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# EFFECTS OF MICROGRAVITY ON RAT BONE, CARTILAGE AND CONNECTIVE TISSUES

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## INTRODUCTION

The response to hypogravity by the skeletal system was originally thought to be the result of a reduction in weight bearing. Thus a reduced rate of new bone formation in the weight-bearing bones was accepted, when found, as an obvious result of hypogravity. However, data on non-weight-bearing tissues have begun to show that other physiological changes can be expected to occur to animals during spaceflight. In this overview of the Cosmos 1887 data we will comment on these results as they pertain to individual bones or tissues because the response seems to depend on the architecture and metabolism of each tissue under study.

## INVESTIGATIONS OF THE FEMUR

The femur was surgically divided into three sections: proximal, distal and central. Biochemical analysis of each section revealed that in the flight animals the central region (i.e., diaphyseal bone) showed a reduction in bone mineral and content of osteocalcin, but no change in collagen content. The collagen results were difficult to analyze because of the wide variation in animal weights and age among the three control groups. Some additional biochemical changes which were noted were a decrease in serum osteocalcin in the flight animals compared to synchronous controls. And adrenal weights were increased in the flight animals suggesting a certain level of stress existed in this group which was not present in the control groups.

Two non-chemical, non-destructive methods were used to analyze the distribution of mineral content in different regions of the femur. In one case, an x-ray microbeam with a ten micron diameter resolution was scanned across the surface of the mineralized cross-sections of femur. Absorption of these x-rays was correlated with mineral content and density relative to the area of bone being scanned. However, because of the extremely long time required to do this analysis, only one femur from the control and the flight group was studied. In the second method, an electron microbeam, generated by a scanning electron microscope, bombarded a cross-section of mineralized femur and generated backscattered electrons which were captured and analyzed. The backscattered electron image generated a density map of the specimen surface and these densities were related to the morphology of the specimen surface. There was considerable variation in these results between groups of animals and no conclusions were reached using these new methods although they offer a significant new approach for future studies.

## INVESTIGATIONS OF THE TIBIA

Morphometric measurements were made on cross-sections of tibia but no differences were found in bone area, marrow area, periosteal perimeter or marrow perimeter between flight and control animals. In previous flights tetracycline was used to indicate areas of new bone formation. This matrix marker was not

used in Cosmos 1887 so it was not possible to distinguish areas of new bone formation from pre-flight bone surfaces. Thus possible alterations in bone formation could not be determined in this flight.

Measurements were made of the surface electrical charge of particles of bone from tibias of the flight and control rats. When uniform particles of bone move through an electrical field (this technique is similar to electrophoresis) information is obtained concerning the surface charge on these particles. Experience from earth-bound control studies suggests that when new bone formation predominates in the skeleton, the bone particles carry a greater than normal negative charge. These same measurements made on bone from the Cosmos 1887 rats indicated that there was new bone formation occurring to a greater degree in the flight animals compared to the controls. This result is contradictory to morphometric data obtained from several previous flight in which bone formation was reduced during spaceflight. The Cosmos 1887 data could be interpreted to mean that the animals were already beginning to recover due to their 55 hours on earth prior to sacrifice. However, this technique has not been applied to bones from spaceflight which were known to have reduced new bone formation so the interpretation of this data is still unclear.

Histochemistry and morphometry of the osteoblast population located along the diaphyseal endosteal surface was done to compare osteoblast function among the different groups. There was no obvious change in alkaline phosphatase activity or overall osteoblast morphology as a result of the spaceflight. This compared well with the biochemical measurements which showed no change in collagen synthesis in the femur diaphysis (1). However, the histochemical demonstration of NADPase activity in the small Golgi vesicles of the osteoblast indicated that there were more vesicles present in flight osteoblasts compared to control osteoblasts. This result could be due to a decrease in cellular energy stores needed to move the vesicles out of the cytoplasm (thus their accumulation when energy is decreased) or it could be due to an acute increase in procollagen synthesis resulting from the 55 hours at 1g prior to sacrifice.

The vascularity of the diaphyseal bone was studied by morphology and histochemistry. The flight animals showed a reduction in enzyme activity of the vascular endothelial cells, an increase in numbers of vessels per bone cross-section area, some lipid accumulation in vessels near the periosteum, and occasional degenerate osteocytes in the vicinity of these same vessels. These results strongly suggest that the vascular supply which nourished the compact bone of the tibia had been damaged either due to spaceflight conditions or to the effects of re-entry from the flight. These same observations could explain the reduction in some mechanical properties seen in the humerus following flight (2) although the vascularity of the humerus was not studied in this flight.

