A SERUM FACTOR INHIBITS IRON INCORPORATION (ANTI-ERYTHROPOIETIN) ASSOCIATED WITH MESENTERIC LYMPHOID HAMARTOMA. E. Omer Burgert, Jr., Gerald S. Gilchrist, Virgil F. Fairbanks, and Hugh B. Lynn, Mayo Clinic and Mayo Fndn., Rochester, MN, and Peter P. Dukes, Childrens Hosp. of Los Angeles, Los Angeles, CA.

An 11-year-old girl had a refractory, hypochromic, microcytic anemia; hypoferremia; normoblastic hyperplasia (marrow); hypergammaglobulinemia; and growth retardation (bone age 6 yr). Isotope ferrokinetic studies revealed impaired absorption, rapid plasma clearance, and impaired incorporation of ^{59}Fe. Treatment with oral and parenteral iron, pyridoxine, folic acid, vitamin B_{12}, and gluten-free diet failed to produce any improvement. A ^{99m}Tc-sulfur colloid scintigram of the abdomen failed to demonstrate abnormal isotope uptake. The abdomen was explored, and a lymphoid hamartoma was removed from the gastrolienal ligament. Follow-up studies at 3 and 6 months revealed complete correction of the anemia, 2.0 cm increase in height within 3 months, and normal serum γ-globulin levels.

Serum obtained preoperatively inhibited the ^{59}Fe incorporation induced by a standard dose of erythropoietin in the exhypoxic polycythemic mouse assay system. This effect persisted in serum obtained 3 days after surgery but disappeared by 7 days. These observations indicate that the tumor forms a substance which inhibits iron incorporation, especially synthesis of heme. Further studies of this inhibitor are in progress.

ANTENATAL DIAGNOSIS OF THE β-THALASSEMIA GENE (β-thal). H. Chang, C.B. Modell, E.R. Huehns and D.G. Nathan. Child. Hosp. Med. Center and Harvard Med. Sch., Boston, Mass. 02115, and University College Hospital, London, England.

Antenatal diagnosis of β-thal depends on safe acquisition of fetal RBC and detectability of β-thal in midtrimester. To approach these requirements, a normal nomogram of the β/γ globin chain synthetic ratio in fetal RBC obtained at hysterotomy was derived by incubation of the cells with ^{3}H-leucine followed by urea-CMC radiochromatography. This revealed that the β/γ ratio increases from 0.075 ± 0.02 at 6 wks to 0.09 ± 0.02 at 16 wks of gestation. Secondly, globin chain synthesis was measured in fetal RBC collected in utero with a 2.7 mm aspiration fetoscope from midtrimester chorionic plate vessels of 4 normal women by the methods of Hobbins and Mahoney. All samples gave normal β/γ ratios for gestational age. Finally, to detect the presence of β-thal in the first trimester, fetal blood from vacuum-extracted abortuses of 3 Cypriot women, 2 of whom had β-thalassemia trait, was examined by similar methods which also included subtraction of β chain radioactivity contributed by contaminating maternal RBC. In 1 fetus of a β-thalassemia trait mother, the β/γ ratio was 0.04 at 10 1/2 wks, a 50% reduction consistent with fetal β-thalassemia trait. The others were normal. These results suggest that fetal cells can be acquired at midtrimester and that β-thal may be detected as early as the first trimester; hence, the antenatal diagnosis of β-thal is becoming a biological and technical reality.

RAPID DIAGNOSIS OF Fe DEFICIENCY BY MEASUREMENT OF FREE ERYTHROCYTE PORPHYRINS (FEP). E. Carlos, G. Gay, P. Young, S. Piomelli, Dept. of Ped., NYU Sch. Med., N.Y.

Inadequate Fe supply to the bone marrow results in an increased level of FEP in the peripheral blood. FEP concentration may be measured easily in blood samples collected on Guthrie paper by a micromethod developed in this laboratory (Ped. Res. 6: 366, 1972). This study investigated the use of FEP to assess the body Fe stores.

Normal values in 48 adult males were 1.42 ± .45 µg FEP/g Hgb. Values above 2.8 µg FEP/g Hgb (mean + 3 s.d.) were considered elevated. FEP were measured in 151 children with normal blood Pb (< 30 µg/100 ml) and transferrin saturation (TS) between 2 and 35%. FEP were found elevated in 21/21 children with Fe deficiency anemia (Hgb < 9 g%); in 13/14 children with TS 2-8%; in 40/53 children with TS 9-15%; in 18/43 children with TS 16-25% and in 5/20 children with TS 26-35%. The logarithm of the FEP concentration was inversely correlated with the TS, indicating that the FEP level increases exponentially as the body Fe stores become depleted. The FEP levels were elevated also in 7/12 cases of sickle cell anemia and in 5/16 cases of chronic infections, but normal in 11/11 cases of β-thalassemia trait.

