EXPERIMENT K-6-05

THE MATURATION OF BONE AND DENTIN MATRICES IN RATS FLOWN ON COSMOS 1887

Principal Investigator:

D. Simmons
University of Texas Medical Branch
Department of Surgery
Division of Orthopedic Surgery
Galveston, Texas 77551

Co-Investigators:

M. Grynpas
University of Toronto
Mt. Sinai Hospital
Department of Pathology
Toronto, Ontario, Canada

G. Rosenberg
Indiana University- Purdue University
Department of Geology
Indianapolis, Indiana 46202

G. Durnova
Institute of Biomedical Problems
Moscow, USSR

SUMMARY

The chemistry, hydroxyapatite crystal size, and maturation of the bone and dentin is characterized in rats exposed to microgravity for 12.5d in a Soviet Biosatellite (Cosmos-1887). Calvarial and vertebral bone ash was subnormal, but contained a normal percent composition of Ca, P, and Mg. These tissues varied from the norm by having lower Ca/P and higher Ca/Mg ratios than any of their age-matched controls [Vivarium and Synchronous Groups]. Gradient density analyses [calvaria] indicated a strong shift to the lower sp.gr. fractions which was commensurate with impaired rates of matrix-mineral maturation. X-ray diffraction data were confirmatory. Bone hydroxyapatite crystal growth in Flight rats was preferentially altered in a way to reduce the dimension of their C-axis. Flight rat dentin was normal with respect to age-matched control Ca, P, Mg, and Zn concentrations and their Ca/P and Ca/Mg ratios. These observations affirm the concept that microgravity adversely affects the maturation of newly formed matrix and mineral moieties in bone.

INTRODUCTION

There is much concern to maintain musculo-skeletal integrity in astronauts during prolonged spaceflight. This concern translates to questions about the role of gravity in calcium-mediated physiological mechanisms. The 'side effects' of spaceflight include impaired bone modeling and remodeling, loss of [trabecular] bone mass and reduced capacity of the vertebrae and long bones to withstand certain loads. These changes are the species independent common thread of experience in space biology research (Jee et al 1983; Kazarian 1981; Roberts et al 1981; Simmons et al 1983).

Our particular interest in the skeletal status of rats flown in space focuses upon the normality of the matrix and mineral moieties deposited in the bones and teeth. Gradient density analyses have shown that the environment of space retards the maturation of newly synthesized collagen and hydroxyapatite [see Cosmos-1129 (Rosenberg et al 1984; Simmons et al 1983)] and Spacelab-3 Missions (Simmons et al 1986). A larger than the normal percent of total calcium, inorganic phosphorus and matrix hydroxyproline and osteocalcin is found in the lowest density fractions which include the most recently formed osseous and dentinal substance. Importantly, we were unable to duplicate the spaceflight density profiles in a ground based 1G model of hypokinesia--the jaws of tail-suspended rats (Simmons 1983a), and the jaws of primates whose postcranial skeleton was immobilized in plaster (Simmons et al 1984). We have since determined that the vertebral, femoral, and mandibular bone mineral in biosatellite flown rats differs from normal in having a smaller crystal size (Grynpas et al 1986; Simmons et al 1986). All of these changes indicate that the maturational deficit in spaceflight has a special gravity component.

Judgments about the effects of gravity on bone maturation are necessarily tentative since our experiences are limitted to a single "long term Mission [18 5d Cosmos-1129] and a single mid-deck Space Shuttle flight [7d Spacelab-3]. This report deals with the analyses of various weight and non-weight bearing bones and incisor dentin from rats carried on a biosatellite flight of intermediate duration--the 12.5d joint Soviet-U.S.A. Cosmos-1887 Mission. In this work, we sought to extend our observations on how microgravity affects the skeletal maturational and compositional changes.

MATERIALS AND METHODS

The study involved 12 week old male Wistar rats bred and reared at the Czechoslovakian Academy of Sciences [325 g average body weight]. The rats were segregated into 4 groups of 6 animals each.

Group	Sacrificed	Wt.at Sacrifice (g)
I Basal C	5d Preflight	325 ± 5
II Vivarium Controls	3-4d Postflight	318.6± 6
III Synchronous Co	5d Postflight	392 ± 9
IV Biosatellite	2.5d Postflight	352 ± 6

The Synchronous Controls (Group III) differed from the other Control rats (Groups I and II) by being artificially exposed to a level of noise and vibration equivalent to that experienced by the Flight rats (Group IV) during the launch, reentry, landing and recovery periods.

