PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 175, 429–437 (1984)

The Effects of Spaceflight on the Mineralization of Rat Incisor Dentin[1] (41816)

GARY D. ROSENBERG,* SUSAN C. CAMPBELL,* AND DAVID J. SIMMONS†

*Indiana University–Purdue University, Department of Geology, Indianapolis, Indiana 46202, and †Washington University School of Medicine, Department of Surgery, Divisions of Surgery and Bone and Mineral Research, St. Louis, Missouri 63100

Abstract. The lower incisors of Male Wistar rats flown for 18.5 days on the Soviet Cosmos-1129 Biosatellite were sectioned and chemically analyzed with an electron microprobe in order to determine whether there were specific effects of spaceflight on dentin formation/mineralization. Control tissues were obtained from rats housed under identical conditions in a land-based mock-up of the Biosatellite. The profiles of calcium (Ca), phosphorus (P), and sulfur (S) concentrations in dentin were measured in continuous traverses (1.0 μm intervals) from the pulp to the dentinoenamel junction. The incisor dentin formed during spaceflight had higher than normal (at 1G) concentrations of Ca (+10–15%) and P (+20–30%), particularly in the temporally youngest tissues within 80 μm of the pulp which had been least affected by secondary mineralization. The S-concentration profiles tended to decrease with tissue age. Fourier analysis (to determine the growth rhythms) revealed abnormal distributional patterns of S in the recently formed dentin from the Flight rats. The sulfur fluctuations in Flight animals alone periodically peaked above the irregular background fluctuations. These observations indicate that spaceflight has measureable effects on dentinogenesis, and they may also bear on the problem of the regulatory role of proteoglycans in mineralization and in the maturation of mineral and matrix moieties in skeletal tissue.

The weightless condition attending spaceflight is known to alter calcium homeostasis in mammals. Astronauts commonly develop hypercalciuria (1–3) and an important fraction of the excreted calcium is thought to be of bone origin. That hypothesis is supported by an observed decrease in mineral density in the highly trabecular calcaneal bones of Skylab astronauts (4). A somewhat similar effect of spaceflight has been reported for rats, an animal whose skeleton lacks some of the specialized histological features (i.e., osteons) of human bone. Rats flown on three Soviet biosatellite missions (Cosmos 782, 936, and 1129) exhibited a diminished mass of trabecular bone in the metaphyseal regions of their knee joints and humeri (5, 6), and they suffered a marked reduction of periosteal bone formation at the level of the tibia–fibula synostosis (6).

In the Cosmos 1129 flight discussed in this paper, we had an opportunity to contrast those effects of null-gravity with the responses in the non-weight bearing bones supplied by "functional" respiratory and masticatory muscles (the ribs and regions of the mandible). In the non-weight bearing bones, the rates of bone formation/mineralization and their mineral content were normal, except at sites which lacked contiguous muscle attachments (area of the mandibular molar teeth) (7). Gradient density fractionation studies indicated that in the jaw—but not in the ribs, weightlessness caused a delay in the maturation of recently formed bone mineral and matrix moieties; these anomalies were corrected after a 29-day postflight recovery period at earth's gravity. The teeth, on the other hand, seemed highly conserved. There was no evidence from analyses of the distribution of calcium, phosphorus, and sulfur in incisor dentin and enamel to make one suspect that tooth formation/mineralization and maturation had become abnormal during spaceflight. However, time series analyses across tooth sections did suggest that the patterns of sulfur concentration were more labile than those of calcium and phosphorus (8).

The purpose of the study was to determine if the effects of spaceflight could be detected in a (recently deposited 80-μm) zone of the dentin containing the mineralizing predentin.

[1] Work supported by a grant from NASA NAGW-301.

0037-9727/84 $1.50

It was felt that our inability to discern an effect of weightlessness across the entire dentin and enamel could have been due to the rapidity of initial calcification and secondary mineralization (crystal growth) processes.

