

Area of Research: **Liver Diseases**
Mentor: **Sawkat Anwer**

Project Description

Regulation of bile acid uptake by phosphorylation of bile acid transporter:

Functional abnormalities associated with various liver diseases lead to the accumulation of toxic products (such as bilirubin in jaundice and bile acids) in blood and premature death of liver cells. One of the major determinants is the inability of the liver to properly regulate bile acid transport from blood to bile. Studies are conducted to better understand the mechanisms regulating bile acid transport. The overall goal is to determine regulatory pathways that are derailed and then find ways to reestablish normal regulation. Studies so far showed that cyclic AMP stimulates bile acid uptake by translocating bile acid transporter to the plasma membrane and this involves dephosphorylation of the transporter. However, it is not known which amino acids of the transporter are phosphorylated. The project will involve determination of phosphorylation site. Potential phosphorylation sites will be mutated and the mutated proteins will be expressed in cells followed by determination of bile acid uptake and transporter phosphorylation. These studies should allow determination of phosphorylation sites and the role of phosphorylation in bile acid uptake and translocation of the transporter. Techniques to be used are cell culture, cell transfections, solute uptake using radiolabeled bile acid, western blots, protein phosphorylation, etc.

Area of research: **Liver disease**
Name of mentor: **Cynthia RL Webster**

Project Description:

Our lab is interested in the study of pathologic mechanisms involved in hepatocyte cell death in cholestatic liver disease. Cholestasis, the slowing of bile flow, accompanies most major hepatic diseases. During cholestasis the liver accumulates many possible endogenous toxins that are normally excreted in bile. Among these toxins are bile acids. We and others have shown that bile acids cause hepatocyte apoptosis. Our efforts are now focused on determining the cellular signaling mechanisms involved in bile acid apoptosis. We are particularly interested in determining how increasing intracellular cAMP levels protects against bile acid induced apoptosis. We have evidence to suggest that cAMP survival effects are controlled by activation of lipid and protein kinases. Elucidation of hepatocyte survival mechanisms may have potential application to the development of therapeutic strategies to slow the pathologic progression in cholestatic liver disorders.

Our work involves the isolation of primary hepatocytes and investigation of kinase driven phosphorylation events involved in cAMP mediated cytoprotection. We also have a human hepatocellular carcinoma cell line that we work with in

order to do genetic studies on cAMP signaling. The techniques a student might be involved in a project in our laboratory would include but not necessarily limited to Tissue culture; Transient transfections of mammalian cells; SDS-PAGE electrophoresis; Western blotting; Immunoprecipitation; Protein kinase assays.

As a boarded small animal internist I am also interested in the pathophysiology of chronic cholestatic disorders in dogs and cats. Currently veterinary hepatology is still in its descriptive era. It is well accepted that Cocker spaniels and Labrador retrievers have a breed specific hepatopathy, but both syndromes have not been formally described in the literature. A retrospective study using our large hospital case load should be possible. This project would involve familiarizing the investigator with the current literature on chronic hepatitis in the dog and subsequently retrieving, organizing and analyzing data from hospital medical records.