EXPERIMENT K-6-27

ANALYSIS OF RADIOGRAPHS AND BIOSAMPLES FROM PRIMATE STUDIES

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SUMMARY

Serial high-contrast radiographs were obtained of both arms and the right leg of two flight and four control monkeys for the period L-60 to S+16. Longitudinal growth of the tibia, radius and ulna was linear over this period in the control monkeys. In the flight monkey for whom the feeder malfunctioned, there were significant decreases in growth of the long bones. There were also hypermineralized growth arrest lines produced in the distal radial and ulnar metaphyses following resumption of growth. In the other flight monkey, there was a suggestion of decreased long bone growth during flight and immediate postflight periods, but this recovered by the end of the postflight control experiment. There was also an increase in intracortical resorption, indicative of skeletal activation. No major changes in cortical thickness or other parameters were noted, but modification of the techniques to obtain very high quality radiographs in further studies should allow subtle changes in these processes to be quantified.

INTRODUCTION

Previous studies of primate skeletal changes on Cosmos 1514 indicated that a short (5-day) spaceflight may activate the skeleton of juvenile primates causing an increase in bone resorption as indicated by intracortical resorption on high-contrast skeletal radiographs. However, in these early studies no preflight radiographs were available for comparison, so no quantitative changes due to the flight could be determined. The present experiment was designed to study serially the growth and development of the juvenile primate peripheral skeleton and to determine if a 2-week period of spaceflight affected this development. This design was chosen because in the juvenile primate (3-5 kg) the skeleton is still undergoing rapid development at this stage, with longitudinal growth at the unfused metaphyseal-epiphyseal junctions and periosteal and endosteal architectural modeling, providing the possibility to detect an effect of microgravity by a change in the normal rate of growth and modeling. Both quantitative and qualitative parameters were assessed from radiographs of the arms and legs of the monkeys, and will be used in conjunction with single photon absorptiometry of the same limbs obtained by U.S.S.R. investigators to understand the effect of spaceflight on skeletal development.

This investigation included a significant amount of preflight testing and development of optimal radiographic techniques in the U.S.S.R., and serial radiographs of flight candidate, flight, and control monkeys from a period 60 days prior to launch to 2 weeks after the postflight control ("synchronous") experiment.

METHODS AND RESULTS

Technique Development Studies

During the 1 year prior to the launch of Cosmos 1887, U.S. and U.S.S.R. investigators collaborated on a development effort to determine optimal equipment and methods for obtaining high quality radiographs in the primate laboratory in Moscow. In Cosmos 1514, the U.S. had supplied a portable x-ray unit, x-ray film and intensifying screens, and a container to transport the exposed film back to the U.S. for automatic development and analysis. Subsequent to these studies, the institute acquired an x-ray unit of its own and access to x-ray development facilities, and both sides agreed to work to determine if these facilities could be used to obtain the radiographs needed for this study.

In February, 1987, the U.S. and U.S.S.R. investigators inspected the x-ray unit at the institute to determine its characteristics, but because it had not yet been installed it was difficult to determine operating parameters. In addition, the U.S. side suggested that it could supply a portable automatic x-ray processor to be used at the institute for development of the x-rays instead of having to take them to the clinic facilities. Prior studies in Cosmos 1514 had used an x-ray unit with a fixed kilovoltage of 65 kVp, and a primary test was to determine if the U.S.S.R. unit could be used at lower kVp (eg 50-

55 kVp) to improve contrast in the bone radiographs. In addition, the U.S.S.R. unit had variable filtration which might be used to optimize contrast. Initial tests determined that the unit at the institute could be used optimally at 75 kVp, not much different from the point of view of bone-soft tissue contrast than the 65 kVp used previously. However, there were problems with the use of the automatic processor sent to Moscow by the U.S., so it was decided to do one of two procedures: 1) develop the films at the clinic in Moscow, or 2) return the films to the U.S. for processing. The final decision was made to develop the films in Moscow.

Pre and Postflight Radiographs

Radiographs were done on 8 monkeys. Monkeys 1-6 (two flight monkeys and 4 controls) had radiographs taken at L-60, L-30, R + 16, R + 37 and S + 16. The other two controls did not have the last set of x-rays. Films were taken at 75 kVp, 25 mAs, and a focus-film distance of 100 cm, using Kodak Min-R film and a Kodak Min-R Intensifying screen. Four x-rays were taken of each monkey (arms and legs) at each time point in the posterior-anterior (lateral-medial) projection. Films were developed at the clinic in Moscow.