Materials and Methods. Three groups of five to seven specific pathogen-free (SPF) male rats (270–320 g body wt) were loaded into block modules of five cages (singly housed which were then mounted in a modified Vostok spacecraft. The animals were then launched into orbit for a period of 18.5 days. The particular details of the flight have been described by Wronski *et al.* (6, 9). During flight, the animals were exposed to a 12-hr/12-hr light–dark cycle. Water was supplied ad libitum and they were fed 10-g aliquots of a nutritionally adequate paste diet four times per day. A group of Synchronous Control animals was maintained in a land-bound mockup of the biosatellite under nearly identical conditions; these were subjected to simulated stresses of launch and recovery (acceleration, vibration, impact shock, etc.). The conditions of housing were an important environmental influence which was imposed upon all of these rats. Group F designates Flight animals, while Group S designates the Synchronous Controls. Groups 1F-S, and 4F-S were singly housed for the duration of spaceflight (18.5 days), while Groups 4F-S were subsequently multiply housed in a Moscow, U.S.S.R. vivarium. This report concerns the elemental composition of the incisors from Groups 1F–1S and 4F–4S.

All groups had been injected with 1.0 mg/kg Declomycin 3 days prior to being loaded into their block modules. Declomycin binds to amorphous calcium phosphate (ACP) within minutes of being injected; newly deposited dentin appears as a fluorescent band when thin sections of skeletal tissue are inspected by ultraviolet microscopy. Thus, the Declomycin serves as a time-marker to enable identification of all dentin deposited after the beginning of the experiment. The average rate of apposition during the experiment can also be calculated, and this figure helps to establish the temporal significance of compositional cycles determined by Fourier analysis (see below).

Groups of 5 Flight rats (Group F) and their Synchronous Controls (Group S) were sacrificed within hours after recovery of the Biosatellite from space (= Groups 1F, 1S) without a subsequent injection of Declomycin. Other groups were injected with Declomycin 6 (Groups 2F, 2S) and 29 days (Groups 4F, 4S) after recovery, and they were sacrificed 24 or 48 h thereafter. The second Declomycin injection in Groups 2 and 4 confirmed that the average rate of apposition in Flight animals and Synchronous Controls was uniform during and after the period of spaceflight.

At autopsy, the mandibles of the rats (Groups 1F, 1S) were removed and fixed in 70% ethyl alcohol. The diastemal area of the mandible was cut transversely into a 2.0-mm-thick slab, and its (molar-facing) surface was polished for electron microprobe analysis. The incisors were identified in cross section, and the distribution of calcium, phosphorus, and sulfur were recorded using an electron microprobe (MAC-5, Monsanto Chemical Co., St. Louis, Mo.) with a 1.0-μm beam diameter at contiguous 1.0-μm intervals from the dentin–pulp interface to the labial surface of the enamel (Fig. 1). The gun potential was 7.5 kV, and the specimen current was 10^{-6} A

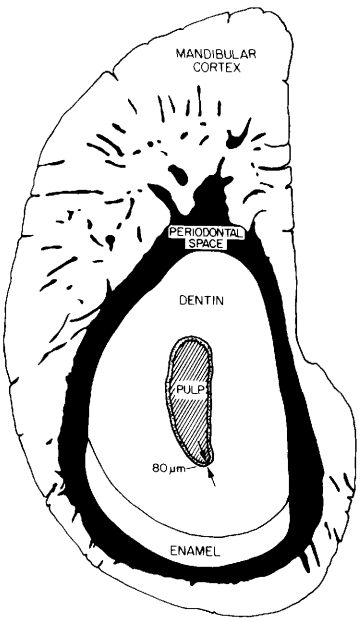

FIG. 1. Diagram of a cross section of the lower incisor of the rat in the area of the mandibular diastema, showing the approximate location of the microprobe traverses (arrow). The 80-μm zone of newly formed dentin is shown by shading.

measured on benitoite. $Ca_{k\alpha}$ was measured using an LiF crystal and a CaF_2 standard. $Sulfur_{k\alpha}$ was measured using a PET crystal with a FeS_2 standard. $Phosphorus_{k\alpha}$ was measured using a PET crystal with a calcium fluoroapatite standard. Readings were made for 30 sec on peak at each position, and for 15 sec on either side of peak to determine background. Concentrations were determined from count rates and corrections were made using the FRAME-B Program developed by the National Bureau of Standards.

