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Effects of Spaceflight on Rat Pituitary Cell Function
Preflight and Flight Experiment for Pituitary Gland Study on COSMOS, 1989

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Effects of Spaceflight on Rat Pituitary Cell Function⁺⁺

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Running Head: Growth Hormone and Spaceflight

ABSTRACT

The secretory capacity of growth hormone (GH) and prolactin (PRL) cells prepared from rats flown in space on the 12.5 day mission of Cosmos 1887 and the 14 day mission of Cosmos 2044 was evaluated in several post-flight tests on Earth. The results showed statistically significant and repeatable decrements in hormone release, especially when biological assays (rather than immunological assays) were used in the tests. Significant and repeatable intracellular changes in GH cells from the flight animals were also found; most important were increases in the GH-specific cytoplasmic staining intensities and cytoplasmic areas occupied by hormone. Tail suspension of rats for 14 days, an established model for mimicking musculo-skeletal changes seen in spaceflown rats, resulted in some changes in GH and PRL cell function that were similar to those from spaceflown animals. Our results add to a growing body of data that describe deconditioning of physiological systems in spaceflight and provide insights into the time frame that might be required for readaptation of the GH/PRL cell system upon return to Earth.

Index terms: Growth hormone, prolactin, microgravity

INTRODUCTION

Changes in the musculoskeletal, immune, vascular and endocrine systems of the rat occur as a result of short-term spaceflight (10). Since pituitary gland growth hormone (GH) plays a role in the control of these systems, and since the results of an earlier spaceflight mission (Spacelab 3, 1985) showed that GH cell function was compromised in a number of post-flight tests (13), we repeated and extended the 1985 experiment in two subsequent spaceflights: the 12.5 day mission of Cosmos 1887 (in 1987) and the 14 day mission of Cosmos 2044 (in 1989). The results of these later two flight experiments are the subject of this report. They document repeatable and significant changes in the GH cell system of the spaceflown rat in several post-flight tests. Results from the Cosmos 2044 flight show changes in the prolactin (PRL) cell system as well.

Methods and Materials

Details of the 1887 and 2044 missions, hereafter referred to as experiments 1 and 2, respectively, are described elsewhere (10, 11, 30). In each experiment, anterior pituitary glands from 5 flight (F) and 5 synchronous control rats (C) were removed aseptically and transported in individual teflon vials from the landing site to the laboratory in Moscow. Results of extensive pre-flight testing indicated that storage of intact pituitary glands for 24-26 hrs at room temperature in Spinners Minimum Essential Medium (SMEM)

containing 0.1% bovine serum albumin, 25 mM Hepes buffer (pH 7.4), 0.2% NaHCO₃, gentamycin sulfate (10 µg/ml), and penicillin (100 U/ml)/streptomycin (100 µg/ml) was optimal for subsequent preparation of cells with high viability (>90%) and good yield ($\approx 1.7 \times 10^6$) (data not shown). Each of these parameters is within the range of those reported for preparation of fresh cells from the rat pituitary (16). During transport of the glands in the above medium (22 ml/vial) the temperature was maintained between 23-24°C.

Cells were prepared from individual glands by trypsinization (8) and kept separate during culture tests to permit statistical analysis of the data. However, some tests required large numbers of cells, so samples from each gland within a group were pooled. The protocol for both flight experiments was similar except that in Exp. 2 an additional group of animals was subjected to tail suspension on Earth for 14 days in order to see how well the results from this approach (22) mimicked actual spaceflight (Figure 1). In each control experiment, 5 Wistar male rats/group were housed under conditions detailed in (10, 30).

Specific methods (individual glands)

Cell preparation: Cells were prepared by trypsinization, counted by hemocytometry and their viability determined as described previously (15).

Cell culture: Cells (2.5×10^5 - Exp. 1, 5×10^4 - Exp. 2) were cultured in 250 µl of modified Eagle's medium (α MEM) containing 5% calf serum and antibiotics (27) or in chemically defined medium containing insulin, transferrin, selenium and other trace elements in

MEM base (27) in 96-well plates for 3 days in a humidified atmosphere of 5% CO₂:95% air at 37°C. There were 3 or 4 wells of cells from each gland. After 3 days the media were saved, stored at -20°C and fresh media added for an additional 3 day culture.