The U.S. received the developed radiographs in February, 1988 when the U.S. specialists visited Moscow. Films of the left and right arms and the right leg were received. The planned analysis of the left leg could not be done because those developed films were reserved by U.S.S.R. investigators for analysis of the EMG implants done in that leg for the muscle studies. A number of films (especially those taken at L-30) were lost in the development process and were unavailable for analysis. In addition, even though aluminum step wedges were included in the exposure of the films, the wide variability in film density precludes any quantitative analysis of bone density. It appears that this variability is due to the development process. Most of the films were of good quality, with a few either overexposed or slightly underexposed, but it is fairly easy to compensate for these exposure differences by viewing with a variable intensity light source. Several films were slightly blurred, due to slight motion of the monkey during the exposure, and in a few cases an arm or leg was rotated slightly in one exposure of the series, so the projected bone was in a different orientation.

The analysis of the radiographs has been done with two approaches, one quantitative and the other qualitative or semi-quantitative. The primary quantitative measurements are bone length and the combined cortical thickness of the bones at 25% and 50% of the length from the distal metaphysis (radius, ulna) or proximal metaphysis (tibia). These data are given in Table 1. The change in length and cortical thickness as a function of time is shown in figures 1-9. In the table and figures, monkey 1 is Drema, monkey 2 is Erosha, and monkeys 3-6 are control animals. The length is measured from metaphysis to metaphysis in each of the bones, so is indicative of growth of the shaft of the long bones. The change in thickness of the cortex at the midshaft can be due to normal periosteal expansion (about 0.2 - 0.4 mm in these animals) as well as a change due to erosion of the endosteal surface as an effect of spaceflight. At the sites closer to the metaphyses (the 25% sites in the table), the periosteal and endosteal surfaces are still undergoing modeling, and this process may be perturbed by flight.

Figures 1a-1c show the cumulative change in length (i.e. growth) from the baseline x-ray at L-60 through the end of the postflight control experiment. For the control monkeys, all three bones are growing at a linear rate, 0.041 ± 0.017, 0.045 ± 0.020, 0,51 ± 0.019 mm/day for the tibia, radiUS and ulna, respectively (mean ± SD). For flight monkey 1 (Drema), longitudinal growth was similar in tibia and radius, with a suggestion that ulnar growth might have been slowed by flight but then increased again so that by the end of the experiment it had reached the same level as the controls. The missing film at L-30 would have been valuable in confirming this. For monkey 2 (Erosha), there was a significant decrease in growth of both tibia and ulna. Toward the end of the postflight period, the rate of growth was the same as in the control monkeys, but the bones remained shorter than would have been expected. Again, the film at L-30 would have allowed quantification of the actual deficit in growth during this period.

Figures 2a-2f show the change in cortical thickness due to the normal modeling process. In the control monkeys, periosteal expansion at the midshaft is 0.2 - 0.4 mm over the experimental period, and the flight monkeys are not different. In general, there is a slight thinning of the cortices as the bones grow and expand, but the changes are quite small. Monkey 2 again shows a distinctly different pattern, especially in the tibia.

Qualitative evaluation of the radiographs included stage of metaphyseal closure, mineralization of the tibial tuberosity, intracortical resorption and endosteal resorption. In the 4 control monkeys, no significant qualitative changes were noted over the experimental period. The tibial tuberosity started to mineralize in monkeys 5 and 6 before L-60 and continued during the experiment. In monkey 4 it was unmineralized at the start, but partially mineralized by S+16; in monkey 3 it was not mineralized at all. In the flight animals, monkey 1 showed continuing mineralization like 5 and 6, while in monkey 2, like monkey 3, it was not mineralized. Other qualitative characteristics included an apparent widening of the fibula medullary canal at the proximal region and increased intracortical resorption in the proximal tibia in monkey 1; this resorption was evident at R+16, still evident at R+37, but starting to resolve at S+16. Monkey 2 did not receive the paste food for most of the flight, and there was a clear response of the skeleton. The radius and ulna each have a clear hypermineralized cement line in the distal metaphysis at R+37 and more clearly at S+16, but which was not evident at R+16. Apparently the deprivation of food slowed down longitudinal growth of the radius and ulna, and when growth started again a hypermineralized junction resulted. This growth arrest line was not evident in the tibial metaphysis, but may have been more subtle at this site.

DISCUSSION

Growth and modeling of the juvenile primate skeleton can be affected by many factors. The monkeys used in these experiments were at a stage in skeletal development where longitudinal bone growth was linear over the 5 month period of the study. Normal modeling at the periosteum and endosteum led to variable cortical thickness changes over the study period, not necessarily the same at each site examined. Metaphyseal closure was not evident in any of the monkeys. Consequently, it would be expected that the stage of mineralization of the cortical bone would be relatively uniform during the experiment. No attempt was made to quantify cortical density from the radiographs obtained, but single photon absorptiometry (SPA) measurements made by U.S.S.R. investigators can be normalized by the periosteal diameter measured on the radiographs to eliminate the variable of bone size from their interpretation of the SPA results.

