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ELECTROPHORETIC SEPARATION OF CELLS
AND PARTICLES FROM RAT PITUITARY

NASA GRANT - NAG8-953

MID TERM PROGRESS REPORT

BY

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TABLE OF CONTENTS

I. Introduction	1
Experimental Design as Proposed	1a
Experimental Design as Modified	1b
II. CCK #1	2-13
CCK #1, bottom line	14-18
III. CCK #2	1-8
CCK #2, bottom line	9
IV. CCK #3	1-11
CCK #3 bottom line	12
V. CCK #4	1-8
CCK #4, bottom line	9
VI. Statement of Work	1
2/8/95-2/7/96	

I. INTRODUCTION

MID TERM PROGRESS REPORT

ANALYSIS OF PITUITARY CELL ELECTROPHORESIS EXPERIMENT DONE ON IML-2 (7/94)

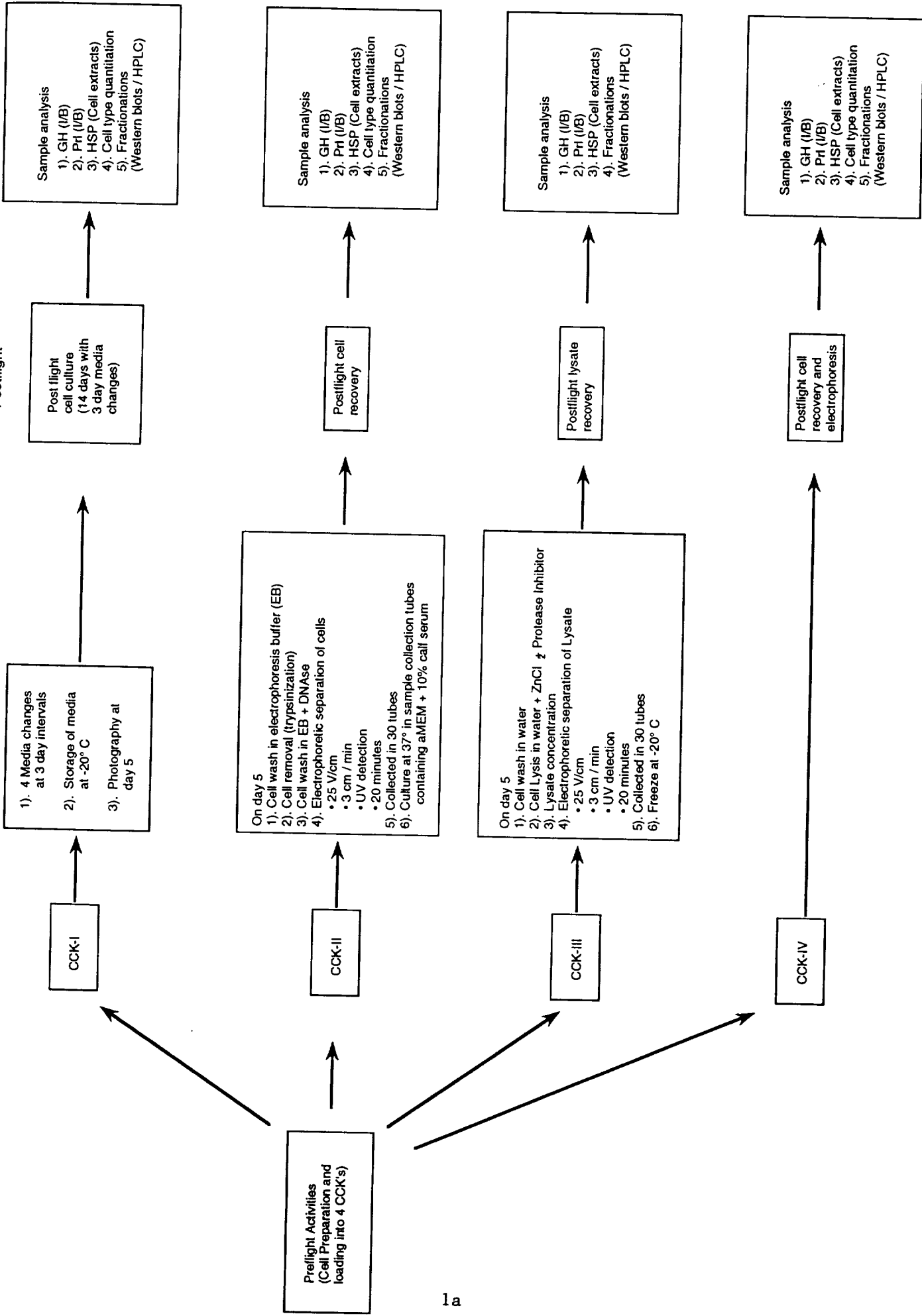
In spite of the fact that a vast majority of the electrophoresis effort (~90%) could not be done on this mission due to failure of FFEU hardware, we find some interesting differences in flight samples obtained from other parts of the experiment. These differences are entirely novel and sometimes unexpected. This report is organized into 4 parts. Each part describes the data collected thus far from each of the 4 cell culture kits (CCK) which flew in space. Each CCK was loaded with 40×10^6 fresh pituitary cells; all CCK's were identical at the start of the experiment because we prepared one pool of cells.

Design of Entire Experiment

The original design is shown on page 1a; modifications to this design were required due to the hardware failure. These modifications are highlighted on page 1b.

Inflight

Postflight



Experiment as performed

Inflight

Postflight

Sample analysis

- 1). GH (I/B)
- 2). Pri (I/B)
- 3). HSP (Cell extracts)
- 4). Cell type quantitation
- 5). Fractionations (Western blots / HPLC)

Sample analysis

- 1). GH (I/B)
- 2). Pri (I/B)
- 3). HSP (**Cell lysates**)
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Sample analysis

- 1). GH (I/B)
- 2). Pri (I/B)
- 3). Cell type quantitation
- 4). Fractionations (Western blots / HPLC)

Post flight electrophoresis and culture of fractions for 6 days with media change on day 3

Postflight cell lysis

Postflight lysate recovery

Post flight cell recovery and electrophoresis

- 1). 4 Media changes at 3 day intervals
- 2). Storage of media at -20° C
- 3). Photography at day 5

On day 8

- 1). Cell wash in electrophoresis buffer (EB)
- 2). Cell removal (trypsinization)
- 3). Cell wash in EB + DNase
- 4). **Return of cells to CCK with fresh media**
- 5). **Culture at 37°**

On day 9

- 1). Cell wash in water
- 2). Cell Lysis in water + ZnCl₂ + Protease inhibitor
- 3). Lysate concentration
- 4). Electrophoretic separation of Lysate
 - 25 V/cm
 - 3 cm / min
 - UV detection
 - 20 minutes
- 5). Collected in 30 tubes
- 6). Freeze at -20° C

CCK-I

CCK-II

CCK-III

CCK-IV

Preflight Activities
(Cell Preparation and loading into 4 CCK's)

Italicized type indicates differences from original experimental plan.

II. CCK #1

CCK #1.

Cell culture manipulations in flight were carried out *exactly* as planned. Both the astronauts in space and our technical crew on the ground at KSC performed these tasks flawlessly. No contamination was encountered. Because cell electrophoresis was a focus of this experiment, a decision was made to remove the flight cells from CCK #1 and electrophorese them at KSC postflight. This was done to determine if microgravity had any effect on cell mobility.

Synchronous ground control cells from CCK #1 could not be electrophoresed because the ground unit at KSC failed after the flight cells were run (the ground unit clogged). Therefore, the asynchronous ground control portion of this entire experiment remains to be done.

The statements listed below are based on the data obtained from samples prepared from CCK #1. A battery of analytic techniques is being used. These include (1) growth hormone (GH) immunoassay, (2) GH bioassay (bone growth in live animals); (3) HPLC (size exclusion and ion exchange); (4) image analysis of cells (flow cytometry/immunocytochemistry/cytoplasmic area hormone occupancy).

Key results thus far show that:

- In flight, cells released more immunoreactive growth hormone (GH)/day than controls (pg. 6 top).
- In flight, cells sometimes released more biologically active GH than controls (pg. 6 bottom) except in the day 2 and 11 sample. Recall that the day 2 sample actually represents 5 days of culture (i.e. 3 days pre-flight and 2 days in flight). The major loss of bioactive GH (6x) in the flight sample at day 2 (But no loss in immunoactivity) could be due to suppression induced by stress of entry into microgravity(?). Clearly, the cells are capable of secreting quality hormone in flight (i.e. they recover from this presumed entry stress). Note however that reentry had no effect on release of bioactive GH.
- Analysis of culture media by HPLC gel exclusion indicates that the major peak of immunoreactive GH (iGH) is between 59,000 and 18,000 MW (i.e. fractions 15-20, the size of monomeric and dimeric GH). Higher MW GH (>59K, fractions 7-14) is probably due to association of monomer/dimer GH with serum proteins in the culture medium. In virtually all cases the amounts of iGH were greater in F than G (pg. 7). Samples were too dilute to permit testing for biological activity.
- Analysis of culture media by HPLC ion exchange chromatography showed that all of the iGH emerged from the column quickly, indicating apparent highly positively charged species. Flight had no effect on this general behavior (pg. 8); however, note that the amounts of iGH recovered from flight samples, relative to ground, were often quite different.

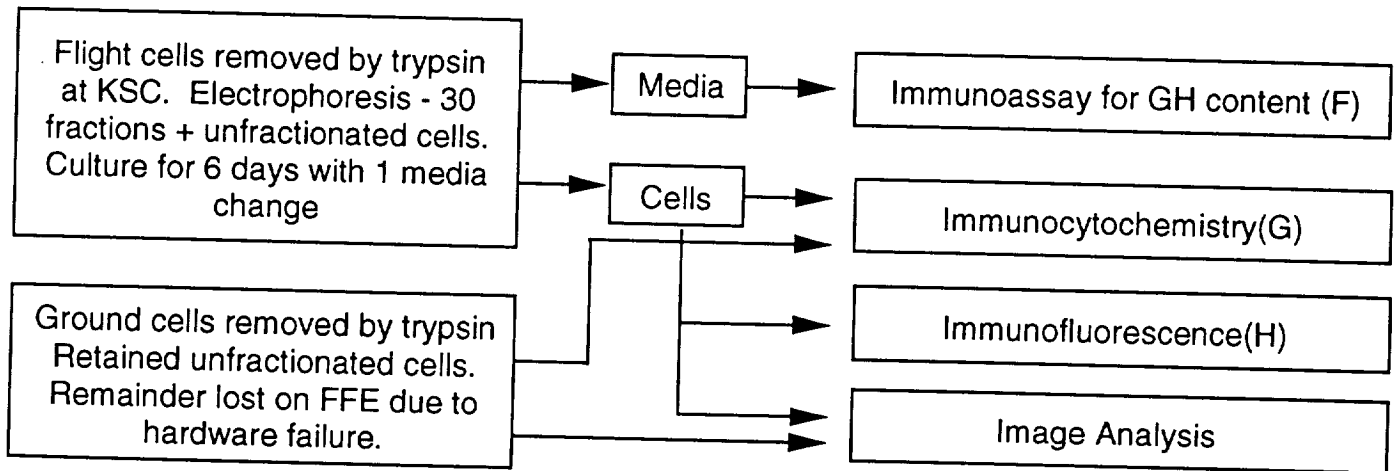
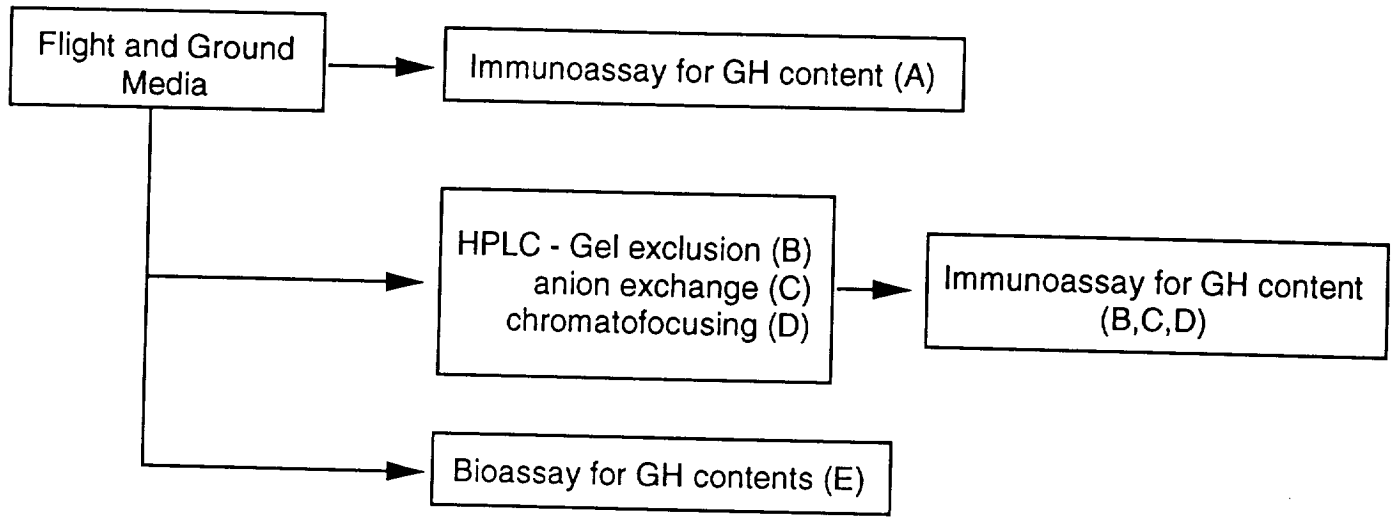
- Analysis of the culture media by HPLC chromatofocusing indicates that flight had no major effect on the isoelectric point(s) of released iGH (pg. 9).
- Postflight recovery of flight cells from CCK #1 by trypsinization at KSC yielded healthy cells which were electrophoresed on a small FFEU made for us by NASDA. The distribution profiles of total cell population (- □ -) and GH cells - ■ -) indicate that (1) anodal migration had occurred; (2) that some evidence for GH cell enrichment was found, but this was only modest at best (pg. 14). The most interesting finding was that mobility of flight cells from CCK #4 (see section 4) was very different from those in CCK #1. This result suggests either the frequency of medium change affects cell migration or (2) that flight (in conjunction with medium change frequency) affects cell migration. This issue will not be resolved further until we do the asynchronous ground control experiment (pg. 10).
- Some of the cells from flight CCK # 1 were also cultured at KSC after electrophoresis. Culture was for 6 days with no media change. Analysis of the 6 day culture media clearly show that viable hormone secreting cells were present after flight and postflight electrophoresis (pg. 10 bottom). Note that hormone producing cells are enriched in certain regions.
- Flow cytometric profiles of ground and flight cells from CCK #1 are complex (pgs. 11/12) and their full interpretation will not be given here. However, a major difference between the microscopic appearance of cells in CCK #1 vs. #4 was the marked proliferation of pituitary fibroblasts in CCK #1 but not in CCK #4. Our 3 month report to Bob Snyder offered photos of this difference. It is confirmed by the DNA distribution profiles shown on pgs. 11/12 vs. equivalent profiles from CCK #4 on pgs. 6/7 (box marked X).

The percentage area of the cytoplasm in a GH cell occupied by GH was studied on cells from CCK #1 using techniques we have developed in previous spaceflight experiments. The data on pg. 13 were collected on 200 cells/group on coded slides to avoid investigator bias. Note that ~40% of the cytoplasm of the GH cell is occupied by hormone at the beginning of the experiment (see "starts" - pg. 13). After the 14 day culture period, GH cells from both ground CCK #1 and CCK #4 had significantly lower hormone area occupancy than that initially present. However, this reduction did not happen when cells were cultured for 14 days in conventional T25 flasks. This result shows that biophysical/cytoplasmic organizational character of the GH cell on the polycarbonate surface of the CCK is very different from that on conventional plastic surfaces. Flight caused an additional (and statistically significant, $p < 0.02$) reduction in cytoplasmic area hormone occupancy that was made even more obvious by each of media changeouts.

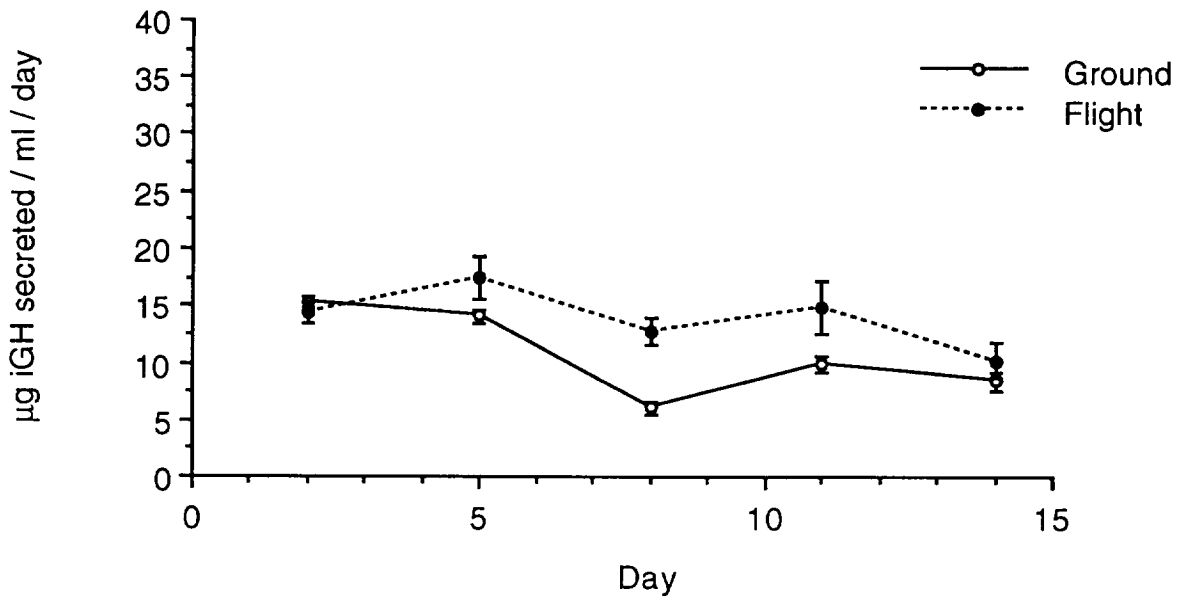
Activities in Microgravity

Proposed	Actual	% of objective achieved
CCK #1		
• Change out of media 5 X	Media changed out 5 X	100%
• Freeze spent media	Media frozen	100%
• Maintain ground operations identical to flight (eg. media change outs, temperature)	Operations at KSC were done within 15 minutes of space ops. Incubator temp within 0.2° C of flight unit.	100%
• Remove culture media on landing	Media removed	100%
• Continue culture for 14 days on ground with media changes. then extract intracellular hormone.	Flight cells recovered. Some of the cells were cultured and the rest subjected to FFE at KSC followed by 6 days in culture with media change at day 3. Cells were then stained for intracellular GH. Ground control cells were processed as the flight but failure of the FFE and loss of the cells precluded separation and continued culture. Unseparated cells were stained for intracellular GH.	5%

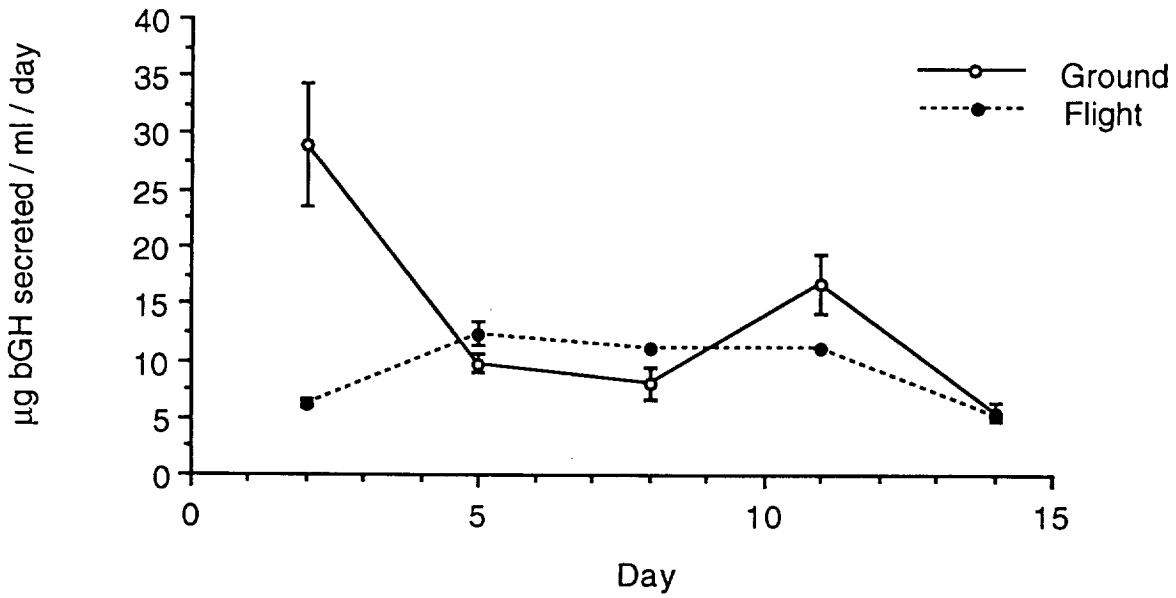
CCK #1 Growth Hormone Analysis



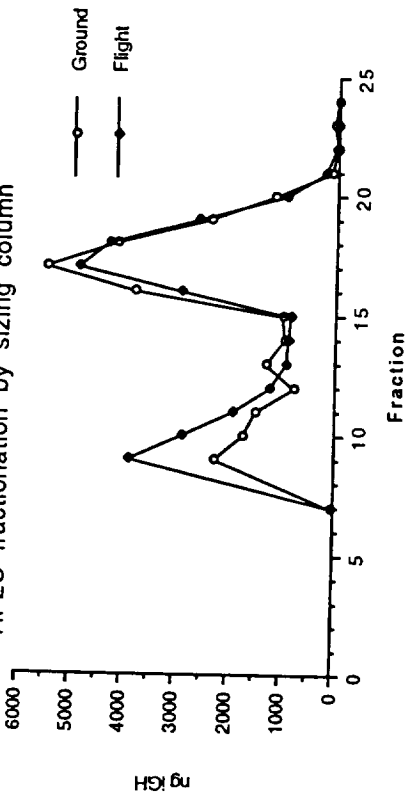
CCK #1
Media exchange
Immunoactive Growth Hormone secreted / ml / day



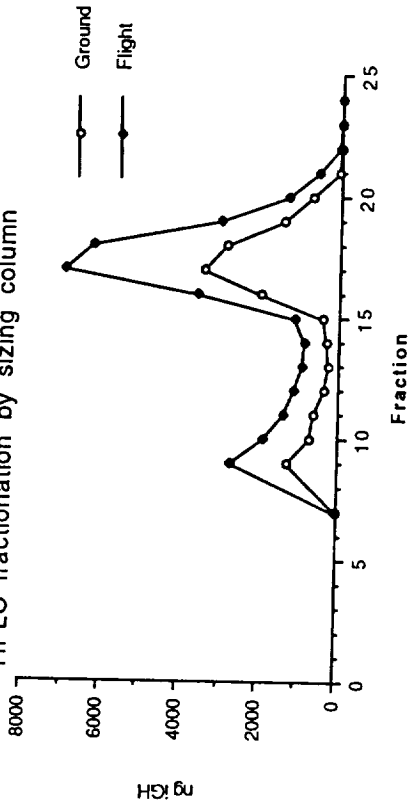
CCK #1
Media exchange
Bioactive Growth Hormone secreted / ml / day



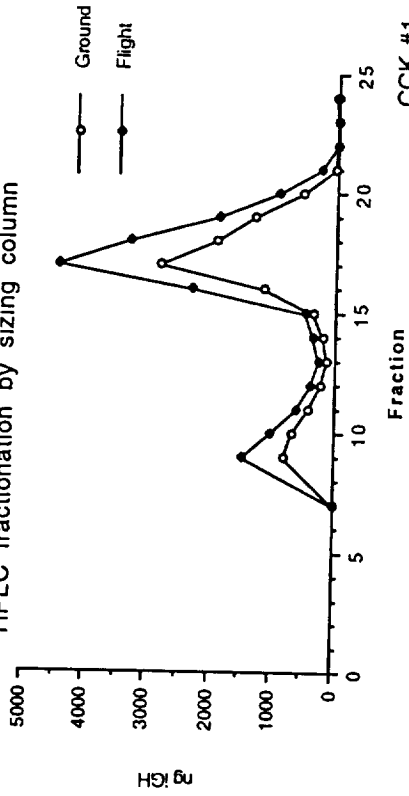
CCK #1
Media changeout on mission day 2
HPLC fractionation by sizing column



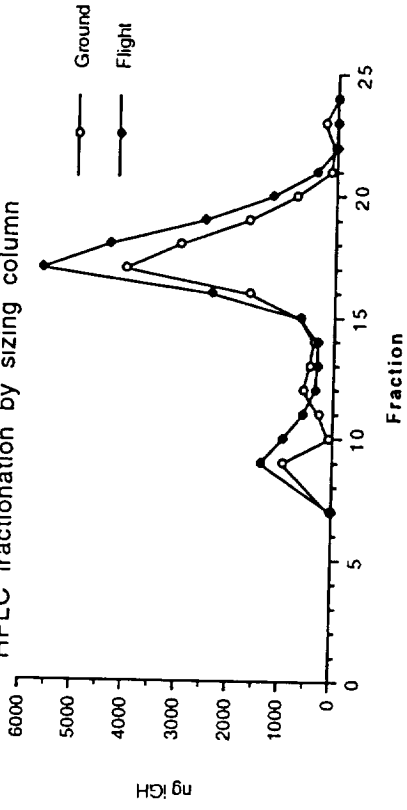
CCK #1
Media changeout on mission day 5
HPLC fractionation by sizing column



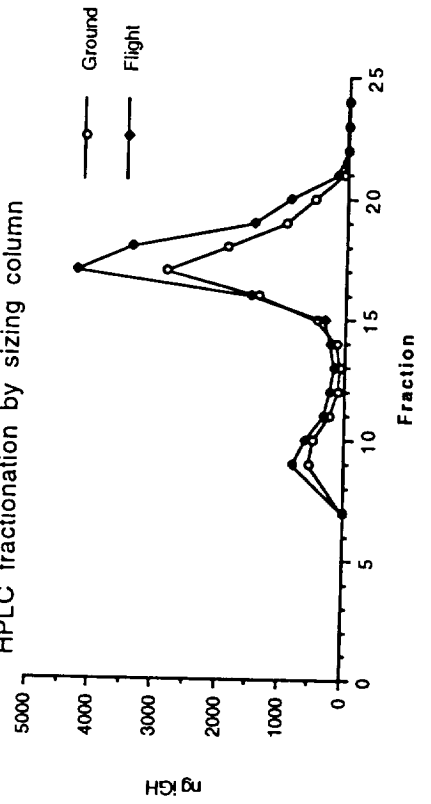
CCK #1
Media changeout on mission day 8
HPLC fractionation by sizing column

