619 A FAMILIAL GLANZMAN'S-LIKE PLATELET DYSFUNCTION WITH NORMAL MEMBRANE PROTEINS. Alton L. Lightsey, Robert Mc Millan, Harold M. Koenig, Sharleen Pfifer. Naval Naval Regional Medical Center and Scripps Clinic, San Diego. (Spon by James D. Connor).

Several recent reports have shown some patients with Glanzman's thrombasthenia (GT) to have a defect in platelet membrane glycoproteins. We have followed three siblings in a Guamanian family with severe qualitative platelet disorder clinically diagnosed as GT. All three have manifested spontaneous bruising and mucous membrane bleeding since birth. Major bleeding associated with minimal trauma has required repeated platelet and blood transfusions. The lab data have been identical in these three patients on repeated testing in two separate laboratories. All three have shown prolonged bleeding times, normal platelet counts and platelet morphology, no clot retraction, no aggregation to epinephrine, ADP, or thrombin and normal aggregation to ristocetin. Slight aggregation was seen with full strength collagen. Platelet membrane proteins from our patients were labeled with ^{125}I using the lactoperoxidase technique. Solubilized labeled and unlabeled membrane proteins were subjected to polyacrylamide gel electrophoresis and evaluated by protein and glycoprotein staining as well as determining the sequential radioactivity of 1 mm gel slices. Normal staining and radioactive patterns were noted in every instance. We conclude that patients with the clinical and laboratory profile of GT reflects a full spectrum of variable molecular abnormalities, since we could not demonstrate abnormal membrane proteins.

620 THE ROLE OF THE HUMORAL IMMUNE SYSTEM IN CHILDHOOD IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP). A.L. Lightsey, R. McMillan, H.M. Koenig, I. Schulman, Naval Regional Medical Center and Scripps Clinic, San Diego, and Stanford University, Stanford. (Spon. by J.D. Connor).

The pathogenesis of childhood ITP is not well understood. To explore the role of the humoral immune system in ITP, bone marrow (BM) and splenic (Sp) lymphocytes from ITP and control children were cultured and in vitro IgG production was measured; in some cases platelet specificity was assessed. Platelet-associated IgG (PAIgG) was also determined as a measure of platelet-bound antibody or complexes. BM and Sp cells were cultured in 20% fetal calf serum, Dulbecco's medium, for 10 days. The Fab-anti-Fab immunoassay was used in all IgG determinations. Platelet specificity was determined using an IgG absorption technique. PAIgG of circulating platelets from control and ITP subjects was assayed directly after washing the platelets by gel filtration. Mean BM synthesis rates (ngIgG/10^6 lymphocytes/day) of acute and chronic ITP cases were 219±92 and 619±216; both were significantly greater than control values of 98±62 ($p < 0.01$ and 0.001 respectively). SpIgG synthesis rates of chronic cases and controls were 85.9±52 and 22.9±11, respectively, and differed significantly ($p < 0.001$). Four of 4 splenic samples showed significant binding to target platelets which averaged 1232 ngIgG/10^9 platelets. Blood PAIgG values (ngIgG/10^9 platelets) of acute cases (range 5588-56,250) and chronic cases (range 2425-16,800) were greater than control values of 1089±213 ($p < 0.001$) and the two ITP groups appeared to reflect different statistical populations. We conclude these data support immune-mediated platelet destruction in both acute and chronic childhood ITP. Differences in PAIgG values, although preliminary, may be of diagnostic importance and suggest different mechanisms may be involved.

621 QUANTITATION OF HUMAN PROTHROMBIN BY RADIOIMMUNOASSAY (RIA). Charles D. Lox, Truda Mikel, G. Henry Strohm and James J. Corrigan, Jr. University of Arizona Health Sciences Center, Tucson, Arizona.

