## <u>Intracellular Electrolytes of Muscle and Epithelial Cells in</u> <u>Simulated Microgra</u>vity

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### INTRODUCTION

NASA -IntraCellular Diagnostics, Inc. Study

Both immobilization and space flight are commonly associated with redistribution of ions and electrolytes.

The effects of these changes in soft tissues is not known.

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#### **OBJECTIVES:**

- 1. To quantify the concentration of intracellular ions in soft tissues from bed rest subjects in simulated micro gravity
- 2. To determine any alterations of intracellular ions induced by electromyostimulation (EMS), during bed rest.
- 3. To compare the effects of bed rest and EMS on intracellular ions in sublingual cells and skeletal muscle.
- 4. To compare the intracellular electrolytes in skeletal muscle to oral sublingual cells using energy dispersive X-ray analysis.

#### **EXPERIMENTAL DESIGN**

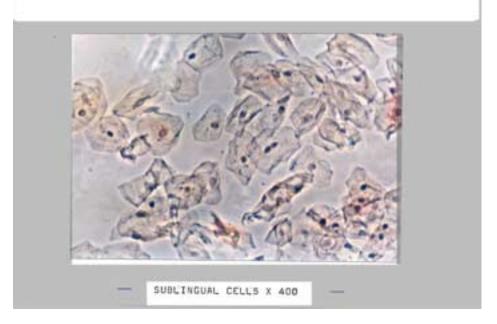
11 NORMAL MALES WERE PLACED IN A -6 DEGREE HEAD DOWN BEDREST FOR 30 DAYS. THREE SUBJECTS WERE SUBJECTED TO ELECTRO-MYOSTIMULATION (EMS) TO THE RIGHT LEG EACH DAY, EMS WAS APPLIED 2X/DAY FOR 1 HOUR DURATION EACH.

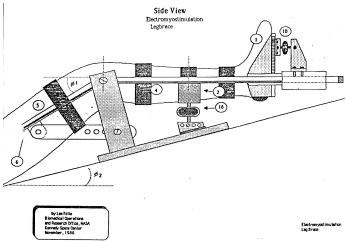
EIGHT SUBJECTS SERVED AS CONTROLS, THESE WERE NOT STIMULATED BY EMS.

SUBLINGUAL CELLS WERE TAKEN FROM ALL SUBJECTS ON FOUR OCCASIONS, PRE-MID, AND POST BED REST

MUSCLE BIOPSIES FROM THE VASUTUS LATERALIS AND SOLEUS MUSCLES WERE TAKEN FROM BOTH LEGS OF THE EMS SUBJECTS, AND OF THE THE DOMINANT LEG OF THE CONTROL SUBJECTS

ALL TISSUES WERE ANALYZED FOR INTRACELLULAR ION CONCENTRATIONS





#### **METHODS- MUSCLE TISSUES**

- Muscle biopsies were taken from the thigh and soleus muscles and immediately frozen in liquid nitrogen
- Muscle tissue was sectioned while frozen. Sections were mounted on carbon slides. No chemical solutions or fixation were used.
- Frozen sections were further freeze dried and carbon coated to eliminate charging effects in the analytical scanning electron microscope.
- The C-coated sections were imaged for muscle fibers in a Philips scanning analytical microscope using energy dispersive X-ray analysis
- Intracellular mineral-electrolyte concentration was quantified from the x-ray fluorescent pattern of the cellular contents. The X-ray counts were compared to known electrolyte standards, and expressed in mEq/l.

