) OD 280 profile of lysate material being processed by CFE in microgravity. This profile was down linked from space ~10 minutes into the CFE trial. (B) Fractions chosen for collection in microgravity after the 21 minute lysate CFE run had been completed. (C) Volumes collected in each of the 30 fractions after Shuttle return. Incomplete tube filling is attributed to bubbles in the separation chamber (see Discussion).

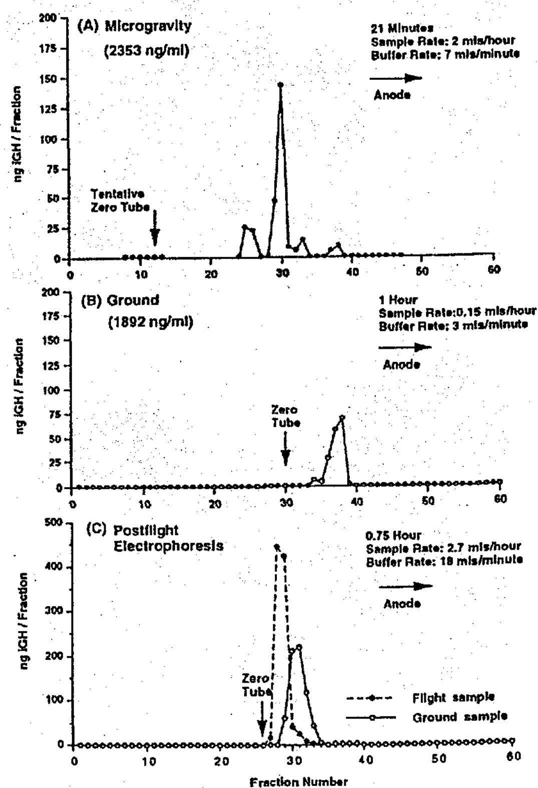

Fig. 6 Mobility profiles of GH containing material in lysate after CFE processing in microgravity (A) or on earth (B). As shown in Fig. 1, some of the initial sample from A and B was frozen and later electrophoresed (C) (see Table 1).

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ground processing, only 5 of 60 fractions contained detectable GH (Fig. 6A vs. B). Three GH peaks were found after flight processing, but only one was present in the ground trial. Note that the zero outlet tube (i.e. the position where material emerges from the chamber when no field is applied) is identified as "tentative" in Fig. 6A (see Discussion). When frozen aliquots of these same concentrated lysates were subsequently processed on earth, the distribution profiles of GH were essentially identical (Fig. 6C).

In order to determine if there were any differences in apparent molecular weight of GH variants that might be contained in these fractions, Western blots were prepared on those fractions containing sufficient GH. The complex profile of GH variants that emerges when SDS-PAGE is used under non-reducing conditions is reasonably well documented[18]; their complexity from both the ground and flight CFE fractions is therefore not unexpected (Fig. 7). Densitometry of these same blots revealed that there were some differences in GH variants between these fractions; those from flight were the most obvious (Fig. 8, shaded areas). These differences were in GH molecular weight regions (based on the mobilities of pre-stained molecular weight protein standards shown in Fig. 7) identified at the top of Fig. 8.

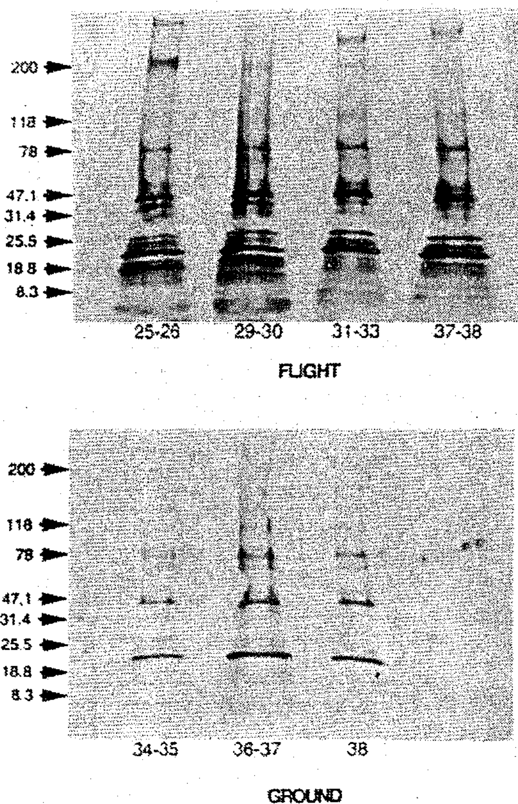

Fig. 7 Western blots of GH molecules contained in different CFE fractions after cell lysate processing; flight (top) or ground (bottom). See Fig. 6 for mobility profiles of GH fractions from which these blots were prepared. SDS-PAGE in 4-15% gradient gels under non-reducing conditions reveal large numbers of GH variants attributed to aggregation, cleavage, glycosylation, etc.[14) 18)] Markers show positions of co-migrating pre-stained molecular weight standards.

