# CPB 69700 RESEARCH SEMINAR

## DEPARTMENT OF COMPARATIVE PATHOBIOLOGY

M. Florenta Sega, M.S.
Departments of Comparative Pathobiology and Chemistry
Purdue University

Thurs., December 10, 2009
VPTH 112
3:30 pm

"Hemoglobin-Band 3 Interactions: Role In Regulation Of Erythrocyte Functions"

Abstract:

The erythrocyte has been used extensively as a model for cellular research due to its perceived simplicity, consisting of a membrane, a membrane associated spectrin skeleton and a cytoplasm dominated by hemoglobin. This cell is primarily known for its essential role in gas exchange by hemoglobin (Hb)-mediated transport of oxygen and carbon dioxide between the lungs and tissues.

Over the years the concept that erythrocytes are "simple" cells has been dispelled by evidence of impressive complexity in structure and function. For example, oxygenation status has been implicated in such diverse functions as volume control, glycolytic enzyme activity and membrane deformability. In our lab, we have shown that under deoxygenation conditions certain glycolytic enzymes and ankyrin (a membrane-spectrin skeleton linking protein) are dislocated from the red blood cell membrane. Dislocation of the glycolytic enzymes leads to increased activity. Under oxygenation conditions the enzymes are not displaced which renders them inactive. This interplay is important because it would explain how that under high oxygenation conditions the cell uses glucose as an alternative pathway to glycolysis to make more reductive power (NADPH) that fights oxidative stress. Binding sites both for the specific glycolytic enzymes and for ankyrin have been mapped to the cytoplasmic domain of transmembrane protein band 3. Deoxyhemoglobin also binds to band 3 and the binding site overlaps that of the glycolytic enzymes, suggesting competitive binding. However, competitive displacement of the enzymes from band 3 by deoxyhemoglobin has not been experimentally verified.

Regarding ankyrin, in addition to deoxygenation conditions displacing it from the membrane, antibodies against the cytoplasmic domain of band 3 that compete with glycolytic enzyme binding sites also block ankyrin binding to band 3 (probably through steric hindrance, not competitive binding). Ankyrin is a main bridge between the skeleton and the membrane. Taken together, we hypothesize that physiologic displacement of ankyrin from band 3, presumably by deoxyhemoglobin, contributes to greater flexibility in the membrane - spectrin skeleton assembly and thereby enhances the ability of erythrocytes to squeeze through very narrow openings of capillaries and splenic sinusoids that are associated with low oxygen tension.

To further study these interactions we are planning to make mutant mice with either no affinity or very high affinity of hemoglobin for the cytoplasmic domain of band 3. This will allow us to answer if indeed hemoglobin-band 3 interactions play a role in mediating erythrocyte responses to different oxygen tensions.