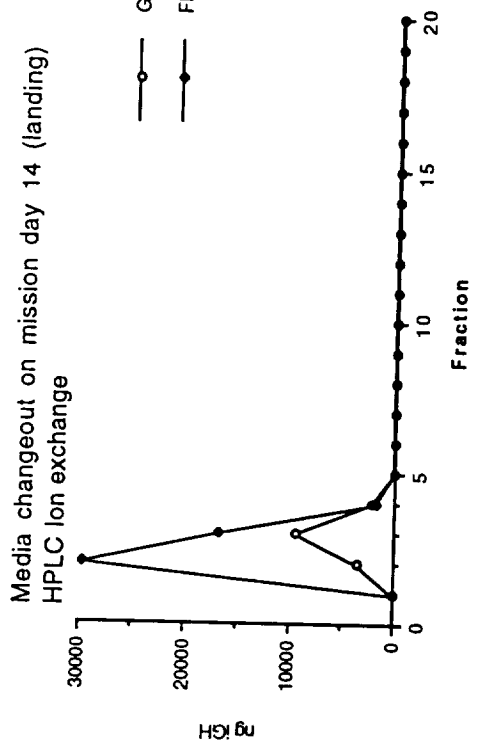
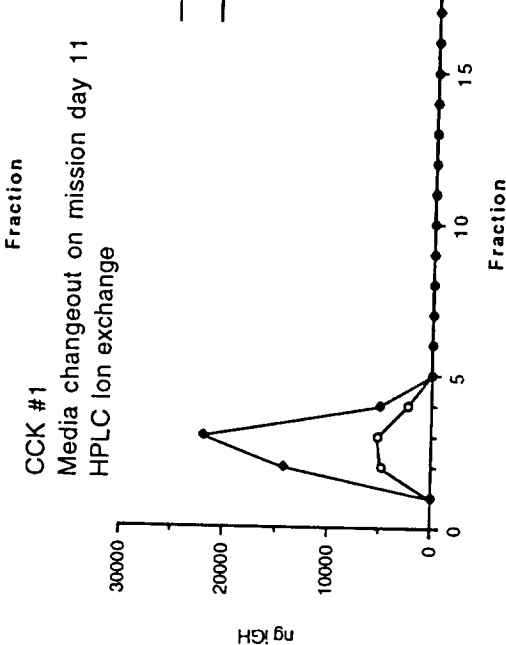
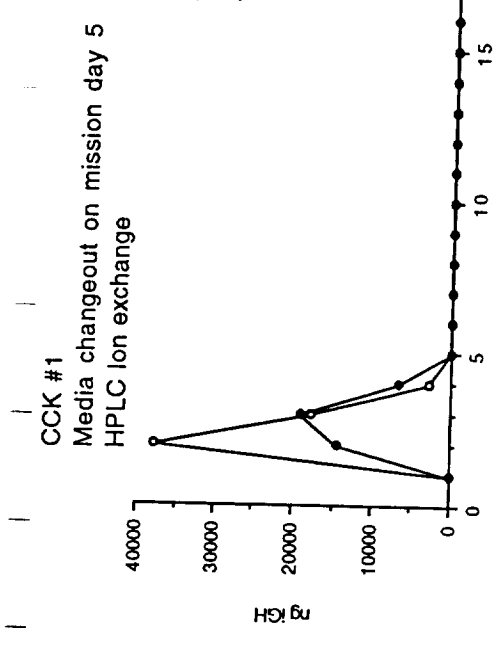
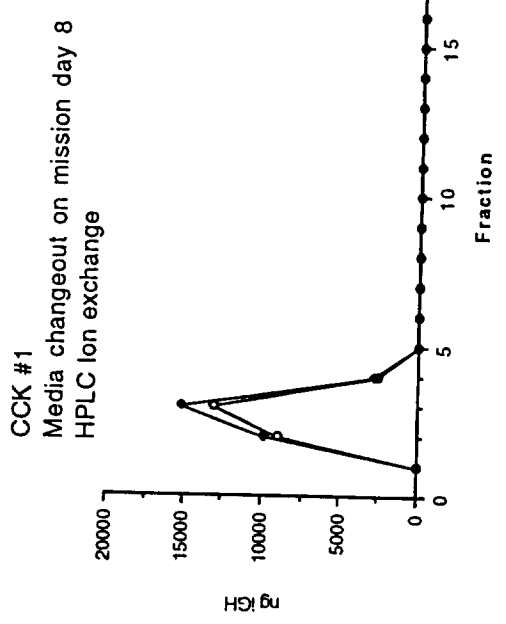
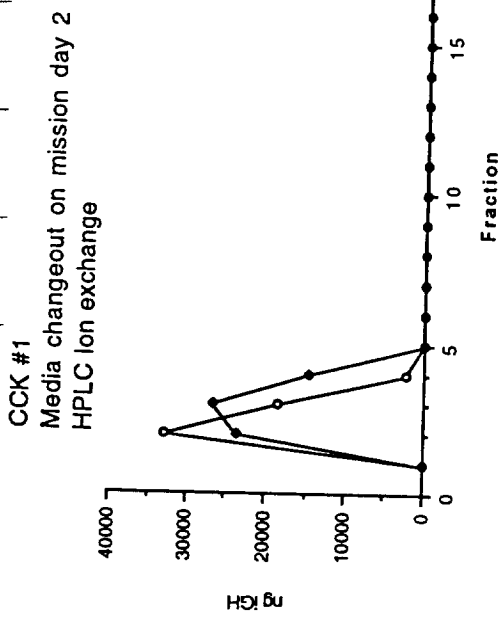


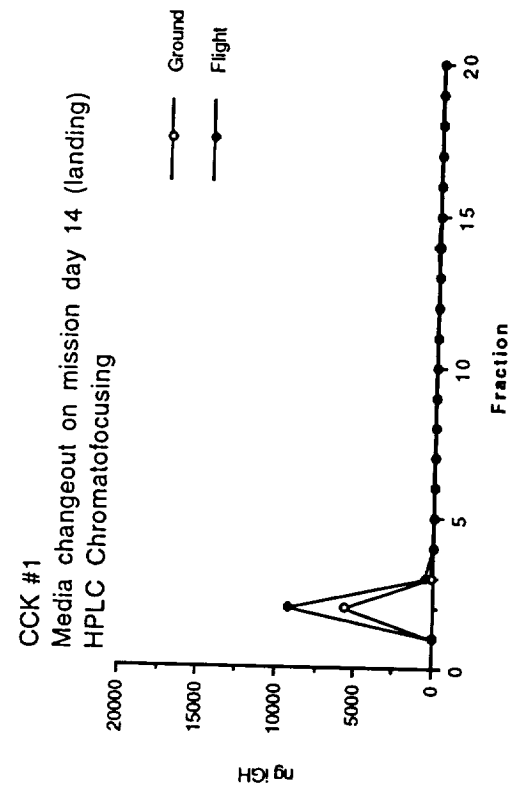
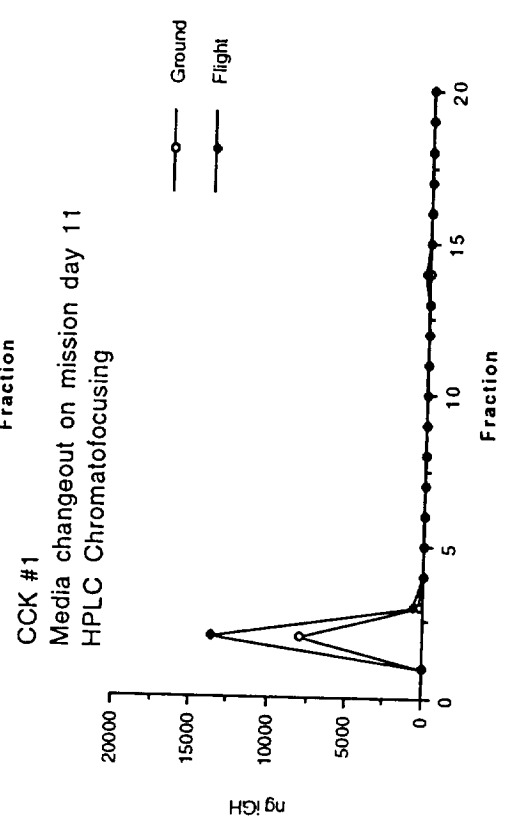
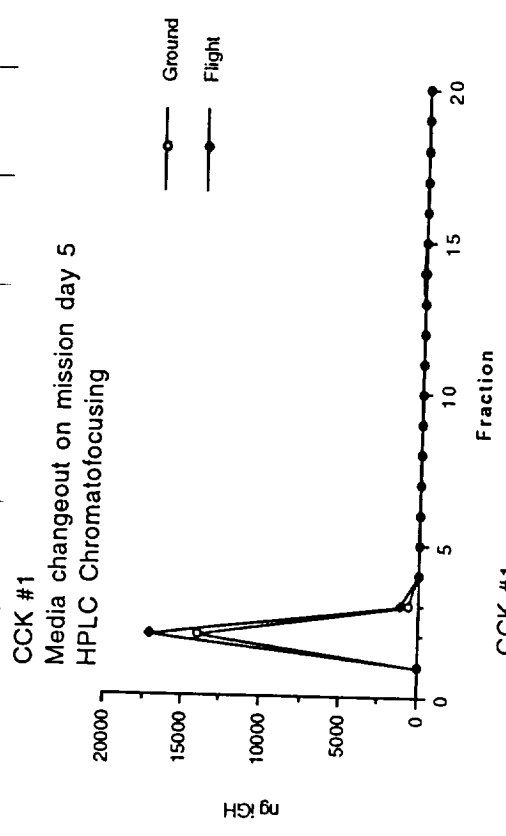
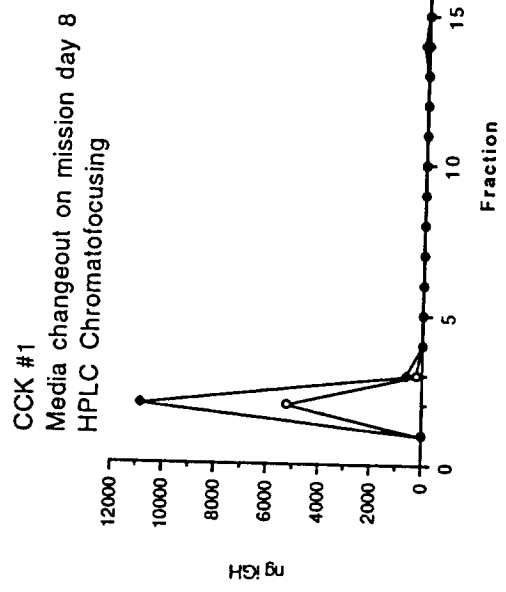
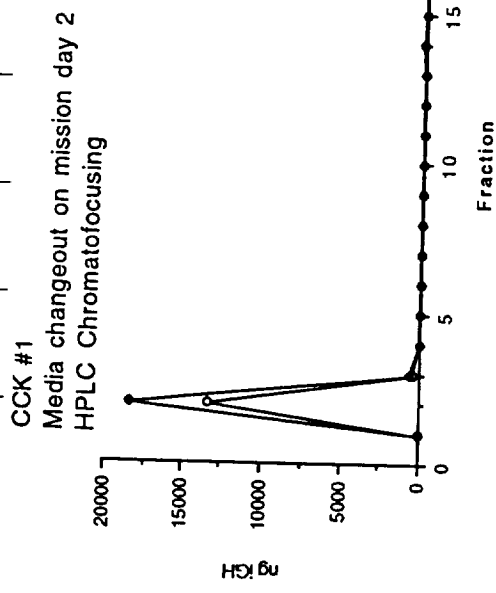
CCK #1
Media changeout on mission day 11
HPLC fractionation by sizing column



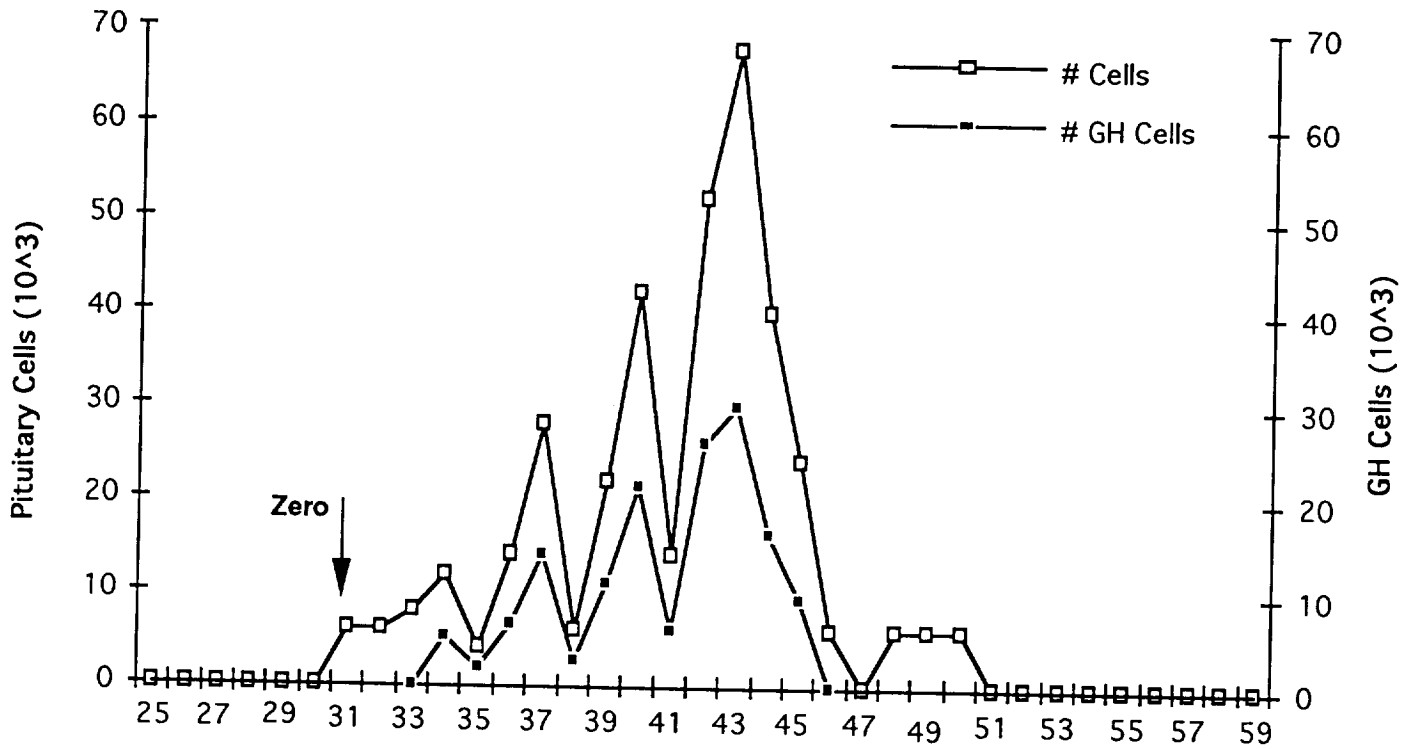
CCK #1
Media changeout on mission day 14 (landing)
HPLC fractionation by sizing column



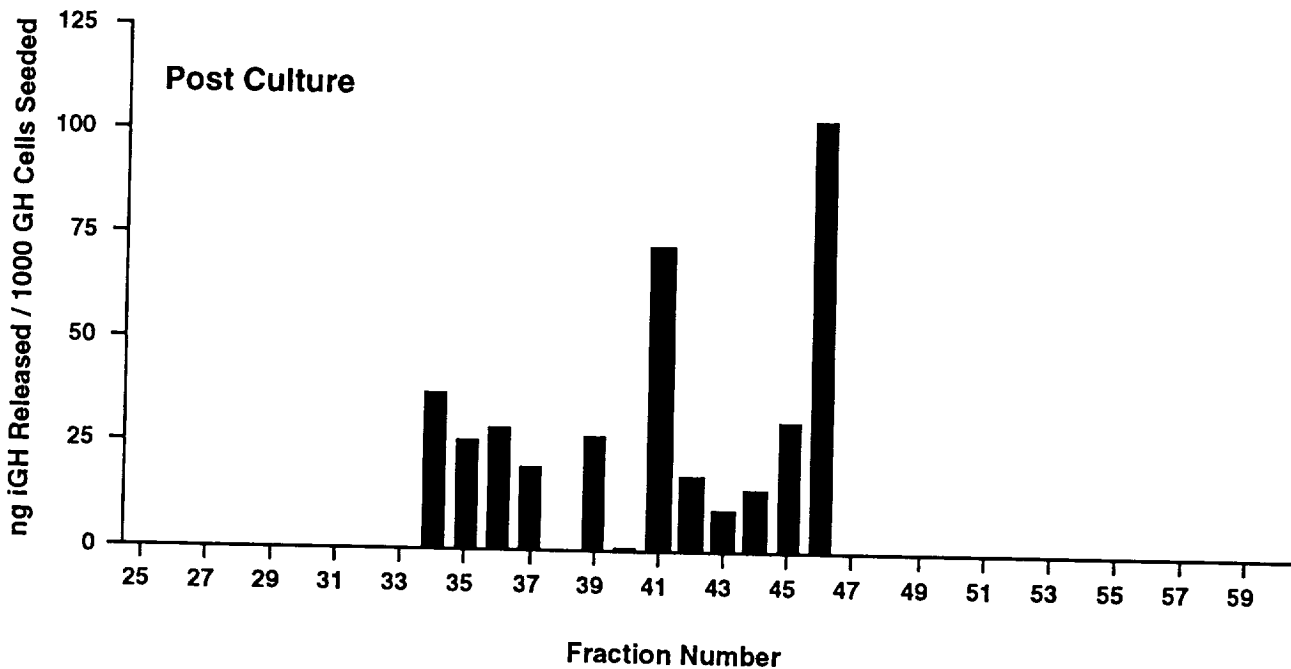




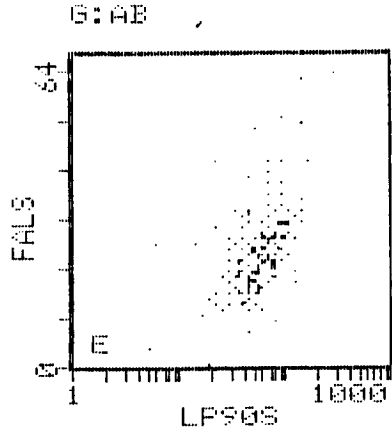
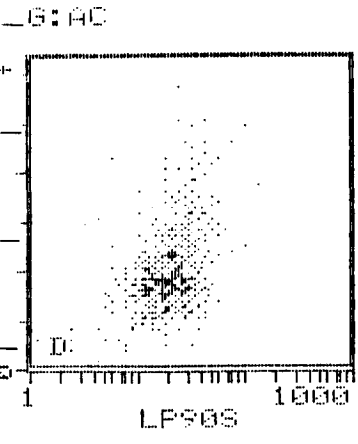
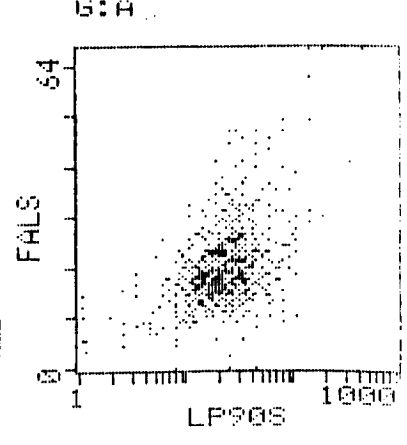
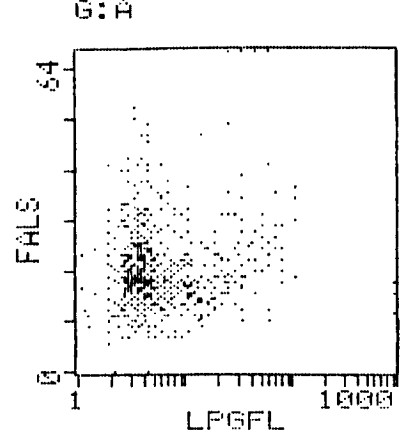
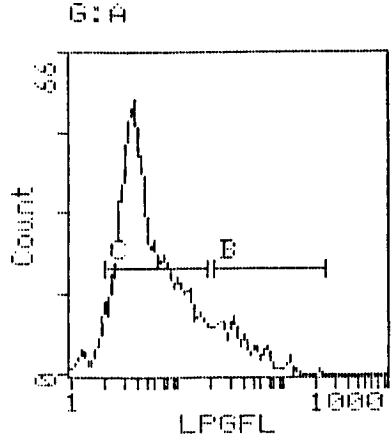
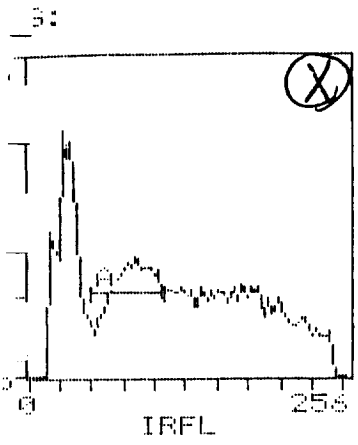
Flight CCK 1 Pituitary Cell Run Profile from ground Free Flow Electrophoresis Unit



Release of iGH from Flight Cells of CCK 1 after Ground Free Flow Electrophoresis and Ground Cell Culture



GROUND



----- SAMPLE INFO -----

PROTOCOL	: TOM	TOTAL COUNT	: 10,001	LAST HIST	:
DATA RATE	: Unknown			LIST FILE	: 251094P4.L04
INSTRUMENT	: MDADS			PROT FILE	: T0000007.PRO
SAMPLE NAME	: IML2			SAMPLE DATE	: 25Oct94
SAMPLE NUMBER	: 4			SAMPLE TIME	: 00:24
COMMENTS	: G1M				

----- STATISTICS -----

DUAL PARAMETER STATISTICS

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			Position	Height	Mean	SD	CV	Mean	SD	CV
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	100	402	50, 19	8	58.5	35.7	60.9	24.1	10.4	43.0

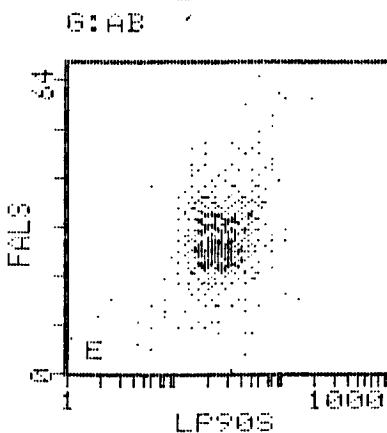
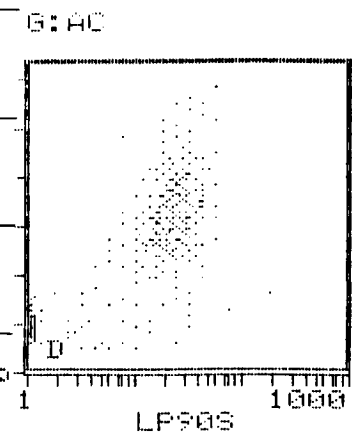
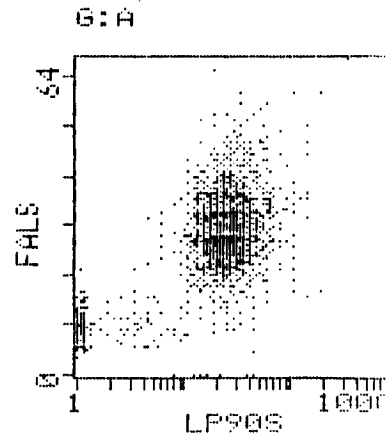
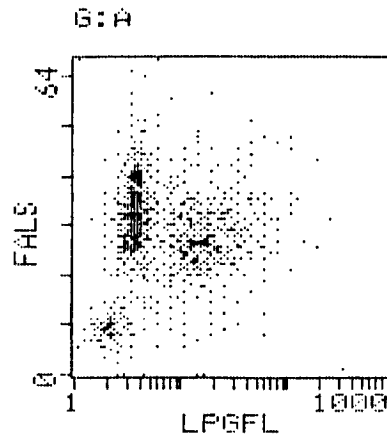
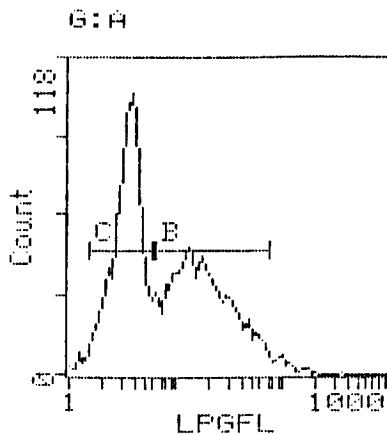
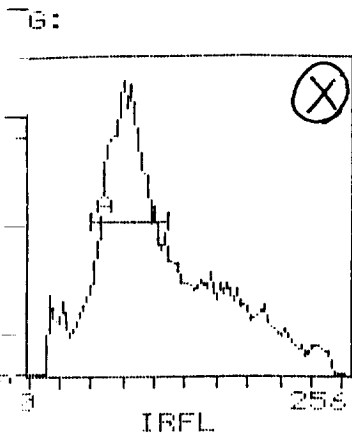
D: Low X = 1.0, High X = 1024, Low Y = 0, High Y = 63 D.

