

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 ⁵¹Cr 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 μ moles/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 pyridine 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. SHOHEET, 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 redistribution 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 $G\gamma$ and $A\gamma$ 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 $G\gamma$ to $A\gamma$ chains, about 3 to 1 at birth, changes

steadily during the first 3 to 4 months, and by 5 months of age approximates 2 to 3, which is similar to the ratio of the Hb-F from normal adults. These observations suggest that the switch from γ chain to β (and δ) chain production after birth apparently involves an unequal repression of the multiple γ structural genes at the time the β and δ structural genes are activated.

124 *Hemoglobin-Oxygen Affinity and Erythrocyte 2,3-Diphosphoglycerate (DPG) Content in Hyaline Membrane Disease (HMD) and Cardiac Malformations (CM)*. GABRIEL V. DUC and KNUD ENGEL, Dept. of Ped., Coll. of Physicians and Surgeons, Columbia Univ., New York (introduced by John C. Sinclair).

The effect of DPG on the affinity of hemoglobin for oxygen, expressed as P^{50} (the PO_2 at which Hb is 50% saturated with O_2), is well known. In the present investigation whole blood from newborns with hyaline membrane disease (HMD) and cardiac malformations (CM) revealed a statistically significant difference between the two groups in both P^{50} and DPG content (HMD: P^{50} 24.6 mm Hg \pm 3.5 S.D., DPG 0.78 mol/mol Hb \pm 0.16; CM: P^{50} 29.1 mm Hg \pm 3.4, DPG 1.31 mol/mol Hb \pm 0.30). Patients with CM were more mature with higher birth weights and had lower arterial PO_2 and O_2 content (10.2 vol% O_2 \pm 4.0 vs. 16.7 \pm 4.9). BE, pH, $P\bar{C}O_2$, Hb and HbF were comparable. The effect of the observed difference in P^{50} between babies with HMD and CM can possibly explain the greater tolerance to hypoxemia of infants with CM since blood with P^{50} of 29.1 mm Hg can unload 15% more O_2 to the tissues than blood with P^{50} of 24.1 assuming the same $P\bar{V}O_2$, CaO_2 and cardiac output. Such differences in P^{50} have almost no effect on the amount of O_2 loaded in the lungs. Further observations showed that DPG concentration varied directly with pH in normoxic patients (>90% saturation, >15 vol% O_2). Low pH could cause a decrease in DPG by inhibition of enzymes responsible for the synthesis, but also low DPG could produce a decrease in pH by impairment of unloading of O_2 to the tissues. The hypoxemic patients showed a higher DPG concentration than predicted from their Hp, again suggesting that hypoxemia causes an increase in DPG concentration.

125 *Molecular Interactions of Normal and Abnormal Hemoglobin in Cell-free Systems*. JAMES G. WHITE and BEATRICE HEAGAN, Univ. of Minnesota Sch. of Med., Minneapolis.

Increased viscosity, fragility and distortion of erythrocytes from patients with inherited defects of hemoglobin (Hb) have been related to sol-gel and sol-crystal transformation. A method has been developed in this laboratory which permits definition of the nature of molecular assembly underlying abnormal hemoglobin interactions. Cell free solutions of HbAA, HbAS, HbSS, HbSC, HbCC, and HbF combined with phosphate buffer, or phosphate buffer containing sodium metabisulfite, formed solid gels, crystals or amorphous precipitates. Gels of reduced HbSS, HbAS and HbSC consisted of masses of 170 Å rods identical to those observed in intact sickled erythrocytes. Gels of oxy HbSS, HbAS, HbAA and reduced HbAA contained 240 Å hollow tubular polymers similar to microtubules. Fresh samples of oxy HbSC and HbCC rapidly formed crystals, but a gel stage could be demonstrated by stirring solutions during transformation. Solutions of oxy and reduced HbF developed amorphous precipi-

tates, but did not gel or form crystals. Inability of HbF to form polymers may relate to its inhibitory effect on the assembly of HbS into rods. The capacity of oxy or reduced HbAA to transform into microtubules, on the other hand, reflects its ability to combine with HbS in rod-like polymers which distort cells containing HbAS. Molecular interactions of HbC favor crystallization, but as in all other examples, the gel state appears to precede crystallization. These findings indicate that hemoglobin molecules in intact normal and abnormal erythrocytes are in a high degree of molecular association or pre-gel state. Slight alterations in hemoglobin molecular structure can produce rapid and profound changes in the organization of hemoglobin into gels or crystals.

126 *Chronic Myelogenous Leukemia: Cyclic Leukocytosis and Identical Twin Discordance*. RICHARD A. GATTI, AMOS S. DEINARD, MARK E. NESBIT, WILLIAM A. ROBINSON and ROBERT A. GOOD, Univ. of Minnesota, Dept. of Ped., Minneapolis; Univ. of Colorado, Dept. of Med., Denver.

A cyclic leukocytosis with cycles of approximately 72 days and counts ranging from 4,000 to 145,000 cells/mm³ has been followed for 15 months with no treatment, in a 13-year-old white female with chronic myelogenous leukemia (Ph¹ positive). An identical twin has no clinical, morphologic or cytogenetic evidence of leukemia. Differential counts of per. bl. and marrow remain unchanged during the patient's cycles although marrow cellularity is increased during peaks. Studies which were normal include: Rebutck skin windows, phagocytic and bactericidal functions of neutrophils, glutathione peroxidase levels, fetal hemoglobin and immunologic responses. Epinephrine stimulation produced a total release of marginal pool cells at the high point with only partial release of cells at a low point. Endotoxin and steroid stimulation studies indicate that mechanisms for delivery of cells from marrow are intact and of similar magnitude at both high and low points. Ph¹ positive cells were seen in 81% of mitoses from per. bl. cells taken at a peak while in per. bl. specimens taken at a low point such Ph¹ positive cells were absent. At both high and low points over 90% of mitoses of bone marrow cells were Ph¹ positive. Mixed leukocyte cultures showed a non-reciprocal response of the patient's leukocytes to cells of her identical twin, suggesting partial loss of transplantation antigens in some of the patient's cells. Leukokinetics with DFP³²-labeled cells, alkaline phosphatase and granulopoietin levels and marrow proliferation studies with H₃T were measured at high and low points. These studies would be compatible with the view that controls of leukocyte production and release as well as in immunogenetic characteristics of cells are involved in this leukemic process.

127 *An Unusual Neutrophil Cycle in Down's Syndrome and Acute Leukemia*. RENATO MASTRANGELO, DOUGLAS E. COX, WOLF W. ZUELZER and JAMIL KHEDER, Child Res. Center of Mich. and Dept. of Ped., Wayne State Univ. Sch. of Med., Detroit, and Plymouth State Home and Tr. Sch., Northville, MI.

A short-term cyclic fluctuation (14 to 24 days) of the neutrophil count in normal adults was shown by MORLEY [Lancet ii: 1220, 1966]. In contrast, a longer duration cyclic fluctuation (30 to 120 days) was seen by MORLEY, BAIKIE and GALTON in 4 patients with