Introduction
Erythrocytes play a crucial role in carrying oxygen to various tissues and organs of the body and their concentration in blood circulation is finely regulated by multiple mechanisms that are not as yet clearly defined. Life span of circulating human and murine erythrocytes has been estimated to be 120 and 50 days respectively. About 1% and 2% of erythrocytes are removed daily in human and mice respectively and are replaced with an equal number of fresh erythrocytes. Bulk of the erythrocyte clearance activity takes place in the reticulo-endothelial system (RES) in spleen, bone marrow and liver. Markers that earmark erythrocyte for destruction are not clearly understood.

It is crucial to understand the factors that regulate erythrocyte turnover in blood. This turnover has two components, one being the erythropoietic mechanisms that are responsible for regular infusion of fresh and young erythrocytes in the blood circulation, and the other being mechanisms of erythrocyte clearance that are responsible for removal of aged and or stress damaged erythrocytes from circulation. Even slight imbalances in these two basic components of erythrocyte turnover may cause immediate increase (e.g. in polycythemia) or decrease (e.g. in a variety of anemias) in blood count of erythrocytes. Polycythemia generally occurs in high altitude where oxygen concentration in the air is relatively low and higher hemoglobin carrying erythrocytes are needed to augment the efficiency of carrying oxygen. Anemias are more serious conditions that compromise the ability of blood to supply oxygen to tissues and organs that may prove to be lethal.

In the present thesis, we have focused on studying the mechanisms that are responsible for clearing the aged and damaged erythrocytes from the blood. At the very beginning of this work, we realized that there existed no good technique to accurately and objectively estimate the survival kinetics of blood erythrocytes. The most promising and at that time recently introduced technique was that of in vivo biotinylation of blood erythrocytes that labeled 100% of erythrocytes with covalently linked biotin. Fresh erythrocytes that enter the blood stream after in vivo biotinylation step was complete, were biotin negative and this provided a good parameter for distinguishing the blood erythrocytes that existed before the biotinylation step from those that were introduced in blood after the biotinylation step. Thus the biotin-negative population of erythrocytes in blood isolated a few days after biotinylation
represented very young erythrocytes and their properties vis-à-vis the rest of the erythrocytes could be studied. Similarly, biotin positive erythrocytes isolated from animals after several weeks of biotinylation step would represent very old surviving erythrocytes that could be examined for markers of aging. While this technique provided a good way of identifying and studying very young or very old erythrocyte population, it was not possible to use this technique to study the two extreme populations simultaneously in an animal, or to identify erythrocyte populations of intermediate age groups in blood.

We devised a modification of the single step biotinylation technique by adding a second step of *in vivo* biotinylation with a lower dose of the biotinylation agent, shortly after the first biotinylation step. This modification divided the blood erythrocytes into three discrete populations (Biotin$^{\text{high}}$, Biotin$^{\text{low}}$, and Biotin$^{\text{ve}}$ populations), rather than two populations (Biotin$^{\text{ve}}$ and Biotin$^{\text{ve}}$) that resulted from one step biotinylation technique. The new double *in vivo* biotinylation (DIB) technique thus allowed us to identify flow cytometrically, a cohort of erythrocytes (Biotin$^{\text{low}}$ population) generated in a defined time window that could be tracked in circulation. DIB technique thus enabled us for the first time to study age related changes in a variety of parameters of blood erythrocytes. Using the DIB technique we have been able to accurately define the survival kinetics of blood erythrocytes as well as the kinetics of changes in several important markers like CD147, CD47 and phosphatidylserine (PS) expression on erythrocytes. Based upon all this data, we conclude that erythrocytes in blood circulation undergo both random as well as age dependent clearance. Random killing may eliminate stress damaged erythrocytes through mechanisms that induce PS externalization in erythrocytes. On the other hand, the clearance of aged erythrocyte populations may be regulated by factors like reduction in expression of membrane markers like CD47 and CD147 that facilitate the entrapment of old erythrocytes in spleen and their phagocytosis by macrophages.