Intracellular hormone extraction: Cells were incubated overnight in 0.01 N NaHCO₃ at 4°C, particulate material centrifuged (1000 xg, 30 min) and the supernatant fraction frozen for later hormone analyses. Greater than 90% of intracellular GH and prolactin (PRL) is extracted under these conditions (8).

Hormone assays: Concentrations of immunoreactive GH and PRL in culture media and extracts were determined by established enzyme immunoassays (6, 7, 26). The antisera for GH and PRL have been validated; their cross reactivities are <0.3% at the final dilutions used (1:80,000-GH; 1:40,000-PRL) (15). The tibial line bioassay of Greenspan (9) was used to measure concentrations of bioactive GH. These were 4 point assays using a bovine GH (1.5 IU/mg) preparation as standard. These data are expressed in terms of rat GH (3.0 IU/mg). PRL bioassays used were either the NB-2 lymphoma cell division assay originally described by Tanaka *et al.* (29) or the IL-2 receptor assay of Mukherjee *et al.* (24). Results of all these assays are expressed in terms of rat GH Standard (B-11) or PRL Standard (B-6) kindly provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the National Hormone and Pituitary Program (University of Maryland School of Medicine).

Morphology: Immunocytochemistry for GH cells (2.5×10^4) attached to poly-l-lysine-coated coverslips was done using a diaminobenzidine procedure (15). These preparations were used for

1) determining GH cell percentages (Exp. 2 only) using a scoring method on coded samples by 6 investigators, and 2) determining the cytoplasmic area occupied by GH, a procedure which involved image analysis that uses a digitized color image of the stained cell to quantify both the area of the cell stained and the intensity of the stain. An OASYS optical analysis system (Lemont Scientific, State College, PA) was used to digitize the image and colorize according to intensities within the system's 256 gray levels (19) so that the area of GH-specific cytoplasmic staining could be quantitated. Care was taken to maintain constant light settings, condenser height, and aperture size in addition to normalizing variations in light using a light meter placed at the level of the camera.

Specific Methods (pooled cells)

Cell implants: In order to determine the amount of bioactive GH secreted from cells of flight animals after implantation into hypophysectomized rats, the hollow fiber method of Hymer *et al.* was used (18). In this case, 2×10^5 cells were loaded into 10 mm long XM 50 Amicon fibers ($n=10$ /treatment group) and implanted into the lateral cerebral ventricles of 100 g male rats 7 days after pituitary gland removal. Ten days post-implantation, the tibia of the host animals were prepared for staining and measurements of epiphyseal cartilage width taken with an ocular micrometer. Fiber placement was verified by visual inspection of brain slices. This implantation procedure was done only in Exp. 1.

Flow cytometry: Cells (2×10^5) were fixed in suspension (PBS/azide buffered 4.0% formalin), their membranes permeabilized

with 0.1% Triton X-100, incubated in GH or PRL antisera (1:10,000) overnight, followed by incubation in FITC-conjugated antisera, counterstained with propidium iodide and analyzed on an Epics flow cytometer (Model 753) exactly as described (15). Analysis included the determination of GH and PRL cell percentages (30,000 cells/trial), intensity of hormone fluorescence, size (forward angle light scatter, FALS) and cytoplasmic granularity (perpendicular light scatter, PLS).

Statistical analysis: When appropriate, data were analyzed by ANOVA and the Students t-test using $p < 0.05$ as a significant difference.

Results

Hormone contents of storage (transport) media

GH: The concentrations of GH and PRL in the 30 hr. storage media of F, C, and tail suspended (TS) animal groups are shown in Table 1. These levels reflect hormone release from the glands into the media during tissue transportation. In Exp. 1 there was a significant increase in levels of immunoreactive GH (i-rGH) in F vials relative to the C group; in Exp. 2 levels of i-rGH were significantly decreased in the flight group. However, in the F groups of both experiments, there was a marked reduction in levels of biologically active GH (b-rGH) secreted into the medium. Transport media from glands of the TS animals were markedly elevated in i-rGH, but were again significantly lower in b-rGH content.