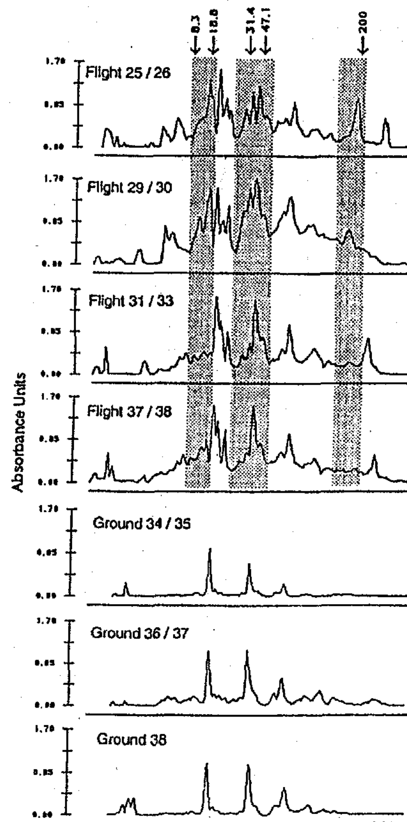

Fig. 8 Optical density tracings of GH Western blots shown in Fig. 7. Highest molecular weight material is at far right. Shaded areas show positions where changes in GH forms in the different CFE fractions from the flight trial are most apparent. Molecular weight markers are identified at the top.

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Small (0.1 ml) aliquots of these lysate fractions obtained after CFE were also fractionated by HPLC gel filtration or anion exchange chromatography to obtain information concerning separation of general pituitary protein. Shown in Fig. 9 are the OD 280 gel filtration profiles of pH10 solubilized material that was contained in (1) the original lysates before CFE (Fig. 9A; molecular weight standards are shown in insert); (2) the fractions making up the GH peak after CFE processing of the synchronous ground control lysate (Fig. 9B) and (3) the fractions making up the three GH peaks after CFE processing in microgravity (Fig. 9C; also shown is another area which did not contain GH, viz. fractions 8-12). While the general protein profiles of the starting lysates were similar, there was a tendency for the flights sample to contain more high molecular weight material (>~29,000) (Fig. 9A). Differences in OD 280 patterns between fractions from the ground lysates were very minor (Fig. 9B); those from the flight lysates were also relatively minor (Fig. 9C). For example, material emerging from the column at ~40 minutes (an elution time similar to the ribonuclease standard) was less in fractions 37-38 than fractions 8-12. The reason(s) for the absence of OD 280 material in CFE fractions eluting between 15 and 35 minutes, material that was present in the starting preparation, is unknown; it may reflect differential susceptibility to proteolysis.

