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 $CaCl_2$ and KCl_2, 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 Porphyria: 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 prophyria 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.

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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 PO_2 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 ^{51}Cr = 33 days), but bilirubin turnover (^{3}H bilirubin) was 5 × normal yielding mean RBC life span of only 20 days. Ferrokinetics with ^{59}Fe showed plasma iron turnover 5 × normal; rapid marrow ^{59}Fe uptake, but low cumulative RBC ^{59}Fe 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