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Spaceflight Results in Formation of Defective Bone[1] (42215)

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Abstract. Growing rats were flown on 19 day spaceflights aboard Cosmos 782 and 936 biosatellites. Spaceflight resulted in a prominent skeletal defect at the periosteal surface of the tibia diaphysis. The defect, termed an arrest line, was approximately 3 μm across and separated the bone formed in space from that formed following spaceflight. The bone matrix at the arrest line region was abnormal in that collagen fibers were preferentially orientated parallel to the periosteal surface. In addition, the bone matrix was hypomineralized. The altered bone was inferior to normal bone in resistance to abrasion and may be partially responsible for the decrease in torsional strength observed after spaceflight.

Bone formation is decreased in growing rats during spaceflight, but quickly returns to normal upon termination of the flight (1–3). The underlying mechanism for this phenomenon is uncertain but is certainly associated with the reduced mechanical loading of the skeleton occurring during near weightlessness (4, 5). Since it is not known whether the bone formed during spaceflight is abnormal, we studied the morphology of bone made before, during, and immediately after spaceflight.

Materials and Methods. We obtained tibiae from rats flown aboard Cosmos 782 and 936 biosatellites. Care, preflight, and postflight activities of animals undergoing spaceflight have been previously described (1). Briefly, a flight control group was maintained for each experiment aboard an identical earth-based spacecraft and was exposed, as closely as possible, to flight conditions, including acceleration, noise, shock and vibration of launch and reentry (1). All rats received tetracycline one day prior to spaceflight and again either 3 (Cosmos 782) or 4 days (Cosmos 936) following completion of the 19-day flights. Rats were sacrificed 25 days postflight. Unfixed ground sections from the two groups were prepared from the left tibia at the tibia–fibula junction (6). The sections were microradiographed (7), and then viewed by visible light microscopy, fluorescence microscopy, and scanning electron microscopy (SEM). These ground sections were subsequently embedded in plastic in preparation for ultramicrotomy and transmission electron microscopy (TEM) (8). Thin sections were made with an ultramicrotome using either collidine buffer or water as the trough solution for the diamond knife. Sections were viewed both unstained and stained with uranyl acetate and lead citrate.

Results. A prominent skeletal defect was observed near the periosteal surface of flight animals. The defect was superimposed on or in some cases lay just within the second tetracycline label; thus, it was located at the boundary between the bone formed during the flight and postflight periods. This defect, previously termed an arrest line (1), was visible by conventional light microscopy as a smooth line that paralleled the periosteal surface (Fig. 1). The defect did not extend the entire perimeter of the periosteum and never appeared in the origin of the posterior eminence (5). Arrest lines were apparent in all tibiae of flight rats killed postflight but were either not present or were much less extensive in tibiae of flight

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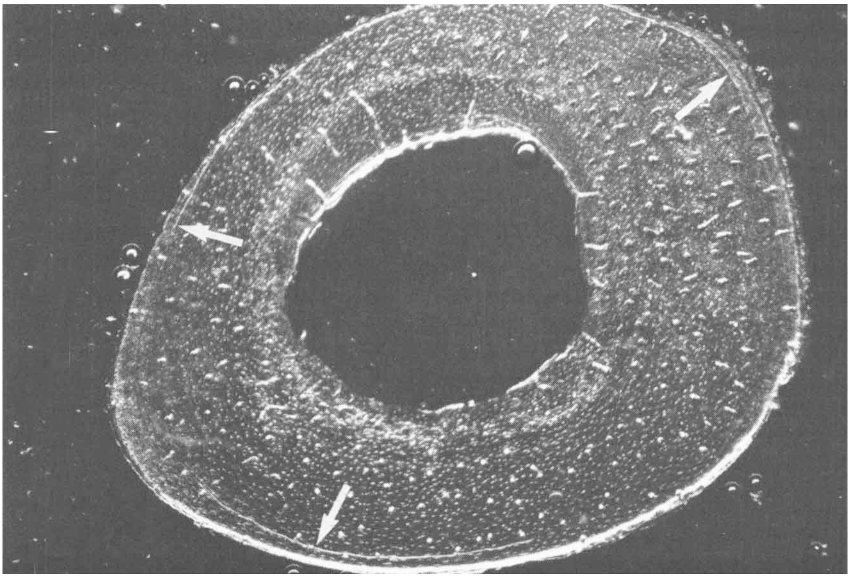

FIG. 1. Photomicrograph of a cross section through the tibia at the tibia–fibula junction from a rat flown in space for 19 days aboard Cosmos 936 and killed 25 days following termination of the flight. The lighting is oblique. The arrows point to an arrest line.

control animals killed at the same time and they were not present in the tibiae of flight rats and flight control rats killed immediately following spaceflight (1).

