PROTEOMIC RETRIEVAL FROM NUCLEIC ACID DEPLETED SPACE-FLOWN HUMAN CELLS

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Compared to experiments utilizing humans in microgravity, cell-based approaches to questions about subsystems of the human system afford multiple advantages, such as crew safety and the ability to achieve statistical significance. To maximize the science return from flight samples, an optimized method was developed to recover protein from samples depleted of nucleic acid. This technique allows multiple analyses on a single cellular sample and when applied to future cellular investigations could accelerate solutions to significant biomedical barriers to human space exploration. Cell cultures grown in American Fluoroseal bags were treated with an RNA stabilizing agent (RNA*later* – Ambion), which enabled both RNA and immunoreactive protein analyses. RNA was purified using an RNAqueous® kit (Ambion) and the remaining RNA free supernatant was precipitated with 5% trichloroacetic acid. The precipitate was dissolved in SDS running buffer and tested for protein content using a bicinchoninic acid assay (1) (Sigma). Equal loads of protein were placed on SDS-PAGE gels and either stained with CyproOrange (Amersham) or transferred using Western Blotting techniques (2,3,4). Protein recovered from RNA*later*-treated cells and stained with protein stain, was measured using Imagequant volume measurements for rectangles of equal size. BSA treated in this way gave quantitative data over the protein range used (Fig 1). Human renal cortical epithelial (HRCE) cells (5,6,7) grown onboard the International Space Station (ISS) during Increment 3 and in ground control cultures exhibited similar immunoreactivity profiles for antibodies to the Vitamin D receptor (VDR) (Fig 2), the beta isoform of protein kinase C (PKCß) (Fig 3), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Fig 4). Parallel immunohistochemical studies on formalin-fixed flight and ground control cultures also showed positive immunostaining for VDR and other biomarkers (Fig 5). These results are consistent with data from additional antigenic recovery experiments performed on human Mullerian tumor cells cultured in microgravity (8).

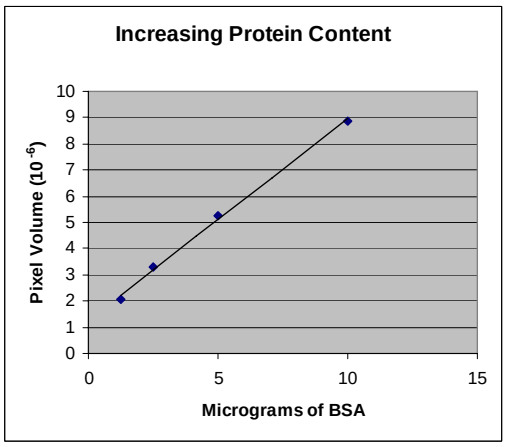

Figure 1 - Increasing Bovine Serum Albumin (BSA) concentrations were used to determine whether volume measurements of the bands on a gel resulted in adequate quantitative data. The SYPRO Orange stained gel containing increasing amounts of BSA was scanned with a Molecular Dynamics Storm and equal sized rectangles were used to collect the pixel volume.

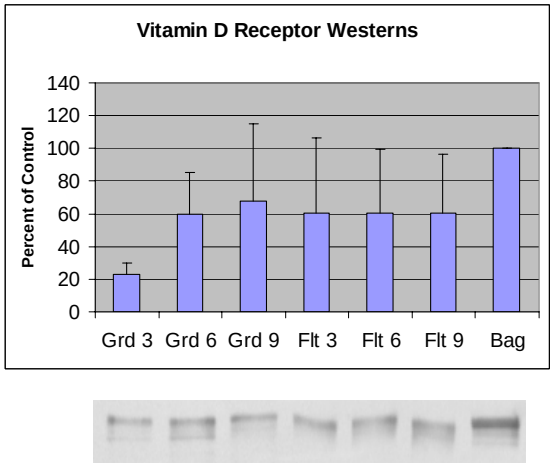

Figure 2 - Vitamin D Receptor (VDR) antibody stained blots, prepared with equal loading of protein (5 µg/lane), measured a protein with a molecular weight of 60-64kD. An example of one of the blots is shown below the graph, which is an average of three blots.

