chronic thrombocytopenia [Blood 16: 943, 1960] provided a unique opportunity to study the functional capacity of young and old platelets. Study cycles were initiated by infusing FFP when the patient's platelet count was less than 20,000/mm³. 'Young' platelet studies were done 4 days after FFP (average platelet count, 200,000/mm³); 'old' platelet studies were done 21 days after FFP (average platelet count, 200,000/mm³). All studies were repeated during several such cycles. In association with a young platelet population, were normal Ivy bleeding times, normal or increased platelet adhesiveness, normal aggregation to ADP and collagen, and normal platelet factor 3 availability (PF-3a). By contrast, when the patient's circulating platelets were old, she was found to have long bleeding times, abnormally low adhesiveness *in vivo* and *in vitro*, and decreased PF-3a. Aggregation to ADP and collagen was slightly decreased but remained within normal limits. No abnormalities were seen by electron microscopy on platelet samples obtained throughout the cycle. (Performed by Dr. JAMES WHITE, Univ. of Minn.) It is of interest that on several occasions the patient experienced mild bleeding manifestations at the end of a cycle but while her platelet count was still normal. These episodes correlated with the findings of long bleeding time and decreased adhesiveness.

- 116 *Bilirubin-induced Platelet Staining, Aggregation, and Adenine Nucleotide Release.* HAROLD M. MAURER and JOYCE CAUL, Med. Coll. of Virginia, Richmond, Va. (introduced by W.E. Laupus).

Our previous studies have shown that free indirect-reacting bilirubin (B) even at low concentration (0.5 mg%) causes yellow staining and aggregation of washed platelets, whereas B bound to albumin and photoxidized B have no demonstrable platelet effect. The effect of B on platelet adenine nucleotide content was studied. Washed human platelets were incubated with either B (4 mg%) or buffered saline in the presence of CaCl₂ and KCl₂, and then platelets and supernatant were separately assayed for ADP and ATP. Approximately 40–80% of ADP and ATP was released from the platelets with bilirubin. Released ADP and ATP were present in the supernatants. B bound to albumin had no effect on platelet ADP and ATP content. The results indicate that platelet staining and aggregation by free B are associated with adenine nucleotide release. Released ADP is probably the underlying mechanism in B-induced aggregation.

- 117 *The Enzymatic Defect in Congenital Erythropoietic Porphyrria: Demonstration in Heterozygotes and in Non-erythropoietic Tissue of Homozygotes.* GIOVANNI ROMEO, MICHAEL M. KABACK, BERTIS L. GLENN and EPHRAIM Y. LEVIN, The Johns Hopkins Univ. Sch. of Med., Baltimore, Md., and the Oklahoma State Univ. Coll. of Vet. Med., Stillwater, Okla.

Activity of the enzyme uroporphyrinogen III cosynthetase in hemolysates from human or bovine subjects with congenital erythropoietic porphyria is much lower than the activity in control hemolysates. The partial deficiency of cosynthetase is probably a primary genetic defect in this disease and explains the pathological overproduction of uroporphyrin I. Cosynthetase activities in hemolysates from asymptomatic bovine carriers of porphyria are intermediate to those of normal and porphyric animals. This observation is consistent with the known autosomal recessive mode

of inheritance of the disorder. Similarly, in two human families parents or children of the porphyric propositus had the cosynthetase activities expected for a heterozygote state; but in a third family, these activities were in the normal range. Hence, there may be some genetic heterogeneity in the human disease. Also, the specific activity of cosynthetase is lower in extracts of fibroblasts grown in tissue culture from skin biopsies taken from patients with porphyria than in similar extracts from non-porphyric cells. This indicates that although the metabolic error in porphyrin formation is expressed only in erythropoietic tissue, the hereditary defect in the enzyme activity occurs in other tissues as well.

- 118 *Erythrocyte Sodium Flux in Hereditary Spherocytosis: Its Significance.* ALVIN ZIPURSKY and LYONEL G. ISRAELS, Dept. of Ped., McMaster Univ., and Dept. of Med., Univ. of Manitoba, Canada.

We have studied five families in whom one or more children have hereditary spherocytosis (h.s.). In the affected patients, erythrocyte ouabain-insensitive sodium efflux was significantly greater than normal in intact cells and isolated membranes. This is consistent with an increased permeability to sodium of these cells.

In each of the five families, neither parent had clinical or hematological evidence of h.s. Four of ten parents had erythrocyte sodium efflux patterns identical to that found in classical h.s. compared to three of thirty normal controls with similarly elevated values ($X^2 = 4.6$; $p < 0.05$).

These findings suggest:

1. The increased sodium permeability of h.s. erythrocytes is unrelated to their shortened life span, since similar sodium flux can be found in erythrocytes with normal life span.

2. The gene for h.s. can manifest as an increase in erythrocyte sodium permeability with no abnormality in morphology or life span of the red blood cell. (Supported by a grant from the Medical Research Council of Canada.)

- 119 *Selective Destruction of Reticulocytes (Retics) in Pyruvate Kinase (PK) Deficiency.* WILLIAM C. MENTZER, LAWRENCE N. BUTTON, STEPHEN H. ROBINSON and DAVID G. NATHAN, Children's Hosp. Med. Center, Boston, Mass.

PK retics lack adequate glycolysis and are peculiarly susceptible to irreversible damage in the spleen because they are dependent upon mitochondrial function for which splenic venous PO₂ is insufficient [Blood 34: 861, 1969]. The fraction and mass of PK deficient retics immediately trapped in the spleen should therefore influence clinical severity. A 22-year-old male (J.P.) with icterus and splenomegaly and with RBC PK 0.25 units (normal > 2.0 units) Hgb. 13.3 g % and retics 7 % illustrates this mechanism. Mean RBC life span was approximately 100 days (T 1/2 ⁵¹Cr = 33 days), but bilirubin turnover (³H bilirubin) was 5 × normal yielding mean RBC life span of only 20 days. Ferrokinetics with ⁵⁹Fe showed plasma iron turnover 5 × normal; rapid marrow ⁵⁹Fe uptake, but low cumulative RBC ⁵⁹Fe and immediate splenic sequestration of newly labeled cells. These data provide evidence that the majority of J.P.'s newly formed retics were immediately sequestered in the spleen. The remainder survived normally and these were sufficient in number to provide 13.3 g % hemoglobin. That the low PK level in circulating mature J.P. cells did not limit their survival

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 ($T\ 1/2\ ^{51}\text{Cr RBC} = 18$ 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 $\mu\text{moles/ml}$ 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