These data indicate that FEP levels in the peripheral blood reflect the body Fe stores. Elevation of FEP detects Fe deficiency before anemia develops; normal FEP level discriminates thalassemia trait from Fe deficiency. FEP levels provide a diagnosis of Fe deficiency by finger puncture.

RED CELL (RBC) GLUCOSE PHOSPHATE ISOMERASE DEFICIENCY (GPI): CLINICAL AND LABORATORY EVIDENCE OF INCREASED BLOOD VISCOSITY. Chilcote, R.R. and Baehner, R.L. Indiana Univ., Dept. of Ped., Indianapolis.

GPI deficiency is the 3rd most common RBC enzyme defect and results in severe chronic hemolytic anemia. A 9 year old developed priapism six years after splenectomy had improved his unexplained hemolytic anemia. RBC studies showed: Hgb. 10 gm%, reticulocytes 20%, ^{51}Cr $t_{1/2}$-4 days, autohemolysis-normal; glucose utilization, lactate production, and 2,3 DPG were normal for cell age. GPI activity was 15% and fell to 4% of normal on exposure of hemolysate to 50°C for 10'. The proportion of $^{14}CO_2$ from glucose-1-^{14}C and glucose-2-^{14}C in patient RBC's at rest and after stimulation with 2 mM methylene blue was normal indicating glucose-2-^{14}C was capable of recycling past GPI to re-enter the hexose monophosphate shunt. Thus, the GPI deficiency did not inhibit reversed flow of glucose-2-^{14}C. However, phase microscopy of 1% suspensions of patient RBC's in normal saline (NS) revealed 10 to 15% echinocytes. Filtration of 1% suspensions of patient RBC's in Tris-NS through 3 micron pore polycarbonate membranes was impaired at pH 7.4 and ceased at pH 6.8. These studies suggest that despite apparently normal glycolysis, GPI RBC's have decreased filterability at physiologic pH and are totally impeded by severe acidosis. This likely accounts for the splenic sequestration and priapism observed in this patient.

IN VITRO STUDIES OF THE RELATIONSHIP BETWEEN INTRALIPID AND BILIRUBIN-ALBUMIN BINDING. George Chan and David Schiff, Depts. of Pediatrics, Obstetrics & Gynecology, University of Alberta, Edmonton, Canada.

Lipoidal material binds to bilirubin in vitro. It has been suggested that its use may augment the bilirubin binding properties of albumin giving further protection against kernicterus. In vitro studies of the effects of bilirubin-albumin binding of Intralipid, a commercially prepared soy bean fat emulsion for use in intravenous feed in human parenteral nutrition, were carried out in human serum albumin, human plasma and red blood cells. Utilizing a modified Sephadex G-25 elution technique, it was shown that in a prepared bilirubin-albumin solution, Intralipid in concentrations of 0.25 - 1.00 gms% binds bilirubin only after the primary albumin binding site is saturated with bilirubin. This effect was not reproducible in artificially jaundiced human adult plasma. Intralipid does not alter the dynamic equilibrium between the bilirubin in plasma and red blood cells at bilirubin-albumin molar ratios of <1. At molar ratios of >2 the fat competes with the red cell membrane for bilirubin. Thus, although Intralipid itself does not have any adverse affects on bilirubin-albumin binding, it does not provide any beneficial effect in the prevention of kernicterus. It is suggested that the in vivo metabolism of fat which leads to an increase of free fatty acid in plasma, may have a deleterious affect on bilirubin-albumin binding and as such may be potentially dangerous.

SEPARATION OF RBCs WITH IMPROVED SURVIVAL FROM NORMAL BLOOD. L. Corash, C. Seaman, C. Camisa, J. Reibman, S. Piomelli, Dept. Ped., NYU, New York.

This study investigated the use of density gradients to prepare RBCs with greater potential "in vivo" survival.

Solutions of the desired density, osmolarity, salt composition and pH were easily prepared with Stractan II (S-II), an inexpensive water soluble arabino-galactan polymer of 30,000 daltons. RBCs were centrifuged on parallel discontinuous gradients of S-II and crystalline albumin. Similar age dependent separation in both systems was indicated by the same rates of decline of reticulocytes, glucose-6-phosphate-dehydrogenase, pyruvate-kinase and hexokinase. Rabbit RBCs were divided on single layers of S-II into light (young) and heavy (old) fractions of varying proportions. Either fraction or both or unfractionated RBCs were labelled with ^{51}Cr alone or with ^{125}I also and reinjected into the donor. The rate of decline of radioactivity was followed until total disappearance. When compared to the unfractionated cells, the rate of decline of radioactivity was similar in the combined fractions; it was slowest and fastest in the light and heavy 10% fraction respectively and it was significantly slower and faster in the light and heavy 50% fractions respectively. Corresponding differences were found for the interval to total disappearance thus ruling out differential label elution.

These findings suggest that this technique may be used to obtain sizeable RBCs fractions of improved survival for transfusion to patients with chronic anemia.