In order to know how spaceflight affected the general plan of dentinogenesis, we calculated the relative concentrations of Ca, P, and S in the most recently deposited dentin (a 0- to 80-μm zone from the pulp which includes the predentin), in the youngest inner half of the dentin thickness (Y1/2D), and in the most mature outer half of the dentin thickness (O1/2D). More specific information about dentinogenesis was drawn from an analysis of its biorhythmicity in the youngest 80-μm zone adjacent to the pulp. This chemical series was then Fourier analyzed (cosine waves were fitted to the data) to determine the periods, amplitudes, and phase relationships of the spectra of compositional rhythms. Smoothed versions of the original data series were generated with summations (recombinations) of various numbers of periodicities from the spectra. Cross-correlations of original data series, of smoothed data series, and of the spectra themselves were performed with Program CROSCR as discussed in Davis (10). Specific information about these techniques as they relate to analyses of hard tissues are described in our previous publications (11, 12).

Results. The average dentin thickness in the Synchronous Controls and Flight rats was 543 ± 35 μm. Based upon tetracycline studies (7) which provide an average rate of dentin apposition/mineralization of 15.0 μm/day, 543 μm represents a total growth period of about 36 days. Thus, slightly less than 50% of the dentin thickness in the incisor of the rats killed after recovery of the biosatellite had been formed during the 18.5-day spaceflight. The oldest most mature half of the dentin in each section represented that tissue formed prior to spaceflight.

Dentin maturation. Table I shows that in

TABLE I. SECTIONAL PROFILES OF THE PERCENTAGE CALCIUM, PHOSPHORUS, AND SULFUR IN THE RAT LOWER INCISOR DENTIN AT THE MANDIBULAR DIASTEMA

Element	Group	Spaceflight					Postflight recovery (29 days)				
		N	PD[a]	Y½D[b]	O½D[c]	TD[d]	*N*	PD[a]	Y½D[b]	O½D[c]	TD[d]
Ca	Synch. control	3	29.86 ± 0.32	30.73 ± 0.18	31.30 ± 0.21	31.02 ± 0.26	3	32.57 ± 2.81	32.20 ± 2.96	32.20 ± 3.23	32.16 ± 3.10
	Flight	3	35.63 ± 1.27	35.61 ± 1.23	35.84 ± 1.32	35.73 ± 1.27	1	34.87	35.01	35.54	35.27
	P value		0.01	0.01	0.01	0.01					
P	Synch. control	3	16.11 ± 0.58	17.00 ± 1.08	18.50 ± 2.70	17.87 ± 2.08	3	26.41 ± 2.85	23.51 ± 0.54	23.44 ± 0.57	23.47 ± 0.55
	Flight	3	26.41 ± 2.85	23.51 ± 0.54	23.44 ± 0.57	23.47 ± 0.55	1	22.05	23.67	24.34	24.01
	P value		0.01	0.001	N.S.	N.S.					
S	Synch. control	3	0.13 ± 0.01	0.11 ± 0.02	0.10 ± 0.02	0.11 ± 0.02	3	0.10 ± 0.04	0.08 ± 0.03	0.07 ± 0.03	0.08 ± 0.03
	Flight	3	0.15 ± 0.10	0.15 ± 0.03	0.13 ± 0.03	0.14 ± 0.03	1	0.12	0.12	0.07	0.12
	P value		N.S.	N.S.	N.S.	N.S.					

[a] PD = 0–80 μm from the pulp cavity, including the predentin.
[b] Y½D = area representing the youngest half of the total dentin thickness, includes PD-rich zone (formed during spaceflight in Group 1F).
[c] O½D = area representing the oldest half of the total dentin thickness (formed during the preflight period in Group 1F).
[d] TD = total dentin thickness.

the Control rats the relative concentrations of Ca (and probably P) tend to increase during aging (from the predentine to the O1/2D). The teeth of the Flight rats did not display equivalent zonal changes. The Ca and P content of the dentin formed before (O1/2D region) and during spaceflight (PD-rich and Y1/2D regions), was significantly greater than that recorded in the controls. The Ca/P ratios (Table II) revealed that the abnormalities in dentin mineralization were restricted to those zones which had been formed during spaceflight (PD and Y1/2D zones). The dentin in those zones was relatively Ca deficient; the "preflight dentin" (O1/2D zone) retained a normal Ca/P ratio. These zonal differences were not apparent after a 29-day postflight recovery period at 1G conditions; in both groups, the Ca/P ratios were generally 1.4–1.5.

Dentin aging also appeared to be associated with a general decrease in average S concentrations, but this trend was not statistically significant either within or between the Synchronous Control and Flight groups.