U.S. patent 4,717,826

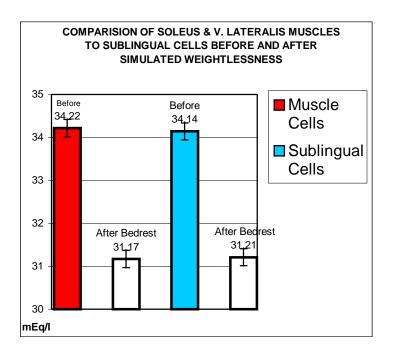
#### **METHODS-SUBLINGUAL CELLS**

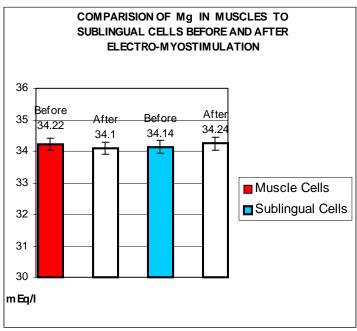
- Sublingual cells are obtained from cleansed sublingual areas of the buccal cavity with an applicator stick and spread on low background carbon slides.
- The smears are immediately fixed with cytology fixative and allowed to air dry
- Once fixed the smear requires no further manipulation and can be stored at room temperature or transported with no loss of elements.
- Smears are observed in a Scanning analytical microscope using energy dispersive X-ray analysis.
- Between 10-15 cells are selected for examination for consistent, reproducible readings.
- Intracellular ion contents from the generated spectra are compared to known external standards. The quantitated results are expressed as mEq/l (as known by calculated cell volumes) © IntraCellular Diagnostics, Inc.

### **SUMMARY**

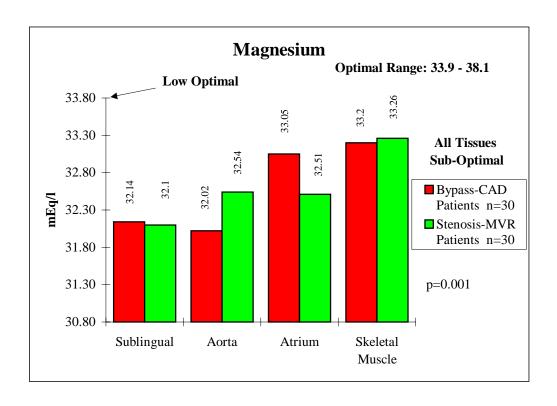
Homogenous sublingual epithelial cell smears were fixed to low background carbon slides. The cells were analyzed and selected due to their unique characteristics.

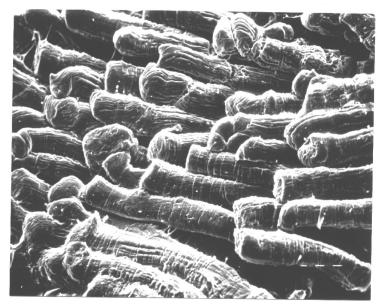
- Cells are non- cornified, may be stored and transported easily
- Cells are aerobic
- Cells have nuclei and rapid metabolism
- *Cells have rapid metabolism*
- Cells are easily accessible and require minimal preparation



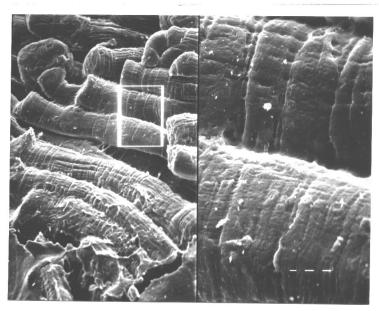


# Correlation Study : Sublingual Cells to Cardiac and Skeletal Muscle Tissue



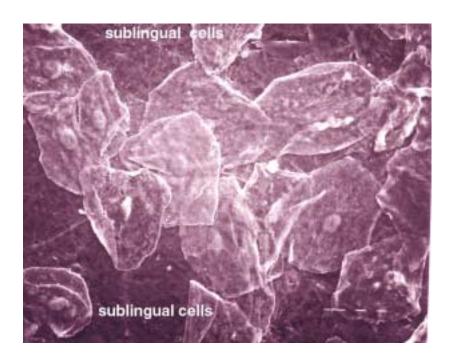


180 X



180 x

900 X



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Burton Silver

IntraCellular Diagnostics, Inc.

Signed and sealed at Washington, D.C. this seventeenth day of March Nineteen Hundred and Ninety-four

ABMINISTRATOR, MASA