A surface feature was visible on the ground sections by SEM that corresponded exactly to the position of the arrest line (Fig. 2). Upon viewing specimens in a tilted position by SEM it was apparent that the surface feature seen by conventional light microscopy and SEM

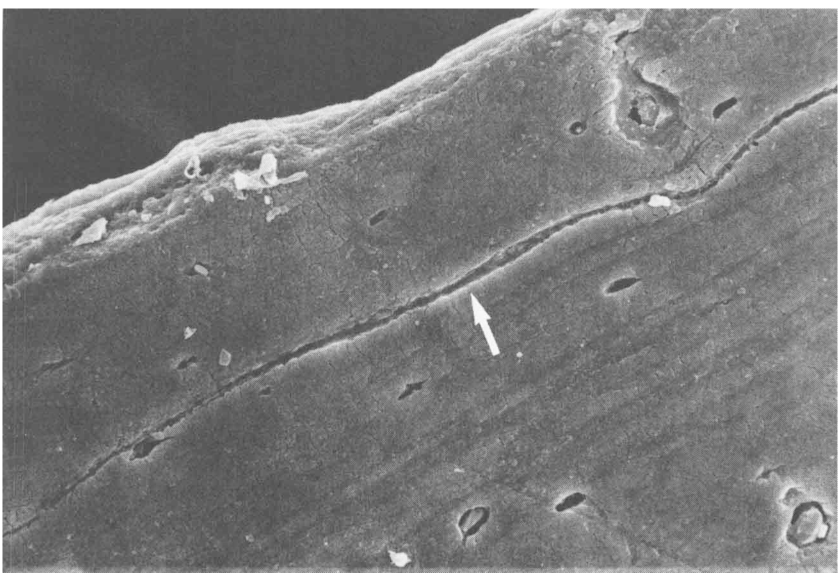

FIG. 2. Scanning electron micrograph of a ground section showing the arrest line (arrow) 600×.

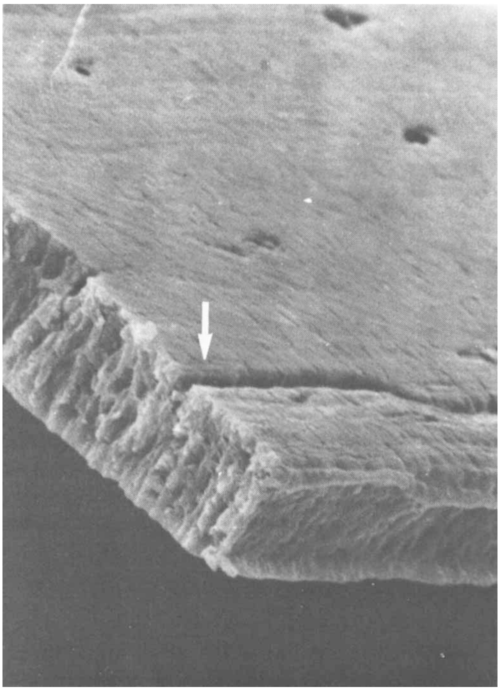

FIG. 3. Scanning electron micrograph of a ground section. The section was divided in two with a razor blade and viewed by SEM in a tilted position. The arrow points to the arrest line. 1100×

was a trench that extended into the specimen to a depth of approximately 2 μm which was <10% of the specimen thickness (Fig. 3). This trench was created during grinding of the specimen and was never observed on unground surfaces or in embedded and sectioned specimens.

When visualized by microradiography (Fig. 4), the arrest line was more radiolucent than the bone on either side of that feature indicating reduced mineral density. Excluding the arrest line region, no differences were found in the relative x-ray absorption of bone formed during the flight, preflight and postflight periods.

The arrest line was located in the thin sections prepared for TEM by using the surface trench as a marker and by making measurements of the distance from the periosteal surface to the arrest line location as seen in SEM of the same specimen. TEM of thin sections collected on collidine buffer to minimize demineralization revealed the arrest line to be a zone approximately 3.0 μm wide (Fig. 5). The bone in this zone was less electron dense than surrounding flight and postflight bone owing to reduced numbers of bone mineral crystallites. Interestingly, the numbers of crystallites

