was additionally supported by therapeutic transfusion of J.P. cells to a severely anemic homozygous PK deficient female (C.D.) with much higher RBC PK (0.9 units) but with marked splenomegaly. Isologous survival of J.P. cells in C.D. was only modestly reduced (T 1/2 ^{51}Cr RBC = 18 days) whereas the autologous survival of C.D. cells was markedly shortened (T 1/2 = 7 days).

Thus low levels of PK need not limit the survival of mature RBC. Retics are much more vulnerable to the block in glycolysis and become irreversibly sequestered in the hypoxic splenic circulation.

120 *The Sorbitol Pathway of the Human Erythrocyte.* SUSAN F. TRAVIS, ANTHONY D. MORRISON, REX S. CLEMENTS, Jr., ALFRED I. WINEGRAD and FRANK A. OSKI, Univ. of Pennsylvania Sch. of Med., Depts. of Ped. and Med., Philadelphia, PA.

The polyol pathway appears to be widely distributed in human tissues. In this pathway glucose is reduced to its polyol derivative, sorbitol, by aldose reductase and the sorbitol then converted to fructose. NADPH serves as a co-factor for glucose reduction while NAD is a co-factor in the second step. The K_m for glucose of aldose reductase is sufficiently high so that the intracellular glucose concentration regulates the rate of sorbitol and fructose synthesis. Our studies now demonstrate the presence of a sorbitol pathway in the red cell and its dependence on media glucose concentration. Red cells were suspended in media of varying glucose concentrations (2–50 mM) and red cell and supernatant sorbitol and fructose measured after incubation. Red cell sorbitol and fructose rose as glucose concentration increased and fructose appeared in the media. Red cell sorbitol rose from 16.0 to 99.6 μmoles/ml as media glucose rose from 2 to 50 mM. Associated with this increase was a rise in the cell lactate to pyruvate ratio (69:1 to 193:1), an increase in the percent of glucose metabolized to CO_2, and an accumulation of triose phosphates and a fall of the DPG within the cell. These alterations appear to reflect changes in the pyrudine nucleotide ratios within the cell secondary to the increased metabolism of glucose to fructose. At high glucose concentration a significant fraction of all glucose metabolized traverses this pathway and is the first evidence that high plasma glucose concentration serves to regulate red cell metabolism. *In vivo* confirmation of the regulatory role of plasma glucose was provided by the demonstration of increased red cell sorbitol in patients with diabetes and hyperglycemia.

121 *Alterations in Membrane Fatty Acid (FA) Turnover in Vitamin E Deficient Erythrocytes (E-RBC) During Exposure to Hydrogen Peroxide.* BERTRAM H. LUBIN and STEPHEN B. SHOHET, Children's Hosp. Med. Center, Boston, Mass. (introduced by Fred S. Rosen).

Human E-RBC are susceptible to membrane damage by 1% H_2O_2. They undergo initial cation loss and shrinkage followed by eventual swelling and osmotic lysis. In order to study alterations of phospholipid metabolism in response to this membrane insult and to evaluate the role of such alterations in the hemolytic process phospholipid FA turnover and redisposition was investigated during H_2O_2 exposure of E-RBC prelabeled with radioactive FA. E-RBC were preincubated for 2 h in buffer with glucose and with saturated and/or unsaturated radioactive fatty acids. The cells were washed and reincubated in Krebs-Henseleit buffer, pH 7.4, with 1% H_2O_2. Lipids were extracted at various intervals prior to eventual lysis and their radioactivities and total quantities determined after TLC isolation. During the prelytic period neutral lipids and phosphatidyl choline (PC) lost radioactivity although the quantity of PC remained stable. Conversely, phosphatidyl ethanolamine (PE) though usually slightly reduced in quantity always gained radioactivity. The increased PE radioactivity was most marked when saturated FA was used for the preincubation. These results indicate that in response to H_2O_2, E-RBC utilize FA from PC and neutral lipid to replace PE-FA which is especially susceptible to peroxidation. This compensatory mechanism can insert saturated FA (H_2O_2 resistant) into PE. Though adequate to prevent bulk PE loss, this mechanism does not prevent lysis. We conclude that major quantitative changes in RBC PE are not necessary prerequisites for H_2O_2 induced lysis. Subtle qualitative alterations in FA composition or of lipid:protein interrelationships appear to be more significant.

122 *The Shape of the Red Blood Cell: A Result of Intracellular Electrostatic Forces?* JOSEPH A. KOCHEN, Dept. of Ped., Albert Einstein Coll. of Med. and Montefiore Hosp. and Med. Center, New York (introduced by Laurence Finberg).

It has recently been proposed that the biconcave shape of the human red cell is maintained by inwardly directed electrostatic forces of attraction between the two opposing membrane surfaces of the cell. Treatment of red cells with hypertonic acidified media would be expected to augment these forces by decreasing the distance between the opposing surfaces and increasing the number of centers of opposite charge. When red cells treated in this manner are returned to an isotonic medium, a membranous structure bridging the biconcavity is demonstrable by phase-contrast microscopy. This structure remains intact during osmotic swelling and hemolysis and is clearly visible in the resulting red cell ghosts. Spherocytes (hereditary spherocytosis) demonstrate no such structure under these conditions, whereas leptocytes (β-thalassemia) show an enhanced tendency to internal bridging in isotonic acidified media. These findings suggest that an increase in the long-range electrostatic forces within the cell promotes the formation of a distinct morphologic structure which, under these conditions, contributes to the preservation of the biconcave shape of the cell.

123 *Changes in the Nature of Human Fetal Hemoglobin During the First Year of Life.* TITUS H. J. HUISMAN, WALTER A. SCHROEDER and AUDREY K. BROWN, Lab. of Protein Chem., and Dept. of Ped., Med. Coll. of Georgia, Augusta, Ga., Div. Chem. and Chemical Engineering, Calif. Inst. Tech., Pasadena, CA.

Two fetal hemoglobins have been identified in the blood of the newborn; they differ in the nature of the amino acid residue at position 136 of the γ chain. The change in the ratio of these two fetal hb chains (termed Gγ and Aγ for the chains with a glycine or an alanine residue in this position, respectively) has been studied in the blood of seven infants at varying intervals after birth. The babies studied included two normals, two Hb-S heterozygotes, two Hb-C heterozygotes, and one infant with Hb-S-Hb-C disease. The ratio of Gγ to Aγ chains, about 3 to 1 at birth, changes