Spectral analysis. Spectral analyses revealed important differences in distribution of the elements within the recently deposited dentin. In both the Flight rats and their controls (Groups 1F and 1S), Ca and P_i concentrations began to oscillate in-phase within 50 μm of the pulp cavity. There was no difference between Flight animals and controls in the fluctuation of Ca concentration, nor in the fluctuation of P_i concentration. On the other hand, dentinal S rhythms were less uniform, and they possessed the greater percentage of the variance in the middle to high frequencies in the Flight animals. For this reason, we are focusing on the spectra for the S rhythms in this report.

Sulfur spectra. Figure 2a represents the mean sulfur spectrum, calculated from the sulfur spectra of each of four Synchronous Controls sacrificed immediately after spaceflight (Group 1S). The mean sulfur spectrum obtained for the four Synchronous Controls sacrificed 29 days later (Group 4S) was very similar (spectrum not shown: $r = 0.813$, $P < 0.001$).

Low frequencies beginning with those of 1 cycle over the entire data length (80 μm) are plotted at the left end of the abscissa; the high frequencies ending with those of 1 cycle/2 μm

TABLE II. SECTIONAL PROFILES OF THE Ca/P RATIOS IN THE RAT LOWER INCISOR DENTIN AT THE MANDIBULAR DIASTEMA

	Spaceflight					Postflight recovery (29 days)				
Group	Rat no.	PD[a]	Y½D[b]	O½D[c]	TD[d]	Rat no.	PD[a]	Y½D[b]	O½D[c]	TD[d]
Synch. control	1S1	1.79	1.87	1.86	1.91	4S1	1.57	1.34	1.19	1.36
	1S3	1.75	1.55	1.31	1.42	4S2	1.44	1.42	1.40	1.41
	1S4	1.83	1.87	—	—	4S3	1.76	1.72	1.69	1.71
	1S6	2.06	2.01	1.97	1.99	—	—	—	—	—
	Mean	1.86	1.83	1.716	1.77	Mean	1.59	1.49	1.42	1.46
	SE	0.07	0.09	0.22	0.18	SE	0.09	0.12	0.14	0.13
Flight	1F1	1.59	1.54	1.56	1.55	4F2	1.57	1.48	1.46	1.47
	1F3	1.46	1.47	1.47	1.47					
	1F4	1.55	1.53	1.55	1.54					
	Mean	1.53	1.51	1.53	1.52					
	SE	0.04	0.02	0.03	0.03					
	P value	0.01	0.02	N.S.	N.S.					

[a] PD = 0–80 μm from the pulp cavity, includes the predentin.

[b] Y½D = area representing the youngest half of the total dentin thickness, including the PD-rich zone (formed during the spaceflight in Group 1F).

[c] O½D = area representing the oldest half of the total dentin thickness (formed during the preflight period in Group 1F).

[d] TD = total dentin thickness.