PRL: Concentrations of PRL in the transport media, measured by immunoassay (i-rPRL), NB-2 bioassay or IL-2R assay (b-rPRL)

were usually similar between all treatment groups; however, transport media from the TS group usually contained lower amounts of PRL (Table 1).

Characteristics of GH and PRL cells: The yield of cells from individual glands of most groups averaged 1.9×10^6 with viabilities $>90\%$ (Table 2). The reason for lower yield and viability in the TS group is unknown. Relative to the GH cells in the C group, F GH cells were a) lower in frequency in Exp. 2 but not affected in Exp. 1; b) markedly and consistently increased (2-fold) in GH-specific immunofluorescence staining intensities; c) slightly larger, as evidenced by increased FALS signal; d) not different in PLS signal, and e) consistently and significantly different in the cytoplasmic area occupied by GH (Table 2). As with F cells, GH cells from the TS group were less frequent than those in the C group; however, in the other parameters they were more like C cells. An example of the increased GH-specific cytoplasmic staining intensity seen in F cells from Exp. 1 is shown in Fig. 2.

Relative to the PRL cells in the C group, F PRL cells were a) inconsistent in differences in their frequency distribution and PLS laser light scatter signals; b) somewhat larger (FALS), and c) not different in staining intensities nor in cytoplasmic area occupied by PRL. PRL cells from the TS group again tended to be lower in these characteristics than those in the F and C groups.

In both experiments the intracellular concentrations of i-rGH before or after culture tended to be similar between all treatment groups; however, the intracellular concentrations of b-rGH in the F group, both before and after the culture, were always lower than the

corresponding C group. This was also true for cells from the TS group. Similar measurements for intracellular PRL were not possible due to limited sample size; however no difference in initial PRL content was seen between the F and C groups in Exp. 1.

Release of GH from F cells post-flight: In general, levels of i-rGH in media of the first 3 day and second 3 day cultures were not significantly different between treatment groups. The limited sensitivity of the tibial line bioassay precluded analysis of b-rGH in media of individual culture wells. However, the levels of b-rGH in pooled culture media from F or TS cells were so low that they were either below the assay sensitivity (Exp. 1) or were reduced by $\approx 50\%$ relative to those in the C media (Exp. 2).

The design of Exp. 1, but not Exp. 2, also allowed us to test the ability of the F cells to secrete b-rGH upon implantation into the cerebral ventricles of hypophysectomized rats. As seen in Table 1, GH secretion from post-flight F cells in this *in vivo* test was one-half of that produced by the C cell implants.

Release of PRL from F cells post-flight: Release of PRL, by either immunoassay or the lymphoma cell bioassay, was not significantly different between the cells in the F, C, and TS groups. However, cells from both the F and TS groups released significantly less PRL when the IL-2R bioassay was used.

DISCUSSION

The first objective of experiments 1 (Cosmos 1887) and 2 (Cosmos 2044) was to determine whether our earlier findings on the SL-3 mission done in 1985 (13) were repeatable and statistically significant. A second objective was to determine whether the biological (B) and immunological (I) potencies of the GH and PRL molecules secreted from the flight cells during various post-flight trials were similar. The rationale for this objective is based on reports that the B/I activity ratio of hormone preparations are not necessarily one (5); therefore a marked change in the activity of one form as a result of spaceflight might help identify one hormonal mechanism underlying the cause(s) of microgravity-induced osteopenia, muscle atrophy, suppression of the immune system, etc. A third objective of experiment 2 was to test the effects of tail suspension on the same parameters that we chose to test with the flight cells. Tail suspension is an established model for mimicked microgravity (22) but the fidelity of the mimic is not completely defined. Previous studies from our laboratories show that tail suspension results in partial suppression of *in vitro* release of GH from pituitary GH cells prepared post suspension (14, 23). The Cosmos 2044 mission offered us the unique opportunity to test within the same experiment the effects of tail suspension on the same functional and morphological parameters that we did on the flight cells so that the results could be compared directly with actual flight data.

