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Adrenocorticotropin Hormone Expression in the Developing Chicken Limb

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Optimization of Fatty Acid and Cholera Toxin Concentrations for Treatments of Epithelial Cells: Can Fatty Acids Provide Mucosal Immunity against Cholera Infections? Joanna Tychowski¹, Paula Cobos², Laura Lorentzen¹ and Farshad Tamari³, ¹New Jersey Center for Science Technology Mathematics Education, ²Kean University, Union, NJ and ³Kingsborough Community College, Brooklyn, NY.

Cholera is caused by infection of the small intestine by *Vibrio cholera*. In developing countries it can be severe or fatal. The two subunit Cholera Toxin (CT) binds to the cell surface allowing one subunit to enter the cell. In response, cyclic AMP levels are elevated, influencing electrolyte and cytokine balances. Fatty acids (FAs) such as oleic, linoleic, and linolenic acids, found in flax (*Linum*) seeds, have medicinal properties. Our ultimate objective is to explore whether metabolites of the above FAs can provide any degree of mucosal immunity, as determined by cytokine dynamics in response to CT challenge. Our first goal is to determine the maximum and minimum concentrations of FAs and CT, respectively, that murine and human epithelial cell can be exposed to. The following control (C) and experimental (E) treatments will then be performed and cytokine levels will be quantified and compared using ELISA: 1. No FA or CT treatment (C), 2. FA treatment only (C), 3. CT treatment only (C), and 4. Pre- and simultaneous treatments with both FAs followed by CT challenge (E). Thus far, using mouse macrophages and MTT assays, the optimum concentrations for oleic, linoleic, and linolenic have been determined at 5-50ng/ μ L.

Genome-wide Distribution of G-quadruplexes in the Transcribed Regions of Human Genes. Viktor Vasilev, Lawrence D'Antonio, and Paramjeet S. Bagga, Ramapo College of New Jersey, Mahwah, NJ.

G-rich DNA and RNA G-quadruplexes can play significant biological roles in important cellular processes and human disease. The goal of current studies in our lab has been to investigate the role of G-quadruplexes in post transcriptional regulation of gene expression. We have used a bioinformatics approach to study the composition and distribution patterns of G-quadruplex forming motifs in the transcribed regions of >17,000 protein coding human genes. G-quadruplex motifs were found in almost all of the >500,000 of exons and introns that were analyzed. Our studies revealed the prevalence of G-quadruplexes with high putative stability near 5' splice sites in the introns. Stable RNA G-quadruplexes in the vicinity of 5' splice site may be involved in modulating splicing via interactions with regulatory proteins that bind G-rich sequences and influence alternative and tissue specific splicing events. We also found a very strong correlation between the distribution of the positions of ESEs (Exonic Splicing Enhancers) and G-quadruplexes, especially in the exons. Further investigation revealed overlaps between the predicted ESEs and G-quadruplexes mapped near the splice sites. ESE mediated regulated splicing may in fact involve the G-quadruplex structure. Our findings suggest that G-quadruplexes play a regulatory role in splicing of the human pre-mRNAs.

Study on the Effect of Cupric Chloride and Cadmium Chloride on Cyanobacteria *Synechococcus* sp. IU 625. Vico Viggiano¹, Shyam Patel¹, Jose L. Perez¹, Tin-Chun Chu² and Lee H. Lee¹, ¹Montclair State University, Montclair, NJ and ²Seton Hall University, South Orange, NJ.

Cyanobacteria, *Synechococcus* sp. IU 625, were used because they are good indicators of water contamination by heavy metals. In this experiment, the effect of CuCl₂ (0, 5, 10, 15 and 30 mg/L) and CdCl₂ (0, 10, 15, 25, and 30 mg/L) on the growth of cyanobacteria *S. IU 625* were studied. Growth was monitored by direct count using hemocytometer and turbidity study using spectrophotometer at wavelength 750 nm. The content of CuCl₂ and CdCl₂ in the cells and media was analyzed by using ICP (Inductively Coupled Plasma) spectrometer. In the cultures containing CuCl₂, the growths were similar except 30 mg/L, where the growth was inhibited. ICP study indicated that 87 to 100% of CuCl₂ stays outside of the cells. In the 5 mg/L CdCl₂ culture, the growth was the same as the control and in 15mg/L it was slightly inhibited. At 30 mg/L, the growth was almost completely inhibited. ICP study indicated that 70 to 80 % of the metal stays in the media. This study suggested that the cells have low permeability to CuCl₂ and CdCl₂ and permeability may be one of the reasons that the cells are able to tolerate the metal contamination.

Adrenocorticotropin Hormone Expression in the Developing Chicken Limb. Michele J. Vigiotti and Jodi F. Evans, Molloy College, Rockville Centre, NY.

In previous studies using mammalian models we have found both clinical and laboratory evidence of a role for melanocortins in endochondral ossification. The melanocortin system has remarkable conservation among vertebrates and melanocortin receptors are expressed with significant sequence homology in teleosts to mammals. The overall goal of these studies is to provide a more accessible model of melanocortin involvement in endochondral growth. We hope to determine if melanocortins play a role during endochondral ossification of the developing chicken limb. Like in mammals melanocortins are widely distributed throughout the body of chicken and participate in a wide range of physiological functions with the peripheral tissue distribution of melanocortin receptors in chicken more widespread. Our first step was to examine melanocortin expression in the developing limbs of the chick embryo. Using immunohistochemistry techniques, we detected ACTH (1-24) in the limbs of embryonic day 9 chick embryos. This initial data indicates that the chick embryo is a viable model that can be used to determine a role for melanocortin in endochondral growth. Melanocortin expression shows remarkable sequence homology, therefore results of these studies can be extrapolated to many vertebrate models.

Development of Purification of Valproic Acid and Butyric Acid for Positron Emission Tomography Studies. Khaing Win¹ and Sunny Kim², ¹St. Joseph's College, Brooklyn, NY and ²Brookhaven National Laboratory, Upton, NY.

Valproic acid (VPA) and butyric acid (BA) are two epigenetic drugs used for seizures and neurocognitive disorders. While the two acids have been known to bind histone deacetylases that suppresses gene expression, their pharmacokinetics, biodistribution, and the blood brain barrier penetrability remain an enigma. Positron Emission Tomography (PET) using [¹¹C]VPA and [¹¹C]BA could potentially solve these issues. Before [¹¹C]radiosynthesis, purification methods for unlabeled VPA and BA, generated via Grignard precursors, as impure mixtures were developed. High Performance Liquid Chromatography (HPLC) with C18- Gemini column under the isocratic system (acetonitrile (MeCN) and formic acid (FA)) is used. The following optimum purification conditions were found: a 50% MeCN/50% FA for VPA and a 15% MeCN/85% FA for BA. Respective HPLC (flow rate=1ml/min) retention times for BA and VPA were 8.55 minutes and 11.76 minutes. Our preliminary radiosynthesis and purification of [¹¹C]BA was completed within 40 min after the End of Bombardment. [¹¹C]BA was obtained in moderate radiochemical yield (>40%) and high purity (>99%). Radiosynthesis of [¹¹C]VPA is still to be attempted. We have successfully developed conditions for the synthesis and purification of both unlabelled VPA and BA for preparation of the radiolabeled acids to be used for PET studies.