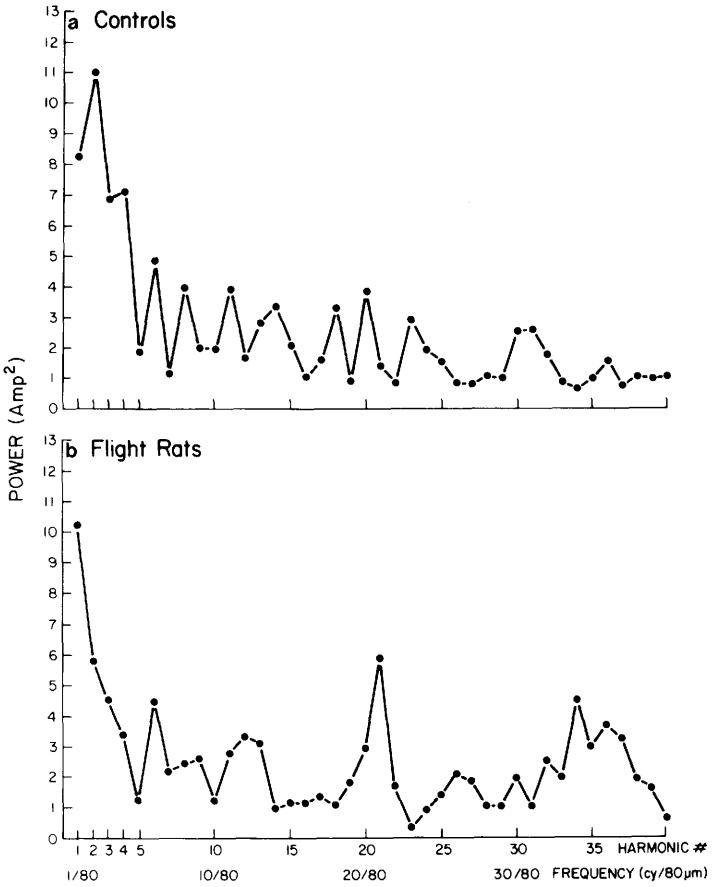

FIG. 2. (a) Plot of the mean spectrum of sulfur rhythms in the most recently deposited 80 μm of dentin vs normalized amplitude (power) in Synchronous Control rats (Group 1S). Low frequencies are graphed at the left; high frequencies at the right (=harmonic number divided by the total number of data points). Note the uniform decrease in power from low to high frequencies. (b) Plot of the mean spectrum of sulfur rhythms in the most recently deposited 80 μm of dentin vs normalized amplitude (power) in the Flight rats (Group 1F). Note the peaks in the spectrum at the 21st and 34th harmonics. The power in the harmonics does not decline uniformly to the high frequencies.

are plotted at the right end of the abscissa.[2] The normalized power within each harmonic is plotted on the ordinate. The normalized power represents the percentage of the total variance in the amplitudes possessed by the respective harmonics. Note that the amplitudes of the harmonics decline uniformly from the low to the high frequencies in the mean spectrum from these Control rats (Group 1S).

In contrast to the S patterns in the Controls, Fig. 2b shows that the power within the mean sulfur spectrum for the Flight animals does not decrease uniformly from low to high frequencies. Peaks in this spectrum appear at the 21st harmonic (frequency = 1 cycle/3.8 μm), and near the 34th–36th harmonic (frequency = 1 cycle/2.2 μm). In other terms, the spectrum of the Flight animals contain nearly 50% of the power in the middle to high frequencies, in contrast to less than 30% within the same range in the Control spectrum.

Sulfur distribution. The distribution of sulfur within the 80-μm zone of dentin from the Control animals (Group 1S) is shown in Fig.

[2] The frequency of a given harmonic is determined by dividing the harmonic number by 80 μm, the total number of measurements.

3a. Figure 3b shows the distribution of sulfur in the Flight rat (Group 1F). The position of the pulp cavity is to the left in both instances; the dentin tissue "ages" progressively to the right. Both graphs represent original data, with retention of both the highest and lowest frequencies. Note that the sulfur concentrations in the dentin from the Flight rat oscillated in episodes of high-amplitude, high-frequency "spikes" across the specimen. The appearance of these spikes is not entirely arhythmic, as the large peak in the 21st harmonic (representing about 4 S cycles per day) in the mean spectrum reveals (Fig. 2b). Moreover, these peaks are modulated with a low-frequency oscillation (harmonic 6, Fig. 2b), representing a modulation of about one cycle/12 μm). The result is episodes of high-amplitude, high-frequency S oscillation alternating with periods of low-amplitude S oscillation. In contrast,