In Figs. 3 and 4, a comparison of the post-flight secretory activities of GH and PRL cells with that of their ground-based counterparts is presented as ratios of F/C responses. It is interesting that in every spaceflight sample in which a GH bioassay measurement was made the F/C ratio was always <1 (7/7), and the magnitude of the suppression was usually on the order of 50-75%. On the other hand, GH secretory responses measured by immunoassay were obviously inconsistent; about half of the time they were lower (4/8) than responses from the control groups. It is also interesting that the GH secretory responses patterns between the TS and flight cells in Exp. 2 are very similar (cf. Fig 3C vs. 3D). This similarity (together with data of previous studies (14, 23)) clearly establishes the utility of the tail suspension model as a means of mimicking spaceflight on hormone release and this experimental approach can therefore be expected to aid considerably in the design of future spaceflight experiments.

The issue of the dichotomy between the activities of secreted b-rGH and i-rGH, initially reported in 1974 (5), is obviously important to understanding the mechanism(s) underlying these microgravity-induced changes. Ongoing studies from our laboratories have established a) that a subpopulation of GH cells preferentially releases b-rGH *in vitro* and *in vivo*, (12) and b) that aggregate forms of the 22 K GH molecule have enhanced hormone biopotency (8). We hypothesize that exposure to microgravity could alter the secretory activity of a subpopulation of GH cells, perhaps mediated through changes in intracellular packaging of the hormone molecule. The Cosmos experiments offer three clues to unraveling

mechanism(s) underlying the GH secretory defect. *First*, the cerebral ventricles of the hypophysectomized rat contain GRF, a peptide which normally regulates GH release from the somatotroph via cell surface receptors. The finding that F cells did not respond to GRF in the rat ventricles as well as the S cells (as evidenced by tibial line responses in the hypophysectomized host) argues that a receptor or post-receptor defect occurs in a GH cell subpopulation which does not recover under the conditions of Exp. 1. This idea is supported by results of a previous study which showed that GH cells prepared from tail suspended animals did not respond to physiological concentrations of GRF in terms of *in vitro* release of b-rGH as well as cells from non-suspended animals (14). *Second*, cytoplasmic secretory GH granules are associated with a complex network of microtubules (25). An increase in the cytoplasmic area occupied by GH (and presumably secretory granules) in F cells (Table 2) could be interpreted to reflect "relaxation" of that network. *Third*, high molecular weight aggregates of GH are weakly immunoreactive; however, chemical reduction with mercaptoethanol enhances immunoreactivity several-fold (8). The increased immunofluorescent staining intensity of F cells (Table 2) could reflect less S-S bridging between GH molecules; the consequence would be a loss in GH biopotency.

Most of the intracellular GH in the mammalian pituitary gland is stored in 0.3 μm secretory granules as disulfide-linked aggregates of high molecular weight. These granules are capable of stimulating long bone growth when they are injected into hypophysectomized rats (3,17). If microgravity decreases the packing density of the

intragranular GH molecules, increased exposure of epitopes would result in increased cytoplasmic GH immunofluorescence and decreased biopotency of the hormone. In this regard, the repeatability of experiments 1 and 2 is striking and is considered significant. Two recently published theoretical studies suggest the plausibility of our hypothesis that low gravity could affect hormone packaging within cytoplasmic secretory granules. In one (1) Albrecht-Buehler shows how polymerization forces within a cell could be sufficiently large to "sense" perturbations in the cell environment directly. In the other (21) Kondepudi shows the importance of treating cells as systems in non-equilibrium states when considering the issue of gravity and cells. Specifically his analysis also shows that organelles in the size range of pituitary secretory granules may be able to "sense" perturbations in the cell environment (e.g. low gravity).

It is interesting that the post-flight secretory responses of the PRL cells paralleled those of the GH cells in the sense that the biological activity of secreted PRL (IL-2R assay) was also suppressed as a result of flight or tail suspension while the immunological assays gave spurious results (Fig. 4). While differing physiological conditions are known to affect the B/I activity ratio of secreted PRL (28), the molecular mechanisms involved are much less clearly defined. Since no major intracellular changes were found in the PRL cells after flight or tail suspension, or in GH cells after tail suspension, we can only suggest that effects of these treatments on packaging and secretion must be only part of the mechanism(s) that accounts

for the repeatable effect on one endpoint, viz. decreased biological activity of secreted b-rPRL (IL-2R) and b-rGH.