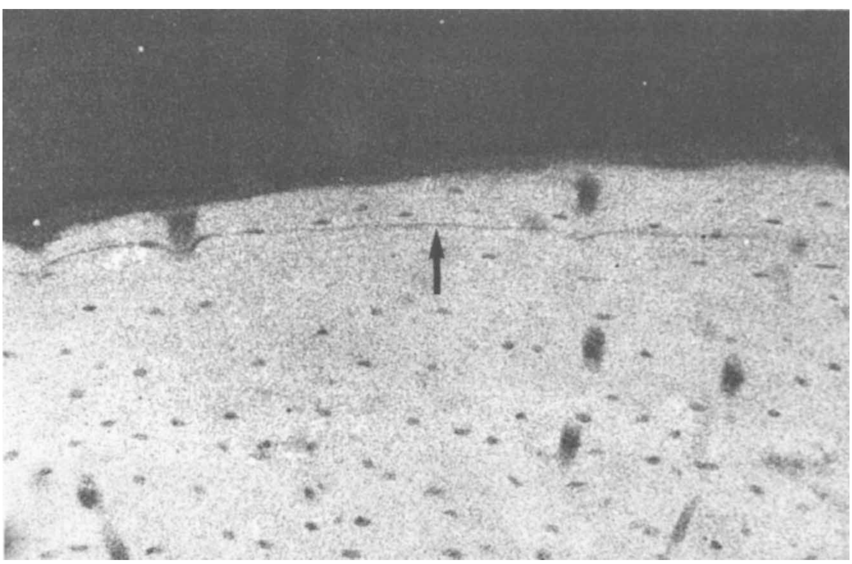

FIG. 4. Microradiograph of a ground section showing an arrest line (arrow) 270×.

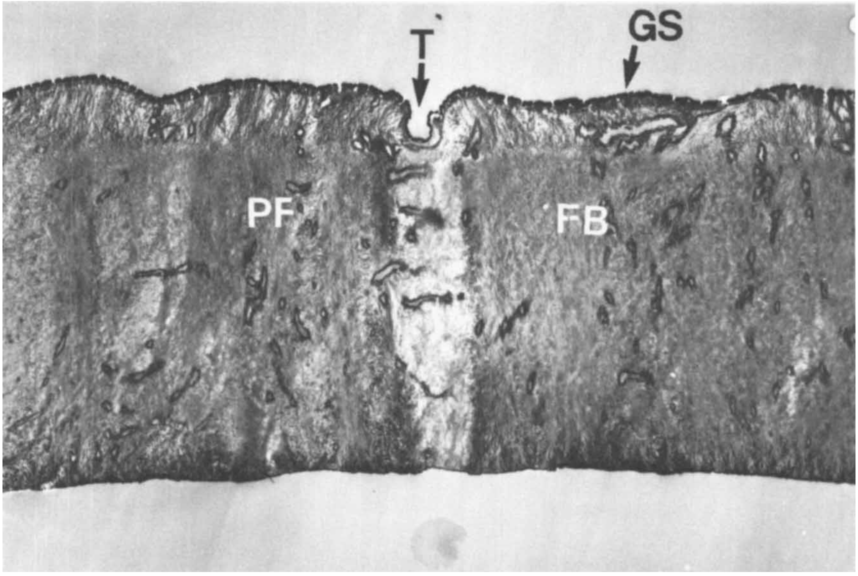

FIG. 5. Transmission electron micrograph showing an arrest line. The section was embedded in Spurr (ERL) medium. Thin sections of undemineralized bone were cut perpendicular to the arrest line. Collidine buffer was the trough solution. GS—ground surface of section; T—trench of the arrest line produced by grinding; F—flight bone; PF—postflight bone 3125×.

in the pericanalicular bone in the arrest zone appeared to be normal. Selected area electron diffraction identified the crystallites in the preflight, flight, and postflight bone matrix as the hydroxyapatite-like material that normally comprises bone matrix (data not shown). Similar, although weaker, patterns were recorded from the sparse crystallites in the arrest zone. All of the hydroxyapatite crystallites appeared in the irregular platelet habit typical of bone mineral. Electron diffraction revealed preferred orientation of the hydroxyapatite platelets in the arrest zone with their crystallographic *c*-axis oriented along the long axis of the constituent collagen fibrils. In contrast, no extensive regions of preferred orientation were observed in bone formed prior to or following spaceflight. There was one exception, preferred orientation of the *c*-axis of the crystallites perpendicular to the surface of the bone was always seen in a zone near the edge of the ground surface of the bone section but not on the unground surface (Fig. 5). This was believed to be an artifact due to surface damage occurring during preparation of ground sections.

The arrest zone generally comprised fiber (presumably collagen) bundles oriented parallel to the zone, i.e., perpendicular to the radial direction of periosteal bone growth of the tibiae (Figs. 6 and 7). An irregular convoluted pattern was always seen just within the arrest zone and normally along the flight period side. Neither oriented fibers nor the convoluted pattern characteristic of the arrest line region was observed in bone formed either prior to or following spaceflight. These patterns were more easily seen in thin sections collected on water since partial demineralization revealed more detail in the ground substance.