The initial results from Cosmos 1514 indicated the need for serial radiographs during the preflight, immediate postflight, and control experiment periods to quantify the effects of spaceflight on the dynamics of bone growth. In this experiment, radiographs were obtained at L-60, L-30, R+16, R+37 and S+16 days relative to launch; most films from L-30 were lost due to technical error, and the other films had a wide variation in radiographic density presumably because of variation in the development process. Even with these technical difficulties, the data were sufficient to quantify changes in bone growth. In the one monkey who did not receive paste food for most of the flight, the effect was clear. In the other flight monkey, ulnar growth was apparently slowed but rebounded in the postflight period. The data also show the time delay in skeletal responses, so that even later films (for example, S+37) might provide more information in future studies. The suggestion of intracortical resorption evidenced in monkey 1 at R+16 and R+37 and refilling of these cavities by S+16 supports the initial results of Cosmos 1514, increased activation of the skeleton by spaceflight. However, because radiographic contrast was limited in the current studies, this effect could not be quantified nor could the existence of more subtle changes in other animals be confirmed or denied. A primary focus of further radiographic studies should be reevaluation of the techniques used to obtain films, with an effort to utilize state-of-the-art equipment (for example, microfocus x-ray sources and fine grain film) and careful animal handling and positioning procedures to optimize image quality. This would provide significant improvements in the quality of both quantitative and qualitative analyses, allowing much more subtle effects of spaceflight to be quantified with a high degree of confidence.

Table 1. Quantitative Analysis of Radiographs

		Tibia (R)			Radius (R+L)			Ulna (R+L)		
		Length[a]	CCT(50)[b]	CCT(25)[c]	Length	CCT(50)	CCT(25)	Length	CCT(50)	CCT(25)
#1	1L-60	12.35	5.60	5.50	11.58	4.10	3.45	13.05	4.05	3.25
	R+16	12.75	4.40	4.70	11.90	3.85	3.85	13.40	3.50	3.15
	R+37	12.85	4.20	5.20	12.05	3.85	3.80	13.60	3.65	3.10
	S+16	12.90	4.50	11.60	12.25	3.95	2.90	13.75	3.80	3.15
#2	L-60	11.10	5.10	5.00	9.85	4.25	3.70	11.05	4.50	3.15
	R+16	11.35	4.60	4.30	10.20	4.40	3.40	11.40	4.20	3.00
	R+37	11.45	4.50	3.30	10.30	4.10	3.45	11.48	4.05	3.00
	S+16	11.60	4.30	3.20	10.50	4.35	3.55	11.68	4.10	3.25
#3	L-60	12.25	4.10	3.30	10.88	4.40	4.70	12.28	3.60	3.35
	R+16	12.70	3.80	3.70	11.45	4.00	4.50	12.82	3.25	3.20
	R+37	12.85	4.20	3.90	11.48	3.70	4.30	12.86	3.05	3.10
	S+16	13.05	4.20	4.20	11.72	4.25	4.95f	13.18	3.30	3.05
#4	L-60	11.85	5.70	6.20	10.95	5.05	4.85	12.02	4.30	3.65
	R+16	12.15	5.00	5.30	11.20	4.65	4.15e	12.38	3.95	3.80
	R+37	12.15	5.30	5.20	11.30	4.50	4.50	12.40	4.10	3.60
	S+16	12.25	5.50	5.70	11.42	4.25	4.20e	12.52	4.10	3.80
#5	L-60	11.80	5.50	6.70	11.15	4.35	3.65	12.30	4.25	3.55
	R+16	12.15	5.50	6.50	11.28	4.25	3.35	12.62	3.45	3.15
	R+37	12.25	5.30	6.80	11.38	4.20	3.35	12.78	3.45	3.35
	S+16	12.15	4.50	6.30e	11.48	4.05	3.35	12.78	3.50	3.30
#6	L-60	11.85	6.00	4.00	10.55	4.45	3.80	11.82	3.80	2.85
	R+16	12.20	5.20	3.80	11.07	4.15	4.15	12.40	3.55	2.80
	R+37	12.35	5.40	5.00	11.22	4.30	4.05	12.62	3.50	2.95
	S+16	12.60	5.40	5.00	11.45	4.50	4.35	12.80	3.70	2.80