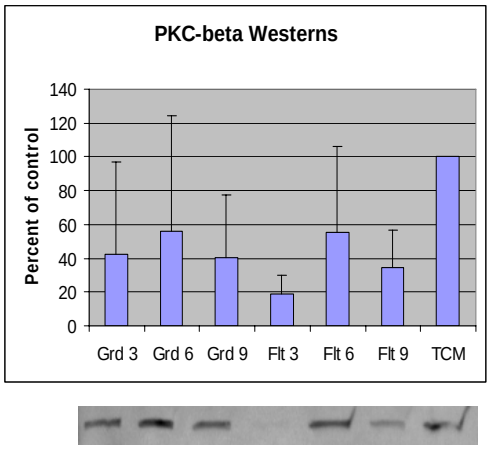

Figure 3 - Protein Kinase CβII (PKCβII) antibody stained blot, prepared with 6 µg/lane of protein, measured a protein with a molecular weight of 80 kD. The graph is an average of four blots with an example below the graph.

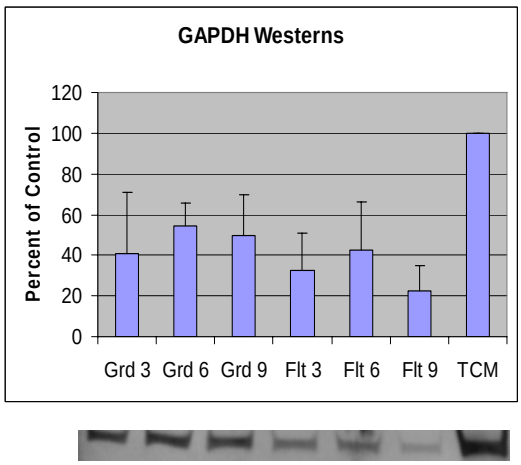

Figure 4 - Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody stained blot, prepared with 6 µg/lane of protein measured a protein with a molecular weight of 42-44 kD. The graph is the average of four blots with an example below the graph.

The protein in the above examples was recovered from RNA*later* treated cells that had the RNA removed by an Ambion RNAqueous kit, using trichloroacetic acid precipitation. Although on a protein per protein basis, the RNA*later* treated cells demonstrated slightly less antigenic protein than

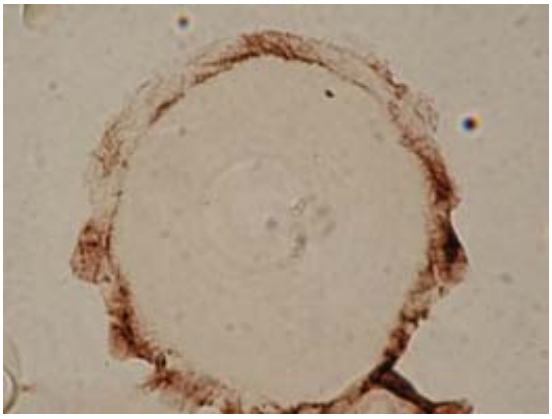

Figure 5 - Light photomicrograph (600x) of Vitamin D receptor immunoreactivity in sectioned formalin fixed HRCE cells cultured on Cytodex-3 microcarrier beads in microgravity for 12 days during ISS Increment 4.

the untreated cells, there was a high percentage of recovery of antigenic protein using three different antibodies in both the ground and flight samples. The data obtained for the vitamin D receptor was further supported by the results of cells fixed in formalin and stained with the same antibody to the vitamin D receptor (Fig 5). This work extends and corroborates previous work done with another cell line (7) and extends the proteins that can be investigated using small amounts of cellular protein after nucleic acids have been removed.

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