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Final Technical Report

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TITLE: INTESTINAL MUCOSA MATURATION & GROWTH IN SPACE

Principal Investigators: Robert W. Phillips
Heywood R. Sawyer

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Institution: Colorado State University
Fort Collins, Colorado 80523

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EFFECTS OF SPACEFLIGHT ON THE PROLIFERATION OF JEJUNAL MUCOSAL CELLS

R.W. Phillips, C.L. Moeller, H.R. Sawyer and K.L. Smirnov *

Department of Physiology
College of Veterinary Medicine and Biomedical Sciences
Colorado State University
Fort Collins, CO 80523

* Institute of Biomedical Problems, USSR Ministry of Health,
Moscow, USSR

SUMMARY

The purpose of this project was to test the hypothesis that the generalized, whole body decrease in synthetic activity due to microgravity conditions encountered during spaceflight would be demonstrable in cells and tissues characterized by a rapid rate of turnover. Jejunal mucosal cells were chosen as a model since these cells are among the most rapidily proliferating in the body. Accordingly, the percentage of mitotic cells present in the crypts of Lieberkuhn in each of 5 rats flown on the COSMOS 2044 mission were compared to the percentage of mitotic cells present in the crypts in rats included in each of 3 ground control groups (i.e., vivarium, synchronous and caudal-elevated). No significant difference ($p>.05$) was detected in mitotic indices between the flight and vivarium group. Although the ability of jejunal mucosal cells to divide by mitosis was not impaired in flight group, there was, however, a reduction in the length of villi and depth of crypts. The concommitant reduction in villus length and crypth depth in the flight group probably reflects changes in connective tissue components within the core of villi.

INTRODUCTION

Although the observed negative nitrogen balance (1,2), muscle atrophy (1-3), loss in bone mass (2) and decreased proliferation of lymphocytes (4) in astronauts exposed to microgravity conditions is thought to reflect a generalized, whole body decrease in synthetic activity (1), the effects of microgravity on rapidly proliferating cells and tissues have not been thoroughly examined. Thus, the purpose of this study was to test the hypothesis that the generalized, whole body decrease in synthetic activity due to microgravity conditions that accompany spaceflight would also be manifested in cells and tissues characterized by a rapid rate of turnover. Accordingly we chose to examine the effects of microgravity on jejunal mucosal cells that line the small intestine. Jejunal mucosal cells are among the most rapidly proliferating in the body and are derived from stem cells present in the crypts of Lieberkühn (5,6). Once formed they migrate out of the crypts onto intestinal villi; are progressively "pushed up" villi as new cells are formed; and, ultimately reach the tips of villi where they are then desquamated. In rats the entire process, from initial formation in crypts to desquamation, normally takes approximately 2 days (5,6). .

To determine the effects of spaceflight on the proliferation of jejunal cells the percentage of mitotic cells present in the crypts of Lieberkühn in each of five rats aboard the COSMOS 2044 flight were compared to the percentage of mitotic cells present in the crypts of Lieberkühn in each of three ground control groups (i.e. vivarium, synchronous and caudal-elevated). In addition, the depth of crypts and length of jejunal villi in each of five rats per group were measured.

MATERIALS AND METHODS

Tissue Samples

Tissue samples (each 1 cm in length) from the proximal, middle and distal regions of the jejunum from each of 5 rats from the vivarium, synchronous, caudal-elevated and flight groups were processed and shipped to Colorado State University per pre- and post-flight protocols described for the COSMOS 2044 mission. Briefly, jejunal regions of interest were removed and flushed with 1 to 2 ml of physiological saline. Immediately thereafter each sample was flushed with 2 ml of a solution of 4% glutaraldehyde-0.1M cacodylate (pH 7.4) containing 5% sucrose and placed into 25 ml screw-top vials containing approximately 20 mls of the same fixative. After 6 - 24 hours of fixation, the samples were rinsed 3 times in 0.1 M cacodylate buffer and shipped to Colorado State University. Upon arrival, each sample was cut into 5 equal segments, post-fixed in 1% osmium tetroxide for 90 min, washed in cacodylate buffer, dehydrated in a graded series of ethanols and embedded in Polybed 812.

Mitotic Index

Sections 1 µm-thick were cut from each of the 5 segments from each of the 3 jejunal regions per animal and stained with toluidine blue. To accurately determine the mitotic index for each respective region, at least 2000 cells per region per animal were examined. Since mitosis is restricted to the crypts of Lieberkühn (5), cells outside the crypts proper were not considered in determining mitotic indices. Prior to evaluation all slides were coded so that the technician reading the slides did not know the region or treatment group being examined. All tissue sections used to determine mitotic index were also evaluated for the presence of microscopic lesions by a pathophysiologist.

Villus Length and Crypt Depth

To determine villus length and crypt depth at least 20 villi and crypts were measured per region per animal. Measurements were obtained using a computerized image analysis system (Bioquant System IV) coupled to a Olympus bright field microscope equipped with a 4X objective and a video camera. Special care was taken to ensure that measurements were taken only on villi and crypts that had been cut in cross-section.

Statistics

All data were statistically analyzed by analysis of variance and differences between means were detected using the Student-Newman-Keuls procedure.

RESULTS

General Observations

With only one exception no microscopic lesions of pathological significance were observed. The exception was limited to the distal region of the jejunum in one rat included in the vivarium group. The lesion observed consisted of a localized fibrotic patch indicative of a previous ulceration that had subsequently healed.

Mitotic Index

The percentage (mean±sem) of mitotic cells present in the crypts of Lieberkühn in the proximal, middle and distal regions of the jejunum for rats included in each of the respective treatment groups is summarized in Table 1. The only significant difference ($p < .05$) detected was that the percentage of mitotic cells present in the middle region of the jejunum of rats included in the synchronous group was slightly higher than in any of the other treatment groups. Similarly, the only statistically significant difference in mitotic index among regions within group was apparent only in the synchronous group where the mitotic index in the proximal region was lower than in either the middle or distal regions.