All animals were multiply housed (10/cage) and fed a semi-synthetic paste diet [40g/d (70% water)], identical in composition to that used on previous biosatellite flights. The diet is adequate in calories [protein= 18.2%, fat= 24.2%], carbohydrates [57.4%], minerals and calciotropic vitamins, but the feed is relatively low in zinc [0.07mg/40g= 39% of the daily requirement (Pace et al 1981)]. Drinking water was freely available. All animals were exposed to temperatures of 23 + 1C and a 16h/8h light-dark cycle. The intensity of light at the cage bottom was 4-8 lux.

The Flight rats were loaded into the biosatellite some 24h prior to launch [Sept. 29, 1987], and they were flown in space for 12.5d. The flight was terminated on October 12, 1987, and the spacecraft landed near Murni, Siberia rather than at the designated recovery site near Kustanei in Kazakhstan. Due to this unforseen occurrence, there was a 56h delay between landing, sacrifice and autopsy.

The rats were sacrificed by decapitation. The skull cap (calvarium), 5th lumbar vertebra, and a mandible from each rat was stripped of soft tissues and fixed in 100% ethyl alcohol. The mandible was transversely sectioned at the diastema to remove a 2.0mm slab for electron microprobe compositional studies. The more anterior and posterior regions of the jaw were reserved for the studies on bone maturation [bone density fractionation, chemistry].

A. Bone Maturation Studies

1. Bone Density Fractionation: Insufficiency of material limited this study to the calvarial samples.

The bone specimens were frozen in liquid nitrogen, lyophilized and pulverized in a percussion mill [Spex Freezer Mill, Metuchen, N.J.], cooled in liquid nitrogen, and sieved in a sonic sifter to isolate bone particle sizes below 20 um. The powder was then fractionated [1.7-2.3 g/ml] in a bromoform-toluene mixture by the stepwise centrifugation method of Grynpas et al (1986). In practice, 200-300 mg of sieved powder was added to a polyallomer tube containing 35ml of a 2.0g/ml density solution [calibrated with sink floats]. The tubes were capped and sonicated to obtain a homogeneous suspension of the powder, and it was centrifuged at 10,000 rpm for 30 min. The density of the supernatant was then modified to 1.9 mg/ml by the addition of toluene,

and the new solution was recentrifuged. Under the same conditions, each precipitate obtained from solutions of progressively decreasing density [at 0.1 g/ml steps] was collected. To obtain a range of mineral densities greater than 2.0 g/ml, the precipitate obtained from the initial 2.0 g/ml density solution was resuspended in a solution of density 2.3 g/ml. Successive centrifugation of precipitates at progressively decreasing densities [0.1 g/ml steps] provided the higher density fractions. The series of specific gravity [sp.gr.] fractions obtained in this way were centrifuged in 100% ethyl alcohol to remove organic solvent and they were dried in a dessicator at room temperature.

The relative contribution of each of the fractions to the original weight of the unfractionated bone powder was calculated from the mineralization profile in each sp.gr. fraction. In our hands, the reproducibility of the method was ± 2.0 % [SD].

2. X-Ray Diffraction: Samples of bone powder from the unfractionated samples were analyzed with a Rigaku microdiffractometer using Ca Ka radiation and using a highly crystalline mineral fluoroapatite as a standard. The values of B 1/2 [002] and [130], the widths at one-half the maximum height of the hydroxyapatite reflections, were measured using a step-scanning procedure with 0.4 degrees/step and 100 sec of counting. Because the instrumental broadening was small compared with sample peak breadths, the measured half-widths were corrected for instrumental broadening by subtracting the square of B[002] and B[130] for the standard [fluoroapatite] from the square of the bone value and taking the square root of the difference. D-values, which are related to the crystal size and strain in the long dimension [002] and the cross section [130] of the apatite crystal, were calculated from the corrected B[002] and B[130] value [B 1/2] using the Sherrer Equation (Simmons et al 1986):

$$D = \frac{K \lambda \text{ radian}}{B_{1/2} \cos \theta}$$

where λ is the X-ray wavelength, $B_{1/2}$ the breadth at half the height of the 002 and 130 peaks, and θ the diffraction angle. K is a constant varying with crystal habit and chosen as 0.9 for the elongated crystallites of bone. Each measurement was repeated 3-times and the results are presented as the ± standard deviation.