As seen in Fig. 3, the times that elapsed between the landing of the vehicle after spaceflight and the preparation of pituitary cells was vastly different between the two missions. A number of other factors have been examined by investigators involved in both the 1887 and 2044 missions to see if certain other variables could affect experimental results. These have included possible effects of launch and vibration, humidity, light, cabin temperatures, cages, water, food consumption and stress. Analyses show that these factors cannot explain the general results reported in the two spaceflight experiments (10,11). The similarity of our results (Fig. 3) clearly establish the repeatability and statistical significance of the effects of microgravity on the pituitary GH cell system postflight. Deconditioning of physiological systems in spaceflight is well documented (10); the time frame for readaptation of these systems on return to Earth's gravity is not. Our data show that the GH cells have not recovered in their ability to secrete b-rGH up to 2 weeks post-flight, the longest period studied thus far.

There is a growing awareness that the biological and immunological potencies of hormones and growth factors are not necessarily the same and that the physiological state of the organism can affect these activity ratios. Recent demonstrations of the importance of heat shock proteins in processing molecules destined for export (2, 4), and their activation by external environment (e.g. heat, exercise, (20)), may provide important clues for probing into the mechanism(s) underlying the changes in the GH cell system reported

here. Equally plausible are microgravity-driven changes in GRF receptors and/or microtubules in the GH cell. The limited opportunities for spaceflight experimentations are partially offset by the fidelity of some of the responses observed in the GH cells prepared from the tail suspended rat. Studies are underway to search for such mechanism(s) in this mimicked model of microgravity.

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Table 1
Release of GH and PRL from Pituitary Cells of Rats
Previously Flown in Space for 12.5 (Exp. 1) or 14 (Exp. 2) Days

Parameter	Experiment 1		Experiment 2		Tail Suspended
	Cosmos 1887 Control	Flight	Cosmos 2044 Control	Flight	
<u>GH</u>					
Transport media					
i-rGH (ng/ml)	1550 ± 190	1950 ± 100*	160.2 ± 10.6	127.5 ± 9.2*	318.8 ± 33.7**
b-rGH (µg/ml)	2.88 (2.76 - 2.99)	1.13*** (1.03 - 1.28)	2.73 (2.38 - 3.14)	0.58* (0.52 - 0.67)	0.60* (0.58 - 0.69)
Basal secretion					
i-rGH (ng/1000 GH cells)-3 day	57.2 ± 4	62.2 ± 5	25.29 ± 3.31	26.81 ± 3.84	36.8 ± 4.0
i-rGH (ng/1000 GH cells)-6 day	142.9 ± 7	73.0 ± 10*	48.13 ± 4.89	61.37 ± 6.95	70.1 ± 7.1*
b-rGH (µg/ml) - 3+6 day	2.34 (2.03 - 2.63)	<<0.780*†	3.86 (3.42 - 4.35)	1.78* (1.63 - 1.94)	2.61* (2.43 - 2.76)
Implant secretion					
b-rGH(µg released)	2.2 (1.7 - 2.7)	1.0 (0.9 - 1.1)	ND	ND	ND
<u>PRL</u>					
Transport media					
i-rPRL (ng/ml)	307 ± 45	351 ± 63	145.4 ± 16.2	113.6 ± 12.4	56.1 ± 7.3***
b-rPRL (NB-2) (ng/ml)	122 ± 26	188 ± 17	203.5 ± 56.4	172.7 ± 23.5	75.0 ± 9.9**
b-rPRL (IL-2R) (ng/ml)	ND	ND	320.3 ± 38.5	203.0 ± 41.3	191.5 ± 26.4
Basal secretion (ng/1000 PRL cells)					
i-rPRL - 3 day	20.4 ± 0.8	10.5 ± 1.0*	27.9 ± 1.9	24.3 ± 2.4	35.1 ± 7.8
i-rPRL - 6 day	29.1 ± 3	16.2 ± 0.2	53.9 ± 4.1	41.8 ± 4.3	79.9 ± 16.2
b-rPRL (NB-2) - 3 day	32.1 ± 6	7.8 ± 0.8	18.4 ± 5.7	16.6 ± 2.9	22.9 ± 6.0
b-rPRL (NB-2) - 6 day	47.7 ± 13.9	12.7 ± 2.5*	14.0 ± 6.2	20.8 ± 5	7.0 ± 2.3
b-rPRL (IL-2R) - 3 day	ND	ND	12.8 ± 1.1	6.3 ± 0.2***	9.0 ± 2.0**

b-rPRL (IL-2R) - 6 day	ND	ND	15.6 ± 0.6	10.5 ± 0.5***	5.5 ± 0.5***
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Footnote to Table 1.