: Low X = 1.0, High X = 1024, Low Y = 0, High Y = 63 E

SAMPLE NAME : IML2
SAMPLE NUMBER: 4

FLIGHT

SAMPLE DATE: 25Oct94
SAMPLE TIME: 00:30



----- SAMPLE INFO -----

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INSTRUMENT	: MDADS			PROT FILE	: T0000007.PRO
SAMPLE NAME	: IML2			SAMPLE DATE	: 25Oct94
SAMPLE NUMBER	: 4			SAMPLE TIME	: 00:30
COMMENTS	: F1P				

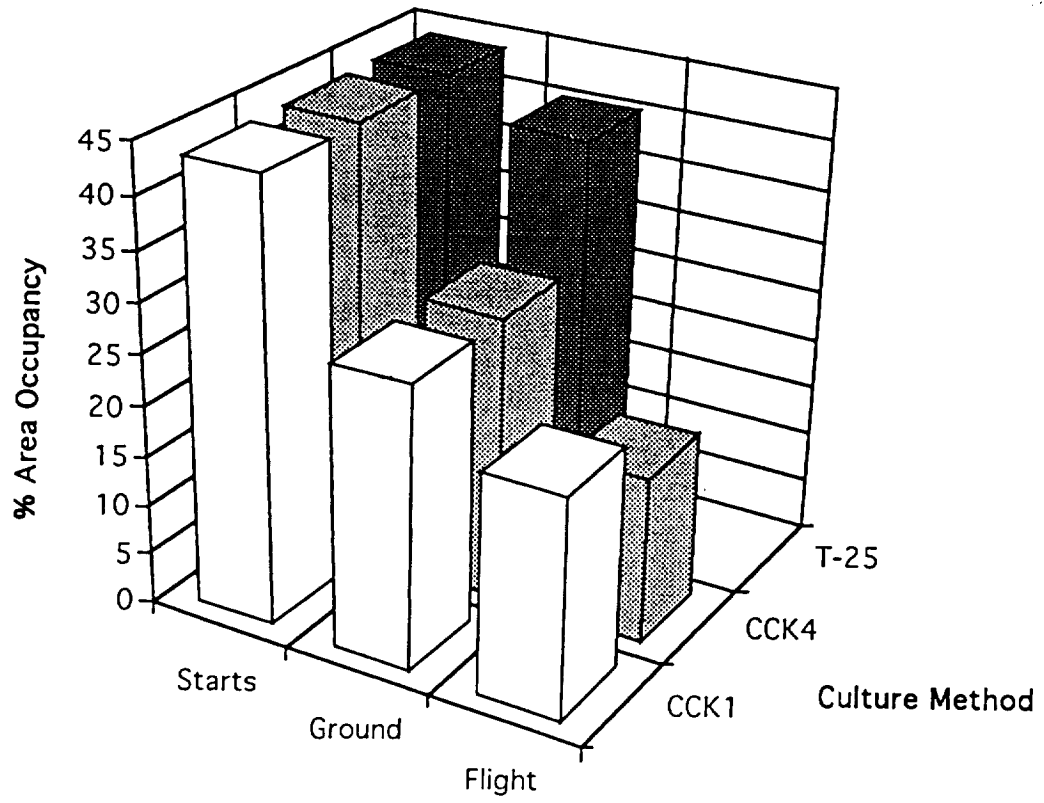
----- STATISTICS -----

DUAL PARAMETER STATISTICS

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			Position	Height	Mean	SD	CV	Mean	SD	CV
D	100	2561	1.1, 11	29	13.2	16.5	125.1	27.6	12.6	45.7
E	100	2715	23, 23	20	27.1	17.0	62.8	27.5	8.5	31.1

Low X = 1.0, High X = 1024, Low Y = 0, High Y = 63 D
 Low X = 1.0, High X = 1024, Low Y = 0, High Y = 63 E

IML-2 Pituitary Cell Area Occupancy



Summary of CCK #1. Results to Date: The Bottom Line and Tentative Conclusions.

- The "stress" of getting into orbit affects the output of bioactive GH.
- Spaceflight appears to modify charges, but not the size, of the secreted immunoreactive GH.
- Cell growth behavior on the polycarbonate surface of the CCK is different than on conventional plasticware.
- Media changes in CCK #1 (on both ground and flight units) resulted in vigorous fibroblast growth that was not seen when media changes were not done.
- The frequency of medium changes affects electrophoretic mobility; we do not yet know if this is a flight effect.
- Frequency of cell feeding on the amount and activity of growth hormone (GH) released from cells in the different CCK's.

The original design of this experiment allowed us to determine microgravity's effect on hormone production as a function of the number of cell culture media changes done during the 14 day flight. It is important to indicate that all of the bioassay data in this report were provided by Dr. R. Grindeland of NASA Ames. These assays are involved in that they require large numbers of animals which have had their pituitary glands surgically removed. Thus far, 180 assay animals have been used. Based on data collected from CCK #1 (4 media changes); CCK #2 (1 medium change) and CCK #4 (0 change), the results thus far allow us to make the following conclusions (see pg. 16):

- On Earth, the amount of bioactive GH (bGH) released is directly related to the number of media changes performed.
- In microgravity, the amount of bGH released is reduced by ~50% (relative to ground controls) when media changes are done.
- Media change frequency in microgravity has relatively little effect on the immunoreactive GH (iGH) but
- When media changes are not done (CCK #4), flight GH cells behave very differently. Spaceflight accentuates the production of iGH. Sometime during the 14 day culture period they apparently "convert" to producing GH that is more immunoreactive. This is not true for ground cells.

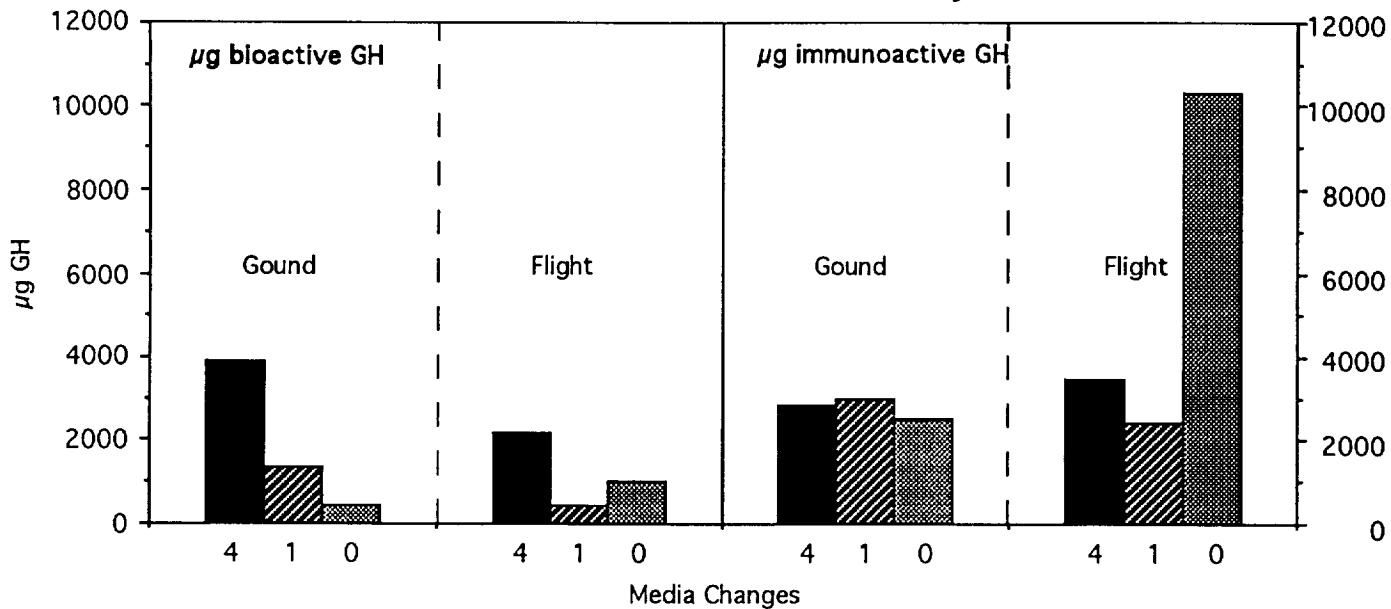
- Spaceflight negatively affects the quality of hormone released. Thus the biological/immunological (B/I) activity ratio of the total secreted GH is usually reduced by at least 50% (bottom panel, pg. 16).
- Variables of both media change frequency and spaceflight affect the B/I of GH released.
- From CCK #1 data it appears that the cyclical nature of GH quality (i.e. B/I) secreted on the ground disappears in spaceflown cells (middle panel, pg. 16).

Characterization of GH released from CCK #1 by HPLC Ion Exchange Chromatography

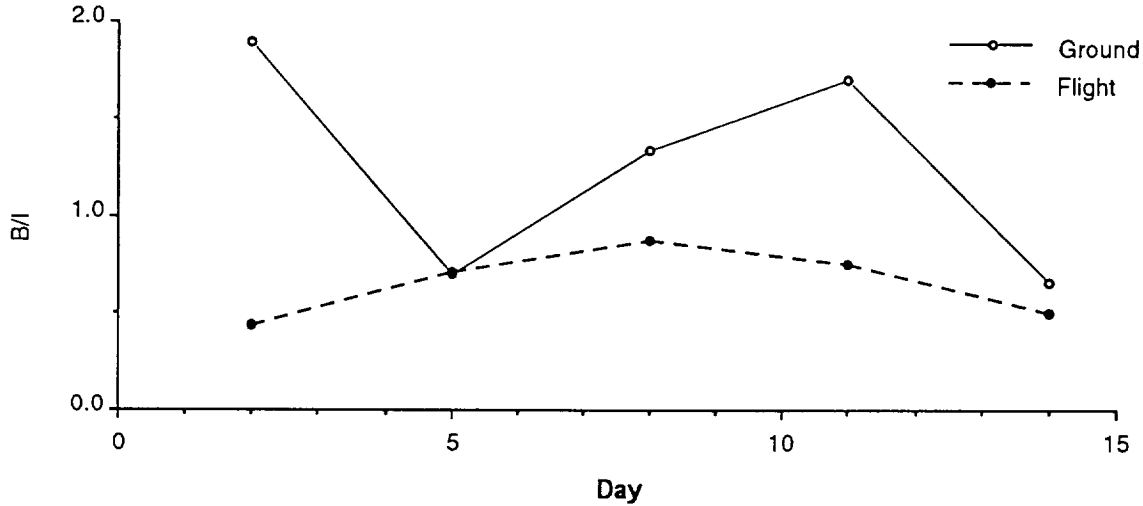
We were somewhat surprised by the elution profiles of iGH which are summarized on page 8. These profiles suggested that the iGH was highly positively charged in both flight and ground samples, but that spaceflight might affect the concentrations of GH in fractions #2 and #3. Accordingly these were subjected to extensive bioassay. These results, and their comparisons with the immunoassay results, are summarized in 4 panels on pg. 17 and the table on pg. 18. Collectively these data show that:

- Early in the culture period (day 5) there is a large increase in bGH output in frs. 2 and 3 from the ground.
- Later into culture (day 14) more bGH is present in these fractions from flight samples.
- The B/I ratios of fraction #2+3 GH are always >1 , usually the ratios of the flight samples are less than the ground samples (pg. 18).

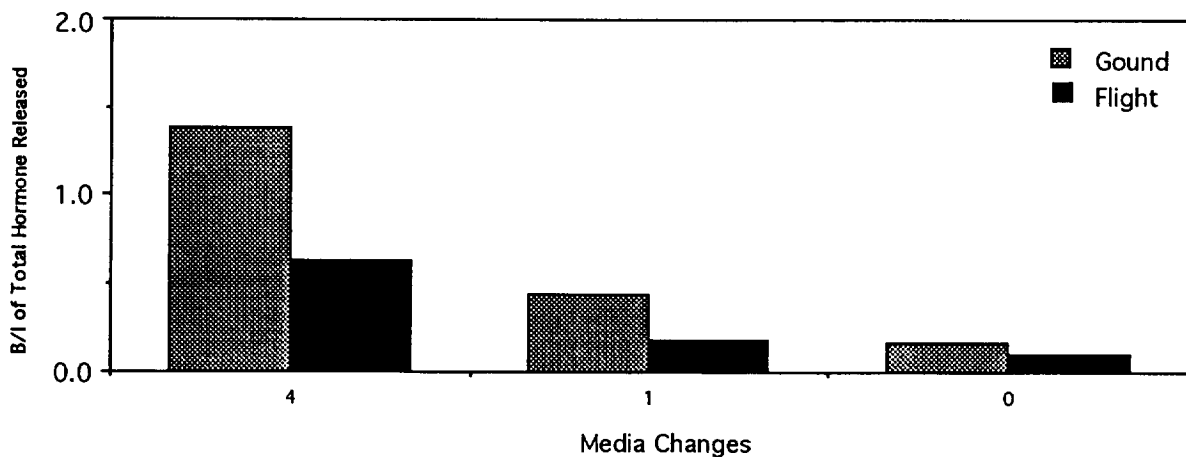
Total Hormone Released over 14 days



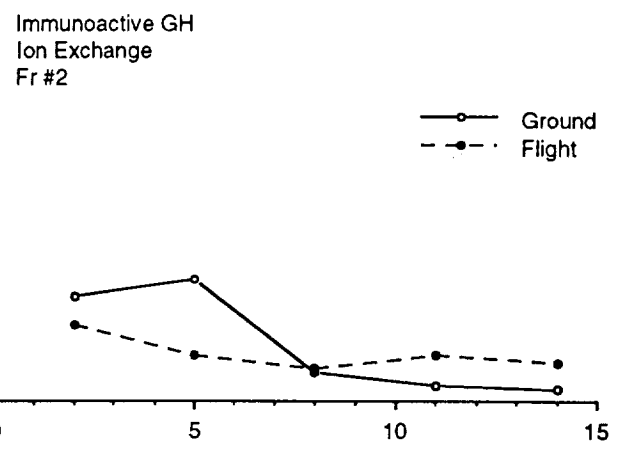
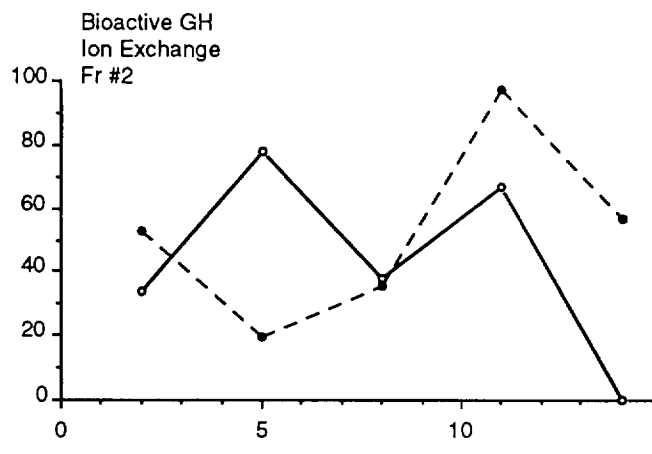
CCK #1
Media exchange
Bioactive / Immunoactive ratio



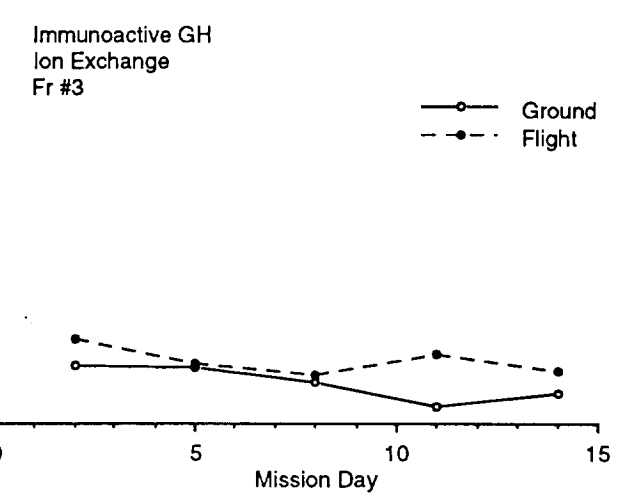
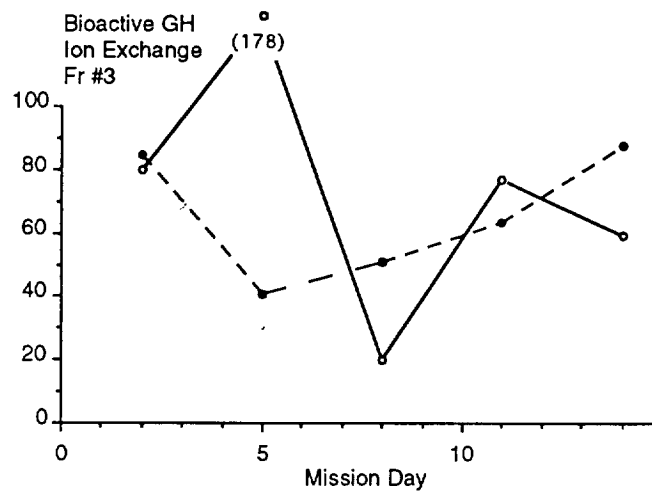
B/I of total hormone released over 14 days



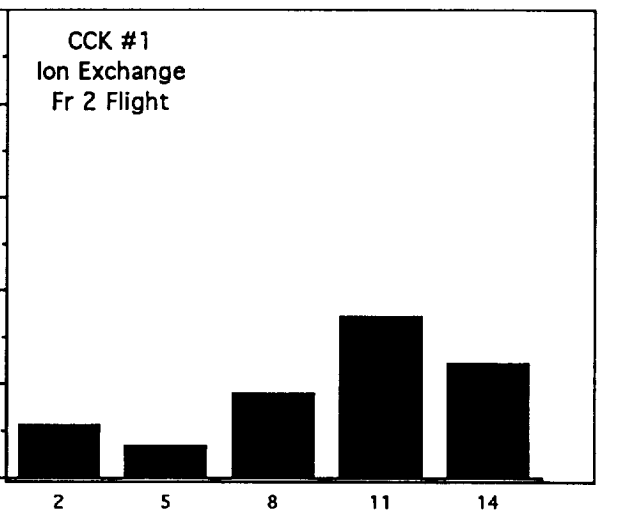
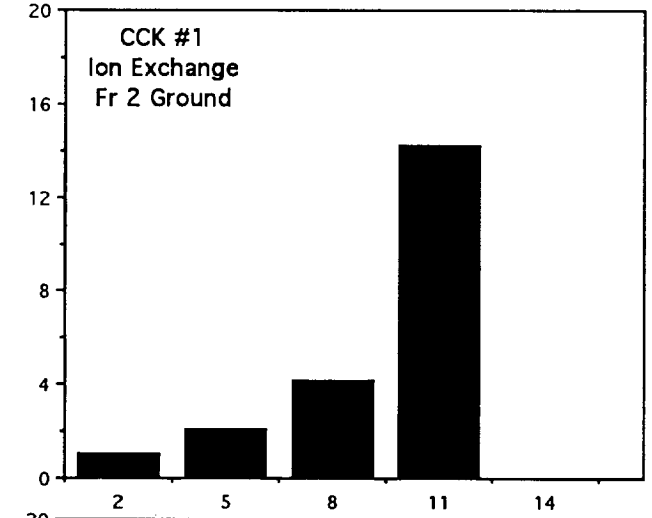
1



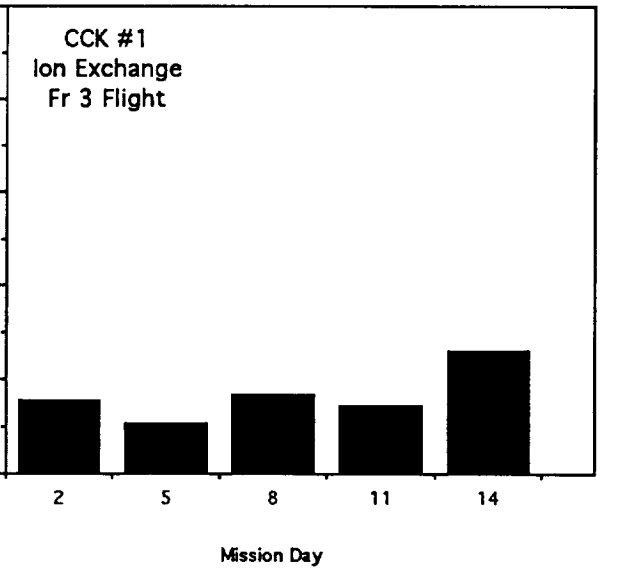
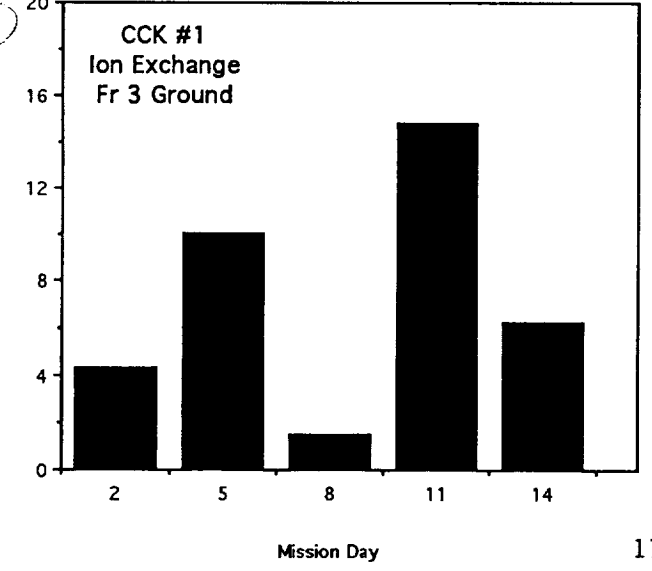
2



3



4



Total Concentrations of bGH and iGH in media from CCK #1
 After HPLC Ion Exchange Chromatography:
 Combined Fractions #2 and #3

Day	Bioactive GH ($\mu\text{g/ml}$)			Immunoreactive GH ($\mu\text{g/ml}$)		
	Flight	Ground	Flight/ Ground	Flight	Ground	Flight/ Ground
2	137	114	1.20	51	52	0.98
8	86	58	1.48	25	22	1.13
11	161	143	1.12	36	10	3.6
14	144	59	2.44	28	13	2.2

B/I Ratio of GH Contained in Combined HPLC Fractions #2 and #3

Day	Flight	Ground
2	2.68	2.18
8	3.45	2.63
11	4.47	14.3
14	5.14	4.54

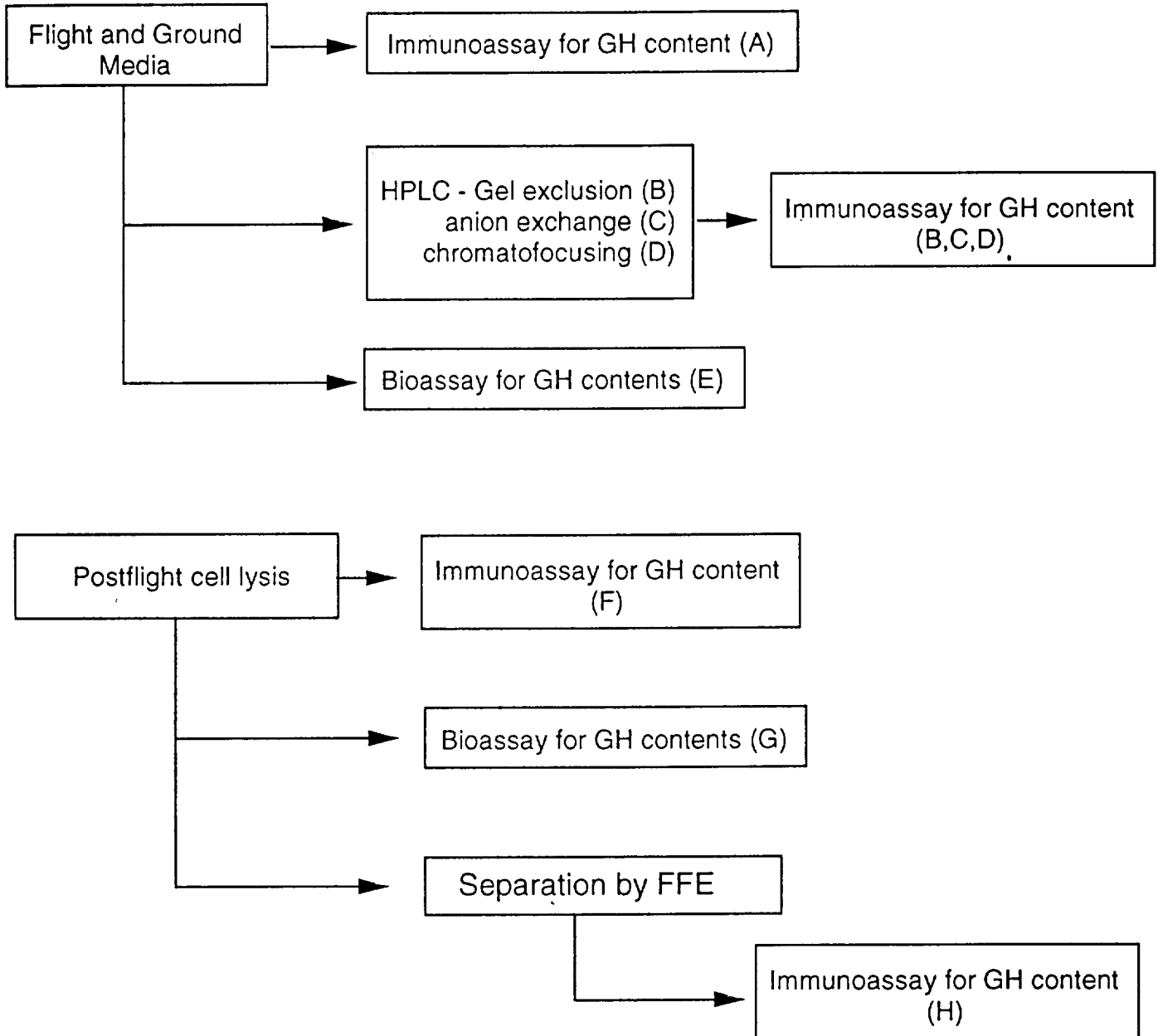
III. CCK #2

CCK #2

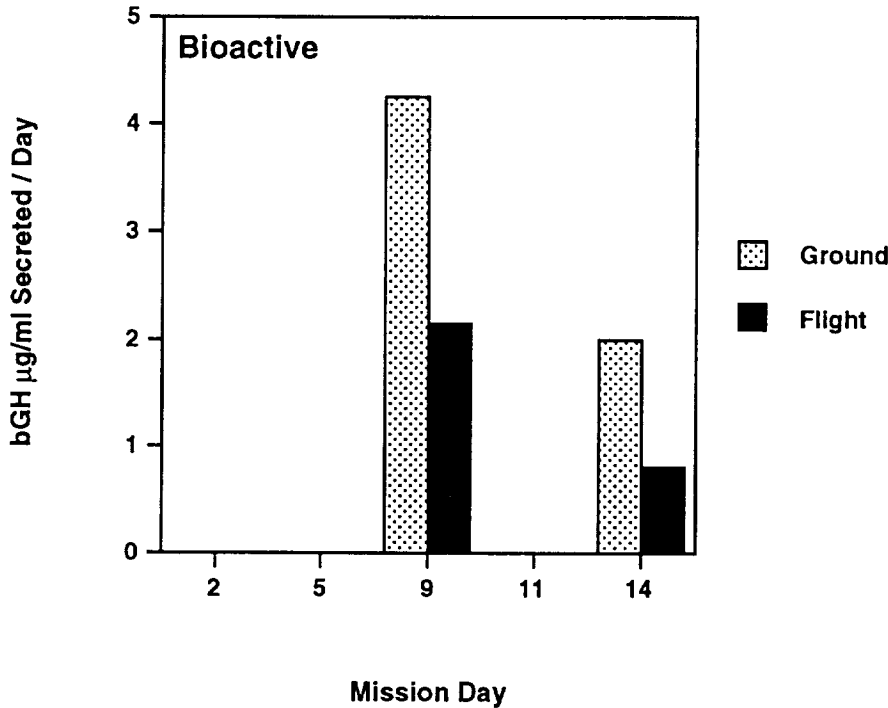
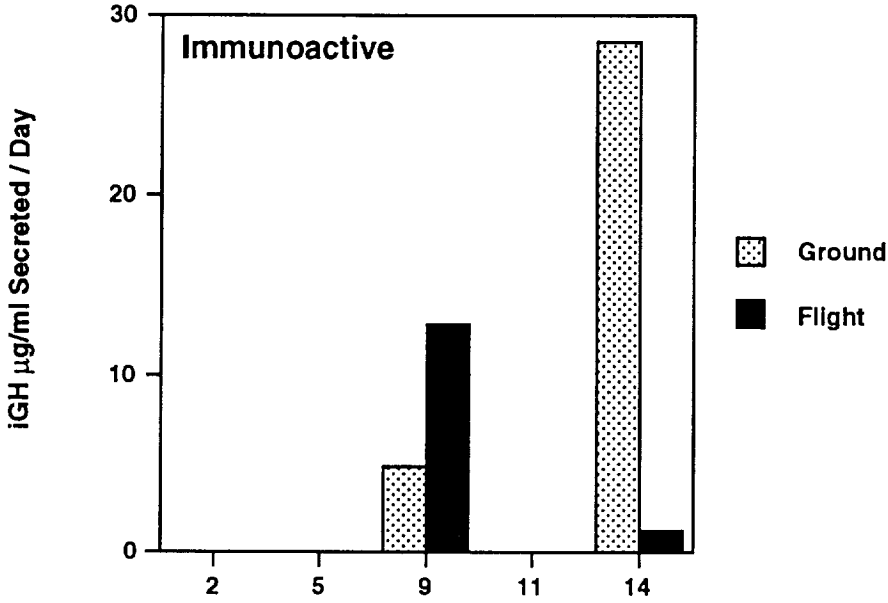
- Because live cells were present in CCK #2 after spaceflight and because no microbial contamination was present on landing, it is obvious that the trypsinization of cells from the CCK and their reinsertion back into the CCK (glovebox operations) were done successfully by the Payload Specialist in microgravity.
- Media changes on day 9 again show that more immunoreactive GH is released from flight cells than ground (compare pg. 4, CCK #2 vs. Pg. 6, CCK #1).
- After cell trypsinization and reinsertion back into CCK #2, ground cells continued to release iGH; however secretion of iGH from flight CCK #2 was greatly reduced (pg. 4). At this point we have no explanation for this result. Release of bGH was lower from flight CCK #2.
- Analysis of mission day 9 media by HPLC sizing chromatography indicated that apparent molecular weights of iGH between ground and flight were not affected, but much more monomeric GH was in the flight media sample (pg. 5).
- Analysis of mission day 14 media by HPLC sizing chromatography shows that while the size profile of the iGH from the ground sample was similar to that of day 9 sample (cf pgs. 5 vs.6), the size profile of the iGH molecules in the flight sample changed. Thus, more high molecular weight material is present in this sample (pg. 6, insert), even though the amount is very much less.
- Osmotic lysis of cells recovered from CCK #2 after landing again shows a difference in apparent sizes (pg. 7) and charge (pg. 8) of iGH.