3. Chemical Studies: Aliquots of tissue weighing an average of 10mg were washed in a 4:1 v/v nitric: percholoric acid mixture. A blank containing only the acid mixture served as a control for the procedure. An external control was provided by including 10 mg phosphate rock [NBS Standard #120b]. The samples placed in covered Teflon beakers were slowly heated and then brought to a boil for 1.5h. The lids were then removed and the digested samples were brought to a final volume of 20 ml with double deionized water.

Calcium and magnesium analyses were performed on ashed samples. For calcium, 1:50 and Mg 1:125 lN 10Mm lanthanum chloride [250 ml 10% La from BDH in 18L distilled water] was added to 100ml of each sample. The samples were thoroughly shaken and analyzed by atomic absorption spectrophotometry [Perkin-Elmer 400]. Ca and Mg were measured thrice and the average was used for further calculations.

For inorganic phosphate, one part 10% ascorbic acid [10g/100 ml distilled water] was mixed with 6 parts 0.42% ammonium molybdate $\cdot 4H_2O$ in 1N H_2SO_4 [28.6 ml concentrated H_2SO_4 and 4.2 g ammonium molybdate$\cdot 4H_2O$ to 1000 ml distilled water], and the solution was kept on ice. 10ml ashed sample and 90 ml distilled water were plated in duplicate in Tetertek Plates. 230 ml of the acid-molybdate solution was added. The plate was covered with paraffin and incubated for 1h at 37C. The plate was then read on a Tetretek Multiskan at 620nm.

B. Electron Microprobe Investigations for Mandibular Bone and Incisor Dentin Composition

The diastemal mandibular slab was embedded in epoxy and the exposed surfaces were polished using successively finer grades of sandpaper and alumina grit. The blocks were then mounted in a MAC-5 Microprobe. Analyses for calcium, phosphorus, magnesium and zinc in bone were made on continuous traverses which passed across the mid-lateral periosteal-endosteal surfaces, the periodontal membrane, and dentine to the pulp-dentine margin using a 3.0-4.0um spot size, with measurement at 4.0um intervals. Analyses for these elements in teeth were made in a traverse which passed from the pulp-dentin border to the enamel surface (Rosenberg et al 1984; Simmons et al 1983). The quantitative analyses were Bence-Albee reductions of 10 measurements made across the bone/teeth. Zinc [Zn] was detected using a LiF crystal. P and Mg were detected using a RAP crystal, and calcium and phosphorus was detected using a PET crystal. Calcium and phosphorus were standardized on an apatite crystal, Mg on a MgO standard, and Zn on the Zn-silicate Willemite. The specimen current was 0.015 uamps. The accelerating potential was 15 kv, and each measurement was made for 10 sec without noticeable deterioration of the specimen. Background and drift were measured and subtracted from each measurement.

In this study, we report only the average composition of the tissues in terms of the ratios of Ca/P, Ca/Mg, and Ca/Zn. The analyses do not compare the compositions of the calcified matrices deposited pre-and postflight. These will be the subject of further study.

RESULTS

1. Bone Maturation Studies (Calvaria)

The sp.gr. mineralization profiles for the calvarial samples are shown in Table 1. Compared to control animals, the profile for the Flight group showed a sharp shift to the lower density fractions. The lower sp.gr. fractions [1.8-1.9] contained the larger percent sample weight, while the higher sp.gr. fractions [2.0 & 2.3] contained the smaller than normal sample weights. This shift was confirmed by the observation that the Flight rat calvaria exhibited the lowest sample ash weight and percentages of dry weight Ca, P and Mg [Table 2].

The size of the samples for the 5th lumbar vertebrae and mandibles were insufficient for gradient density evaluation. However, their chemistries were assessed [Table 2]. While calvarial and vertebral Mg levels were the lowest in the Flight rats, the overall composition of these tissues were not aberrant [ex. Ca/P & Ca/Mg] and thus the data are consonant with the results of all previous investigations (Rosenberg et al 1984; Simmons et al 1983). The results from the electron microprobe studies were very similar (see Tables 4 & 5).

2. X-ray Diffraction
The data consist of analyses from the Synchronous Control and Flight Groups. Table 3 indicates that the hydroxyapatite crystals in the mandibles and calvaria of Flight rats are comparatively much the smaller, but the change is found only in the longer C-axis.