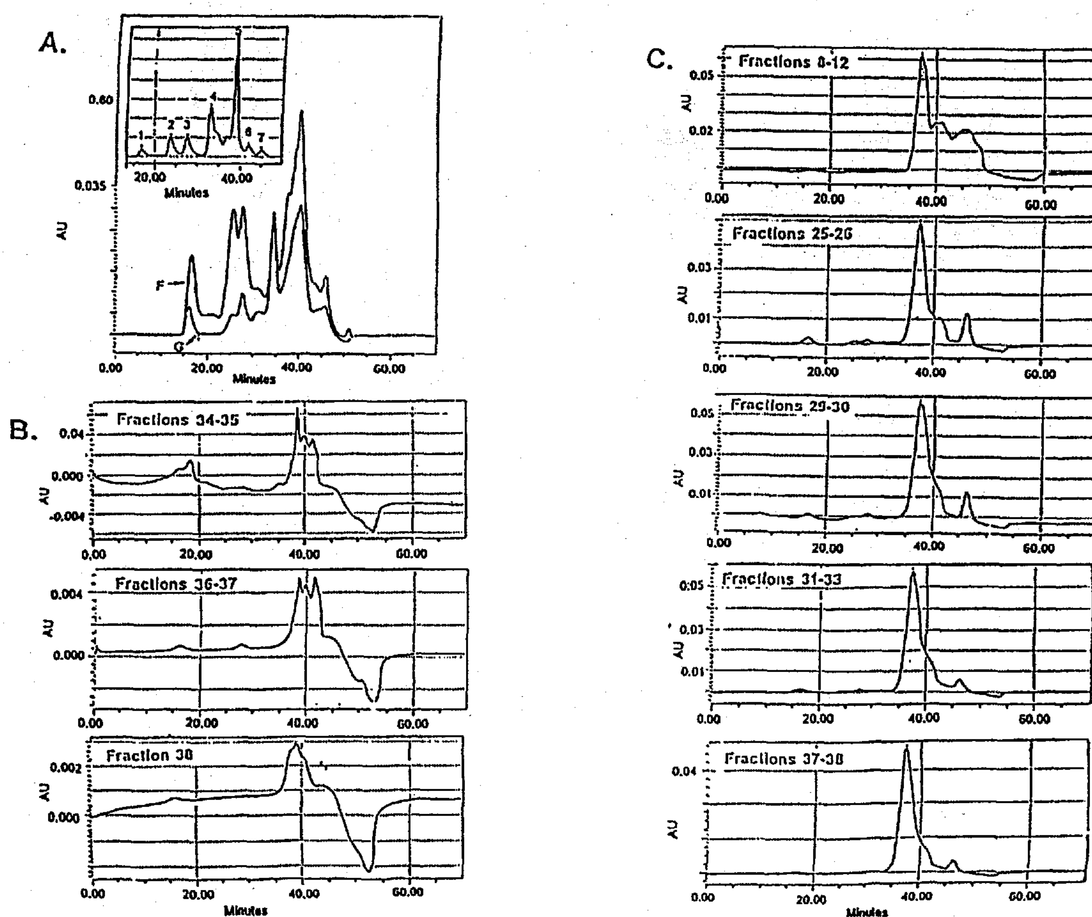

Fig. 9 Gel filtration profiles (OD 280) of pH 10 solubilized material that was contained in (A) the original lysate before CFE (B) fractions making up the GH peak after CFE processing on earth or (C) in microgravity. Protein standards, identified in insert to panel A, were (1) blue dextran, 2×10^6; (2) β-amylase, 2×10^5; (3) BSA, 66×10^3; (4) carbonic anhydrase, 29×10^3; (5) ribonuclease, 13.6×10^3; (6) aprotinin, 6.5×10^3 and (7) vasopressin, 1×10^3.

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Because anion exchange chromatography of cell culture media collected from fed cells in this experiment had shown some interesting results (see Ref. 11, Fig. 9), we also fractionated the CFE lysate material from the CFE flight fractions containing GH by anion exchange HPLC. Only very small amounts of flight material processed due to insufficient sample volume.; the ground samples were not done. The general OD 280 profile of the proteins from the intracellular lysate (Fig. 10) was similar to those proteins contained in the culture medium (Fig. 9, top in Ref. 11) in that a large protein peak eluted from the column ~2 minutes before the salt gradient began followed by major protein peak eluting between 15-20 minutes. As before, minor differences in the OD 280 patterns infers that some protein separation was occurring during CFE processing in microgravity.

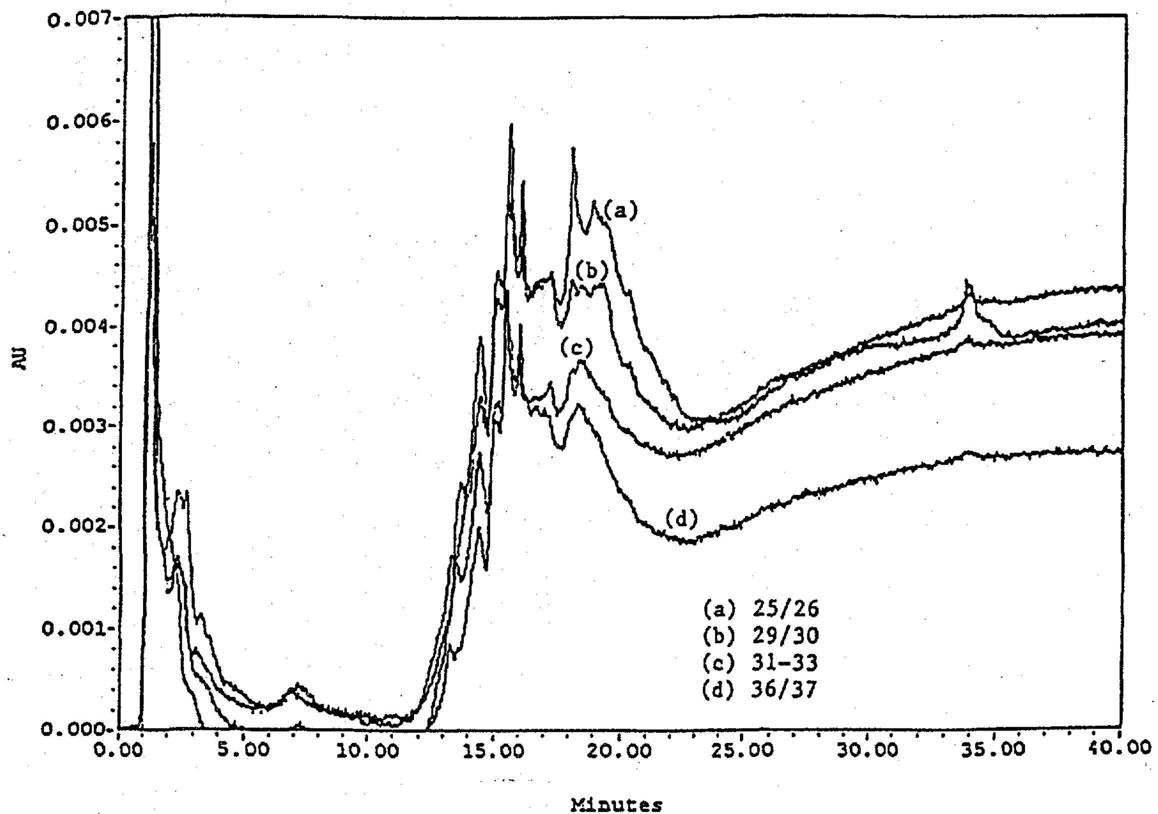

Fig. 10 Anion exchange profiles (OD 280) of pH10 solubilized material that was in GH-containing fractions after CFE processing in microgravity. Flight fractions in Fig. 6 are identified in this figure as a-d.