Transport media contains hormone released from intact pituitary glands during the 24-30 hrs. in transit from the landing site to the laboratory in Moscow.

Basal secretion represents hormone released into culture wells over two consecutive 3 day periods. Data are expressed on the basis of the number of GH or PRL cells seeded.

Implant secretion represents the amount of GH released from cells previously loaded into hollow fibers and implanted into the cerebral ventricle of hypophysectomized rats over a 15 day period. Tibial epiphyseal plate widths were compared to those in other hypophysectomized rats which had received bovine GH for 4 days in order to estimate concentrations of hormone secreted from implant. 95% confidence limits are shown in ().

i-rGH/PRL = immunoreactive growth hormone/prolactin; b-rGH/rPRL = biologically active growth hormone/prolactin; b-rPRL (NB-2) = prolactin activity detected by lymphoma cell bioassay (26); b-rPRL (IL-2R) = prolactin activity detected by interleukin-2 receptor assay (21); ND = assay not done; - indicates insufficient sample volume; data represent averages ± standard error of mean; *p<0.05, **p<0.01, ***p<0.001 relative to control. †Epiphyseal plate widths not different than those from saline-injected control animals.

Table 2
 Characteristics of GH and PRL Cells Prepared From Pituitaries of
 Rats Previously Flown in Space for 12.5 (Exp. 1) or 14 (Exp. 2) Days

Parameter	Experiment 1 Cosmos 1887		Experiment 2 Cosmos 2044		Tail Suspended
	Control	Flight	Control	Flight	
<u>General</u>					
cell yield	1.68 ± 0.1	1.98 ± 0.2	1.8 ± 0.1	2.2 ± 0.1*	1.67 ± 0.08
viability	90 ± 0.9	93 ± 0.7	94.2 ± 0.6	92.3 ± 0.9	83.8 ± 1.3***
<u>GH</u>					
%(flow cytometry)	23	27	36	24	27.6
%(ICC)	ND	ND	26.3 ± 2.8	20.2 ± 2.9	16.1 ± 2.0*
fluorescence intensity (marker index)	13.1	24.5	4.4	8	4.3
FALS	151	181	32	40	31
PLS	145	145	37	41	38
% area occupied by GH	37.1 ± 4.7	49.9 ± 4.1*	36.6 ± 2.5	48.6 ± 2.3*	34.1 ± 1.5
<u>Extracts</u>					
initial i-rGH (ng/1000 GH cells)	61 ± 17	40 ± 17	-	-	-
initial b-rGH (µg/ml)	9.13	4.18§	2.11 (1.63 - 2.74)	<<0.780*†	<<0.780*†
final i-rGH (ng/1000 GH cells)	22 ± 2.0	8.6 ± 0.8	18 ± 2	22 ± 3	18.3 ± 2.3
final b-rGH (µg/ml)	2.05	1.63§	3.31 (3.20 - 3.49)	<<0.780*†	<<0.780*†
<u>PRL</u>					
%(flow cytometry)	32	47	33.3	34.4	22.6
marker index	5.1	4.1	5.7	4.7	3.7
FALS	24	40	12	21	10
PLS	64	92	25	21	21
% area occupied by PRL	-	-	25.5 ± 3.2	32.9 ± 6.1	32.1 ± 7.8

initial intracellular PRL
(ng/1000 PRL cells)

0.24 ± 0.02 0.22 ± 0.05

-

-

-

Footnote to Table 2.

