THE MICROMEASUREMENT OF FREE ERYTHROCYTE PORPHYRIN (FEP) AS A MEANS OF DIFFERENTIATING ALPHA-THALASSEMIA TRAIT FROM IRON DEFICIENCY ANEMIA. H. M. Koenig, and A. L. Lightsey, Jr. (Intr. by Howard A. Pearson), Department of Pediatrics and the Clinical Investigation Center, Naval Hospital, San Diego, Ca.

Microcytosis occurs in children with iron deficiency anemia, lead poisoning and thalassemia. To differentiate these disorders, it has been necessary to quantitatively measure serum iron, transferrin saturation, serum lead levels, hemoglobin A_2 and F levels. Piomelli has screened for lead poisoning and Oski has differentiated beta-thalassemia from iron deficiency anemia by measuring free erythrocyte porphyrins (FEP). We have used the micromeasurement of FEP to differentiate alpha-thalassemia from iron deficiency. The method used is a modification of that described by Piomelli et al. (Pediatrics 51: 254, 1973). Eighteen individuals with previously diagnosed alpha-thalassemia have had FEP and serum iron and transferrin saturation levels determined. Twelve individuals with alpha-thalassemia and normal transferrin saturation studies had FEP below 90 ugms/100 ml of Rbc's. Six individuals with documented iron deficiency and alpha-thalassemia had FEP greater than 130 ugms/100 ml as did individuals with uncomplicated iron deficiency. Micromeasurement of Rbc FEP provides a rapid means of differentiating iron deficiency from thalassemia with normal transferrin saturations. However, when alpha-thalassemia and iron deficiency occur together, alpha-thalassemia cannot be proven until the iron deficiency is corrected.

RED CELL SIZE AND FETAL HEMOGLOBIN CONCENTRATION IN THE NEWBORN INFANT. Masaru Komazawa, Alfredo M. Garcia and Frank A. Oski. State University of New York, Upstate Medical Center, Syracuse, New York.

At birth, the newborn infant has a heterogenous population of erythrocytes; variability exists with respect to size, shape, enzymatic capacity and hemoglobin composition. Employing both differential staining for fetal hemoglobin and cytophotometric techniques, the erythrocytes of term infants were examined in an attempt to relate cell size to hemoglobin composition. One-hundred and twenty cells from each infant were individually sized and subjected to microdensitometry measurements. Sixty of the cells had been eluted and counter-stained for fetal hemoglobin. The staining for fetal hemoglobin did not alter the size of the cells. Analysis revealed that as the red cells increased in size the concentration of fetal hemoglobin within the cells decreased significantly more than did the total hemoglobin concentration. Cells with a diameter of approximately 6 μ had almost exclusively fetal hemoglobin. These findings are consistent with the hypothesis that the largest, and presumably the youngest, erythrocytes are synthesizing less fetal hemoglobin at the time of birth than cells that had been produced some weeks before. The use of cytophotometry provides a technique for following cohorts of cells with varying ages and hemoglobin composition.

SYNOVECTOMY IN HEMOPHILIAC HEMARTHROSIS, Stella B. Kontras and Edward J. Eyring, Ohio State Univ. Coll. of Med. Children's Hosp., Dept. of Ped. and Dept. of Orthoped., Columbus.

Joint bleeding in hemophiliacs initiates a cycle of synovial lesions and repeated hemarthrosis. Disabling hemophiliac arthropathy may be the end result despite adequate plasma component therapy. Synovectomy which has been made feasible by improvements in component therapy may be used to interrupt this vicious cycle in selected cases. Indications for synovectomy include frequent and chronic recurrent hemarthroses not responding to medical management and a cooperative family. Fifteen synovectomies have been performed in 9 patients with hemophilia A. The average age was 12 years. The procedure was performed on 4 knee joints, 4 ankle joints and 7 elbow joints. There were no instances of early or late hemorrhage. The hospital time averaged 11 days. Re-bleeding into the joint has been absent or markedly decreased in an average 3 year period. There was some improvement in joint range of motion in 50% of patients and radiologic improvement was also noted. In selected hemophilia patients, synovectomy may be indicated as a hemostatic procedure.

FLUORESCENT DYE METHOD FOR DETERMINATION OF BILIRUBIN-BINDING CAPACITY OF SERUM ALBUMIN. Kwang-sun Lee and Lawrence M. Gartner, Dept. of Pediatrics, Albert Einstein College of Medicine, Bronx, N.Y.