Cell Processing (Earth).

The electrophoretic mobility profiles of unfed primary rat anterior pituitary cells that had been in culture for 8 days are shown in Fig. 11 (3 exps.). These trials were designed to mimic the cell processing trial that had been originally planned on day 8 for the IML-2 experiments. They show considerable variability. However, it seems clear from these preflight trials that a majority of the cultured, unfed pituitary cells exhibit anodal mobility under these electrophoresis conditions (Fig. 11, bottom panel) while some show no or even cathodal migration.

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Cell Processing (Microgravity).

Due to the technical problems with the flight electrophoresis hardware (see Discussion), it was not possible to carry out the cell electrophoresis trial as planned and the single microgravity CFE trial that was done (as planned) used pituitary cell lysate (Fig. 6). This made two cell culture kits available after Shuttle landing; we therefore tested the effect of microgravity exposure on the electrophoretic mobility of cells that had been in the culture for 17 days. In one case (CCK #1) the cells had been fed four times, in the other (CCK #3) the cells were kept in their original seeding medium. A detailed morphological description of the cells in these two CCK's is given in the companion report[11]. The mobility profiles of cells trypsinized from these two CCK's and electrophoresed within 8 hrs. of Shuttle landing showed that fed cells had greater anodal mobility than unfed cells (Fig. 12, top two panels). This was not true when asychronous ground control cells, cultured for 17 days, were electrophoresed (Fig. 12, bottom panels).

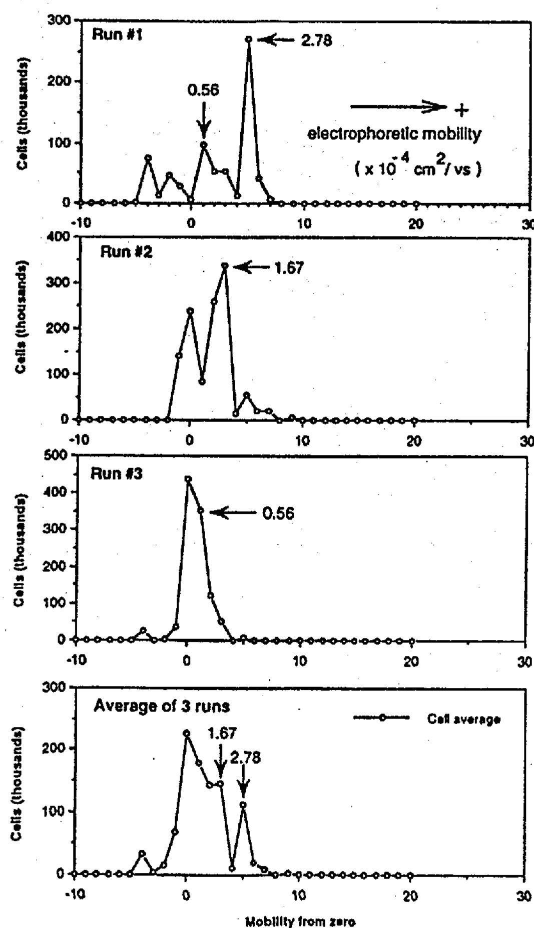

Fig. 11 Electrophoretic mobility profiles of unfed rat anterior pituitary cells that had been in culture for 8 days. (n=3 exps; the average is shown in the bottom panel). Actual electrophoretic mobility units of cell peaks, expressed as 10^{-4} cm²/vs, are identified in this and the next figure.

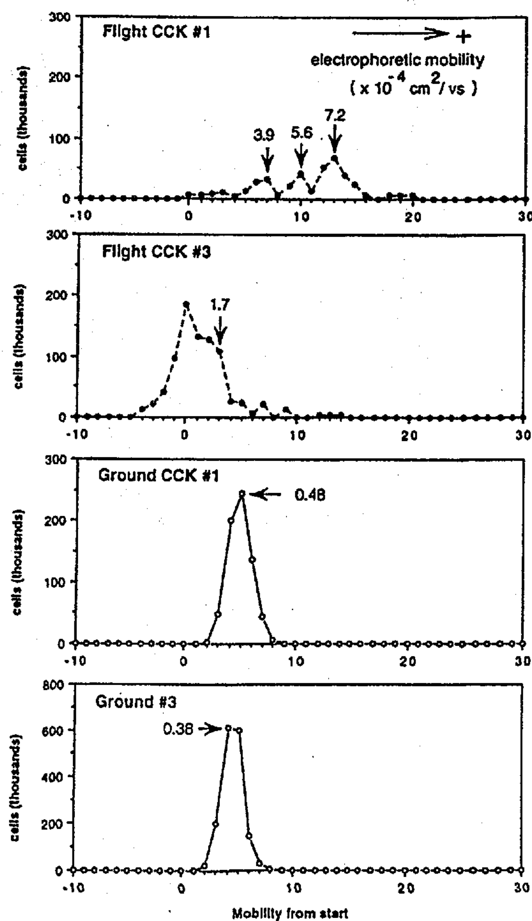

Fig. 12 Cell mobility profiles after trypsinization from flight and ground CCK's. CCK #1--cells fed 4x in microgravity; CCK #3--cells not fed (see Fig. 2 and Table 1 for additional details).