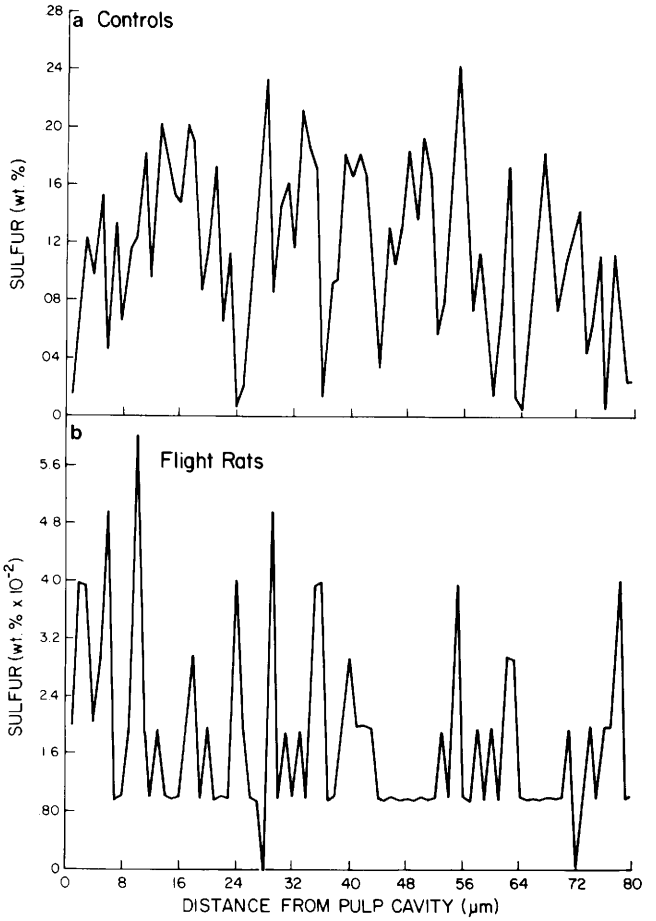

FIG. 3. (a) Distribution of sulfur concentration (weight %) across the dentin of a tooth section from a Control rat. The traverse procedes a distance of 80 μm from the margin of the pulp cavity (at the left). Note that sulfur fluctuates uniformly across the traverse, a consequence of the absence of dominant high to middle frequencies (cf. Fig. 2a). (b) Distribution of sulfur concentration (weight %) across the dentin of a tooth section of a Flight rat (compare with (a)). Note that sulfur does not fluctuate uniformly across the traverse, a consequence of dominant spectral peaks at the 21st and 34th harmonics (cf. Fig. 2b). Oscillations of one cycle per 2–4 μm are modulated by lower frequencies (especially harmonic six with one cycle per 12 μm). Thus, high-amplitude, high-frequency sulfur oscillations ("spikes") alternate with episodes of low-amplitude sulfur oscillation (e.g., between 48 and 64 μm). Note that none of the dentin samples from Control rats showed these high-amplitude, high-frequency sulfur "spikes."

the sulfur distribution fluctuates uniformly across the dentin of the control animal where its mean spectrum reveals no dominant high frequency. It should be noted that none of the Control animals display the array of sulfur spikes to the same degree as the animals flown in space. This difference is independent of the variability in mean S content (Table I). That is, the high spikes of S variation in Flight animals are not artifacts of the statistical techniques used to evaluate microprobe measurement errors.

Discussion. Herein, we have reported that spaceflight results in an abnormal accumulation of Ca and P_i in the most recently formed (0–5 day) dentin of rats, where there are also abnormal sulfur spectra. These data suggest one of two events: (i) that the increased concentrations of dentin Ca and P_i are due to a site-selective translocation of these elements from regions of the skeleton which were being resorbed in response to disuse (weightlessness) and/or (ii) that there might be a defect in the maturation of the bone mineral moiety.

Several observations favor the second hypothesis. First, Table II shows that the Ca/P_i ratios in the PD and Y1/2D sectors are lowest in the Flight rats (Group 1F: 1.52 vs Synchronous Control Group 1S: 1.77). The most mature sector of the dentin (O1/2D) in the Flight rats also has the generally lower Ca/P ratio (vs two of the three rats in Group 1S). Thus, the Ca/P ratios in the dentin of the rats actually flown in space most closely approximates the ratio of 1.3 quoted for the newly formed mineral in the calcifying fronts of dentin, bone, and enamel (13). It is not clear why the Ca/P_i ratios remained as low as 1.2 in the flight rat (Rat 4S1) after 29 days of postflight recovery, but this may reflect a continued failure of maturation and secondary mineralization processes. Conversely, the dentin in the Synchronous Control rats is the relatively more mature tissue.

Second, characterization of fecal/urine calcium by neutron activation showed that the rates of both bone formation and resorption had been decreased during spaceflight (14), but that resorption remained the relatively more active process. Histomorphometric measures in trabecular bone (tibial and humeral metaphyses) of the flight rats (Group 1F) indicated that osteoclasts were not present in abnormally large numbers, and that their resorption cavities were shallower than normal. Nevertheless, if there had been a selective translocation of resorbed mineral, we would have expected to detect it by gradient density fractionation techniques in a rapidly growing nonweight element such as the rib. We did not detect such a change (7). Whenever a high concentration of mineral (Ca and P) was detected in the youngest bone fractions, such as in the areas of the jaw supplied by functional masticatory muscle, the density gradient profiles (15) indicated a failure of maturation of mineral and matrix moieties. This suggests again that the major defect of mineralization during spaceflight was at the level of bone formation (6, 16).