3. Electron Microprobe Compositional Analysis-Diastemal Mandibular Bone [Table 4]:
Flight rat bones exhibited normal Ca/P and Ca/Zn ratios when compared to any of the three control groups. However, the Flight rat bones were relatively richer in Mg than the bones from the Vivarium and Synchronous Controls -- animals which were closest in age at sacrifice. Anomalously, the Ca/P ratio for the Synchronous Controls were subnormal with respect to the Basal Controls.

4. Diastemal Incisor Dentin [Table 5]:
Flight rat dentin exhibited trends which were somewhat similar to the mandibular bone. The tissue from the Flight rat and Synchronous Controls had lower Ca/P ratios than the Basal Controls [$P<0.05$]. The Ca/Zn ratios were similarly equivocal. The Flight rat dentin had significantly less Zn than the Basal Controls [$P<0.01$], but there were no differences in concentration between the Flight and the other two control groups. The Ca/Mg ratios suggested the lack of important intergroup differences in tissue Mg. There was, then, little in the way of compositional changes that could be attributed to spaceflight.

DISCUSSION

Despite the fact that it was not possible to obtain tissue samples from the Flight rats until after they had been exposed to earth's gravity for 2.5d postflight, the bones recorded effects of microgravity which were similar to those observed after shorter and longer Missions. The bone mineral (Ca, Mg, P)-matrix (hydroxyproline) gradient density distributions from a variety of weight bearing and non-weight bearing bones have always shown a delay in tissue maturation. This conclusion is based on three findings: that the bulk of bone [particles] sediments-out in the lower sp.gr. range, that the mineral hydroxyapatite crystals formed in spaceflight remain small/immature, and their low Ca/P ratio and higher than normal sulfur content reflects the protracted adolescence of the forming tissue. The higher than normal Ca/Mg ratios can be interpreted within the context of a literature that recognizes that Mg levels are always higher in immature/newly forming skeletal tissues than in temporally older regions which have mature/achieved full mineralization (Schwartz 1988). Herein, the trend to low Mg was pronounced in the Flight rat bone [vertebrae, calvaria, jaws], indicating a subnormal rate of new bone formation. There was no such trend in the rat dentin where the rate of dentinogenesis has never been demonstrated to deviate from normal-- perhaps, teleologically speaking, a metabolic concession that in space the teeth are more necessary than the weight bearing bones.

The inclusion of zinc determinations in the electron microprobe study was pursued to investigate whether the decrease in skeletal ash might have been related to low zinc nutriture. There are reasons for believing that the 30% of the rat daily Zn requirement provided by the paste diet might have direct and indirect skeletal consequences. A diet high in Ca and P and casein (18%) could interfere with intestinal zinc absorption (Allred et al 1964; Pecoud et al 1975). Indirectly, poor zinc nutriture is associated with decreased circulating levels of plasma somatomedin-C/ insulin-like

growth factor-I, as well as impaired synthesis of DNA, alkaline phosphatase, collagen, non-collagen protein, prostaglandin and mineralization (Cossack 1984). More directly, Zn stimulates differentiated bone cell function -- the ability of osteoblasts to synthesize and export collagen (Yamaguchi et al 1987). In terms of bone resorption, Zn deficiency compromises immune responsiveness and the activity of Zn-containing collagenase, effects which might be considered to interfere with the mobilization of the monocytic/macrophagic precursors of osteoclasts, macrophagic collagen resorption, and even the putative resorptive activity of osteoblasts to abet monocytic homing to calcified bone surfaces. Thus, in severe Zn deficiency, collagen turnover could be decreased (Starcher et al 1980). The fact is that our electron microprobe studies which present tissue-wide average compositions did not indicate that microgravity had an effect on the bioavailability of Zn; the Flight rat mandibular bone and incisor dentin proved to have a normal Ca/Zn ratio. It may be that the high Vitamin D content of the paste diet was adequate to overcome such potentially deleterious effects (Chang et al 1969). Yet, this conclusion may need to be modified upon reanalysis of the Ca/Zn ratios in the tissues formed before and during flight.