Villus Length and Crypt Depth

The length of jejunal villi and depth of crypts of Lieberkühn in the proximal, middle and distal regions of the jejunum are summarized in Tables 2 and 3, respectively. Regardless of region examined jejunal villi in rats included in the vivarium group were greater in length than in rats included in the synchronous and flight groups (Table 2). The length of villi in the middle and distal regions in rats included in the caudal-elevated group was similar ($p > .05$) to that measured in rats in

the vivarium group. However, the length of villi in the proximal region differed (vivarium = 738.2 μm vs. caudal-elevated = 643.6μm). Differences among jejunal regions within group were restricted to the caudal-elevated group where villi were longer in the middle and distal jejunum than in the proximal jejunum.

Treatment effects on the depth of the crypts of Lieberkühn were primarily restricted to the proximal and distal jejunal regions: no significant difference among groups was detected with respect to the depth of crypts in the middle region of the jejunum (Table 3). Thus, with the exception of the distal region in the flight group rats, the depth of crypts in the proximal and distal regions was greater in the vivarium group than in the synchronous, caudal-elevated and flight groups. As evident from the data presented in Table 3, there was no significant variation in the depth of crypts among regions within treatment groups.

DISCUSSION

The percentages of mitotic cells present in the crypts of Lieberkühn in the proximal, middle and distal regions of the jejunum in rats included in the flight group did not significantly differ from the percentage of mitotic cells present in identical regions in rats included in the ground control vivarium group. Thus, while there may be a generalized, whole body decrease in synthetic activity associated with microgravity conditions during spaceflight, the proliferation of jejunal mucosal cells does not appear to be affected. These data also support our previous conclusions from the Cosmos 1887 mission that the effects, if any, of spaceflight on the proliferation of jejunal mucosal cells are minimal and short-lived (7). However, it should be noted that while the ability of cells in the crypts of Lieberkühn to divide by mitosis was not found to be impaired in the present study, the length of villi was significantly reduced in all jejunal regions in flight rats as compared to vivarium rats. Since villi in flight rats were lined by normal mucosal cells, the concomitant reduction in villus height and crypt depth probably reflects changes (e.g, shrinkage) in the connective tissue core of villi and is not due to an impairment in the migration of newly proliferated cells needed to replace those desquamated. At present, the physiological basis and potential significance of this observation is not clear.

SUMMARY AND CONCLUSIONS

To examine the effects of spaceflight on the proliferation and turnover of jejunal mucosal cells, the percentages of mitotic cells present in the crypts of Lieberkuhn in the proximal, middle and distal jejunum in each of 5 rats flown on the COSMOS 2044 mission were compared to percentages in the identical regions in rats included in the vivarium, synchronous and caudal-elevated groups. Based on the data obtained, there was no difference in mitotic indices between animals in the flight and vivarium (ground control) groups. Thus, it appears that the ability of jejunal mucosal cells to proliferate is not affected by microgravity conditions associated with spaceflight. Although the length of villi and depth of crypts were reduced in flight animals as compared to those in the vivarium group, the observed reduction is probably attributable to changes in the connective tissue core of villi, and is not likely due to an impairment of the proliferation and migration of jejunal mucosal cells.

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TABLE 1. Mitotic index for jejunal mucosal cells

Region	Treatment groups[a]			
	Vivarium	Synchronous	Caudal-elevated	Flight
Proximal	3.1 ± .2	3.3 ± .2^{b}	3.3 ± .3	4.0 ± .4
Middle	3.6 ± .4^{AB}	3.9 ± .1^{Aa}	3.3 ± .2^{B}	3.4 ± .1^{B}
Distal	3.3 ± .2	3.9 ± .2^{a}	4.0 ± .1	3.9 ± .2

[a]Means (± SEM) with different uppercase letter superscript within rows are different ($P<0.05$). Means (± SEM) with different lowercase letter superscript within columns are different ($P<0.05$).

TABLE 2. Length (μm) of jejunal villi

	Treatment groups[a]			
Region	Vivarium	Synchronous	Caudal-elevated	Flight
Proximal	738.2 ± 23.7^{A}	634.0 ± 13.0^{B}	643.6 ± 10.9^{Bb}	608.5 ± 19.0^{B}
Middle	730.2 ± 47.0^{A}	632.0 ± 17.9^{B}	689.5 ± 7.5^{ABa}	622.1 ± 10.5^{B}
Distal	717.7 ± 27.1^{A}	623.2 ± 16.0^{B}	672.5 ± 8.1^{ABa}	628.2 ± 20.0^{B}

[a]Means (± SEM) with different uppercase letter superscript within rows are different ($P<0.05$). Means (± SEM) with different lowercase letter superscript within columns are different ($P<0.05$).

TABLE 3. Jejunal crypt (μm)

	Treatment groups[a]			
Region	Vivarium	Synchronous	Caudal-elevated	Flight
Proximal	164.2 ± 1.2^{A}	133.3 ± 2.4^{B}	139.5 ± 4.5^{B}	134.6 ± 3.5^{B}
Middle	143.4 ± 7.5	133.4 ± 3.0	131.8 ± 2.4	$135. \pm 5.1$
Distal	161.6 ± 11.3^{A}	129.6 ± 2.9^{B}	129.1 ± 3.5^{B}	152.0 ± 7.3^{AB}

[a]Means (± SEM) with different uppercase letter superscript within rows are different ($P<0.05$). Means (± SEM) with different lowercase letter superscript within columns are different ($P<0.05$).