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Cells from flight and ground CCK #1, after CFE, were cultured for 6 days to determine if the separated cells released GH and if the CFE process enriched GH producing cells. The data in Fig. 13 establish (1) that microgravity-exposed fed cells released (5x more hormone than corresponding ground controls (reason unknown); (2) that hormone producing cells exposed to space showed greater bandspread than the corresponding ground controls; and (3) that high producer cells after spaceflight tended to be among the most mobile (e.g. those in fractions 14-18).

Because cells were limiting from flight CCK #3 (see Materials and Methods), post CFE culture was not done. However, their intracellular GH concentrations mirrored the general cell profile, a result that was generally similar to the ground sample (Fig. 14). It is important to indicate that the average electrophoretic mobility distribution profiles of unfed cells in the preflight trials (Fig. 11, bottom panel) and those from unfed flight cells (Fig. 14, top panel) are similar. These profiles add support to the concept that there may be specific microgravity- feeding interactions which affect cell electrophoretic mobility.

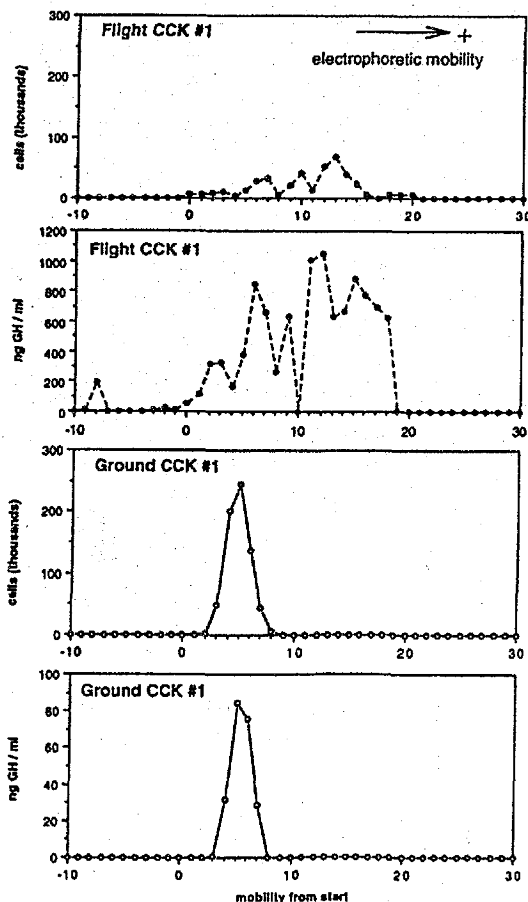

Fig. 13 GH released from cells contained in different CFE fractions after six days in culture. Cells were fed 4x in microgravity on earth; all of the cells in each fraction were placed into the culture well. See Fig. 2 and Table 1 for additional details.

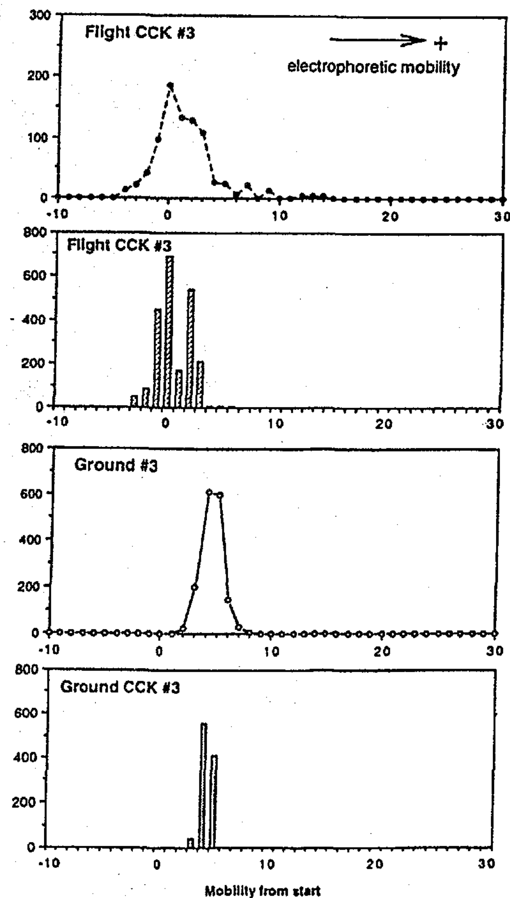

Fig. 14 Intracellular GH contained in different CFE fractions prepared from cells originally contained in CCK #3 (unfed for 17 days; see Fig. 2 and Table 1 for additional details).

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The morphologies of GH cells prepared from ground and flight CCK's before and after CFE processing (Fig. 15) revealed the presence of intact cells which were typical of those seen in other studies.