Proposed	Actual	% of objective achieved
CCK #2		
• Astronaut takes photomicrographs of cells on MD 5	Photomicrographs taken, one roll accidentally exposed on orbit, rest of photo's not useable	2%
• Media removed and stored on MD 5	Media removed and stored on MD 8	100%
• Cells removed from CCK by trypsin on MD 5	Cell removed by trypsin on MD 8 (glovebox)	100%
• Trypsinized cells on flight FFE	Not done, hardware malfunction and crew time limitations	0%
• Separated cells into culture.	Trypsinized cells back into CCK for continued culture.	20%
• Separated cells extracted on landing	Recultured cells lysed on landing because volume of lysate from CCK 3 was limiting. Science required more sample for μg effects on intracellular events.	25%
• Synchronous ground operations.	Ground ops performed within 2 hours of flight operations.	100%

CCK #2 Growth Hormone Analysis



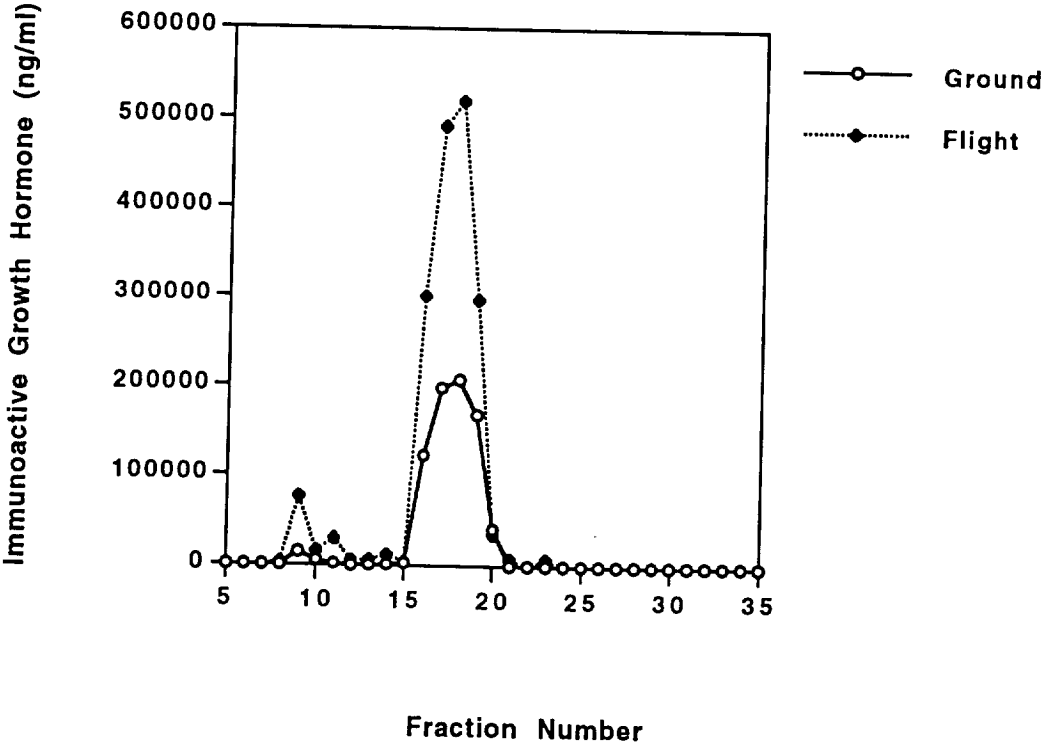
CCK 2 Media Recovered



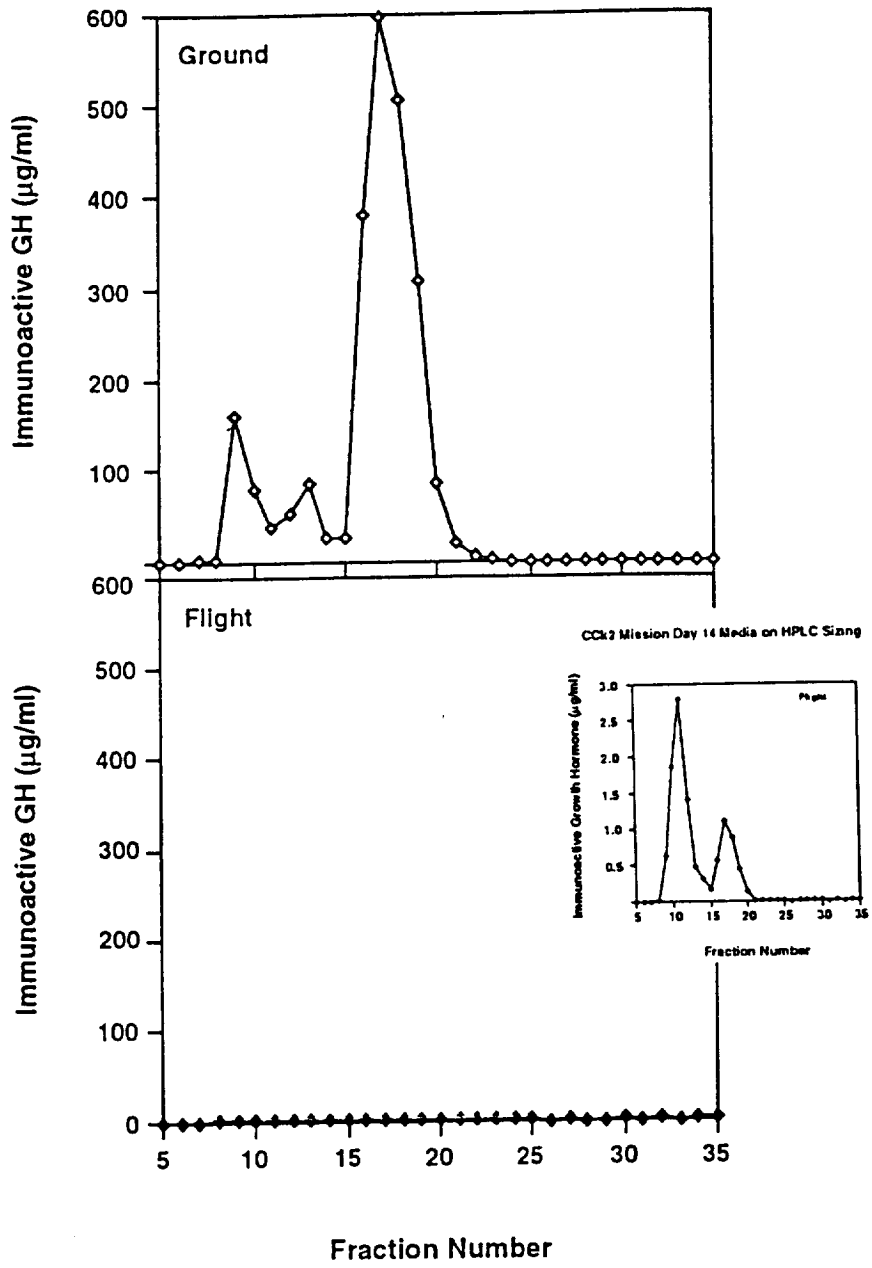
Mission Day

*Note Scale Difference

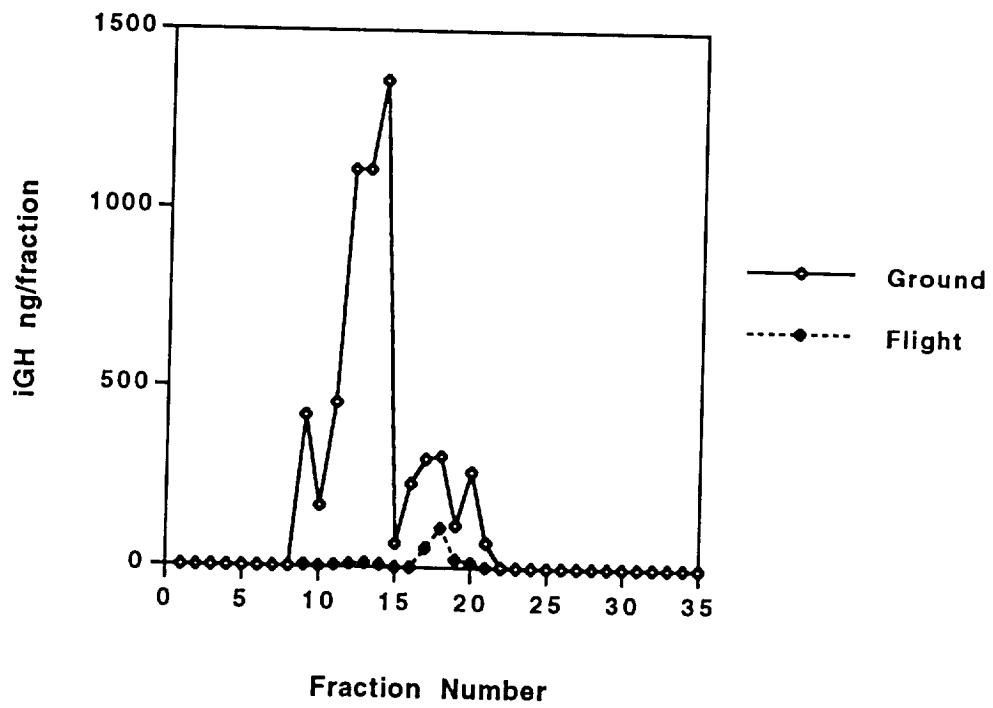
CCK 2 Mission Day 9 Media on HPLC Sizing



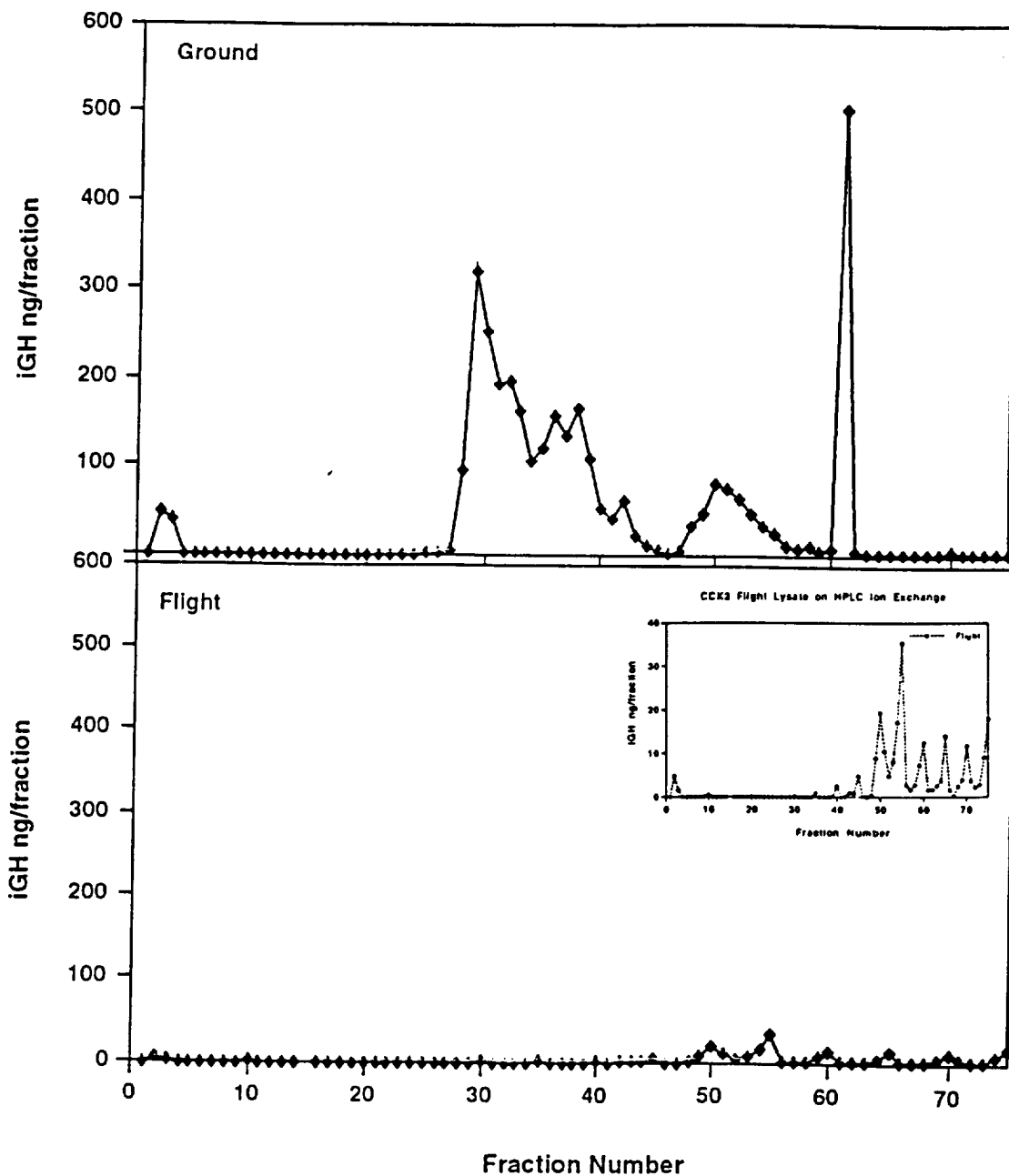
CCK2 Mission Day 14 Media on HPLC Sizing



CCK2 Lysate on HPLC Sizing



CCK2 Lysate on HPLC Ion Exchange



Summary of CCK #2. Results to Date: The Bottom line and Tentative Conclusions.

- The astronaut was highly efficient, effective and successful in performing the many complex manipulations that were required on several pieces of hardware.
- After trypsinization, GH cell behavior appears to be quite different in space than on the ground. These differences are most evident in:
 - a) the amount of iGH released into the culture medium
 - b) the apparent size of the released iGH and
 - c) the apparent size and charge of the iGH retained within the cells.

IV. CCK #3

CCK #3

The reader will understand that (a) electrophoresis of pituitary cells and pituitary subcellular particles in microgravity was the primary goal of this experiment and (b) that the FFEU hardware failed on the IML-2 mission. The reader also knows that a major percentage of the actual experiment time necessarily had to be sacrificed for crew in-flight maintenance procedures. In spite of these severe limitations, one electrophoresis trial on the subcellular particles prepared from CCK #3 was attempted in microgravity. The reader may appreciate that the PI and his team (including NASDA personnel) made quick, real time decisions with regard to selection of the 30 tubes for collection knowing full well that some exit ports might be blocked by air bubbles in the separation chamber. The following descriptions of the data collected on these 30 fractions thus far offer the HINT that some fractionation may have occurred in microgravity.

- Pg. 3 - suggests that apart from the actual FFE run, many experimental objectives dealing with CCK #3 were achieved.
- Pg. 4 - downlinked UV absorption data during electrophoresis run (top panel) led the PI to select frs. 8-13 and frs. 24-47 for collection. On return to Penn State some fractions contained close to their nominal 2.5 ml, while many did not (bottom panel). Is this evidence for bubble blockage??
- Pg. 6 - The concentration of iGH in the culture media of CCK #3 shows that more immunoactive hormone is released from flight cells than ground. This result is entirely consistent (and very similar to) that found in culture media from the CCK #2 sample. However, the result with bGH is different between CCR #2 and #3. We do not know why.
- Pg. 7 - Details of FFE operating conditions demonstrate one advantage of microgravity processing by FFE.
- Pg. 8 - distribution of iGH in FFE fractions. Greater bandspread and more GH peaks suggest better separation in microgravity.
- Pg. 9 - Western blots of iGH molecules contained in different FFE fractions. These are non-reducing SDS-PAGE gels; all bands represent different molecular weight forms of GH. These are the result of aggregation/splicing variants. Some evidence for differences in high MW variants in different flight fractions is seen (arrows). Densitometry will be done on these blots to identify and quantify these differences.
- Pg. 10a,b,c, - HPLC gel sizing chromatograms (OD 280) of pituitary cell lysates prepared in space (10a) or on earth (10b). The difference spectra

(10c) indicate some significant protein profile differences between ground and flight.

- Pg. 10d-k - HPLC sizing chromatograms of proteins contained in FFE fractions prepared in space or on earth. Note that some protein material present in the starting sample (e.g. that at retention time 16.6 min, pg. 10a,b) fails to appear after the FFE run.
- Pg. 10L - Superimposed protein profiles of flight only FFE fractions after HPLC. Clear-cut differences in these profiles support the thesis that separation on the FFEU was occurring in microgravity.
- Pg. 10m-s - HPLC ion exchange chromatographic profiles of proteins extracted from FFE fractions (ground and flight). These have not been evaluated for additional evidence of separation.

Proposed	Actual	% of objective achieved
CCK #3		
• Media removed and stored on MD 5	Media removed and stored on MD 9	100%
• Cell lysis / granule concentration	Accomplished, volume after concentration unknown. †	90%
• Lysate on FFE in μg and collection of 30 fractions with 5 mls each	Lysate on FFE and collection of 30 fractions in microgravity. ††	30%
• Lysate fractions frozen at -20°C in microgravity.	Lysate fractions frozen at -20°C in microgravity.	100%
• Perform synchronous ground operations.	Ground operations performed within 15 minutes of flight operations.	100%

† Estimated volume on basis of recovered fluid.

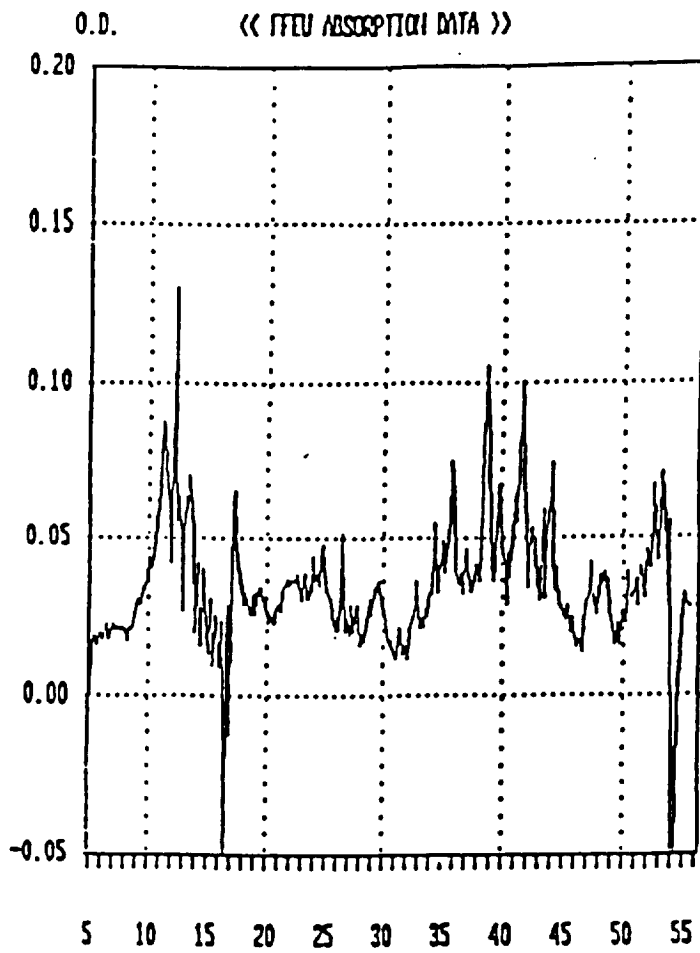
a) 28 minutes of FFE run time (8' no field/20' @ 25 v/cm) \approx 1 ml.

b) Volume in FFE sample syringe \approx 1 ml

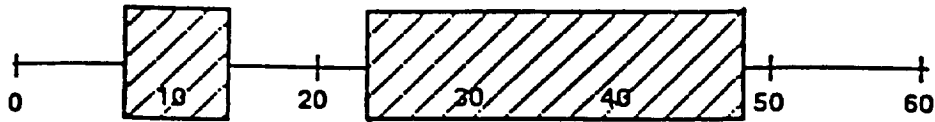
c) Volume remaining in CCK#3 which did not get concentrated \approx 2.5 ml.

†† Bubbles in separation chamber at time of run. Downlink optical density profile (see page 22) suggests sample flow with and without field. See Fig 5. for additional information.

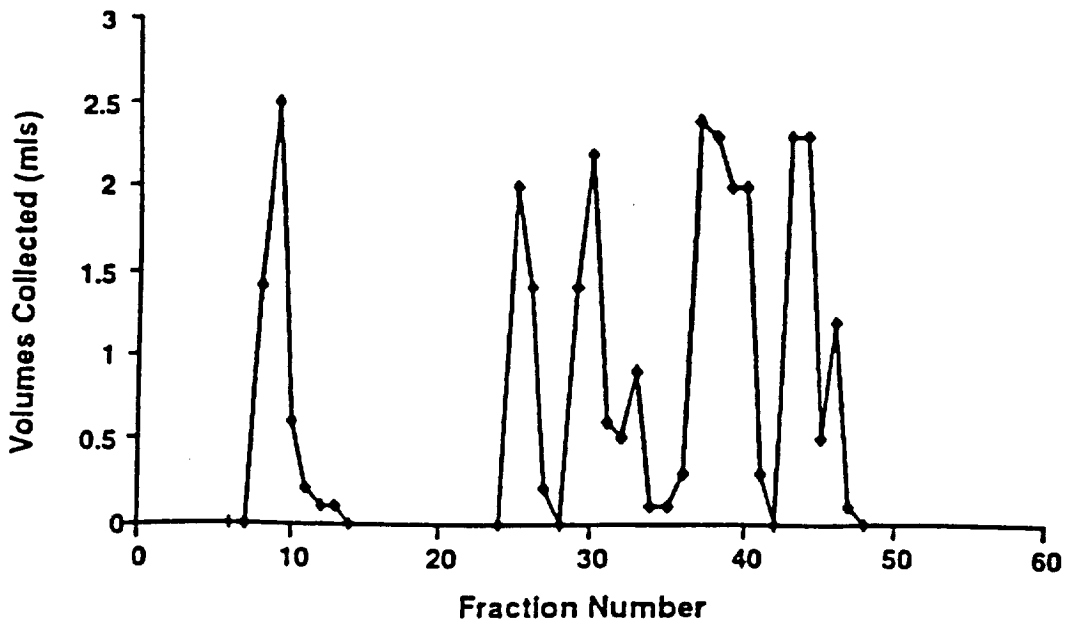
A).