A simple, rapid microfluorometric method for quantitative measurement of serum albumin binding capacity for unconjugated bilirubin (UB), utilizing a fluorescent dye, Direct Yellow 7, has been developed. Sephadex G-200 column chromatographic study revealed exclusive association of serum albumin fraction with the dye. Spectrofluorometric study demonstrated enhanced fluorescence (ΔF) of the dye when bound to serum albumin. The addition of increasing concentrations of UB (5 to 70 mg%) to adult human sera resulted in a progressive inhibition of ΔF with complete inhibition at 55 mg%. Multiple regression analysis yielded two significantly different slopes ($p<.001$), first, from 0 to 27 mg% and second slope, 27 to 55 mg%. UB concentration of 27 mg% corresponded to UB: albumin molar ratio of 0.97, while 55 mg% to 1.97. Enhanced fluorescence of the dye resulted entirely from binding of the dye to two bilirubin-binding sites on albumin, since ΔF was completely inhibited by addition of two moles of UB per mole of albumin. Lowered pH reduced albumin binding capacity and exposure to light increased binding capacity of icteric sera. The binding capacity of purified human serum albumin was 7.1±0.8 ΔF/gm albumin (SD) (n=4), approximately one-half that of the adult human sera, 13.4±0.5 ΔF/gm (SD) (n=4), while human cord sera gave 9.3±1.1 ΔF/gm (SD) (n=41), 30% less than that of the adult sera on albumin molar basis.

THE IMPORTANCE OF DURATION OF THERAPY IN ACUTE LYMPHATIC LEUKEMIA (ALL) by Brigid G. Leventhal, Edward S. Henderson, Robert G. Graw, Jr., Arthur S. Levine, Ronald A. Yankee and Richard Simon, Pediatric Oncology Branch, NIH, Bethesda, Md.

In 1968-1969, previously untreated patients with ALL were given high dose combination chemotherapy (POMP). This included on days 1-5, i.v., prednisolone 1000 mg/m^2; methotrexate (MTX) 7.5 mg/m^2; 6-mercaptopurine (6MP) 125 mg/m^2; and, on day 1, vincristine 2 mg/m^2. Once remission was achieved POMP was given every 2 weeks. The doses of 6-MP and MTX were increased to maximally tolerated levels in all pts. After 6 months, bischloroethylnitrosourea (BCNU) was given for 3 days at 50 mg/m^2 and pts. were randomized to receive POMP monthly for 6 months or no further therapy. Complete remission was achieved in 61/66 pts. (91%). Median remission duration for all 61 pts. was 13 months; 48 pts. completed the initial 6 months of therapy without relapse; 24 were randomized to receive further POMP therapy and 24 to no further therapy. The further therapy group had a median remission of 20 months with 7 pts. still in initial remission. The no further therapy group had a median remission of 11 months with 2 still in initial remission. These differences are significant at the $p < .05$ level by the generalized Wilcoxon test. A comparable group of 35 pts. had an intermediate remission duration of 17 months. These data indicate that even with intensive therapy in the first 6 months, cure of leukemia will not be possible in the majority of pts. Prolonged therapy is required for improved remission duration.

INTRACELLULAR PRECIPITATION OF HEMOGLOBIN BY HEAT: IN VIVO AND IN VITRO STUDIES. Janet D. Liljestrand, Proinnsias O'Croinin, Wm. H. Zinkham. The Johns Hopkins Hosp. Dept. of Ped. Balt.Md.

A patient with hemoglobin Zürich (Hb Z) had a viral-related fever of 39-41°C for 5 days. The hematocrit decreased from 45-25, plasma hemoglobin was 144 mg%, Heinz bodies (H.B.) appeared in 12-18% of the erythrocytes (RBC), and there was moderate reticulocytosis. There was no history of exposure to oxidants. These observations led to a study of the effects of temperatures in the clinical range of fever on the solubilities of Hb Z and normal hemoglobin (Hb A) in intact RBC. Whole blood from 25 normal adults and 4 Hb Z pts was incubated at 37 and 41°C. After incubation, H.B. were defined by mixing equal parts of blood and rhodanile blue for 2 minutes and making dry films. The % of H.B. in Hb A RBC at 37°C for 3,6,12, and 24 hrs was 0,0,0, and 49.5±19.1%; in Hb Z - 0,5.5,63.6, and 99.2%. The %'s at 41°C were 0,8.6,59.4±25.6, and 100 in Hb A RBC; and 24.0, 74.9,100, and 100 for Hb Z RBC. Hb Z in the red cells did not decrease until 3-6 hrs after the appearance of H.B. The % of Hb A_2 remained constant. Progressive precipitation of Hb in Hb Z and Hb A RBC was accompanied by increased formation of methemoglobin, a marked decrease in reduced glutathione and hexokinase activity, and a moderate decrease in glucose-6-phosphate dehydrogenase and pyruvate kinase activities. Thermal denaturation of hemoglobin may be a cause of fever-related anemia in pts with unstable hemoglobins. Also measurement of H.B. formation in heat-treated whole blood is a sensitive and simple technique for defining Hb Z and possibly other unstable hemoglobins.