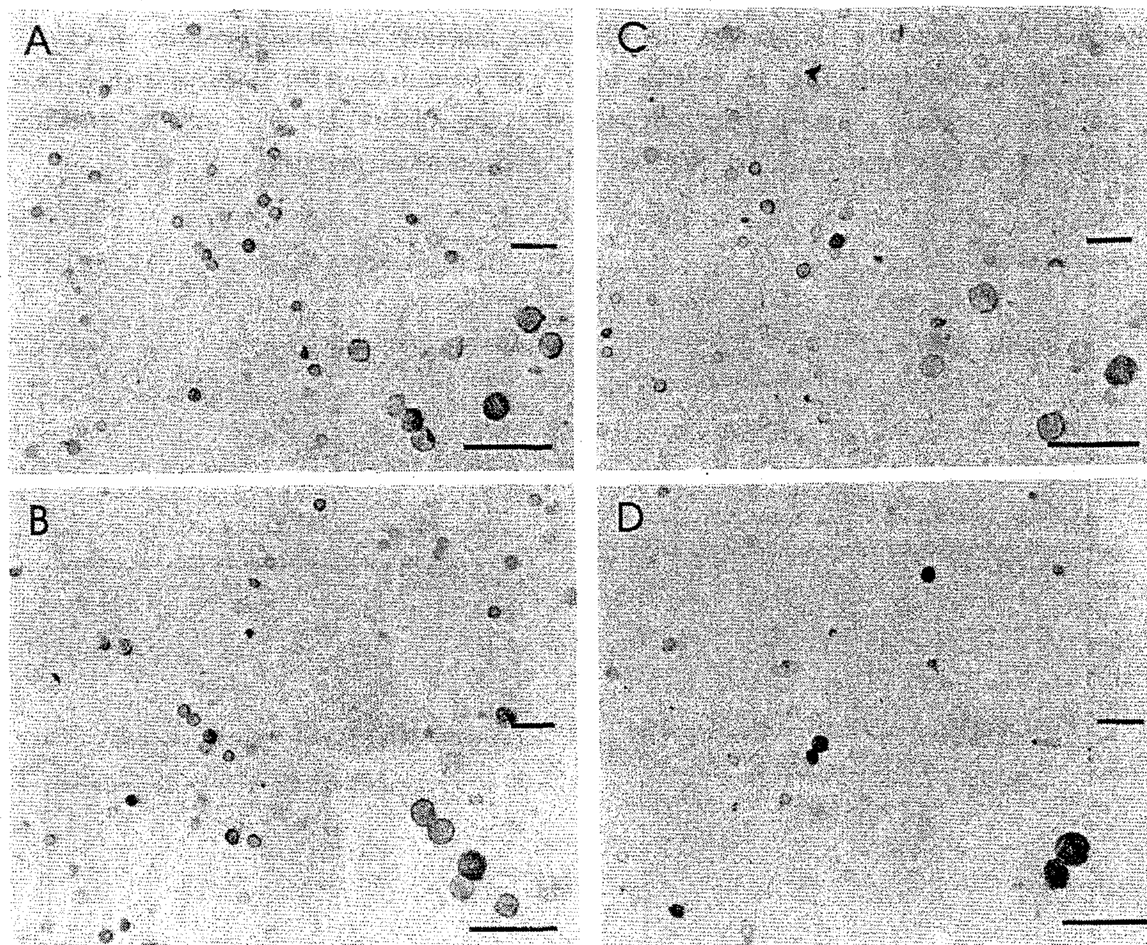

Fig. 15 Photomicrographs of cells recovered from flight CCK #1 and flight CCK #3 before and after electrophoresis postflight processing at Kennedy Spaceflight Center. These cells were removed from the CCK's which had been fed 4x (CCK #1) or not fed (CCK #3) during the course of the 14 day microgravity experiment. Cell removal by trypsinization, electrophoresis by CFE and staining by GH immunohistochemistry was done as described in Methods of this and the companion paper[11]. Cells with dark cytoplasms are GH cells. Inserts are higher magnifications; all micron bars are 100 μm. Sample identification: (A) CCK #1 (fed) cells before CFE fractionation; (B) CCK #1 (fed) cells after CFE fractionation contained in fraction #11 (see Fig. 13); (C) CCK #3 (unfed) cells before CFE fractionation; (D) CCK #3 (unfed) cells after CFE fractionation (see Fig. 14). Cells in B produced high amounts of GH during the 6 day culture after CFE (Fig. 13).

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DISCUSSION

The original objective of this experiment was to determine if microgravity exposure affected the electrophoretic mobility of either cultured rat pituitary GH cells or intracellular GH-containing particles. Our previous experiments which documented microgravity specific changes in GH cells from either spaceflown rats[17) 19)] or spaceflown cells themselves[16) 20)] established the underlying rationale for this objective; i.e. that CFE technology might aid in helping to define mechanisms of gravisensing in the GH cell. Additional secondary objectives emerged automatically; viz. (1) demonstration of CFE processing advantages in microgravity and (2) demonstration of ancillary methods required for these CFE operations (e.g. preparation of fresh solutions, trypsinization, cell lysis).