Previous studies had indicated that the rate of dentin apposition remained normal during spaceflight (7). It is well known that daily feeding patterns and concomitant cycles in occlusal stress affect the growth incrementation and apposition of rodent and canine incisors (11, 12, 17–20). The maintenance of occlusal stress and feeding patterns (rats were fed 10 g paste diet 4× per day) during spaceflight doubtless contributed to what had appeared to us (in an earlier investigation (7)) to be normal dentin mineralization over much of the lower incisor of the rat. Had a narrower density fraction cut been analyzed (i.e., 1.3–1.7, rather than the larger 1.3–1.9 sp gr), we might have detected the Ca and P_i enrichment of the "recent" dentin which we have demonstrated in this report. As noted above, Simmons *et al.* (7) showed an enrichment of Ca and P_i in the lowest density fraction of the mandible (= least mature bone), as well as a decrease of those elements in the highest density fraction (= the most mature bone). Thus, on the basis of elemental analyses alone, there is at least a partial association between the growth characteristics of the mandibles and incisor. The association, in combination with the anomalies in dentin sulfur distribution, suggests an impairment of events leading to maturation of the mineral and matrix moieties.

The anomalies in sulfur distribution and the increased concentrations of Ca and P in the recently formed dentin may have broader implications. There is, for instance, a long history of concern about the role of glycosami-

noglycans in mineralization. Considerable biochemical (21) and histochemical (example (22)) evidence indicates that the proteoglycans and their subunits inhibit mineralization, despite the fact that a recent qualitative immunohistochemical (link protein antibodies) study suggests that mineralization does not require prior proteoglycan degradation (23). Others, such as Smillie (24) consider proteoglycans to be facilitative.

Our detection of anomalous sulfur fluctuations, and increased Ca and P concentrations in the "recent" incisor dentin from the Flight rats does not resolve this controversy. The electron microprobe determines the total weight percentage of each element and not the percentage distribution in the different phases. Sulfur does not only occur in the proteoglycans; it can also be found associated with hydroxyapatite. However, it would not be inconsistent with the suggestion that there is a causal relationship between the concentration of the Ca, P_i, and proteoglycan in dentin . . . but confirmation of such a hypothesis will require quantitative partitioning of total S into its constituent phases. Whether or not there is some facilitative relationship between the high Ca concentration and the unusual distribution of S in the Flight animals is a matter for speculation. However, we have also observed a similar behavior of S in the highly mineralized enamel of these rats (8). On the other hand, it is particularly interesting that several laboratories (25–27) have shown that the progression from predentin-to-(mineralizable) dentin involves the degradation and complete removal of moieties containing chondroitin-6-sulfate and partial degradation of moieties containing chondroitin-4-sulfate. The appearance of sulfur spikes across newly mineralized dentin—with its large Ca and P_i concentrations, may in some way reflect this selective degradative process, but confirmation of such a hypothesis will require additional research.

The authors acknowledge with thanks the cooperation of Dr. O. Gazenko and Dr. E. Ilyin, Institute of Biomedical Problems, Moscow, U.S.S.R., who directed the work of the Cosmos-1129 Soviet team which was responsible for the care of the animals and for the procurement of the biological specimens.