[a] Length in cm

[b] CCT-combined cortical thickness at midshaft, mm

[c] CCT-combined cortical thickness, 25% of length from distal (radius, ulna) or proximal (tibia) metaphysis, mm

[e] Slightly different projection due to rotation

[f] Focal change in endosteal bone at measured site

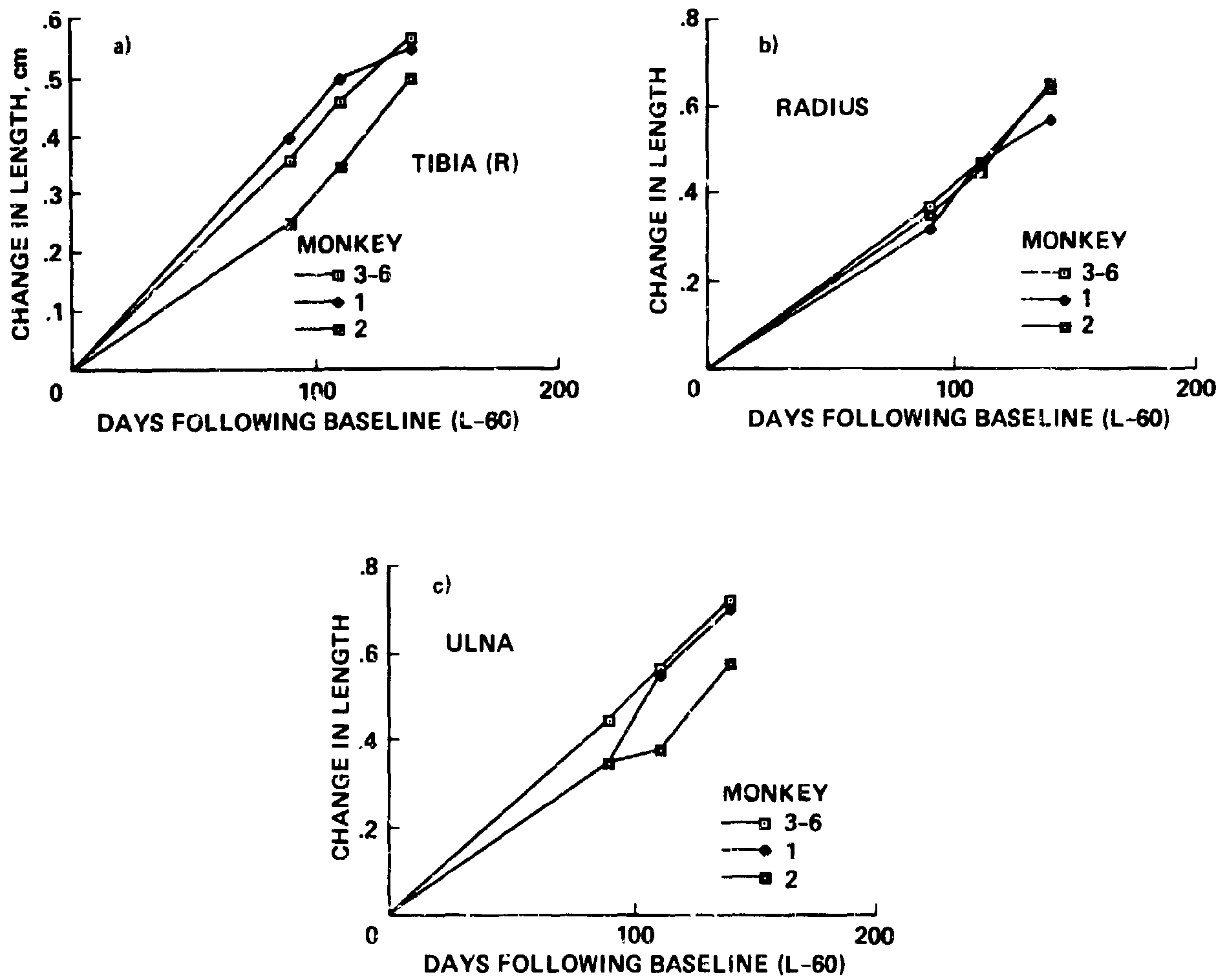

Figure 1. Indicate the cumulative change in length (i.e. growth) from the baseline x-ray at L-60 through the end of the postflight control experiment. For the control monkeys, all three bones are growing at a linear rate, 0.041 ± 0.017, 0.045 ± 0.020, 0,51 ± 0.019 mm/day for the tibia (a), radius (b) and ulna (c), respectively (mean ± SD). For flight monkey 1 (Drema), longitudinal growth was similar in tibia and radius, with a suggestion that ulnar growth might have been slowed by flight but then increased again so that by the end of the experiment it had reached the same level as the controls.