## INVESTIGATIONS OF THE TIBIA EPIPHYSEAL PLATE

The epiphyseal growth plate has been shown to respond to the absence of gravity in the Spacelab-3 experiments. But the growth of long bones is also sensitive to other influences, especially the presence or absence of growth hormone. It has been shown by Hymer and Grindeland (3) that secretion of growth hormone from the pituitary somatotrophs is inhibited in spaceflight.

In flight animals from Cosmos 1887, the proliferative zone at the top of the growth plate was larger than in the synchronous controls, however the hypertrophic/degenerate cell zone was smaller than in the controls. The total number of cells in the growth plate was greater following flight, yet the overall area of the plate was smaller. These data are contradictory to results found in the Spacelab-3 experiment and

suggest that in Cosmos 1887 the proliferation of cartilage cells had resumed following flight due to the 55 hour delay in sacrifice following the return of the biosatellite.

In a parallel study on the tail-suspended non-weight bearing rat model of Morey-Holton there was also a decrease in plate height and decreased numbers of cells per volume of growth plate. However in these suspended animals the greatest effect was seen in the hypertrophic zone, not in the proliferative zone. So the suspended animal model and space flight produce some slightly but significantly different effects on the growth plate of the long bones.

There needs to be a continuation of work in this area to determine which effects on growth are controlled by hormonal changes, by overall change in animal physiology, or by the absence of gravity. This is apparently a complex process of growth and may be controlled by many different biochemical and biomechanical events.

## INVESTIGATIONS OF THE HUMERUS

Morphomctric measurements of cross-sections of the humerus showed a decrease within flight animals compared to synchronous controls and a decrease in periosteal circumference compared to synchronous and vivarium controls. These data are different from the results with the tibia (4) in which no changes in these parameters were seen. The mechanical bending stiffness of the humerus correlated with the morphometry in that there was a decrease in the flight animals compared to synchronous controls. The elastic modulus, which is determined by the material characteristics of the bone, was unchanged due to flight and the biochemistry indicated that there was no difference in Ca, P, or hydroxyproline content of the humerus. However, these measurements were made from whole bones. It might have been interesting to compare the biochemistry of different areas of bone (proximal, distal and central diaphysis) as was done in the study by Arnaud et al (1).

An important study was done by Grindeland at NASA Ames to compare US and USSR flight experimental procedures and how these might affect experimental results. For example, the US uses Taconic-Sprague Dawley rats, on special diet, and housed as one rat per cage. The USSR experiments use Czechoslavakian-Wistar rats, maintained on a different diet, and housed as ten rats per cage. The diet ingredients are included in the Vailas, et al report (2). A comparison was made of morphometric and biomechanical measurements on the humerus and vertebrae under these various experimental conditions and the differences in results were noted in this report.

Another aspect to the Vailas report was a comparison of the effect of spaceflight on the patellar and Achilles tendons. The measurements of collagen content, collagen cross-linking, and DNA content showed a heterogeneous spaceflight response depending on the tendon function during flight.

## INVESTIGATION OF BONE AND ITS COMPARISON TO DENTINE

In this study, bone mineral from the calvaria and vertebrae (L5) was compared to mineral from dentine in the mandible. The bone ash from flight bones was reduced compared to controls, however, the concentrations of Ca Mg and P (expressed as % of dry weight) were normal. The ratio of Ca/P was reduced in the flight group and the ratio of Ca/Mg was higher than the control values. This suggests that the bone was mineralizing but was failing to mature properly. To study these mineral changes in more detail, the

bone was reduced to particles below twenty microns in size and then fractionated through a series of solutions of different specific gravity. The density of the bone particles from the flight animals showed a shift to lower specific gravity fractions compared to controls, again indicating an immaturity in the process of bone mineralization. X-ray diffraction was also used for analysis of bone mineral and dentine. These data showed that there was a reduced growth in the c-axis of the bone mineral crystal from the flight animals. The mineral from dentine did not show this change, indicating normal mineral maturation in this tissue. Because of the small amount of dentine available for study, an electron microprobe was used to determine Ca/P and Ca/Mg ratios, and concentrations of Ca, P, Mg and An. These values for dentine were normal when compared to age-matched controls. Thus the flight effects on the inhibition of mineral maturation seem confined to bone.