Discussion. The current microradiographic and TEM findings indicate that the arrest line region contains abnormal bone matrix and is hypomineralized. As a consequence of the altered morphology, this region is inferior to surrounding bone in resistance to abrasion. Furthermore, the abnormal alignment of collagen fibers and reduced crystallite content would be expected to degrade tensile and shear strength. Space flight results in decreased torsional breaking strength in long bones that is only partially explained by the decrease in cross sectional area (9). Thus, formation of abnormal bone at the arrest line may contribute to reduced strength.

The mechanism responsible for the mineralization defect at the arrest line region is unknown. The availability of calcium and phosphorus at that site is adequate since mineralization of the pericanicular bone appears to be normal.

Appositional bone formation normally undergoes the following sequence of events: Initially, osteoblasts produce collagen and glycosaminoglycans, substances which comprise osteoid. The organic matrix then undergoes a maturation process which favors the precipitation of hydroxyapatite-like crystallites and mineralization occurs. Previous results showed that the periosteal osteoblasts did not cease making bone immediately after launch but once bone formation ceased it remained halted for the duration of spaceflight (4). Apparently, once bone matrix formation ceases the binding properties for the molecules on the surface of the bone are such that newly secreted molecules cannot now be crosslinked to the older molecules, thus requiring a cementing junction, or arrest line. Although the defect resulting in cessation of bone formation is corrected immediately after spaceflight (1), the present findings provide evidence for a morphological defect that is incorporated into the skeleton and not corrected within 25 days after spaceflight and may even be permanent.

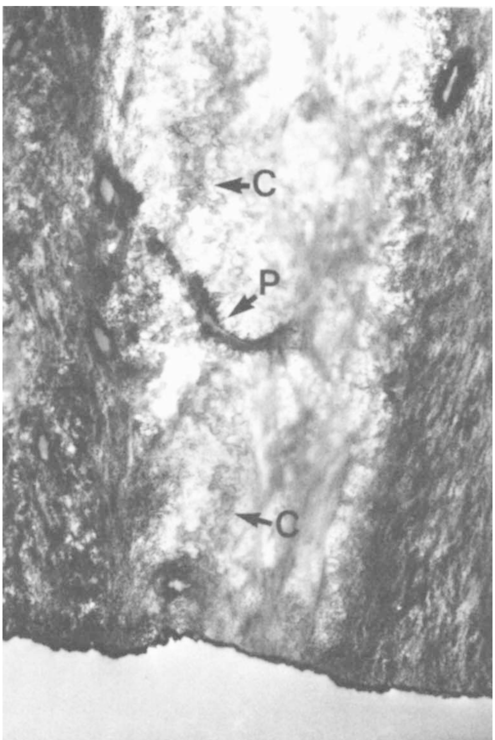

FIG. 6. Higher magnification of the arrest zone in Fig. 5 showing normally mineralized pericanalicular bone (P) and convoluted features (C). 13,000×.

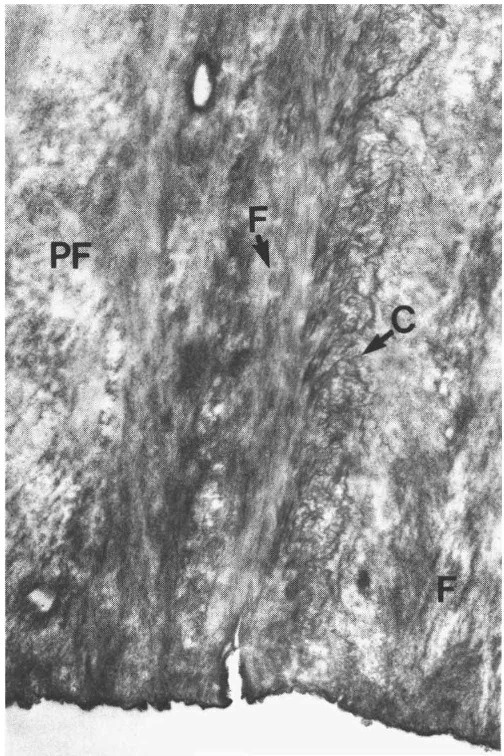

FIG. 7. TEM of section cut with water as a trough solution. Noticeable demineralization had taken place revealing more detail in the ground substance. The collagen fibers (F) and convoluted features (C) are aligned parallel to the arrest line. 17,000×.

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