Even though three different CFE devices were used in this experiment, all utilize essentially the same technology; viz. separation in rectangular chambers using the same low ionic strength buffer. As shown in Table 1, cell recoveries averaged 29 % although there was considerable variability. This average compares favorably with that reported for a previous space CFE experiment using rat pituitary cells (20 %[10)]). The low recovery from the flight lysate trial may be due to the fact that only one half of the fractions were collected and many had low volumes.

Even though all of the original objectives of this experiment could not be met, new results were obtained. For example, evidence for a CFE processing advantage in microgravity is indicated by (1) increased throughput (the flight sample was 5.6x more concentrated than the ground); (2) greater bandspread of GH containing particles in the lysate sample (Fig. 6) and (3) better discrimination of some GH variants within different CFE fractions (Fig. 8). Since this same microgravity sample did not show a mobility difference when it was processed at earth gravity (Fig. 6), we conclude that microgravity exposure has little effect on the net surface charge of intracellular GH-containing particles. The idea that microgravity may effect net cell surface charge, depending upon the cell culture conditions (Fig. 12), is to our knowledge entirely new. Our companion report showed that the frequency of cell feeding in low gravity affected the cell morphology as well as the quantity and quality of certain hormones[11)]. We speculate that the greater mobility of the fed cells may reflect more hormone on their surfaces resulting from increased local concentration gradients caused by lack of microconvection in microgravity. The early report of St. John et al.[21)] which established the presence of hormone on the surface of rat pituitary cells supports the basic mechanism underlying this idea. Cell surface hormone could result from the exocytosis process; its implication to cell function is unknown. However, we have speculated that cell surface hormone could be recycled back to the golgi zone (together with secretory granule membrane) to provide a seed site for the formation of a new granule[17)].

Bauer, in his thoughtful discussion concerning the significance of cell electrophoretic mobility[22)], raises a number of points that are relevant to our current data set. He points out that most mammalian cells have a narrow mobility range (0.5-3.5 x 10^{-4} cm^2/vs) even though several different types of electrophoresis devices have been used under very different conditions of analysis. Bauer also reviews the literature which attempts to relate the electrophoretic mobility value of the cell to its functional state. He concludes that electrophoretic cell mobility may relate to (1) the state of cell differentiation (but not strictly

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so); (2) "switching" as the cell changes from one physiological state to another or (3) ligand binding to its receptor (e.g. such as in the case when a monokine binds to a lymphocyte subclass). The electrophoretic values of cells processed by CFE in this study are identified in Figs. 11 and 12. Note that for all trials except the one using cells from flight CCK #1(Fig. 12, top panel) the EPM's are within the narrow range identified by Bauer. However, a majority of the cells which had been fed in microgravity had mobilities >3.5 x 10^{-4} cm^2/vs; e.g. cell peaks with EPM's of 5.6 and 7.2 x 10^{-4} cm^2/vs are identified in Fig. 12. Because high mobility cell populations were not found in flight CCK #3, the data support the idea that feeding pituitary cells in microgravity alters their net surface charge. Whether this reflects a gravisensing mechanism, or is the result of one, is unknown.

How might our new microgravity data relate to our previous spaceflight experiment[10] and those of others that have been reviewed by Bauer[22]? First, an earlier 1983 study from our laboratory showed that GH cells could be enriched by either density gradient electrophoresis or continuous flow electrophoresis, even through measurements of cell mobilities by two analytical methods (microscopic electrophoresis and laser tracking electrophoresis) revealed little difference between unpurified rat anterior pituitary cell suspensions and GH cell enriched suspensions[8]. At that time we suggested that a microgravity experiment could help to establish whether or not the higher mobility of GH cells seen in ground-based CFE was truly attributable to a difference in their net surface charge or merely attributable to an artifact caused by fallback in one of the GH cell subpopulations having high density ($p > 1.071$ g/cm^3). In fact, evidence for the former explanation was obtained in a 1983 spaceflight experiment which showed that anodal regions of the cell distribution profile contained more GH/cell and that there were 3x more GH cells in these fractions than in those of the slowest moving cells[10]. It is important to note that this earlier microgravity experiment was done by placing freshly prepared cells into a sealed conical tube at Cape Kennedy prior to launch. These cells were kept in a triethanolamine based buffer, low ionic strength buffer until the time of CFE processing in microgravity; only after collection were they exposed to buffers that were more physiologically compatible. Obviously the cell processing environment in our most recent IML-2 experiment was much more physiologically favorable; i.e. cells were maintained in a serum-bicarbonate containing Hepes buffered medium[11] for their entire microgravity exposure before CFE processing and culture at KSC. The data in Figs. 11 and 12 not only establish that high mobility GH producing cells were recovered from flight (but not ground) CCK #1, but also that a microgravity-feeding interactive effect occurred which apparently affects not only hormone output but cell surface charge as well.

What set of conditions might result in microgravity/feeding/hormone release/net surface charge changes of the type seen in this IML-2 experiment? Is the companion report we speculated that autocrine/paracrine interactions play a large role in pituitary cell gravisensing[11]. We suggest that the microgravity-specific cell feeding interactions in the FSH/ACTH cells found in the cell culture portion of this IML-2 experiment might affect the electrophoretic mobility profile of a GH cell subpopulation by exposing these cells to a different hormonal environment.