CONCLUSION

The present study lends additional evidence that long-term exposure to microgravity will compromize the integrity of skeletal structures. Within the context of measurements of young growing rats made during a 7d Space Shuttle Mission and two U.S.S.R. biosatellite flights of 12.5 and 18.5 days duration, the risks of spaceflight entail a decrease in the quantity of hard tissues formed, a delay in maturation of those matrices, and the resorptive loss of preexisting [preflight] trabecular bone structures. The consequences of these changes is a decrease in the biomechanical strength of the rat skeleton. While we did not have access to Cosmos 1887 Flight animals that had been permitted to recover at earth's gravity, our experience indicated that the degree of change was comparable to that observed with the 18.5d Cosmos-1129 Mission, and that the maturational defect would be reversed within a period of 7-14 days.

REFERENCES

1. Allred, J.B., Kratzer, F.N. and Porter, J.W.G.: Some factors affecting the in vitro binding of zinc by isolated soyabean protein and by a-casein. Br. J. Nutr. 18: 575, 1964.

2. Chang, J.H., Harrill, I. and Gifford, E.D.: Influence of zinc and vitamin D on bone constituents of the rat. Metabolism 28: 625, 1969.

3. Cossack, Z.T.: Somatomedin-C in zinc deficiency. Experientia 40: 498, 1984.

4. Grynpas, M.D., Patterson-Allen , P. and Simmons, D.J.: The changes in quality of mandibular bone mineral in otherwise totally immobilized Rhesus monkeys. Calcif. Tissue Int. 39: 57, 1986.

5. Jee, W.S.S., Wronski, T.J., Morey, E.R. and Kimmel, D.B.: Effect of spaceflight on trabecular bone in rats. Amer. J. Physiol. 244: R310, 1983

6. Kazarian, L.E.: Strength characteristics of rat spinal columns. Cosmos 1129. In: Hideg, J. and Gazenko, O. (eds). Advances in Physiological Sciences. Vol. 19, Gravitational Physiology, New York, Pergamon Press, 1981, p. 129.

7. Pace, N., Rahlmann, D.F., Smith, A.H., and Pitts, G.C.: Effects of the Cosmos-1129 Soviet paste diet on body composition in the growing rat. Report of the UCLA-Berkeley Environmental Physiology Laboratory [EPL-81-1], Feb. 16, 1981.

8. Pecoud, A., Donzel, P., and Schelling, J.L.: Effect of foodstuffs on the absorption of zinc sulfate. Clin. Pharmacol. Ther. 17: 459, 1975.

9. Roberts, W.E., Mozsary, P.G. and Morey, E.R.: Suppression of osteoblast differentiation during weightlessness. Physiologist 24: S75, 1981.

10. Rosenberg, G.D., Campbell, S.C. and Simmons, D.J.: The effects of spaceflight on the mineralization of rat incisor dentin. Proc. Soc. Exp. Biol. Med. 175: 429, 1984.

11. Rosenberg, G.D. and Simmons, D.J.: Electron microprobe analyses of Ca, S., Mg, and P distribution in incisors of Spacelab-3 rats. The Physiologist 28 (Suppl 1): S189, 1985.

12. Schwartz, R.: Magnesium metabolism. In: Simmons, D.J. (ed). Nutrition and Bone Development. Oxford Univ. Press, N.Y., 1988 (in press).

13. Simmons, D.J., Russell, J.E., Winter, F., Tran Van, P., Vignery, A., Baron, R., Rosenberg, G.D. and Walker, W.V.: Effect of spaceflight on the non- weight bearing bones in the rat skeleton. Amer. J. Physiol. 244: R319, 1983.

14. Simmons, D.J., Grazman, B., Russell, J.E., Walker, W.V., Bikle, D.D. and Morey, E.R.: Simulating certain aspects of hypogravity effects on bone maturation in the non-weight bearing skeleton. Aviat. Space and Environ. Med. 54: 1080, 1983a.

15. Simmons, D.J., Russell, J.E., Walker, W.V., Grazman, B., Oloff, C. and Kazarian, L.: Growth and maturation of mandibular bone in otherwise totally immobilized Rhesus monkeys. Clin. Orthop. 182: 220, 1984.

16. Simmons, D.J., Russell, J.E. and Grynpas. M.D.: Bone maturation and quality of bone mineral in rats flown on the space shuttle 'Spacelab-3 Mission'. Bone and Mineral 1: 485, 1986.

17. Starcher, B.C., Hill, C.H. and Madras, I.G.: Effect of zinc deficiency on bone collagenase and collagen turnover. J. Nutr. 110: 2095, 1980.

18. Yamaguchi, M., Oishi, H. and Suketa, Y.: Stimulatory effects of zinc on bone formation in tissue culture. Biochem. Pharmacol. 36: 4007, 1987.