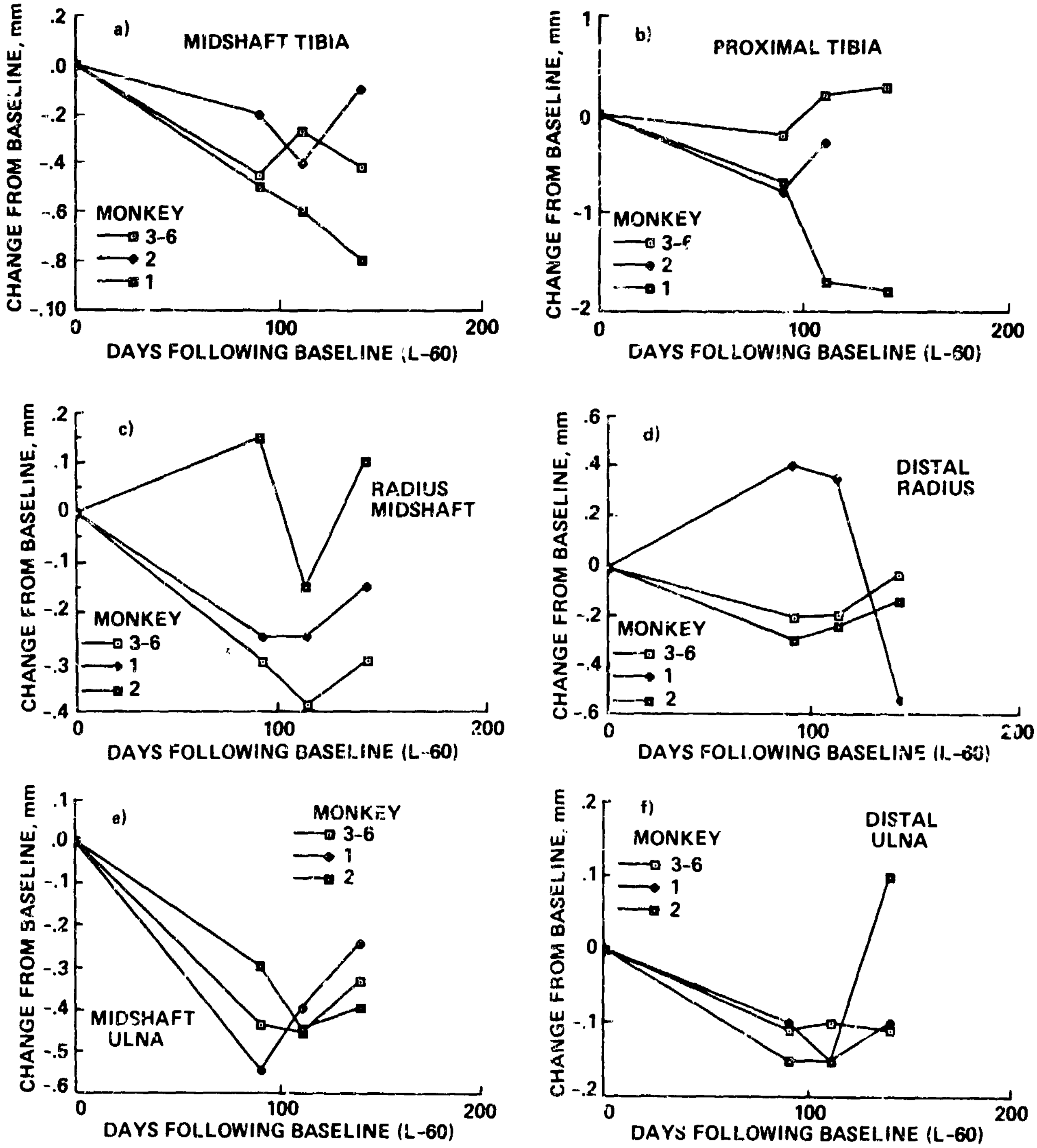

Figure 2. Indicate the change in cortical thickness due to the normal modeling process: (a) midshaft tibia; (b) proximal tibia; (c) radius midshaft; (d) distal radius; e) midshaft ulna; and (f) distal ulna. In the control monkeys, periosteal expansion at the midshaft is 0.2 - 0.4 mm over the experimental period, and the flight monkeys are not different. In general, there is a slight thinning of the cortices as the bones grow and expand, but the changes are quite small. Monkey 2 again shows a distinctly different pattern, especially in the tibia.