

## BONE CULTURE RESEARCH

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Background and Hypothesis

Among the most overt negative changes experienced by man and experimental animals under conditions of weightlessness are the loss of skeletal mass and attendant hypercalciuria. These clearly result from some disruption in the balance between bone formation and bone resorption (i.e., remodeling), but precisely what this disruption is and how it might occur have not been established. Frost (1987) has suggested that the loss of bone under conditions of weightlessness is due to a decrease not just in weight but also in muscle resistance. He has proposed that bone contains a mechanostat which senses these changes and adjusts the mass accordingly. Work in vitro has suggested that osteoblastic cells respond to mechanical stress but there are presently no published studies documenting the presence and function of mechanoreceptors on such cells. Frost has gone on to suggest that changes in the mechanostat may change responsiveness to circulating agents, such as parathyroid hormone (PTH), resulting in increased numbers of remodelling units without any change in circulating hormone. Since the osteoblast is the target cell for bone-resorbing hormones like PTH, it would suggest that in microgravity, the osteoblast may be more sensitive to such agents.

The experiments described in this paper are aimed at exploring PTH regulation of production of collagenase and protein inhibitors of collagenase (tissue inhibitors of metalloproteases, TIMP-1 and -2) by osteoblast-like osteosarcoma cells under conditions of weightlessness. The

results of this work will contribute to information as to whether a microgravity environment alters the functions and responsiveness of the osteoblast.

### Objectives

The more specific objectives of the BCR experiment are:

- To observe the effects of microgravity on the morphology, rate of proliferation, and behavior of the osteoblastic cells, UMR 106-01.
- To determine whether microgravity affects the hormonal sensitivity of osteoblastic cells.
- To measure the secretion of collagenase and its inhibitors into the medium under conditions of microgravity.

### Methods

The methods employed will consist of the following:

- The osteoblast-like cells, UMR-106-01, will be cultured in four NASDA cell culture chambers.
- Two chambers will be subjected to microgravity on SL-J; two chambers will remain on the ground at KSC as ground controls but subjected to an identical set of culture conditions as on the shuttle.

- Media will be changed four times; twice the cells will receive the hormone parathyroid hormone-related protein (PTHrP) and media collected.

- Cells will be photographed under conditions of microgravity.

- Media and photographs will be analyzed upon return to determine whether functions of the cells changed.

#### Post-Flight Analysis

Post-flight analysis will consist of the following:

- The photomicrographs for the two chambers and the various treatment periods will be analyzed for differences in behavior, morphology, and rates of proliferation of the osteoblastic cells in micro- and unit gravity for the field of observation.

- Measurement of collagenase in the media will be conducted by ELISA assay.

- Measurement of TIMPs in the media will be conducted by ELISA assay.

- Cell protein will also be measured.

- Collagenase and TIMP secretion will be expressed per unit cell protein.

### Expected Results

- UMR cells in microgravity will respond more sensitively to PTHrP.
- Growth will be decreased more than at unit gravity.
- Cells will produce greater amounts of collagenase, perhaps lesser amounts of inhibitors.

From the data, we may obtain knowledge at the cellular level of the behavior and functions of osteoblasts under microgravity and thus gain information as to the mechanisms causing microgravity-induced osteopenia.

### Reference

Frost, H. M. (1987), Anat. Rec. 219, 1-9.