TABLE 1

Mineralization Profile in Calvaria of Cosmos 1887 Rats

Percent Sample Weight

Specific Gravity Fraction	Basal Control	Vivarium Control	Synchronous Control	Flight
1.8	1.1	1.4	0.7	16.7 (>15.2-23.8)
1.9	9.7	6.0	7.7	38.2 (> 3.9- 6.3)
2.0	80.7	89.7	89.2	44.2 (< 1.8- 2.0)
2.1	6.8	2.4	1.9	0.6 (< 3.1-11.3)
2.2	0.6	0.3	0.2	0.3
2.3	1.1	0.2	0.2	0.1 (< 2.0-11.0)

Underlining indicates values which are higher or lower than those recorded by control rats. ( )= range of differences.

TABLE 2

Bone Chemistry in Cosmos-1887 Rats
(Unfractionated)

Group	Ca (1)	P (1)	Mg (1)	Ca/P	Ca/Mg(2)	Ash Wt.(3)
Calvaria						
Basal Control	23.0±0.07	10.1±0.09	0.46±0.004	1.75	50.0	54.12
Vivarium Ctrl	24.6±0.06	10.8±0.25	0.49±0.008	1.76	50.2	57.66
Synchronous Ctrl	24.3±0.15	10.5±0.15	0.4650.009	1.78	52.8	56.54
Flight	20.3±0.07	9.6±0.64	0.38±0.004	1.63	50.2	57.66
5th Lumbar Vertebra						
Basal Ctrl	20.5±0.21	9.3±0.72	0.38±0.001	1.71	53.9	48.84
Vivarium Ctrl	23.8±0.06	10.5±0.09	0.46±0.007	1.75	51.7	55.99
Synchronous Ctrl	20.3±0.14	10.2±1.21	0.46±0.041	1.53	44.1	51.65
Flight	20.7±0.01	9.3±0.17	0.37±0.001	1.72	55.9	49.13
Mandible						
Basal Control	25.4±0.07	11.5±0.09	0.48±0.004	1.71	52.9	60.52
Vivarium Ctrl	23.8±0.12	12.6±0.48	0.48±0.001	1.46	49.5	62.40
Synchronous Ctrl	26.6±0.15	12.0±0.20	0.47±0.013	1.72	56.5	63.24
Flight	28.0±0.13	12.1±0.10	0.50±0.005	1.78	56.0	65.11

(1) % of dry weight
(2) Molar ratio
(3) as % ($Ca+PO_4$)

TABLE 3

X-Ray Diffraction of Bone from Cosmos-1887 Rats

Group	$b_{1/2}$	D-002 (*)	$b_{1/2}$	D-130 (*)
Calvaria				
Synchronous Ctrl	0.68±0.01	147± 5	1.70±0.29	55±12
Flight	0.73±0.06	132±13	1.57±0.09	57± 3
5th Lumbar Vertebra				
Flight	0.77±0.01	122±2	1.78±0.19	50±6
Mandible				
Synchronous Ctrl	0.56±0.04	209±29	1.23±0.26	80±20
Flight	0.68±0.01	145± 5	1.32±0.14	71± 5

* In angstrom units

TABLE 4

Composition of Diastemal Mandibular Bone in Cosmos-1887 Rats
(Electron Microprobe Investigations)

Group	N	Ca/P	Ca/Mg	Ca/Zn
Basal Controls	5	1.439±0.049^	43.38±1.01	330.92± 46.14
Vivarium Controls	5	1.315±0.116	44.27±3.59^	290.94± 40.75
Synchronous Ctrls	5	1.300±0.023#	46.28±3.99^	358.35± 97.34
Flight	5	1.318±0.068	39.80±3.34#	337.17±124.00

Dissimilar superscripts are statistically different P<0.01.

TABLE 5

Composition of Diastemal Incisor Dentin in Cosmos 1887 Rats
(Electron Microprobe Investigations)

Group	N	Ca/P	Ca/Mg	Ca/Zn
Basal Control	5	1.331±0.117^	13.79±1.15	198.46±10.03^
Vivarium Control	5	1.130±0.102	16.03±2.17	246.83±35.63
Synchronous Ctrl	5	1.050±0.026#	16.94±2.78	179.26±39.51
Flight	5	1.085±0.050	14.30±1.74	295.42±36.23*

^ vs # = P<0.05 ^ vs * = P<0.01