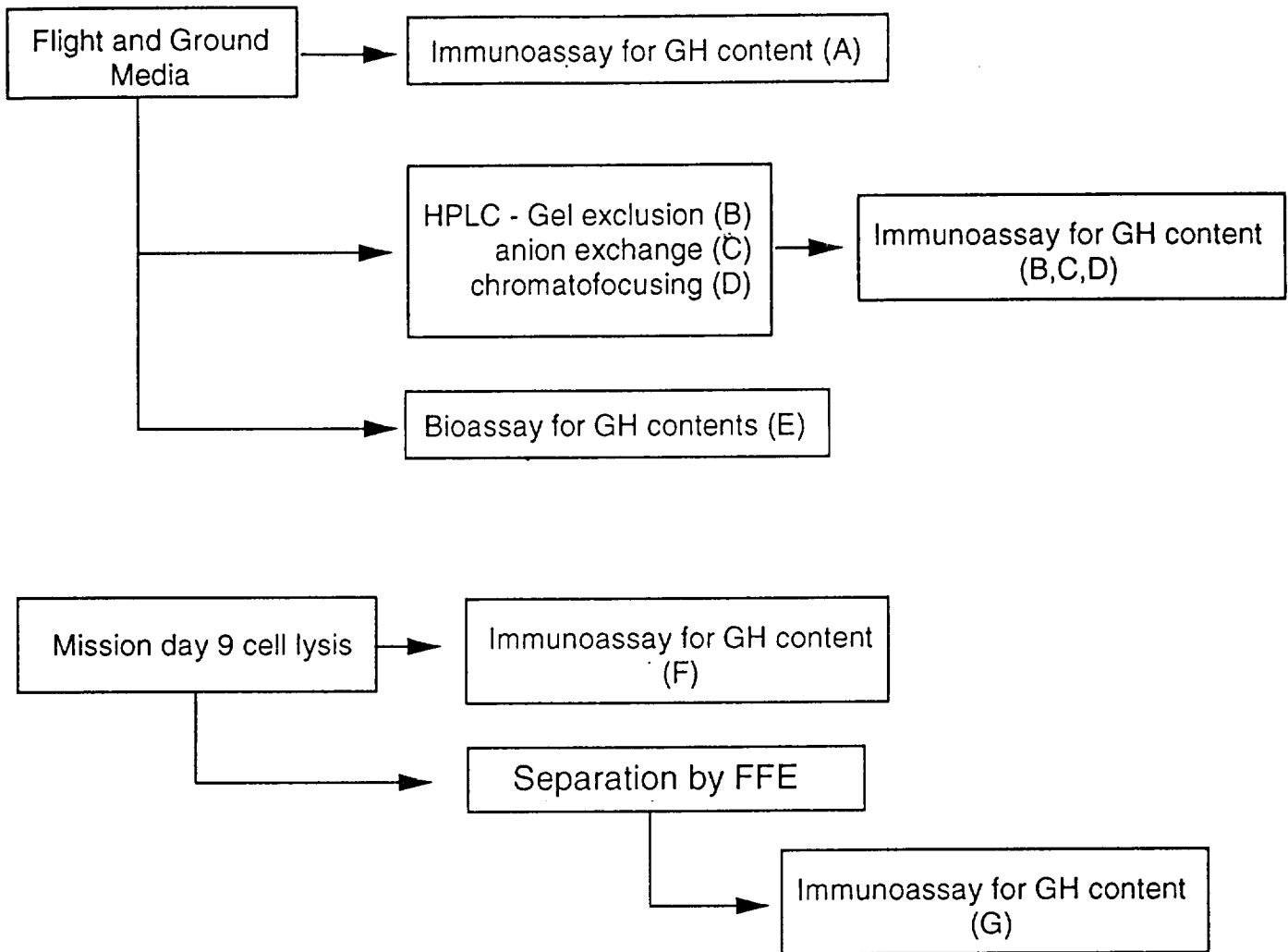
B).



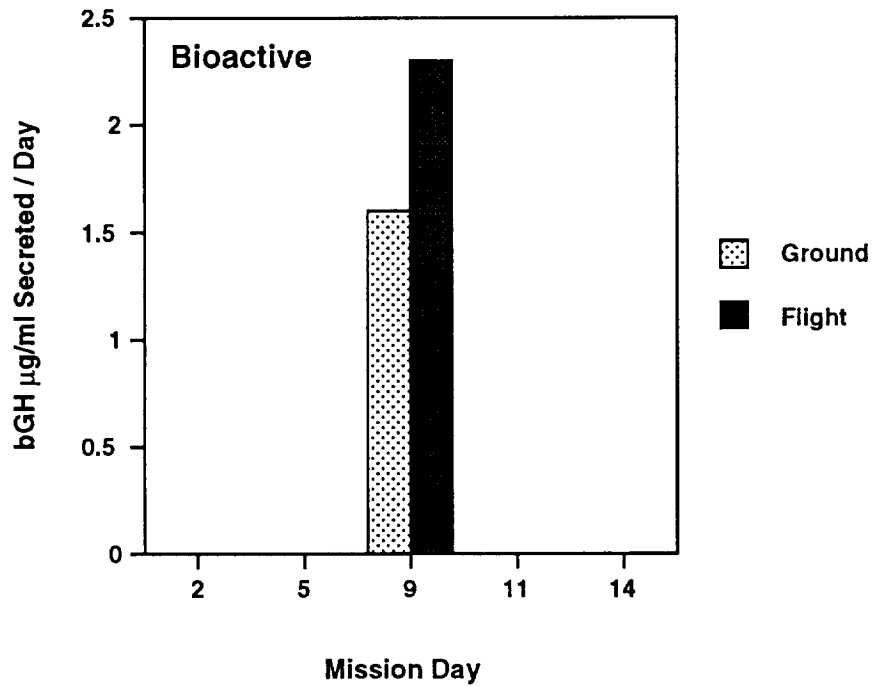
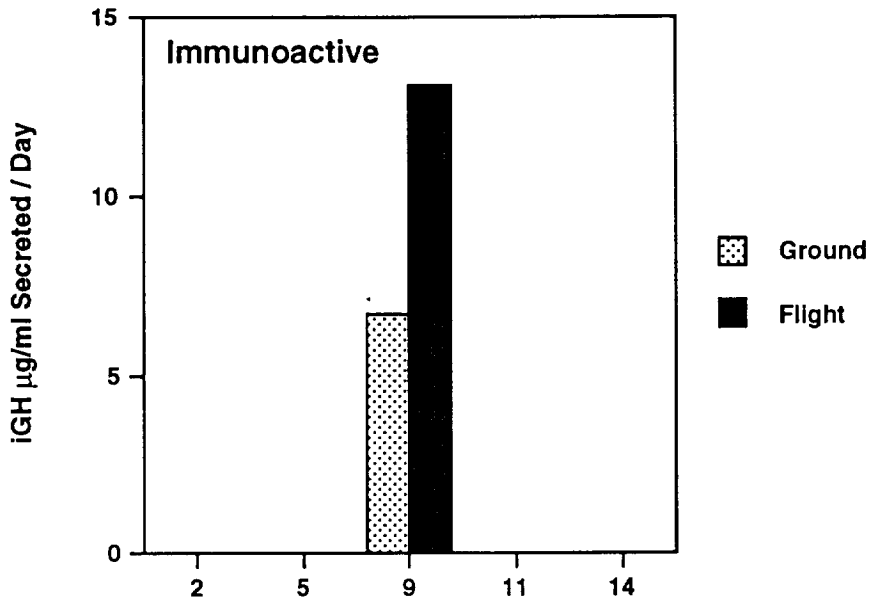
Fractions Collected



CCK #3 Growth Hormone Analysis



CCK 3 Media Recovered



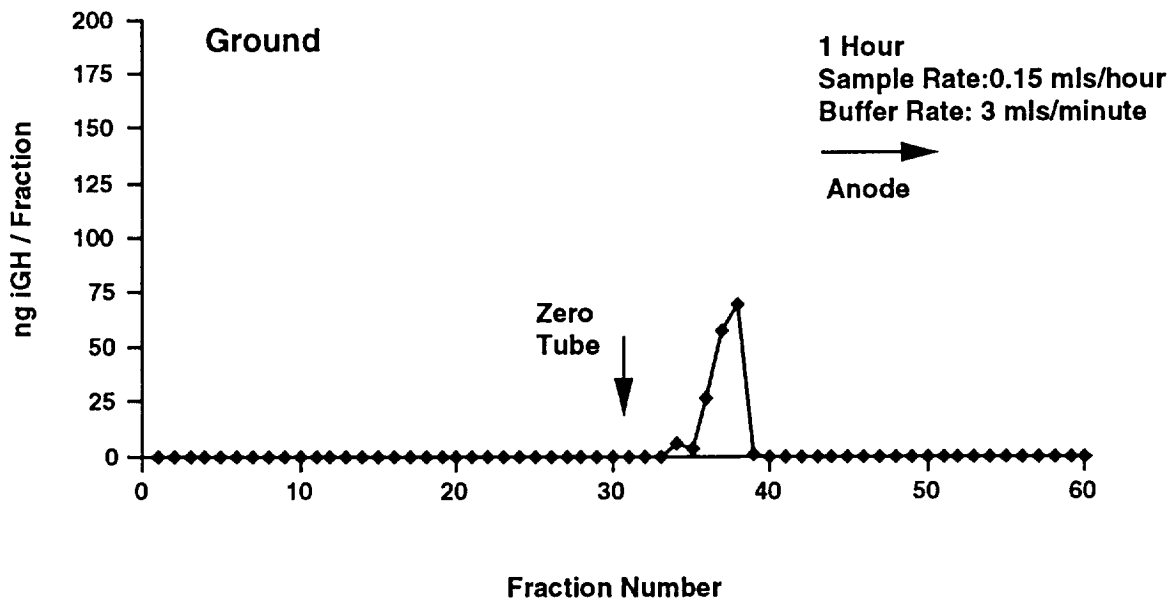
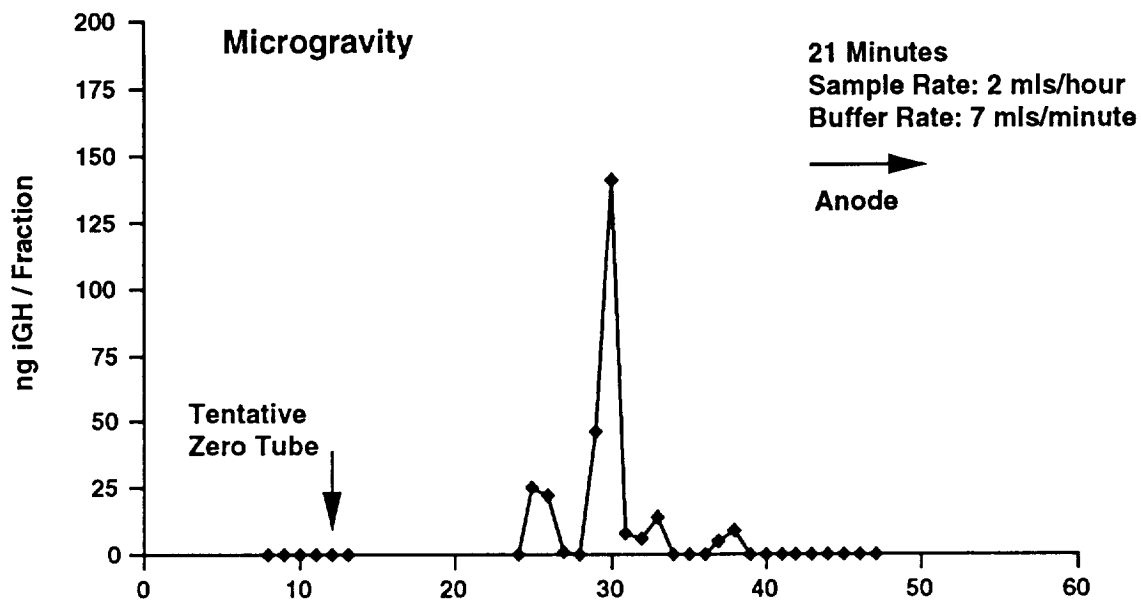
*Note Scale Difference

IML-2 CCK-3 Lysate Processing

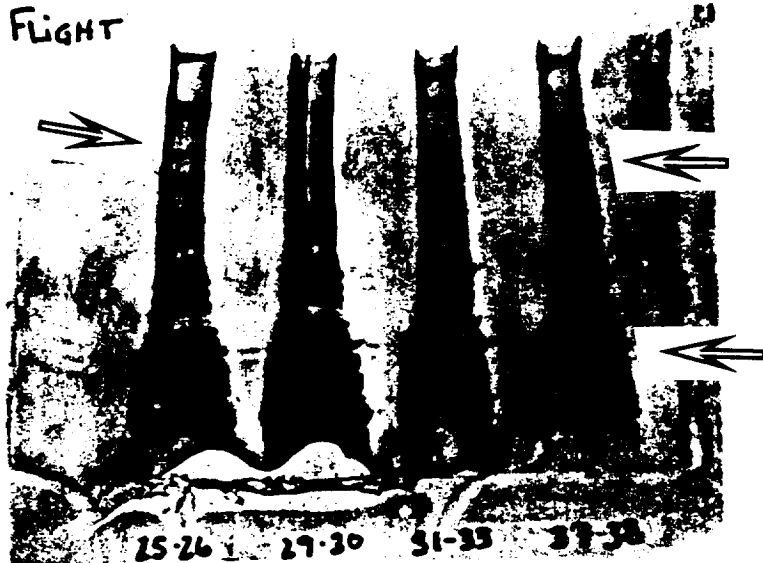
Flight
20 minute processing time
0.7 ml processed
150 ml carrier buffer
21.4 x dilution effect

Ground
60 minute processing time
0.15 ml processed
180 ml carrier buffer
1200 x dilution effect

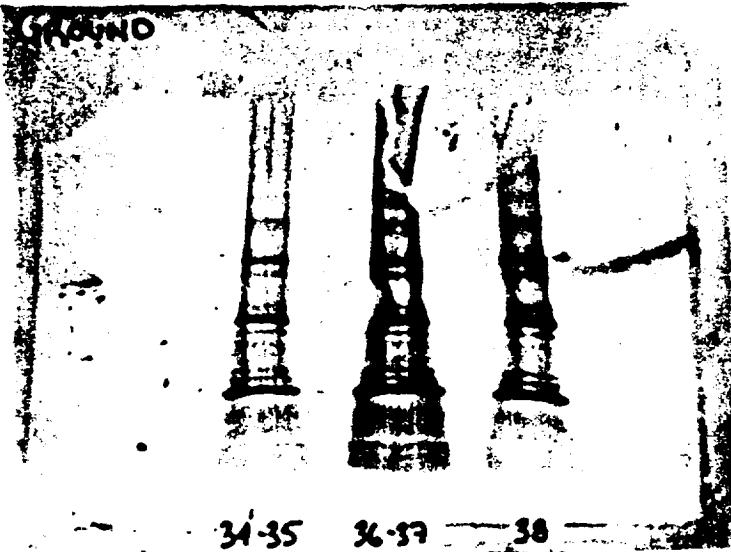
Therefore the flight sample is **56 x** more concentrated than the ground sample



FLIGHT



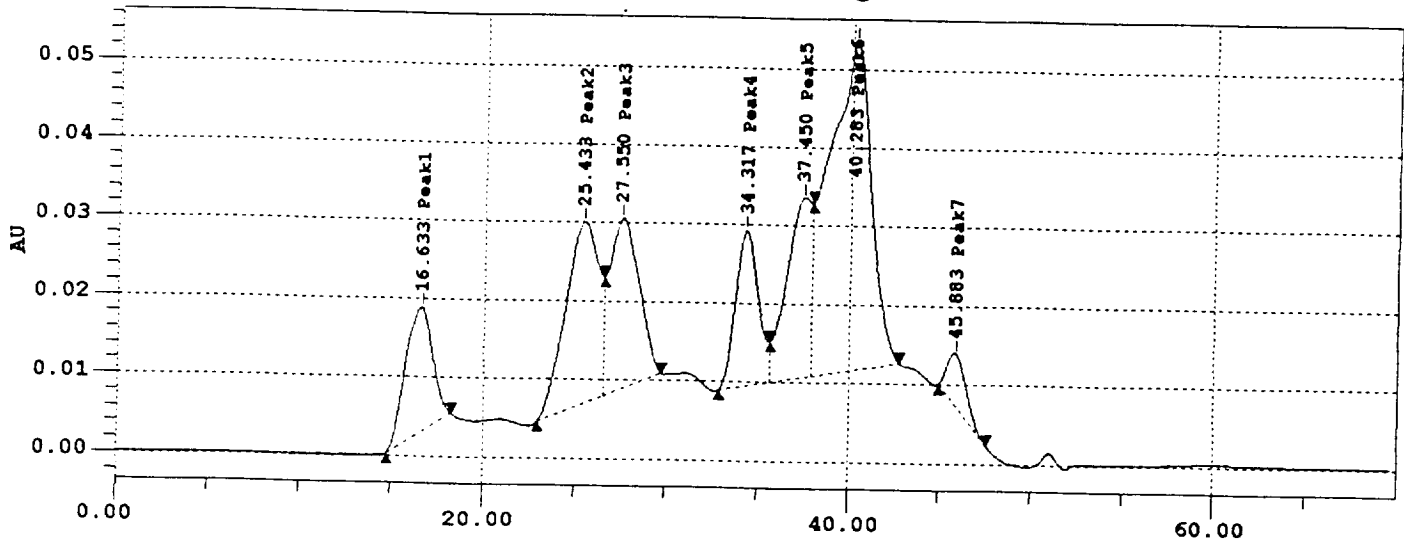
GROUND



Millennium Sample Information

Project Name:	kris	Sample Type:	Unknown
Sample Name:	CCK3-flight-ly	Volume:	300.00
Vial:	4	Run Time:	70.0 min
Injection:	1	Date Processed:	02/28/95 08:55 AM
Channel:	490 Ch1	Dilution:	1.00000
Date Acquired:	01/26/95 01:01 PM		
Scale Factor:	1.00		
Acq Meth Set:	kris_ms		
Processing Method:	cck3Fly		

Flight Lysate - Starting Material



Peak Results

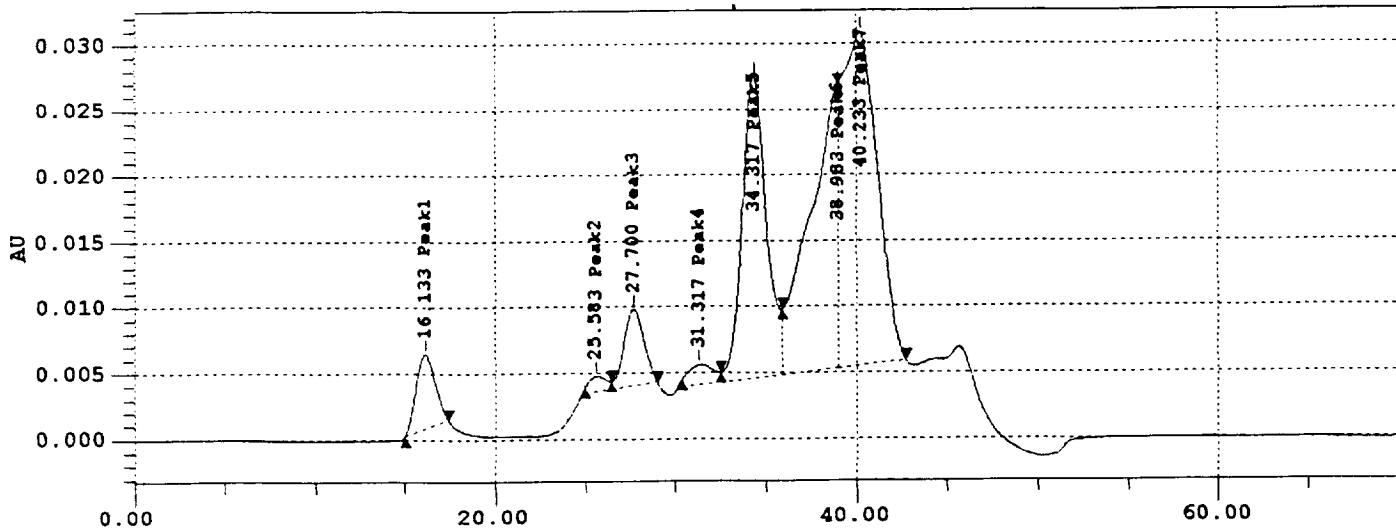
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	Peak1	16.633	1643040	15850		BB
2	Peak2	25.433	2918329	23183		BV
3	Peak3	27.550	2484895	21707		VB
4	Peak4	34.317	1718907	19845		BV
5	Peak5	37.450	2108257	22953		VV
6	Peak6	40.283	6864648	41906		VB
7	Peak7	45.683	485699	6686		BB

Millennium Sample Information

Project Name: kris
 Sample Name: CCK3-GR-lysate
 Vial: 1
 Injection: 1
 Channel: 490 Ch1
 Date Acquired: 01/25/95 01:45 PM
 Scale Factor: 1.00
 Acq Meth Set: kris.ms
 Processing Method: cck3ly

Sample Type: Unknown
 Volume: 300.00
 Run Time: 70.0 min
 Date Processed: 02/28/95 08:28 AM
 Dilution: 1.00000

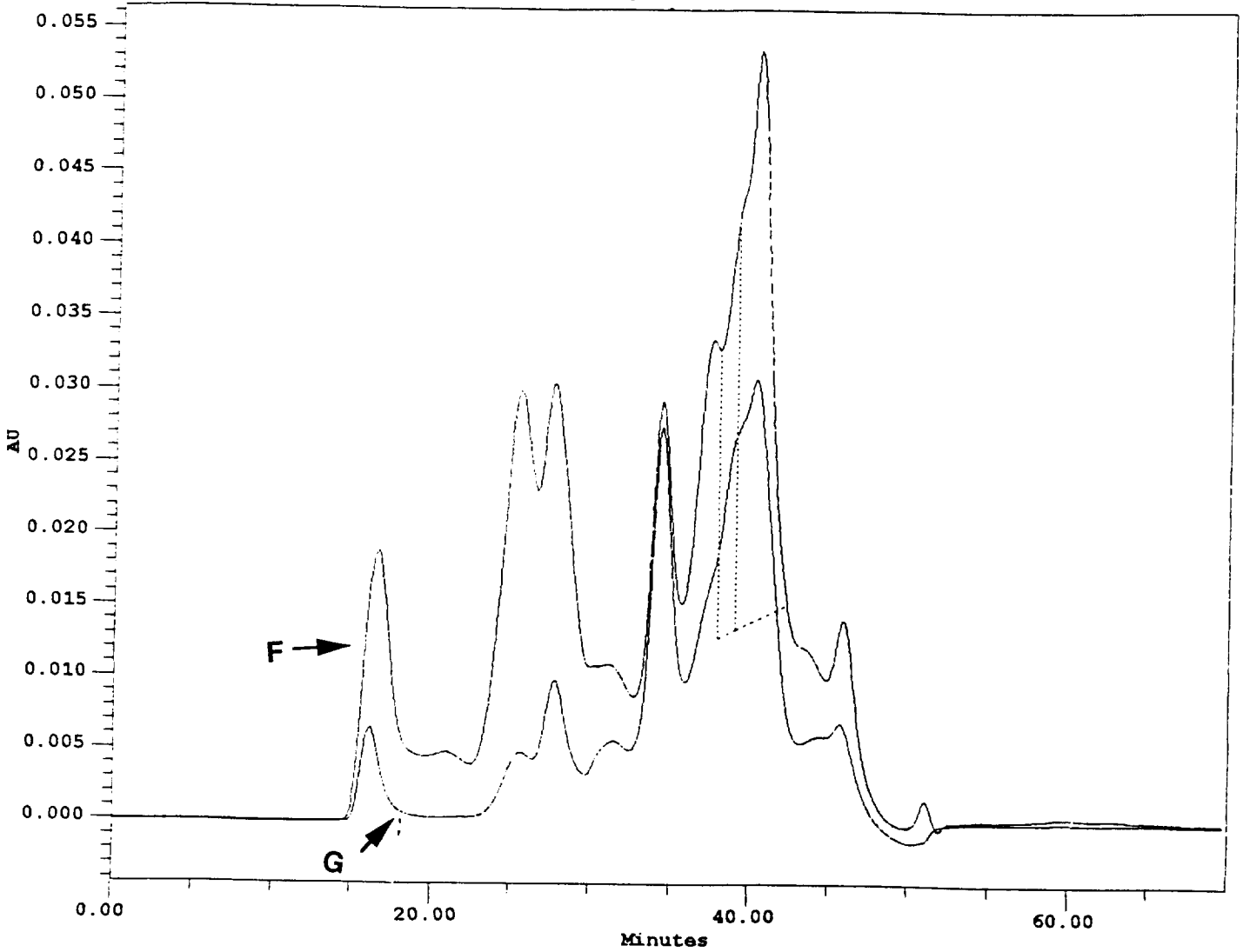
Ground Lysate - Starting Material



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	Peak1	16.133	426331	5686		BB
2	Peak2	25.583	84771	1262		VV
3	Peak3	27.700	451661	5803		VB
4	Peak4	31.317	151576	1580		VV
5	Peak5	34.317	2147813	22976		VV
6	Peak6	38.983	2276186	21506		VV
7	Peak7	40.233	3497883	25384		VB

Start Lysate



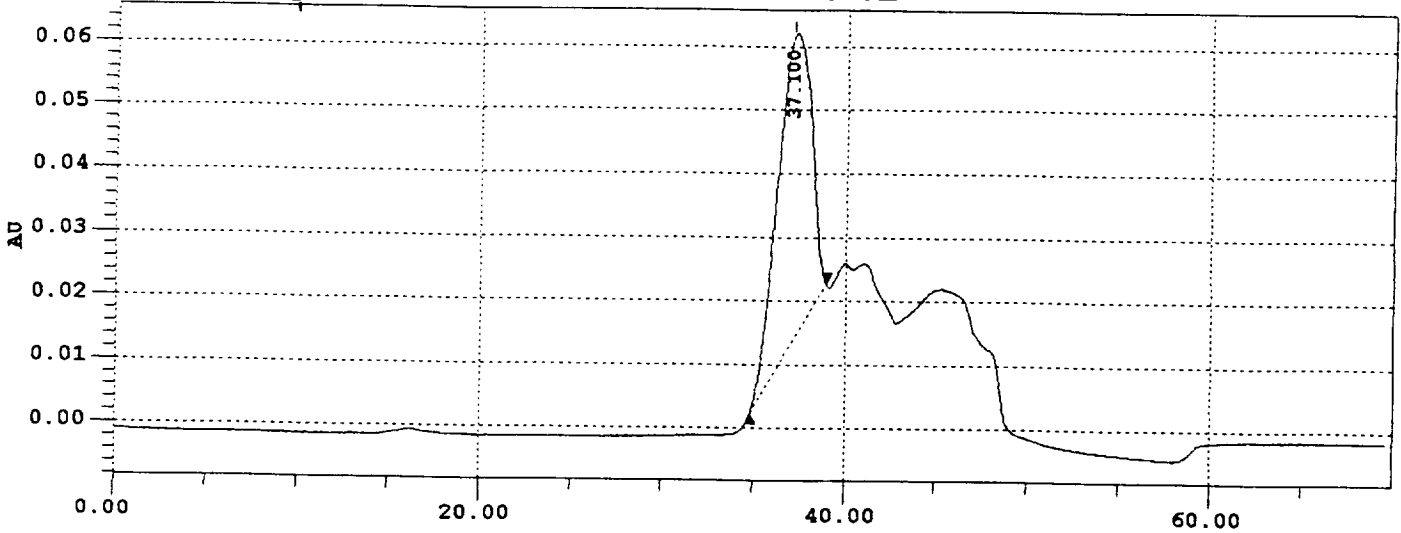
Samples

#	Sample Name	Vial	Inj	Channel	Date Processed
1	CCK3-flight-ly	4	1	490 Ch1	01/26/95 02:13 PM
2	CCK3-GR-lysate	1	1	490 Ch1	01/25/95 02:57 PM