What consequence, if any, a change in cell surface charge might have on the quantity or quality of different hormones released from the pituitary gland of the intact organism

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obviously requires further study. Nevertheless, this IML-2 experiment showed that the rat pituitary cell system lends itself well to microgravity experimentation involving the coupled technologies of cell culture and continuous flow electrophoresis.

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REFERENCES

1) Barrolier, V., Watzke, E., Gibian, H. *Z. Naturforshung.* **13B**, 754 (1958).
2) Hannig, K. *Anal. Chem.* **181**, 244 (1961).
3) Roman, M., Brown, P. Free-flow electrophoresis. *Anal. Chem.* **66**(2), 86A-94A (1994).
4) Dalens, F., Roux-de Balmann, H., Sanchez, V. Improved operating conditions for preaparative separation of proteins by continuous flow zone electrophoresis. *Bioseparation.* **5**, 127-139 (1995).
5) Bauer, J. *Cell electrophoresis*, CRC Press, Inc., Boca Raton, FL (1994).
6) Morrison, D. Cell electrophoresis in microgravity: past and future. In *Cell electrophoresis*, ed. by J. Bauer, CRC Press, Inc., Boca Raton, FL (1994). 283-313.
7) Hannig, K., Kowalski, M., Klöck, G., Zimmerman, U., Mang, V. Free-flow electrophoresis under microgravity: Evidence for enhanced resolution of cell separation. *Electrophoresis.* **11**, 600-604 (1990).
8) Plank, L., Hymer, W., Kunze, E., Marks, G., Lanham, J. and Todd, P. A study of cell electrophoresis as a means of purifying growth hormone secreting cells. *J. Biochem. and Biophys. Methods.* **8**, 275-289 (1983).
9) Hayes, D., Exton, C., Salada, T., Shellenberger, K., Waddle, J. and Hymer, W. Separation of rat pituitary secretory granules by continuous flow electrophoresis. *Electrophoresis.* **11**, 976-978 (1990).
10) Hymer, W., Barlow, G., Blaisdell, S., Cleveland, C., Farrington, M., Feldmeier, M. Grindeland, R., Hatfield, J., Lanham, J., Lewis, M., Morrison, D., Olack, B., Richman, D., Rose, J., Scharp, D., Snyder, R., Swanson, C., Todd, P. and Wilfinger, W. Continuous flow electrophoresis separation of proteins and cells from mammalian tissues. *Cell Biophysics.* **10**, 61-85 (1987).
11) Hymer, W., Grindeland, R., Salada, T., Cenci, R., Krishnan, K., Mukai, C. and Nagaoka, S. Feeding frequency affects cultured rat pituitary cells in low gravity. *Biotechnology.* In submission.
12) Hymer, W. and Hatfield, J. Purification of cells from the anterior pituitary. In *Methods and Enzymology*, ed. by Colowick and Kaplan, Academic Press (1983).

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257-287.

13) Hymer, W. and Kuff, E. Isolation of nuceli from mammalian tissues through the use of Triton X-100. *J. Histochem. Cytochem.* **12**, 359-363 (1964).

14) Farrington, M. and Hymer, W. Growth hormone aggregates in the rat adenohypophysis. *Endocrinology.* **126**, 1630-1638 (1990).

15) Akiba, T., Nishi, A., Takaoki, M., Nagaoka, S., Tomita, F. Electrophoretic free mobility and viability of microbial cells: a preliminary study in preparation for space experiments. *Applied & Theoretical Electrophoresis.* **4**(2), 65-69 (1994).

16) Hymer, W., Grindeland, R., Salada, T., Nye, P., Grossman, E. and Lane, P. Experimental modification of rat pituitary growth hormone cell function during and after spaceflight. *J. Appl. Physiol.* In press (1995).

17) Hymer, W., Grindeland, R., Krasnov, I., Victorov, I., Motter, K., Mukherjee, P., Shellenberger, K. and Vasques, M. Effects of spaceflight on rat pituitary cell function. *J. Appl. Physiol.* **73**(2), 151S-157S (1992).

18) Lewis, U. Growth Hormone: What is it and what does it do. *Trends Endocrinol. Metab.* **3**, 117-121 (1992).

19) Grindeland, R., Hymer, W., Farrington, M., Fast, T., Hayes, C., Motter, K., Patil, L. and Vasques, M. Changes in pituitary growth hormone cells prepared from rats flown on SpaceLab 3. *Am. J. Physiol.* **252**, 209-215 (1987).

20) Hymer, W., Salada, T., Avery, L. and Grindeland, R. Experimental modification of rat pituitary prolactin cell function during and after spaceflight. *J. Appl. Physiol.* In press (1995).

21) St. John, P. Anti-prolactin cell-surface immunoreactivity identifies a subpopulation of lactotrophs from the rat anterior pituitary. *Endocrinology.* **119**(6), 2783-2795 (1986).

22) Bauer, J. The negative surface charge density of cells and their actual state of differentiation or activation. In *Cell Electrophoresis* ed. by J. Bauer, CRC Press, Inc., Boca Raton, FL (1994). 267-280.

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