1. Lutwak L, Whedon GD, LaChance PA, Reid JM, Lipscomb HS. Mineral electrolyte and nitrogen balance studies of the Gemini-VII fourteen day orbital space flight. J Clin Endocrinol 29:1140–1156, 1969.
2. Rambaut PC, Leach CS, Johnson PC. Calcium and phosphorus changers of the Apollo 17 crewmembers. Nutr Metab **18**:62–69, 1975.
3. Whedon GD, Lutwak L, Rambaut PC, Whittle MW, Smith MC, Reid J, Leach C, Stadler CR, Sandford DD. Mineral and nitrogen metabolic studies, Experiment MO71. In: Johnston RS, Dietlein LF, eds. Biomedical Results from Skylab. NASA Document SP-377. Washington, DC, pp164–174, 1977.
4. Vogel JM, Whittle MW, Smith MC, Rambaut PC. Bone mineral measurement experiment MO78. In: Johnston RS, Dietlein LF, eds. Biomedical Results from Skylab. NASA Document SP-377. Washington, DC, pp 183–190, 1977.
5. Yagodovsky VS, Triftanidi LA, Gorokhova GP. Spaceflight effects on skeletal bones of rats (light and electron microscope examinations). Aviat Space Environ Med **47**:734–738, 1976.
6. Wronski TJ, Morey ER. Effect of spaceflight on periosteal bone formation in rats. Amer J Physiol **244**:R305–R309, 1983.
7. Simmons DJ, Russell JE, Winter F, Tran Van P, Vignery A, Baron R, Rosenberg GD, Walker WV. 1982. The effect of spaceflight on the non weight-bearing bones of the rat skeleton. Amer J Physiol **244**:R319–R326, 1983.
8. Rosenberg GD, Simmons DJ. Time-varying calcium, phosphorus, and sulfur concentrations in rat incisors: The effects of spaceflight (Cosmos-1129 Biosatellite). In: Veis A, ed. The Chemistry and Biology of Mineralized Connective Tissues. New York, Elsevier, pp295–297, 1981.
9. Wronski TJ, Morey-Holton E, Jee WSS. Cosmos 1129: Space flight and bone changes. Physiologist **23**(Suppl):79S–82S, 1980.
10. Davis J. Statistics and Data Analysis in Geology. New York, Wiley, 1973.
11. Rosenberg GD, Simmons DJ. Rhythmic dentinogenesis in the rabbit incisor. Circadian, ultradian and infradian periods. Calcif Tissue Int **32**:29–44, 1980.
12. Rosenberg GD, Simmons DJ. Rhythmic dentinogenesis in the rabbit incisor: Allometric aspects. Calcif Tissue Int **32**:45–53, 1980.
13. Matthews JL. Structure, properties, and function of mineralized tissue components: Group report. In: Nancollas GH, ed. Biological Mineralization and Demineralization. Berlin, Springer-Verlag, pp327–348, 1982.
14. Cann CE, Adachi RR, Morey-Holton E. Bone resorption and calcium absorption in rats during spaceflight. Physiologist **23**(Suppl):S83–S86, 1980.
15. Russell JE, Avioli LV. Effect of experimental chronic

renal insufficiency on bone mineral and collagen maturation. J Clin Invest **51**:3072–3079, 1972.
16. Jee WSS, Wronski TJ, Morey ER, Kimmel DB. The effects of space flight on trabecular bone in rats. Amer J Physiol **244**:R310–R314, 1983.
17. Ullman GA, Meyer J, Schneider BJ. Experimental studies on the interrelationships of condylar growth and alveolar bone formation. III. Response to relief of occlusion in aged rats. Angle Orthod **39**:83–92, 1969.
18. Ness AR. The response of the rabbit mandibular incisor to experimental shortening and to the prevention of its eruption. Proc R Soc London, Biol **148**:129–154, 1956.
19. Miani A, Miani C. Circadian advancement rhythm of the calcification front in dog dentin. Panminerva Med **14**:127–136, 1972.
20. Schour I, Medab H. The effect of experimental removal of the apical portion of the upper incisor of the rat upon the rate of eruption and tooth morphology. J Dent Res **30**:521–530, 1951.
21. Blumenthal NC, Posner AS, Silverman LD, Rosenberg LD. Effect of proteoglycans on *in vitro* hydroxyapatite formation. Calcif Tissue Int **27**:75–82, 1979.
22. Frank RM. Electron microscopic autoradiography of calcified tissues. Int Rev Cytol **56**:183–253, 1979.
23. Poole AR, Pidoux I, Rosenberg L. Role of proteoglycans in endochondral ossification: Immunofluorescent localization of link protein monomer in bovine fetal epiphyseal growth plate. J Cell Biol **92**:249–260, 1982.
24. Smillie AC. The chemistry of the organic phase of teeth. In: Zipkin I, ed. Biological Mineralization. New York, Wiley, pp139–164, 1973.
25. Engfeldt B, Hjerpe A. Glycosaminoglycans of dentin and predentin. Calcif Tissue Res **10**:152–159, 1972.
26. Hjerpe A, Engfeldt B. Proteoglycans of dentin and predentin. Calcif Tissue Res **22**:173–182, 1976.
27. Rahemtulla F, Prince C, Caterson B, Christner JE, Baker JR, Butler WT. Studies on the chemistry and metabolism of dentin proteoglycans using an *in vivo* labelling technique. In: Veis A, ed. The Chemistry and Biology of Mineralized Connective Tissues. New York, Elsevier, pp389–393, 1981.

Received September 2, 1983. P.S.E.B.M. 1984, Vol. 175.
Accepted December 6, 1983.