Millennium Sample Information

Project Name:	kris	Sample Type:	Unknown
Sample Name:	CCK3-FR8-12F	Volume:	300.00
Vial:	1	Run Time:	70.0 min
Injection:	1	Date Processed:	02/28/95 02:22 PM
Channel:	490 Ch1	Dilution:	1.00000
Date Acquired:	02/28/95 01:11 PM		
Scale Factor:	1.00		
Acq Meth Set:	kris_ms		
Processing Method:	kris_pm		

Flight - Post FFE Fractions 8-12



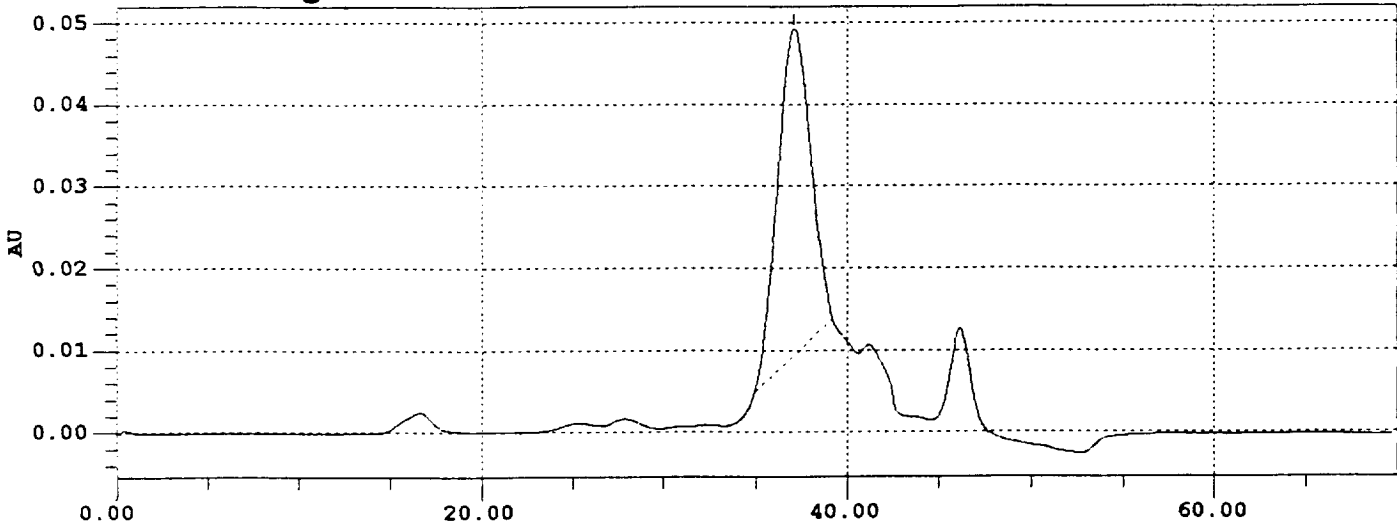
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	Peak1	1.033				Missing
2	Peak2	2.717				Missing
3	Peak3	4.817				Missing
4		37.100	6020124	48072		BB

Millennium Sample Information

Project Name:	kris	Sample Type:	Unknown
Sample Name:	CCK3-F-fr25-26	Volume:	300.00
Vial:	5	Run Time:	70.0 min
Injection:	1	Date Processed:	01/26/95 03:45 PM
Channel:	490 Ch1	Dilution:	1.00000
Date Acquired:	01/26/95 02:33 PM		
Scale Factor:	1.00		
Acq Meth Set:	kris_ms		
Processing Method:	kris_pm		

Flight - Post FFE Fractions 25-26



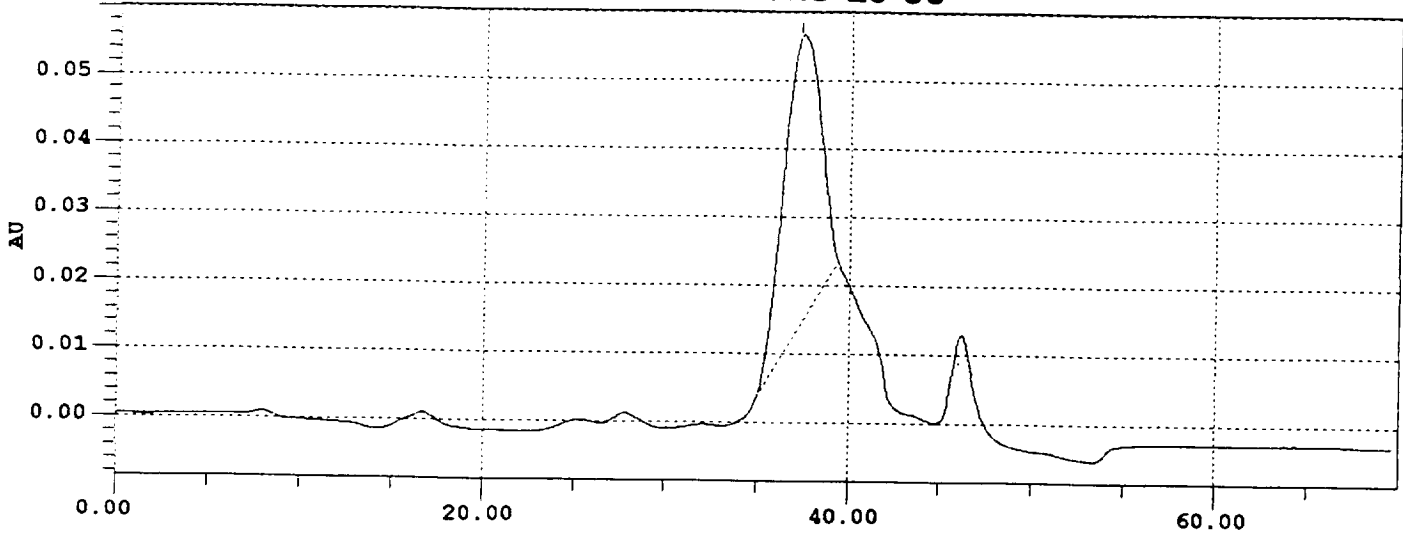
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	Peak1	1.033				Missing
2	Peak2	2.717				Missing
3	Peak3	4.817				Missing
4		37.083	5016891	39976		BB

Millennium Sample Information

Project Name:	kris	Sample Type:	Unknown
Sample Name:	CCK3-F-fr29-30	Volume:	300.00
Vial:	1	Run Time:	70.0 min
Injection:	1	Date Processed:	01/27/95 08:52 AM
Channel:	490 Ch1	Dilution:	1.00000
Date Acquired:	01/27/95 07:40 AM		
Scale Factor:	1.00		
Acq Meth Set:	kris_ms		
Processing Method:	kris_pm		

Flight - Post FFE Fractions 29-30



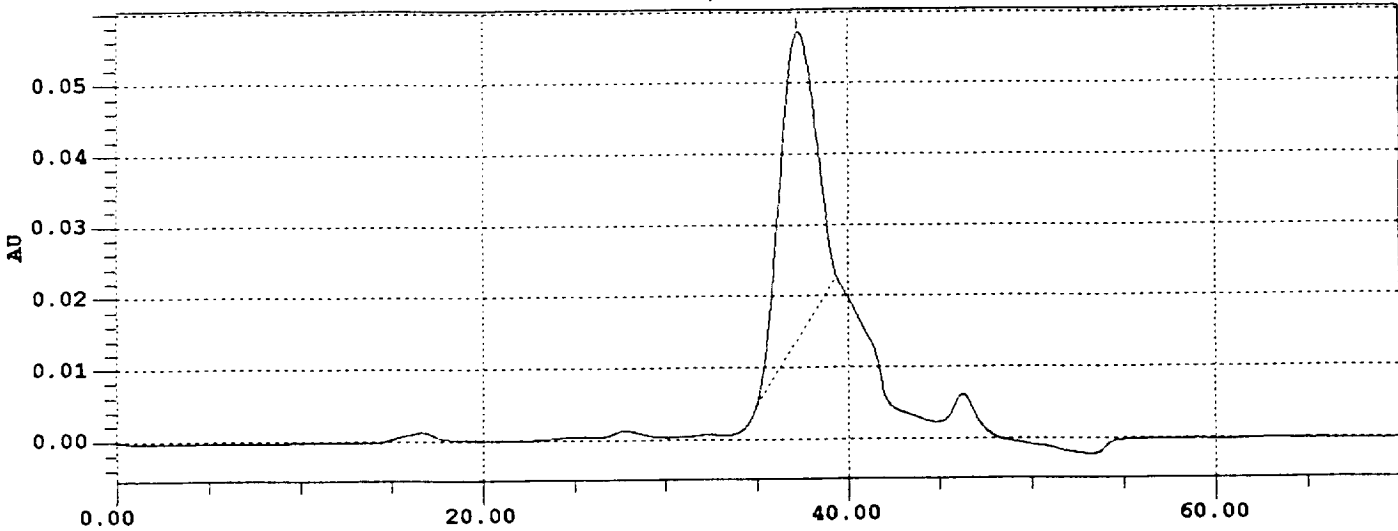
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	Peak1	1.033				Missing
2	Peak2	2.717				Missing
3	Peak3	4.817				Missing
4		37.267	5844754	42478		BB

Millennium Sample Information

Project Name:	kris	Sample Type:	Unknown
Sample Name:	CCK3-F-fr31-33	Volume:	300.00
Vial:	2	Run Time:	70.0 min
Injection:	1	Date Processed:	01/27/95 10:10 AM
Channel:	490 Ch1	Dilution:	1.00000
Date Acquired:	01/27/95 08:58 AM		
Scale Factor:	1.00		
Acq Meth Set:	kris_ms		
Processing Method:	kris_pm		

Flight - Post FFE Fractions 31-33



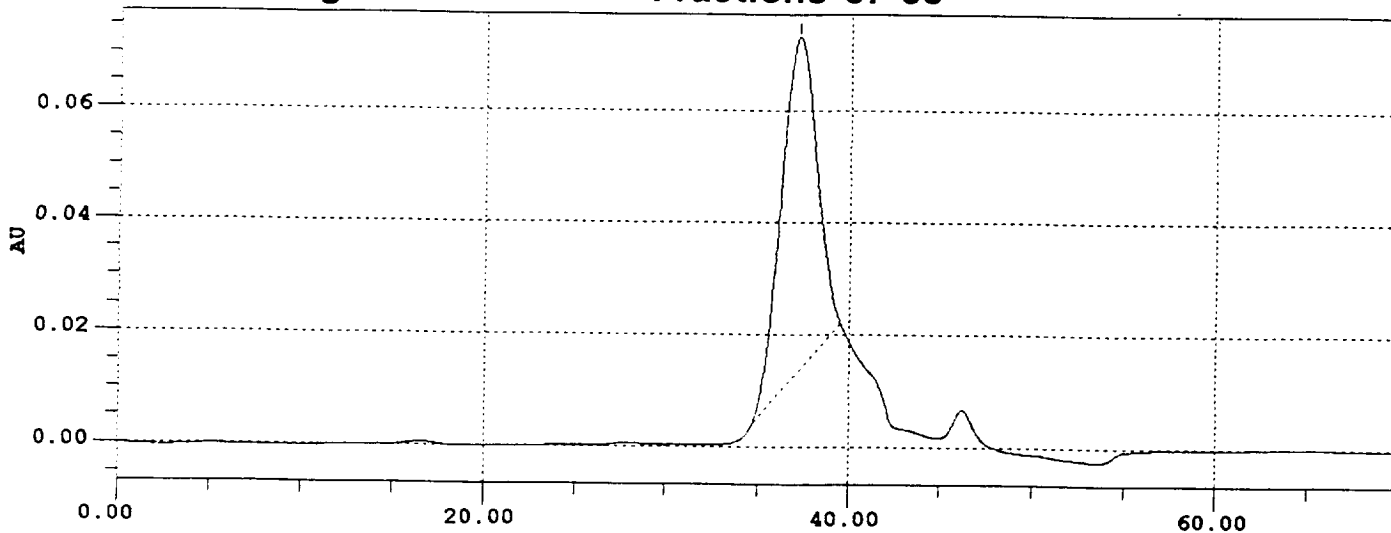
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	Peak1	1.033				Missing
2	Peak2	2.717				Missing
3	Peak3	4.817				Missing
4		37.233	5848591	43213		BB

Millennium Sample Information

Project Name:	kris	Sample Type:	Unknown
Sample Name:	CCK3-F-fr37-38	Volume:	300.00
Vial:	3	Run Time:	70.0 min
Injection:	1	Date Processed:	01/27/95 11:31 AM
Channel:	490 Ch1	Dilution:	1.00000
Date Acquired:	01/27/95 10:20 AM		
Scale Factor:	1.00		
Acq Meth Set:	kris_ms		
Processing Method:	kris_pm		

Flight - Post FFE Fractions 37-38



Peak Results

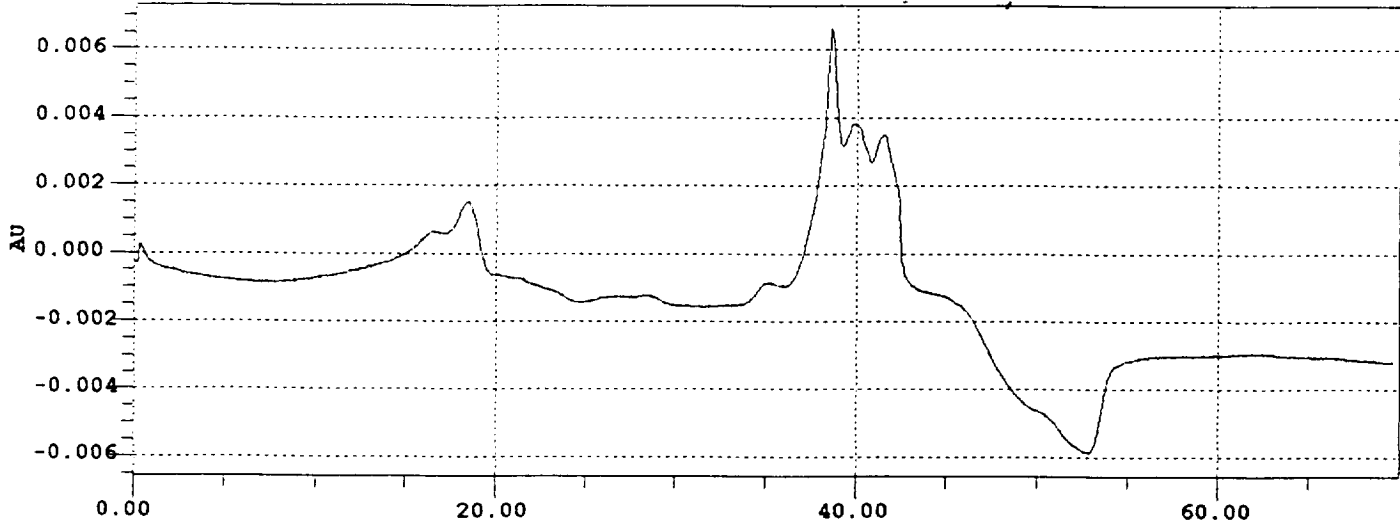
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	Peak1	1.033				Missing
2	Peak2	2.717				Missing
3	Peak3	4.817				Missing
4		37.150	8171734	60268		BB

For Sample: CCK3-G-fr34-35 Vial: 1 Inj: 1 Chan: 490 Ch1 Date Processed 01/26/95 09:18 AM

Millennium Sample Information

Project Name:	kris	Sample Type:	Unknown
Sample Name:	CCK3-G-fr34-35	Volume:	300.00
Vial:	1	Run Time:	70.0 min
Injection:	1	Date Processed:	01/26/95 09:18 AM
Channel:	490 Ch1	Dilution:	1.00000
Date Acquired:	01/26/95 08:06 AM		
Scale Factor:	1.00		
Acq Meth Set:	kris_ms		
Processing Method:	kris_pm		

Ground - Post FFE Fractions 34-35



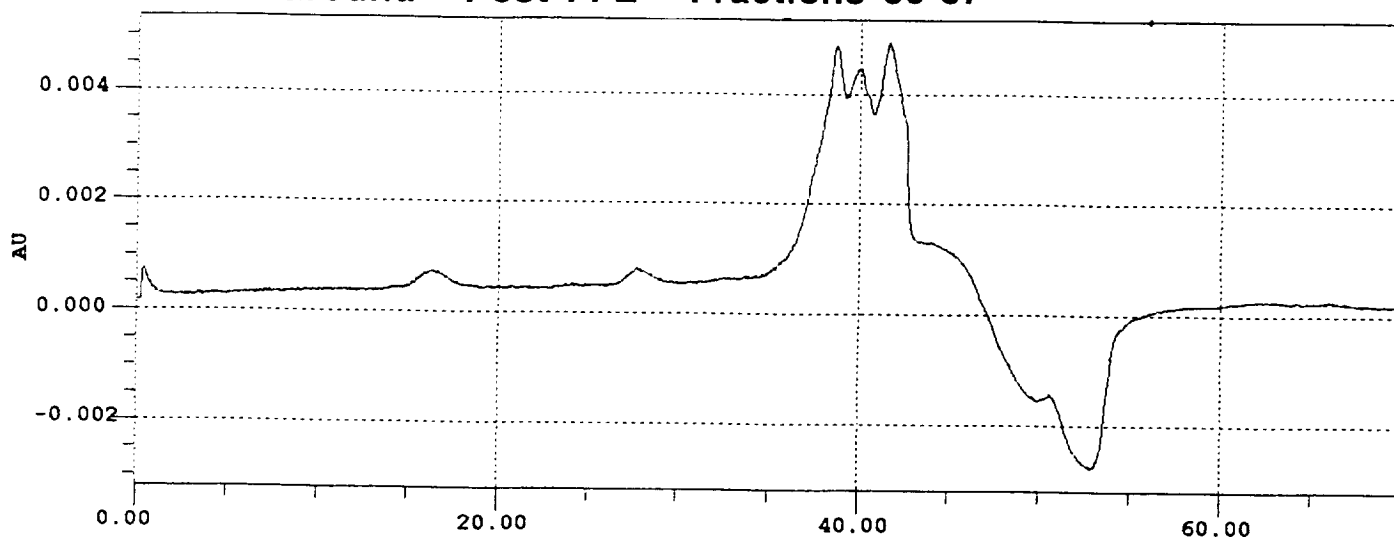
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	Peak1	1.033				Missing
2	Peak2	2.717				Missing
3	Peak3	4.817				Missing

Millennium Sample Information

Project Name:	kris	Sample Type:	Unknown
Sample Name:	CCK3-G-fr36-37	Volume:	300.00
Vial:	2	Run Time:	70.0 min
Injection:	1	Date Processed:	01/26/95 10:48 AM
Channel:	490 Ch1	Dilution:	1.00000
Date Acquired:	01/26/95 09:36 AM		
Scale Factor:	1.00		
Acq Meth Set:	kris_ms		
Processing Method:	kris_pm		

Ground - Post FFE Fractions 36-37



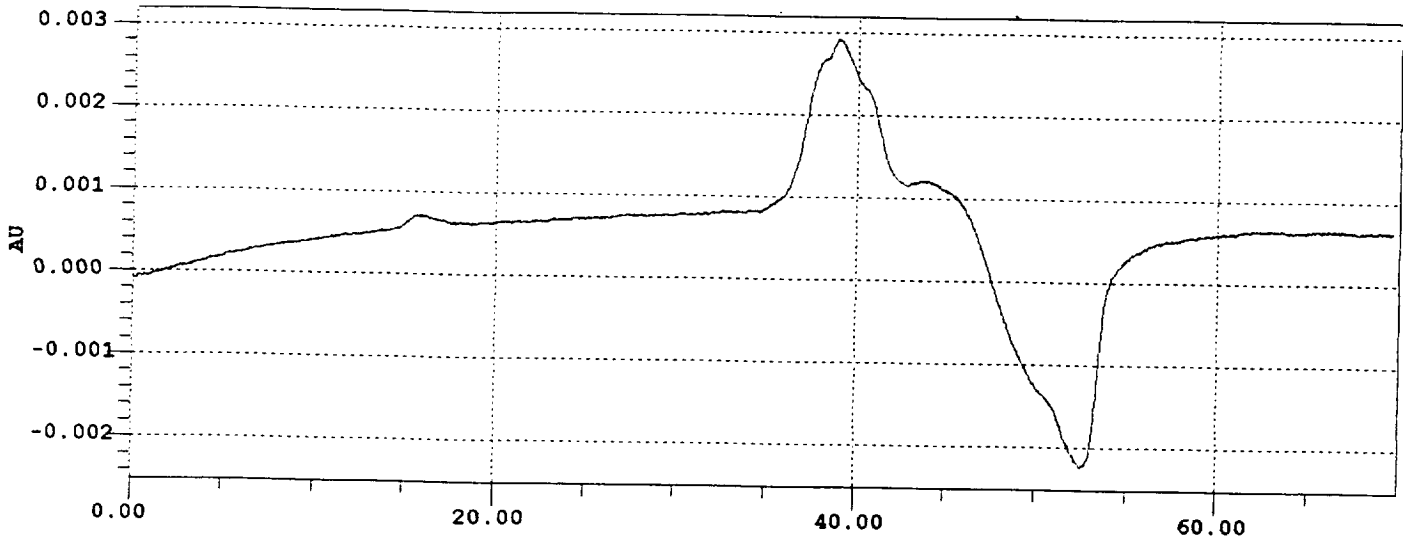
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	Peak1	1.033				Missing
2	Peak2	2.717				Missing
3	Peak3	4.817				Missing

Millennium Sample Information

Project Name:	kris	Sample Type:	Unknown
Sample Name:	CCK3-G-fr38	Volume:	300.00
Vial:	3	Run Time:	70.0 min
Injection:	1	Date Processed:	01/26/95 12:08 PM
Channel:	490 Ch1	Dilution:	1.00000
Date Acquired:	01/26/95 10:56 AM		
Scale Factor:	1.00		
Acq Meth Set:	kris_ms		
Processing Method:	kris_pm		

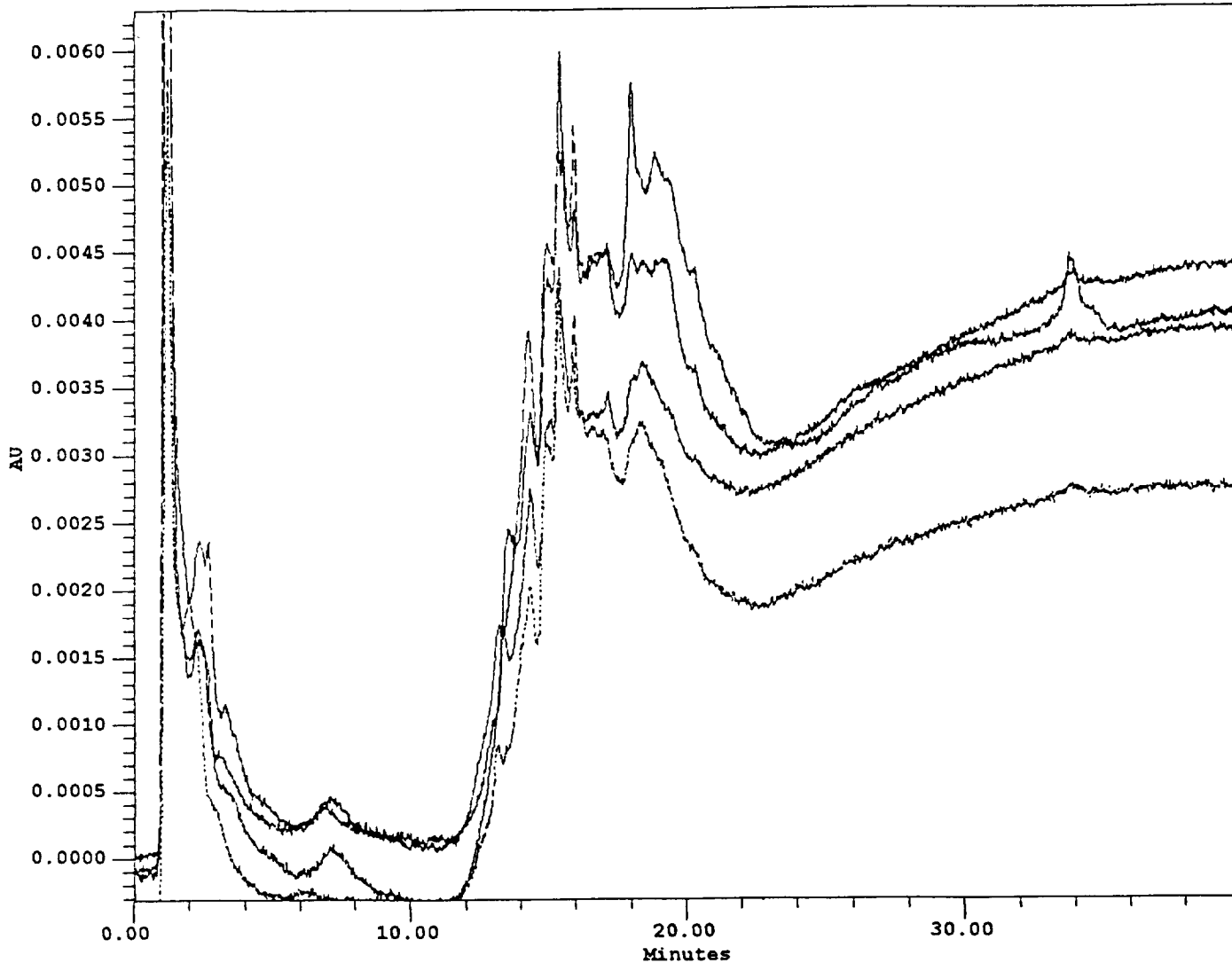
Ground - Post FFE Fraction 38



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	Peak1	1.033				Missing
2	Peak2	2.717				Missing
3	Peak3	4.817				Missing

Comparisons of OD 280 Profiles of Proteins Contained in Different FFE Fractions (Flight Only)