## INVESTIGATION OF THE PERIODONTAL LIGAMENT

The periodontal ligament which stretches between the tooth root and the surrounding alveolar bone, contains bone cell precursors which are responsive to signals for increasing or decreasing bone formation. These cells can be classified by their nuclear morphology into 1) a precursor cell which gives rise to 2) an osteoprogenitor, or committed cell, which gives rise to 3) the preosteoblast, which eventually forms 4) the mature bone-forming osteoblast. Previous spaceflight experiments indicated that the progenitor cell (osteoprogenitor) increased in numbers while the preosteoblasts decreased in numbers, in response to the hypogravity environment. The present Cosmos 1887 data indicated the opposite result, with an increase in the preosteoblast population by 42% over the synchronous controls, and a concomitant decrease in the osteoprogenitor cell population. It would appear that the bone cells had begun to recover from the flight effects during the 55 hour period between the return to earth and the sacrifice of the rats. The fact that matrix mineralization defects were seen in the mandibular bone (5) provides support that a bone matrix defect did occur during the flight, however, the cellular response apparently adjusted quickly to the change in the gravitational environment.

## INVESTIGATIONS OF THE VERTEBRAE

The vertebral body (L6) was biomechanically and biochemically analyzed by Vailas et al (2) following Cosmos 1887. The calcium and hydroxyproline content was unchanged due to flight; however, the number of cross-linkages per collagen molecule were less in samples from flight animals compared to vivarium or synchronous controls. Mechanical testing of the whole vertebra revealed that the compression stiffness was actually reduced as a result of flight.

Studies on the vertebrae (L4) by Cann et al (6) were done by dissecting out the posterior portion from the vertebral body. This is the region, in the rat, that contains the most dense bone and is the major supportive element of the vertebra. The measurements of weight, water content, Ca, P and osteocalcin were not very conclusive when the flight group was compared to the various controls. However, the osteocalcin concentration in the posterior of vertebrae from flight animals tended to be higher than in the control groups. (This result was opposite to the finding of Arnaud et al (1) in measurements from the diaphyseal portion of the femur.) This result suggests, based on previous ground-based studies, that the bone turnover in this region was reduced.

A study of the intervertebral disc was done by Hargens et al (4) to determine if swelling pressures within the discs could explain the back pain experienced by astronauts during space travel. Direct

measurements of swelling pressure in the nucleus pulposus from lumbar vertebrae were done using a newly developed technique. However, no significant difference was found between the flight and control groups. This lack of effect was probably due to the 55-hour recovery period prior to animal sacrifice in this experiment.

## CONCLUSIONS

Various effects were seen in different tissues from the rats flown on Cosmos 1887. The femur showed a reduced bone mineral content but only in the central region of the diaphysis. This same region in the tibia showed changes in the vascularity of bone as well as some osteocytic cell death. The humerus demonstrated reduced morphometric characteristics plus a decrease in mechanical stiffness. Bone mineral crystals did not mature normally as a result of flight suggesting a defect in the matrix mineralization process. Note that these changes relate directly to the matrix portion of the bone or some function of bone which slowly responds to changes in the environment. However, most cellular functions of bone are rapid responders. The stimulation of osteoblast precursor cells, the osteoblast function in collagen synthesis, a change in the proliferation rate of cells in the epiphyseal growth plate, the synthesis and secretion of osteocalcin, and the movement of water into or out of tissues, are all processes which respond rapidly to environmental change. These rapidly responding events produced results from Cosmos 1887 which were frequently quite different from previous space flight data. It was not always possible to know if these results were due to space flight or the recovery from flight during the 55-hour period between flight and animal sacrifice. On the other hand, matrix related events or permanent cellular changes (e.g., matrix mineralization or osteocyte cellular degeneration ) did not have the ability or enough time during the 55 hour delay to "recover" or to return to some other state of activity. Consequently, the more slowly responding biological processes provided a better indication of past biological events and apparently retained the record of the flight effects.

If another biosatellite were to repeat the Cosmos 1887 experiments but were followed by a more rapid satellite retrieval and sacrifice of animals, the Cosmos 1887 data would gain even more in value. We could then retrospectively determine which data indicated a true "recovery" from flight and which data showed more lasting long term effects created by the hypogravity. The flight of Cosmos 1887 and the accidental delay in retrieving the animals has provided data which are very rare in studies of space biology. An appreciation of the cellular or tissue "recovery" aspects of this flight will always remain an important contribution to our scientific understanding of the effects of hypogravity on the skeletal system.

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