Percentages of GH/PRL cells were determined by flow cytometry (30,000 cells counted/trial) or, in one case, by immunocytochemistry as well. Intensity of cytoplasmic fluorescence hormone staining (see Fig. 2) were quantified by flow cytometry using the marker index method, which is based on fluorescence intensity converted to voltage. The ratio of voltages of stained to unstained cells = marker index. FALS and PLS (forward angle light scatter and perpendicular light scatter, respectively) determined by flow cytometry. Values represent peak channels of size (FALS) and cytoplasmic granularity (PLS), respectively (15). Area of GH in cytoplasm of GH cells were determined by image analysis and intracellular hormone levels before and after culture determined on alkaline extracts of cells (see Materials and Methods).

§Significance could not be determined for these samples due to insufficient sample volume.

Figure Legends

- Figure 1 Experimental protocol followed for Experiments 1 (Cosmos 1887) and 2 (Cosmos 2044). See Materials and Methods for specific assays.
- Figure 2 Photomicrographs of GH cells (yellow) from Exp. 1 stained by an immunofluorescence procedure (see Materials and Methods) (magnification = 670x). Top: control; bottom: flight.
- Figure 3 Comparison of post-flight secretory responses of GH cells from rats flown on the Spacelab 3 mission in 1985; Cosmos 1887 (Exp. 1); Cosmos 2044 (Exp. 2); and tail suspended animals (TS-Exp. 2) expressed as the ratios of experimental to control. Data for the SL-3 experiment previously published in (14); permission to use these data obtained from *American J. Physiology*.
- Figure 4 Comparison of post-flight secretory responses of PRL cells from rats flown on the Cosmos 1887 mission (Exp. 1); Cosmos 2044 mission (Exp. 2) and tail suspended animals (TS-Exp. 2) expressed as the ratio of experimental to control.

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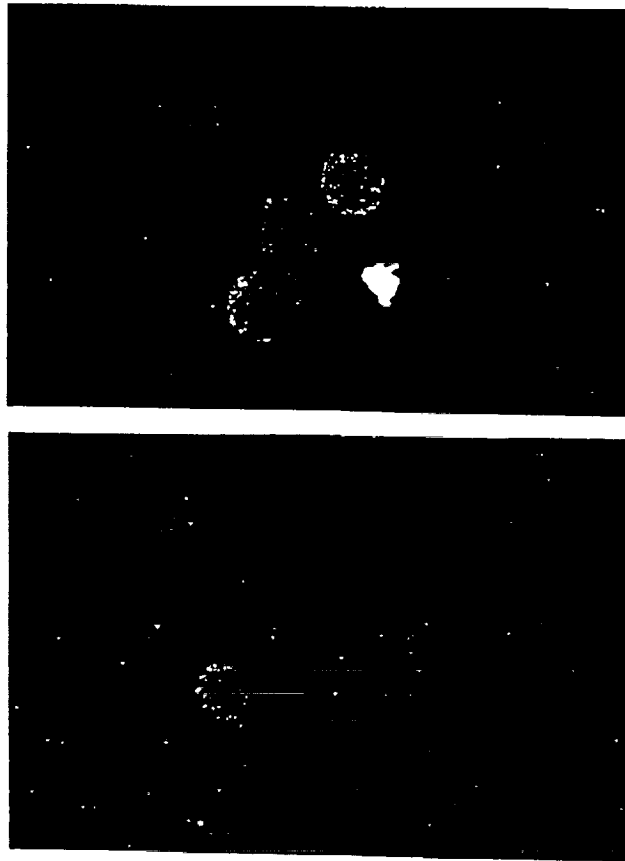


FIGURE 2

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ANTERIOR PITUITARY GLANDS (Flight and synchronous ground control)
(5 glands)

↓ TRANSPORT TO MOSCOW

→ ANALYSIS OF TRANSPORT MEDIA

↓ TRYPSINIZE GLANDS INDIVIDUALLY



- (A) *IN VITRO* CELL CULTURE
- (B) MORPHOLOGY (EXP. 2)
- (C) INTRACELLULAR GH



↓ POOL REMAINING CELLS

- (A) *IN VIVO* CULTURE (EXP. 1)
- (B) MORPHOLOGY (EXP. 1)
- (C) FLOW CYTOMETRY