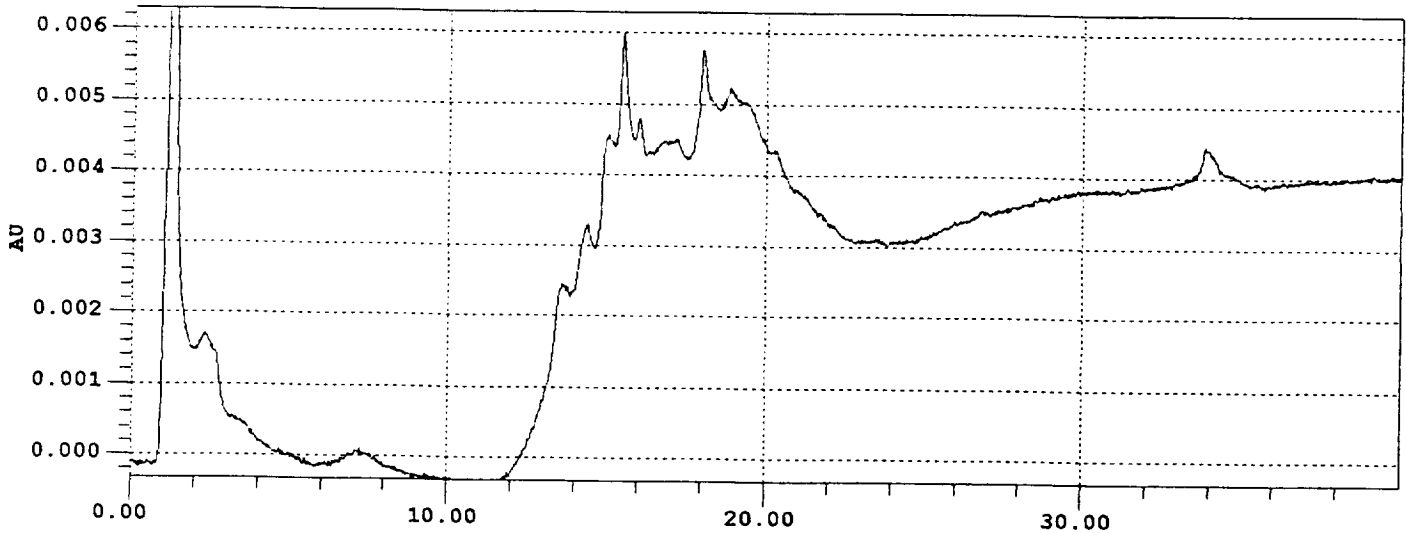


Samples

#	Sample Name	Vial	Inj	Channel	Date Processed
1	cck3fr25-26Fio	1	2	490 Ch1	03/02/95 08:20 AM
2	cck3fr29-30Fio	2	1	490 Ch1	03/02/95 09:16 AM
3	cck3fr31-33Fio	3	1	490 Ch1	03/02/95 10:11 AM
4	cck3fr36-37Fio	4	1	490 Ch1	03/02/95 11:02 AM

Millennium Sample Information

Project Name: kris
 Sample Name: cck3fr25-26Fio
 Vial: 1
 Injection: 2
 Channel: 490 Ch1
 Date Acquired: 03/02/95 07:34 AM
 Scale Factor: 1.00
 Acq Meth Set: kris_ms
 Processing Method: kris_pm
 Sample Type: Unknown
 Volume: 200.00
 Run Time: 45.0 min
 Date Processed: 03/02/95 08:20 AM
 Dilution: 1.00000

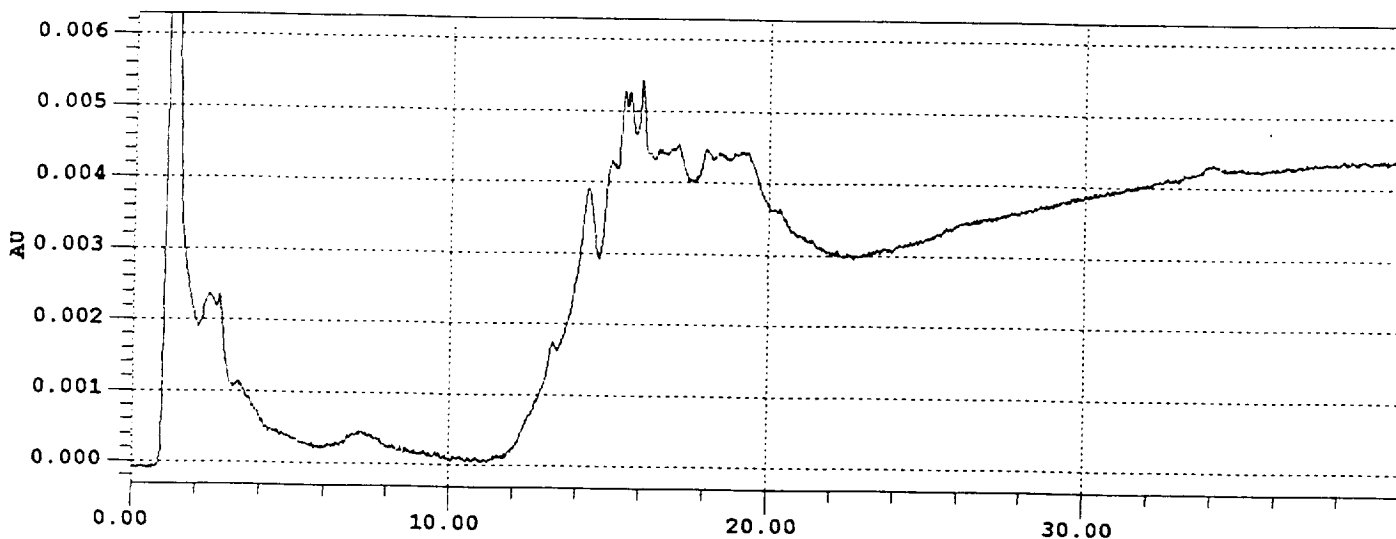


Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	Peak1	1.033				Missing
2	Peak2	2.717				Missing
3	Peak3	4.817				Missing

Millennium Sample Information

Project Name:	kris	Sample Type:	Unknown
Sample Name:	cck3fr29-30F10	Volume:	200.00
Vial:	2	Run Time:	45.0 min
Injection:	1	Date Processed:	03/02/95 04:40 PM
Channel:	490 Ch1	Dilution:	1.00000
Date Acquired:	03/02/95 08:30 AM		
Scale Factor:	1.00		
Acq Meth Set:	kris_ms		
Processing Method:	kris_pm		



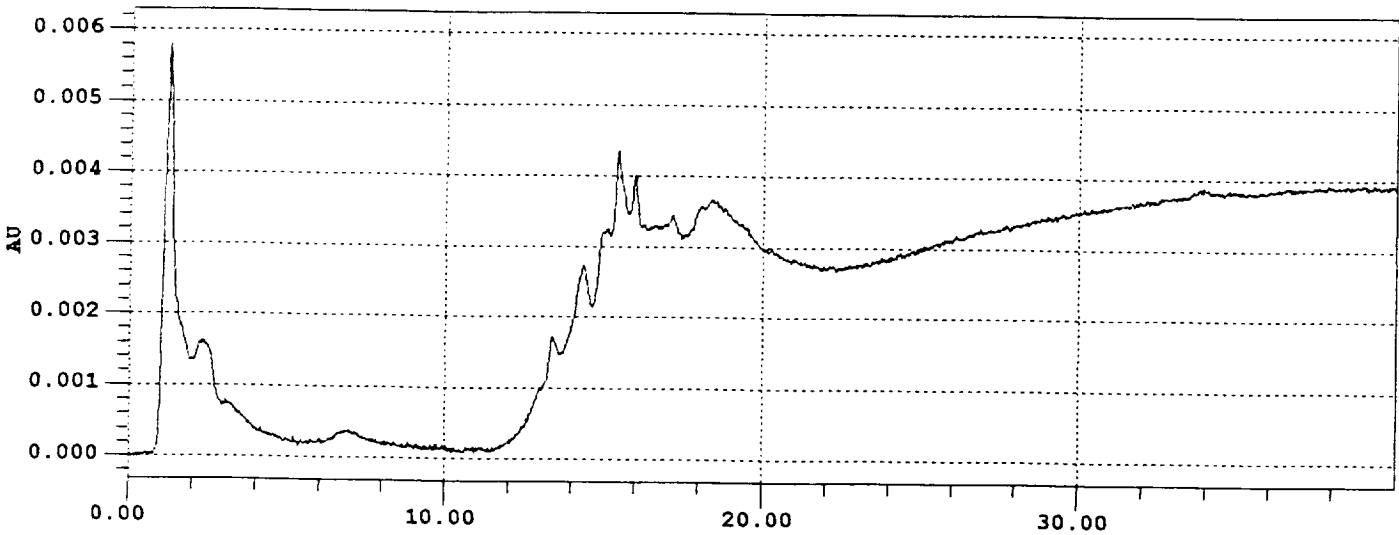
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	Peak1	1.033				Missing
2	Peak2	2.717				Missing
3	Peak3	4.817				Missing

For Sample: cck3fr31-33Fio Vial: 3 Inj: 1 Chan: 490 Ch1 Date Processed 03/02/95 10:11 AM

Millennium Sample Information

Project Name:	kris	Sample Type:	Unknown
Sample Name:	cck3fr31-33Fio	Volume:	200.00
Vial:	3	Run Time:	45.0 min
Injection:	1	Date Acquired:	03/02/95 09:24 AM
Channel:	490 Ch1	Date Processed:	03/02/95 10:11 AM
Date Acquired:	03/02/95 09:24 AM	Dilution:	1.00000
Scale Factor:	1.00		
Acq Meth Set:	kris_ms		
Processing Method:	kris_pm		



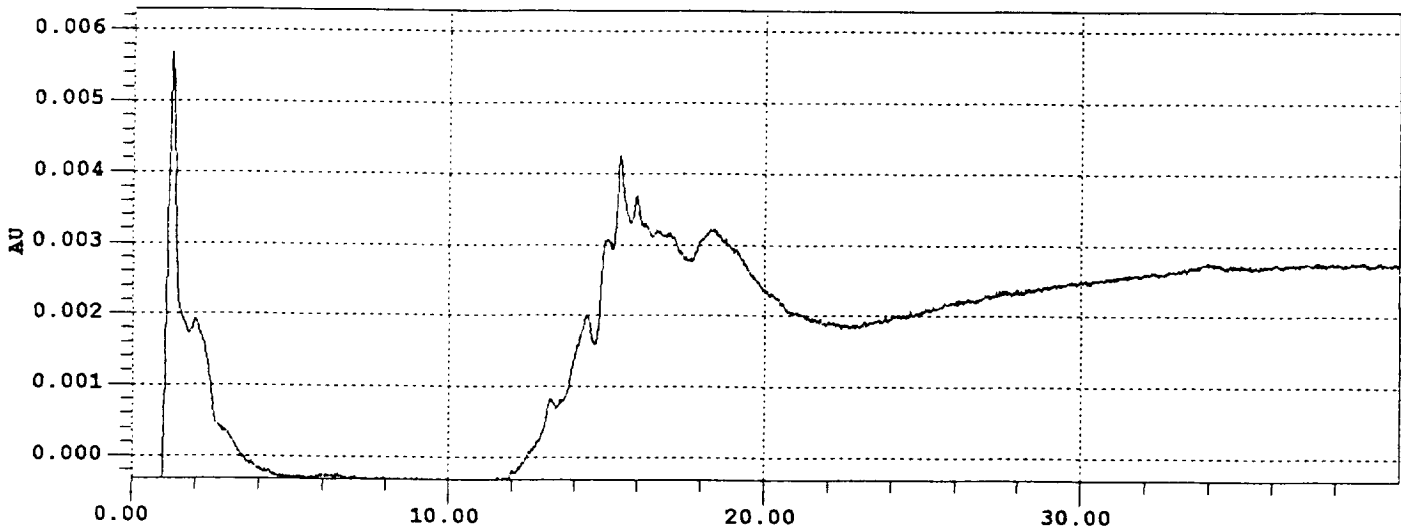
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	Peak1	1.033				Missing
2	Peak2	2.717				Missing
3	Peak3	4.817				Missing

For Sample: cck3fr36-37Fio Vial: 4 Inj: 1 Chan: 490 Ch1 Date Processed 03/02/95 11:02 AM

Millennium Sample Information

Project Name:	kris	Sample Type:	Unknown
Sample Name:	cck3fr36-37Fio	Volume:	200.00
Vial:	4	Run Time:	45.0 min
Injection:	1	Date Processed:	03/02/95 11:02 AM
Channel:	490 Ch1	Dilution:	1.00000
Date Acquired:	03/02/95 10:16 AM		
Scale Factor:	1.00		
Acq Meth Set:	kris_ms		
Processing Method:	kris_pm		



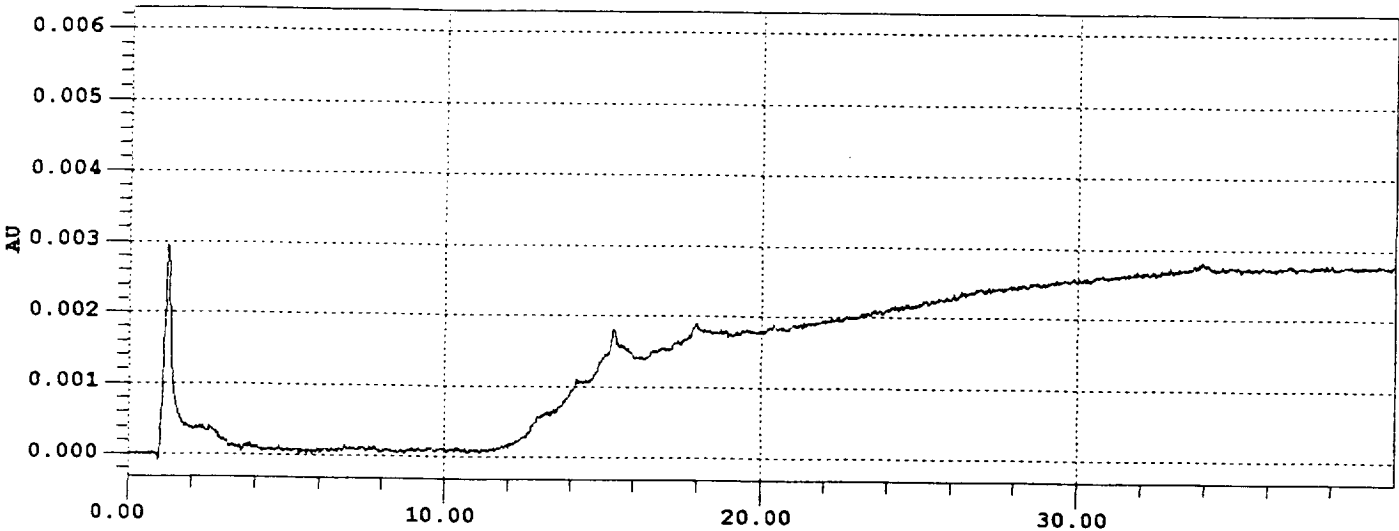
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	Peak1	1.033				Missing
2	Peak2	2.717				Missing
3	Peak3	4.817				Missing

For Sample: cck3fr34-35G10 Vial: 5 Inj: 1 Chan: 490 Ch1 Date Processed 03/02/95 12:00 PM

Millennium Sample Information

Project Name:	kris	Sample Type:	Unknown
Sample Name:	cck3fr34-35G10	Volume:	200.00
Vial:	5	Run Time:	45.0 min
Injection:	1	Date Processed:	03/02/95 12:00 PM
Channel:	490 Ch1	Dilution:	1.00000
Date Acquired:	03/02/95 11:14 AM		
Scale Factor:	1.00		
Acq Meth Set:	kris_ms		
Processing Method:	kris_pm		



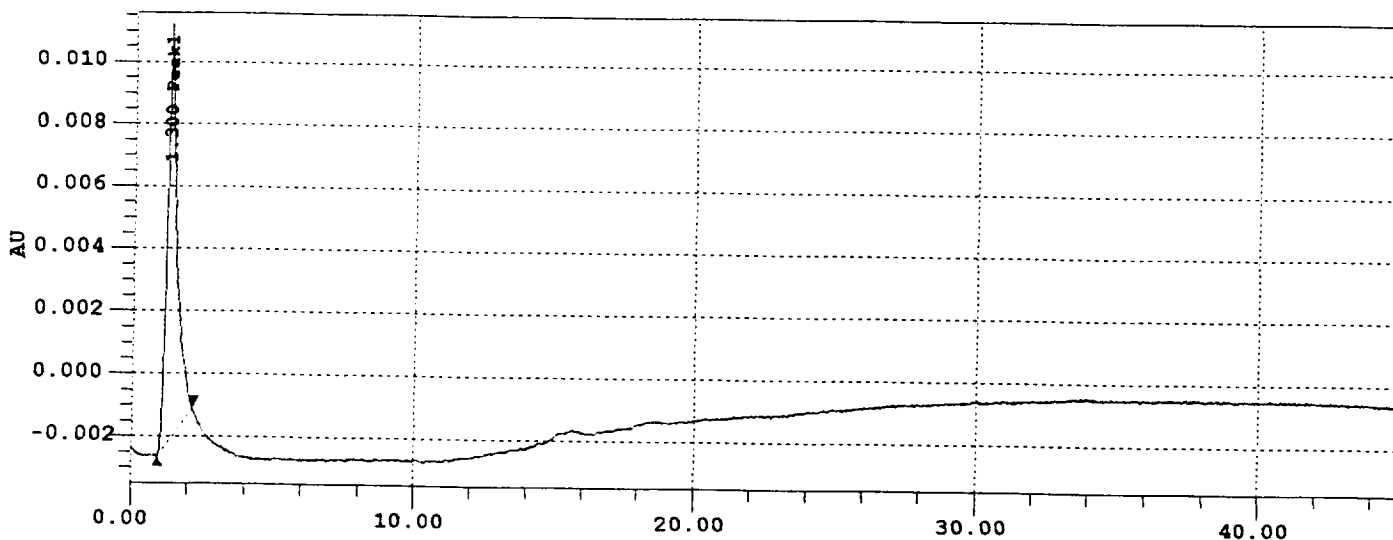
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	Peak1	1.033				Missing
2	Peak2	2.717				Missing
3	Peak3	4.817				Missing

Millennium Sample Information

Project Name: kris
 Sample Name: cck3fr36-37G10
 Vial: 6
 Injection: 1
 Channel: 490 Ch1
 Date Acquired: 03/02/95 12:02 PM
 Scale Factor: 1.00
 Acq Meth Set: kris_ms
 Processing Method: 36g

Sample Type: Unknown
 Volume: 200.00
 Run Time: 45.0 min
 Date Processed: 03/02/95 02:19 PM
 Dilution: 1.00000



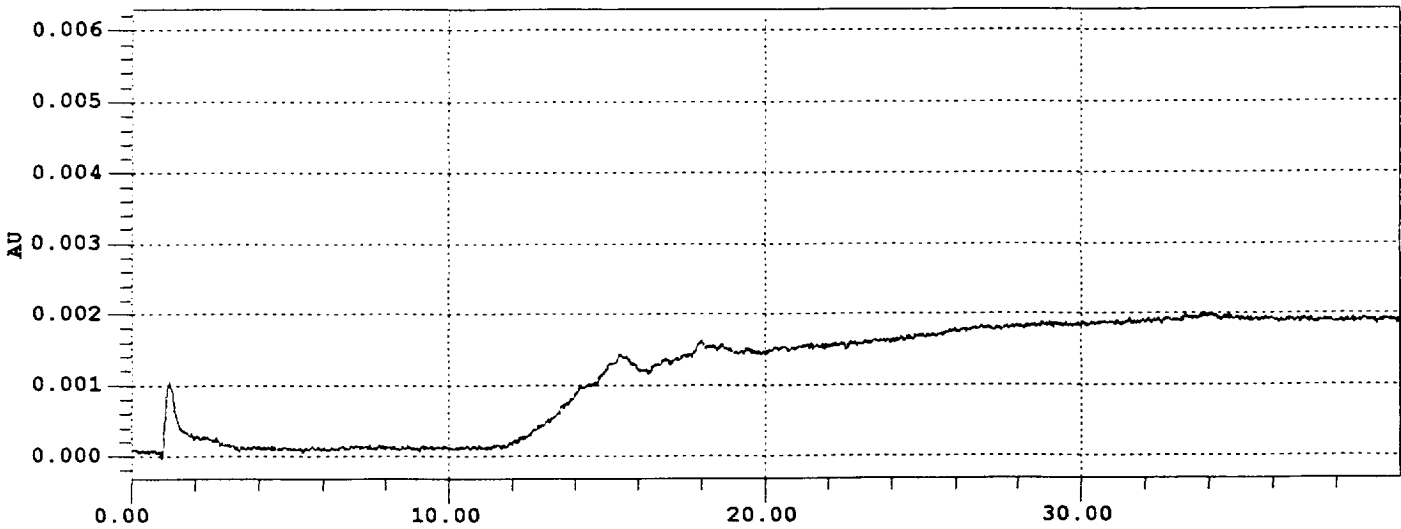
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	Peak1	1.300	312085	13110		BB

For Sample: cck3fr38Gion Vial: 1 Inj: 1 Chan: 490 Ch1 Date Processed 03/02/95 01:47 PM

Millennium Sample Information

Project Name:	kris	Sample Type:	Unknown
Sample Name:	cck3fr38Gion	Volume:	200.00
Vial:	1	Run Time:	45.0 min
Injection:	1	Date Acquired:	03/02/95 01:01 PM
Channel:	490 Ch1	Date Processed:	03/02/95 01:47 PM
Date Acquired:	03/02/95 01:01 PM	Dilution:	1.00000
Scale Factor:	1.00		
Acq Meth Set:	kris_ms		
Processing Method:	kris_pm		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	Peak1	1.033				Missing
2	Peak2	2.717				Missing
3	Peak3	4.817				Missing

Summary of CCR #3. Results to Date: The Bottom Line and Tentative Conclusions.

Several lines of evidence offer support for the idea that the FFEU was separating pituitary GH as well as general pituitary protein that was contained in a fresh cell lysate that was prepared by the astronaut. These include the observations that:

- bandsread and number of GH peaks was greater in the flight sample (pg. 8).
- Western blot analysis show some differences in iGH forms contained in different flight fractions after SDS-PAGE under non-reducing conditions (pg. 9).
- protein profiles of material in the 4 FFE flight fractions, after HPLC-gel exclusion chromatography, show some differences.

It is also important to indicate that flight had a definite effect on intracellular protein profile in the original lysate (before electrophoresis) - pg. 10C. These differences tended to be in areas of higher molecular weight protein. This result implies that microgravity affected the amount of certain proteins inside the cell. This change became apparent, in part, because of in-flight processing steps done with cells in CCK #3.

V. CCK #4

CCK #4

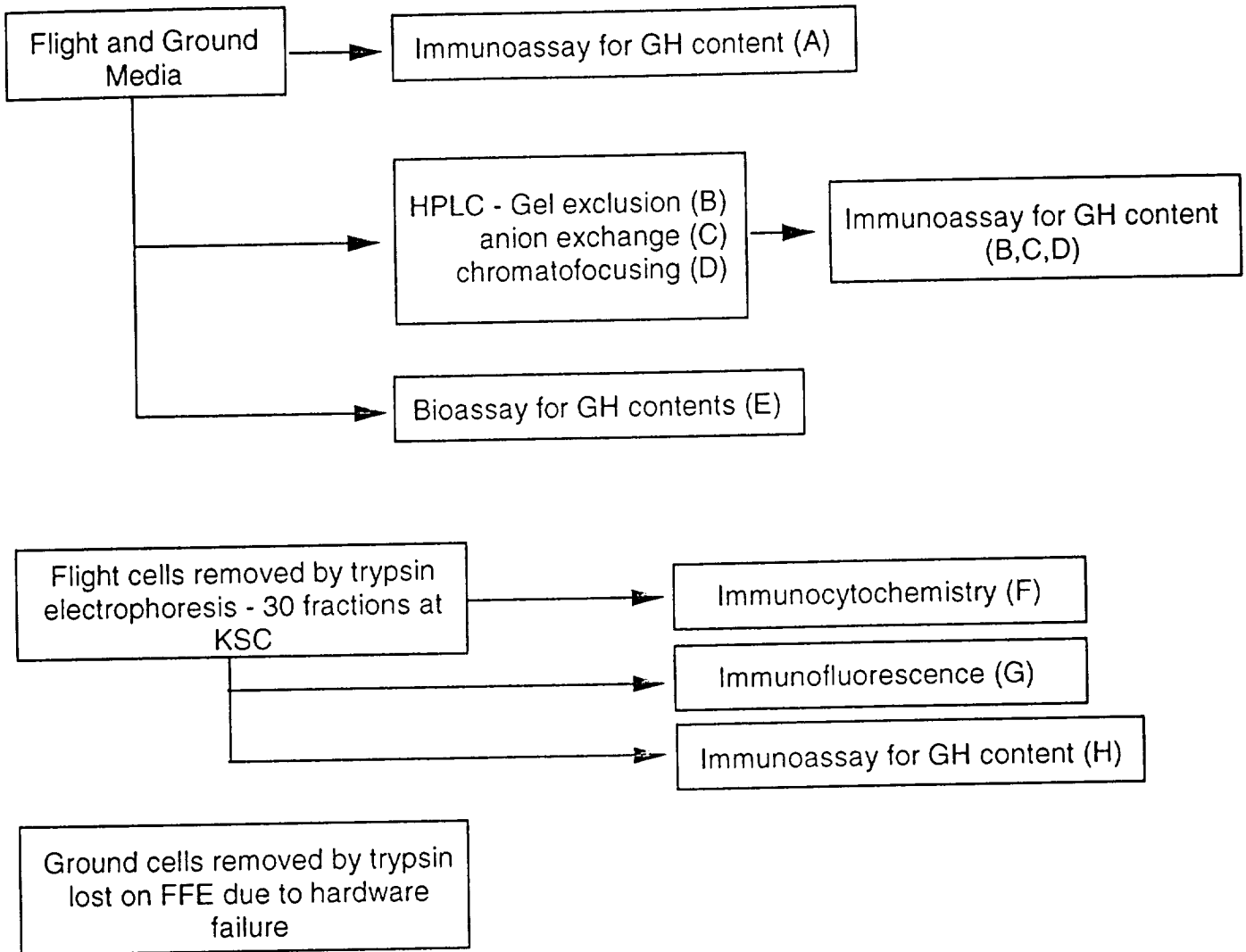
The original purpose for the cells in this CCK changed due to FFE hardware problems; however it did serve an extremely useful purpose for other parts of this experiment. For example, it provided comparative data for the cell culture portion of this experiment (detailed in section describing CCK #1 data).

Other CCK #4 results show:

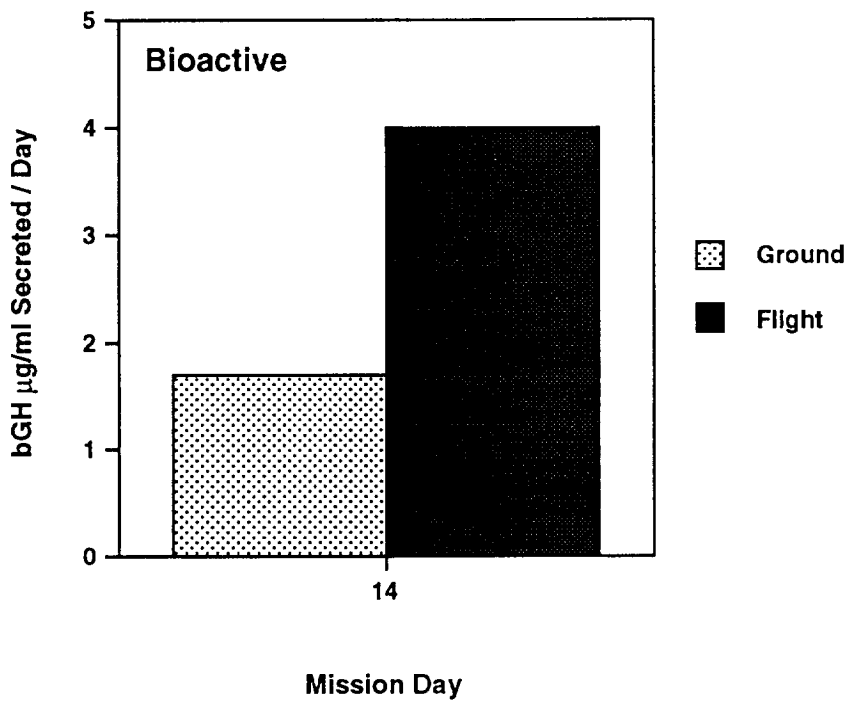
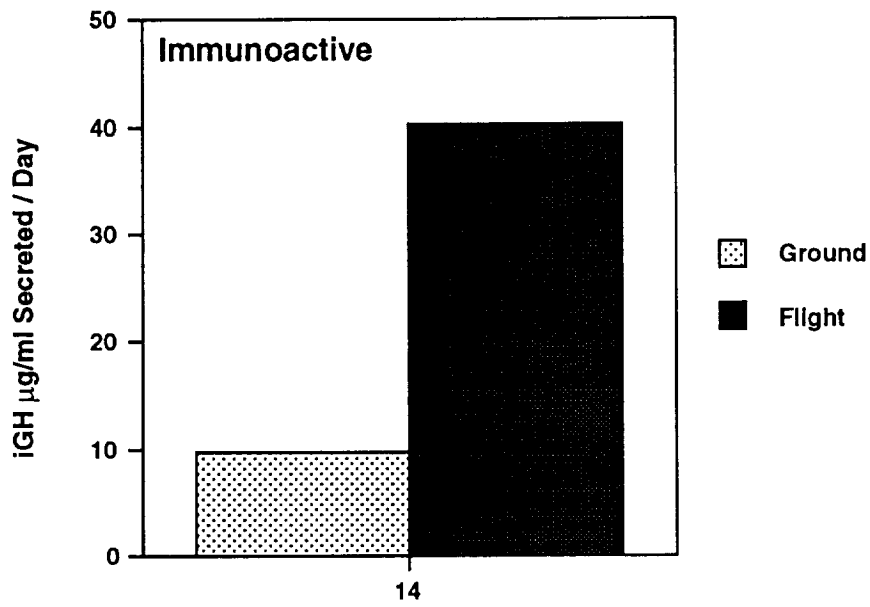
- That not only was there more iGH in media from this CCK than from the other CCK's, there was also 3x more iGH in the flight sample (pg. 4).
- That the electrophoretic mobility of the cells in CCK #4 is much lower than those from CCK #1 (pg. 5) with little evidence for separation of GH cells. Low mobility is tentatively attributed to lack of media change.
- That high intracellular GH in Fr. #30 (see pg. 5 bottom) correlates with the distribution profile showing a peak of GH cells in this fraction (pg. 5 top)
- That fibroblast growth in CCK #4 is minimal (both ground and flight) as determined microscopically and by flow cytometry. Photomicrographs of cells in CCK #1 and #4 immediately on return from space were included in an early report (given in Germany). The flow cytometry profiles of cells from CCK #4 (pgs. 6 and 7) again reflect the major effect of media change on relative lack of fibroblast growth and large numbers of cells with >2C contents of DNA compared to their CCK #1 counterparts (pg. 11/12, CCK #1).
- That the significant effect of spaceflight on cytoplasmic area occupied by GH is most easily seen in CCK #4 (pg. 8). We believe that the decrease in cytoplasmic area occupancy could be explained by the polycarbonate surface of the CCK. Note that this decrease does not occur on conventional tissue culture plasticware surface. How these biophysical changes relate to GH production and secretion remains a question.

Proposed	Actual	% of objective achieved
CCK #4		
<ul style="list-style-type: none"> • Cells maintained in culture for duration of flight without media changes. 	Cells maintained in culture for duration of flight without media changes.	100%
<ul style="list-style-type: none"> • FFE of cells at KSC on return to earth. 	FFE of cells at KSC on return to earth.	100%
<ul style="list-style-type: none"> • Synchronous ground control for FFE separation. 	Hardware failure at KSC prohibited cell separation of ground sample by FFE.	10%

CCK #4 Growth Hormone Analysis

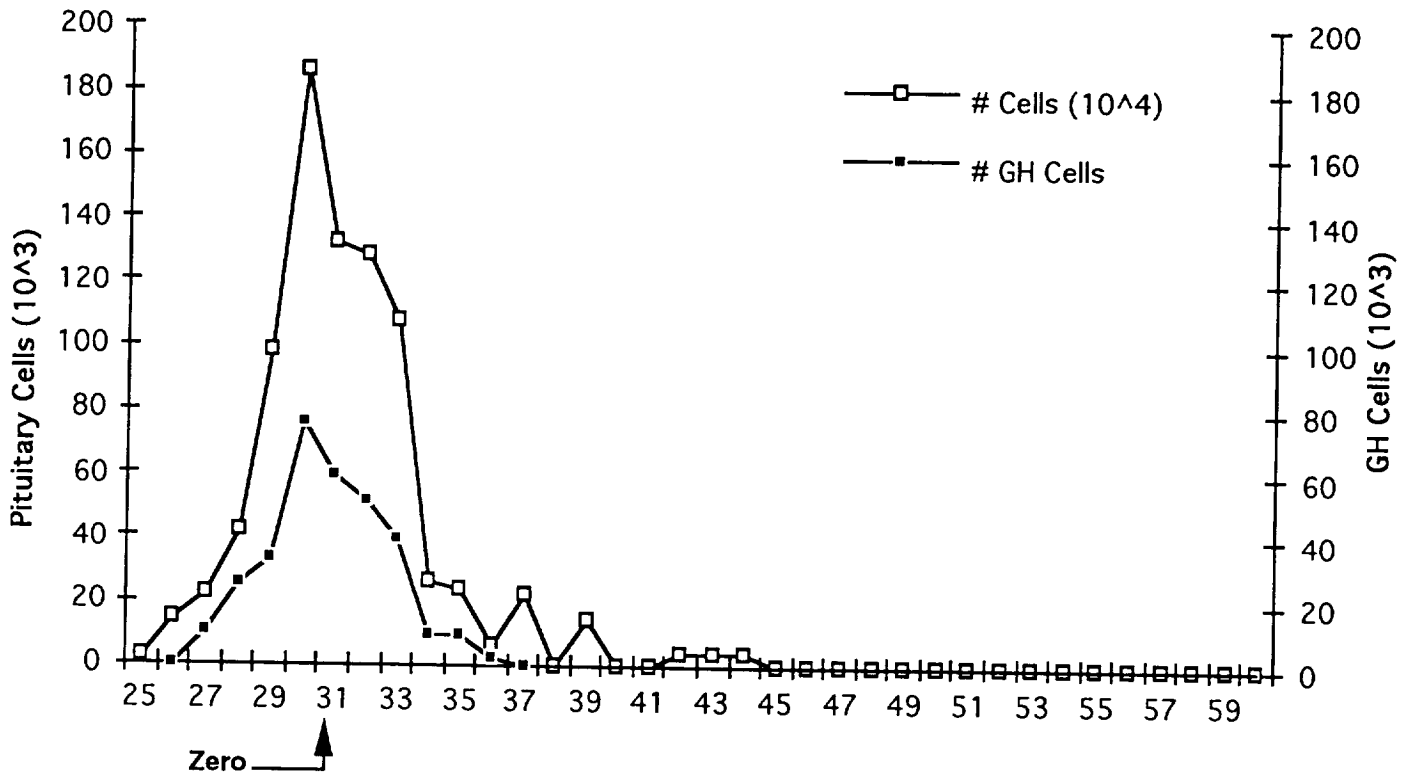


CCK 4 Media Recovered

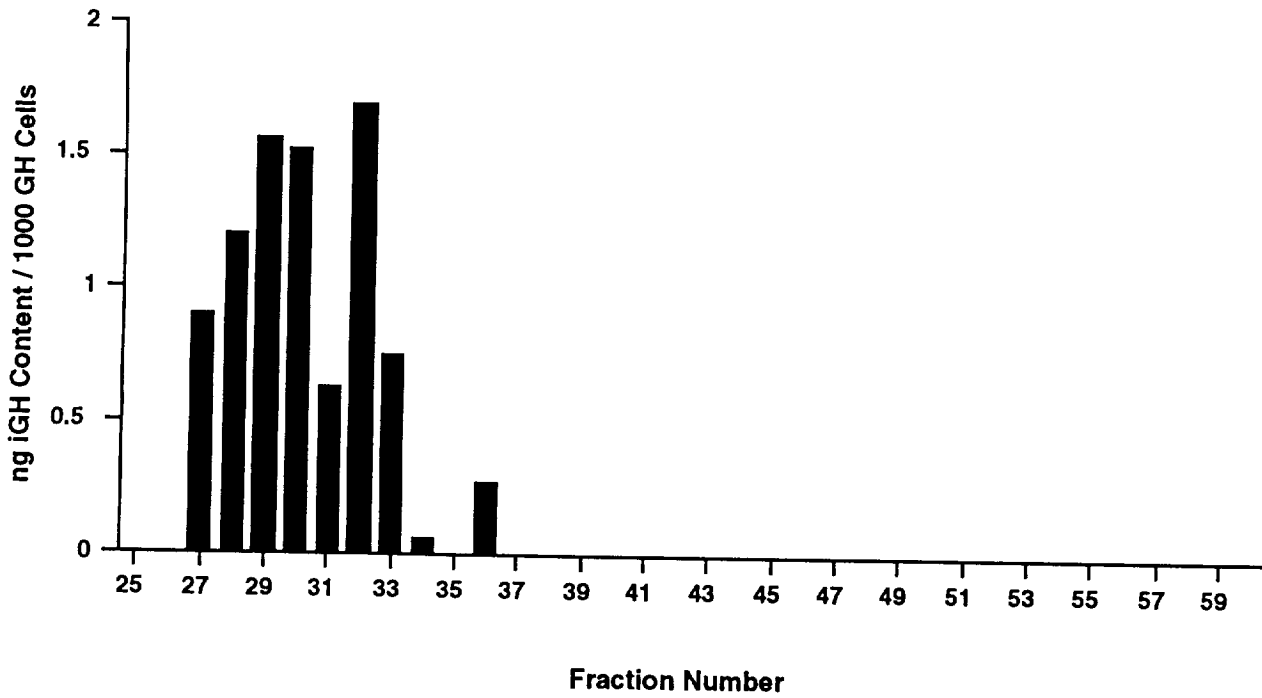


***Note Scale Difference**

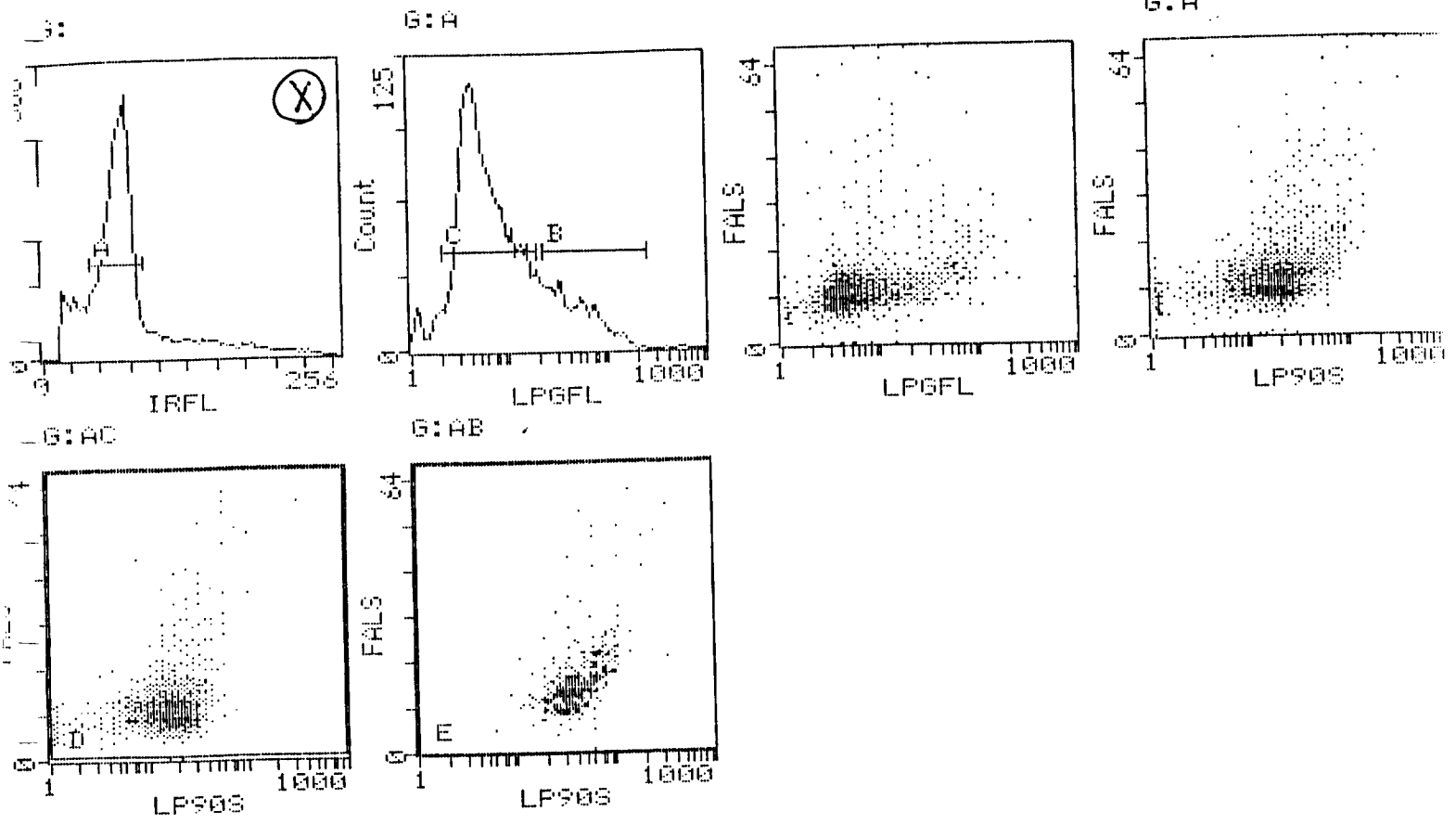
Flight CCK 4 Pituitary Cell Run Profile from ground Free Flow Electrophoresis Unit



Intracellular Growth Hormone Content of CCK 4 Electrophoresed Pituitary Cell Fractions



CCK #4 - Ground



SAMPLE INFO

PROTOCOL : TOM
 DATA RATE : Unknown
 INSTRUMENT : MDADS
 SAMPLE NAME : IML2
 SAMPLE NUMBER : 5
 COMMENTS : G4P
 TOTAL COUNT : 10,001
 LAST HIST :
 LIST FILE : 251094P4.L05
 PROT FILE : T0000007.PRO
 SAMPLE DATE : 25Oct94
 SAMPLE TIME : 00:27

STATISTICS

DUAL PARAMETER STATISTICS

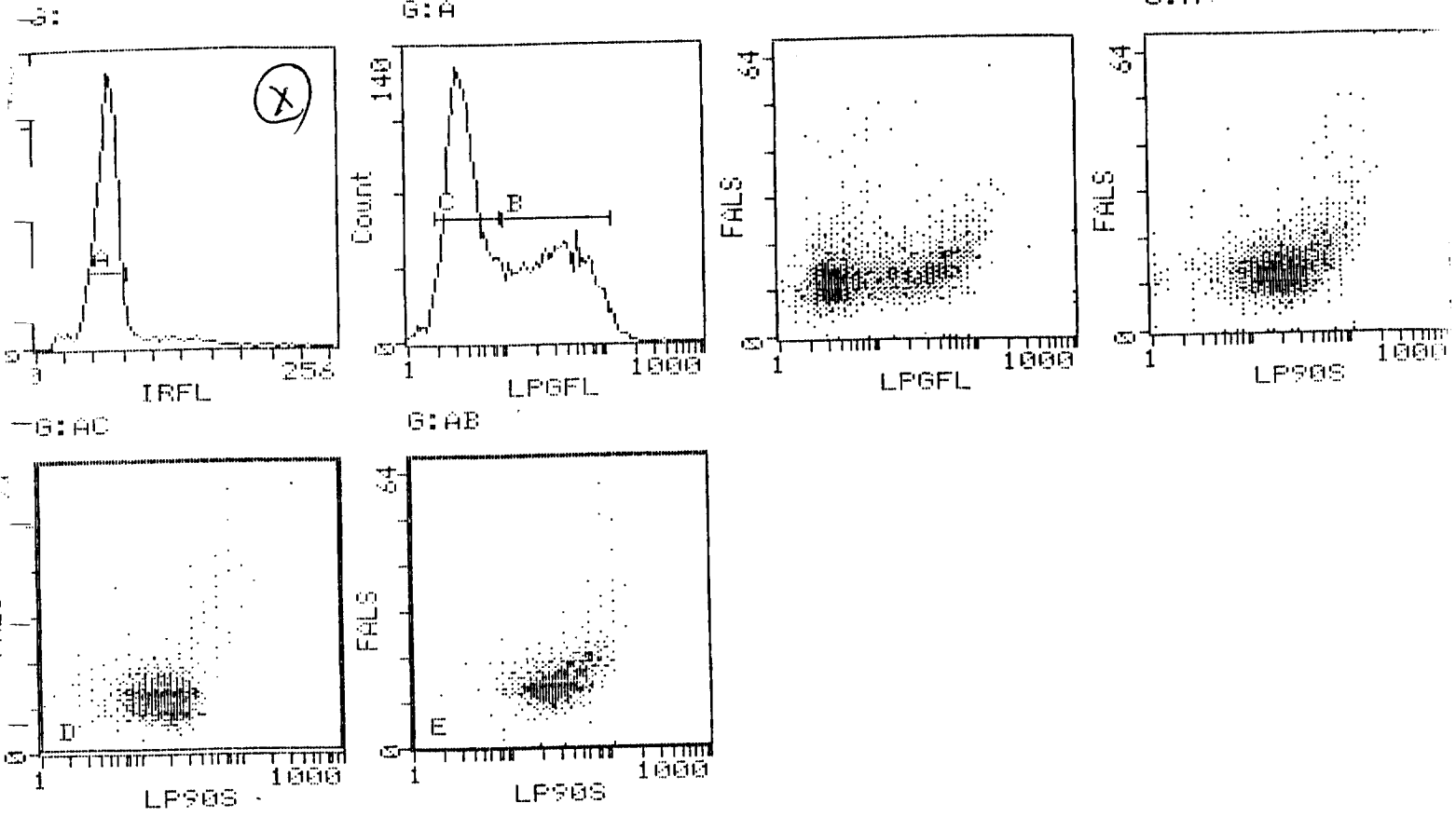
ID	Pcnt	AreaPeak.....	X Channel....		Y Channel....		
			Position	Height	Mean	SD	CV	Mean	SD	CV
1	100	4999	19, 10	67	14.2	11.2	78.8	12.2	7.5	61.3
2	100	1254	40, 13	13	41.3	27.8	67.2	16.7	9.5	56.6

D Low X = 1.0, High X = 1024, Low Y = 0, High Y = 63 D
 E Low X = 1.0, High X = 1024, Low Y = 0, High Y = 63 E

FILE NAME: IML2

CCK #4 - Flight

A (B)



SAMPLE INFO

PROTOCOL : TOM
 DATA RATE : Unknown
 INSTRUMENT : MDADS
 SAMPLE NAME : IML2
 SAMPLE NUMBER : 7
 COMMENTS : F4P
 TOTAL COUNT : 10,001
 LAST HIST :
 LIST FILE : 251094P4.L07
 PROT FILE : T0000007.PRO
 SAMPLE DATE : 250ct94
 SAMPLE TIME : 00:33

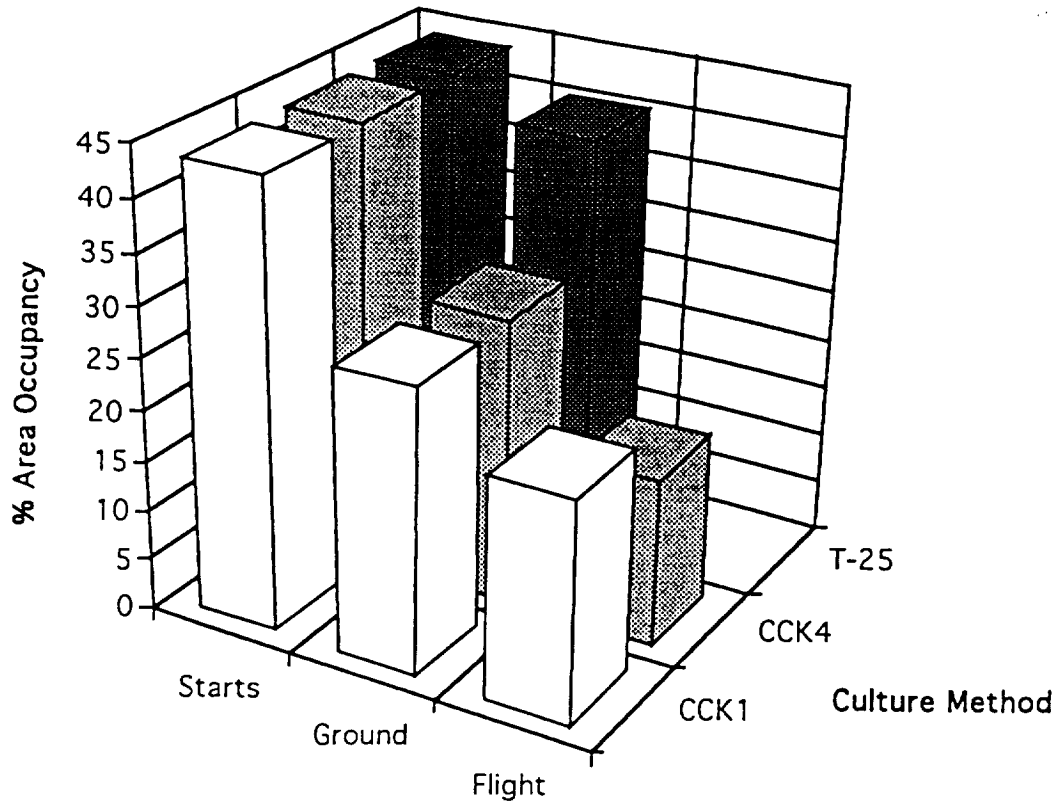
STATISTICS

DUAL PARAMETER STATISTICS

ID	Pent	AreaPeak.....	X Channel....		Y Channel....		
			Position	Height	Mean	SD	CV	Mean	SD	CV
1	100	4235	21, 12	49	15.6	11.1	71.3	12.9	6.0	46.4
2	100	3384	19, 12	28	29.4	20.0	67.9	14.8	5.7	38.1

7 Low X = 1.0, High X = 1024, Low Y = 0, High Y = 63 D
 8 Low X = 1.0, High X = 1024, Low Y = 0, High Y = 63 E

IML-2 Pituitary Cell Area Occupancy



Summary of CCK #4. Results to Date: The Bottom Line and Tentative Conclusions.

Lack of media change had marked consequences on pituitary cell structure and function. Some of these differences were apparent between ground and flight whereas these were not.

- The major difference between cells in CCK #4 vs. #1 was that fibroblast growth was minor in CCK #4 (this was true for both ground and flight).
- Electrophoretic mobility of CCK #4 flight cells was very much less relative to fed cells.
- Flight cells in CCK #4 released 5x more immunoreactive GH than corresponding ground control cells in CCK #4 (pg. 16 of CCK #1 section).
- In order to try to keep cells releasing GH with good bioactivity in flight it appears that frequent media changes will be required.

VI. STATEMENT OF WORK

Statement of Work

NASA grant NAG 8-953

“Electrophoretic Separation of Cells and Particles from Rat Pituitary and Rat Spleen”

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Professor of Biochemistry
006 Althouse Laboratory
Penn State University
University Park, Pa 16802
814 - 865 - 2407
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Period of Performance: 2/8/95 - 2/7/96

Electrophoretic Separation of Cell and Particles from Rat Pituitary and Rat Spleen (NAG8-953)

Statement of Work: 8 Feb. 1995 - 7 Feb. 1996

The objective for the next year's work is to complete the sample analysis outlined in the right hand box of the flow chart (fig. 1). We also need to perform the entire ground based experiment over again because of FFEU hardware failure. Thus we will do an asynchronous ground control experiment, utilizing the same hardware used in the flight experiment at KSC.

Tasks accomplished thus far are detailed in the mid-term progress report (submitted 3/17/95)

Tasks remaining to be accomplished:

CCK#1. Complete analysis of culture media changeouts for Growth Hormone (GH) bioactivity and Prolactin (Prl) immunoactivity and bioactivity. Analyze culture media from electrophoretically fractionated cells for GH and Prl immunoactivity. Perform image analysis on electrophoretically fractionated cells. Analyze extracts from cells for Heat Shock Protein (HSP) presence.

CCK #2. Complete analysis of culture media changeouts and cell lysate for GH bioactivity and Prl immunoactivity and bioactivity. Complete HPLC analysis of culture media and lysate for changes in GH. Perform FFE fractionation of cell lysate. Analyze cell lysate for HSP presence. Complete gel electrophoresis and Western blots of cell lysates for GH and Prl variant analysis.

CCK #3. Complete analysis of culture media and cell lysate for GH and Prl immunoactivity and bioactivity. Continue HPLC analysis of culture media for GH activity. Complete analysis of electrophoretically fractionated lysates. Perform gel electrophoresis and Western blot of cell lysate for GH and Prl variant analysis.

CCK #4. Complete analysis of culture media for GH bioactivity and Prl immunoactivity and bioactivity. Complete HPLC analysis of culture media for GH activity. Analyze cellular extracts for GH and Prl variants and HSP presence.

Asynchronous Ground Control . The requirement for an asynchronous ground control experiment is dictated by the failure of the Free Flow Electrophoresis unit postflight to process the ground control cells from CCK's 1 and 4 (detailed in mid-term progress report).

Prepare manuscripts and present data.