The purpose of this study was to develop a quantitative method for factor II antigen in human plasma utilizing the sensitivity and precision of RIA. Factor II was isolated by chromatographic technique and shared a single band on electrophoresis. I^{125} tagged factor II was prepared by a standard chloramine-T oxidation and sodium metabisulfite reduction. Antigen levels were then determined by classical double antibody radioimmunoassay. A dose response curve was generated by adding known amounts of normal human plasma to human barium sulfate-absorbed oxalated plasma. A straight line relationship existed between 15 to 140% coagulant activity (CA) and 7 to 50 µg/ml antigen content. Plasma factor II antigen in 20 normals was 29.63 ± 4.6 µg/ml (mean ± S.D.). Eight patients with induced vitamin K deficiency revealed normal antigen levels (30.13) and reduced CA (20) whereas three patients with severe hepatocellular disease were found to have reduced antigen and CA levels (6.7 µg/ml and 40% activity). The use of the RIA for measuring prothrombin protein in conjunction with coagulation factor assays could have clinical application in studying the mechanism of action of vitamin K, the developmental aspects of factor II production and activation, and in understanding acquired coagulopathies.

622 SERIAL ASSESSMENT OF BONE MARROW (BM) COLONY FORMING CAPACITY (CFC) IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL). Naomi L.C. Luban and Denis R. Miller, Cornell Univ. Med. Col., N.Y. Hosp., Dept. of Ped., N.Y., N.Y.

In vitro semisolid agar culture has been used to characterize and predict relapse in myeloid leukemias. We evaluated 5 children with ALL on the same drug regimen with sequential BM and CFC on day 0, 14, 28, and 83 or 138 to characterize the CFC in ALL and compare cytologic methods to CFC to predict chemotherapeutic effect and relapse. Feeder underlayers were prepared from $1x10^6$ normal human WBC in 0.3% agar and McCoys media as a source of CSF; $2x10^5$ nucleated marrow cells were overlayered. Colonies were counted at day 14 and compared to the peripheral blood absolute neutrophil count (ANC) and Giemsa stained BM. BM cellularity was ranked from 0-4 (3=normocellular).

DAY	Mean ANC	% Blasts	Av. Cellularity	CFU-C±SEM/$2x10^5$ cells
0	500	60%	1.0	1.33±0.32-9.3±0.32
83/138	2000	1%	3.0	5.0±0-56.0±6.0

There was poor correlation between CFU-C and ANC. However, an increase in CFU-C was noted with impending remission induction as the BM was reconstituted with myeloid precursors. This was most consistently noted when serial determinations were performed on an individual. In one patient no increase in CFU-C was noted despite cytologic evidence of remission; this patient subsequently relapsed. Serial assessment of CFU-C may be helpful in predicting relapse before morphological evidence on routine BM preparations, but does not help in predicting chemotherapeutically induced neutropenia.

623 BONE MARROW CULTURES IN CHILDREN WITH FANCONI'S ANEMIA AND T.A.R. SYNDROME. Victor Lui, Abdelsalam H. Ragab, Harry Findley, and Barbara Frauen, Emory Univ. Dept. of Pediatrics, Atlanta, Ga.

The clinical picture of Fanconi's anemia and the T.A.R. syndrome are very similar. In order to investigate the pathophysiology of these two conditions, we have performed in vitro bone marrow culture studies on two children with Fanconi's anemia and two children with T.A.R. syndrome. The bone marrow cells were cultured for myeloid progenitor cells in agar and for erythroid progenitor cells in methylcellulose. No colonies were observed from the cultures of the two children with Fanconi's anemia. The two children with T.A.R. syndrome grew a normal number of myeloid and erythroid colonies. Our results suggest that the basic defect in Fanconi's anemia resides in the stem cell while the defect in the T.A.R. syndrome resides in the megakaryocytic progenitor cell.

624 IRON SUPPLEMENTATION BY 3 MONTHS OF AGE IS NECESSARY IN LOW BIRTH WEIGHT INFANTS. Ulla Lundström, Martti A. Siimes and Peter R. Dallman. University of Helsinki, Children's Hospital, Helsinki, Finland and University of California, Department of Pediatrics, San Francisco.

Prevention of iron deficiency in low birth weight infants requires iron supplementation before the age when neonatal iron stores are exhausted. In order to determine this age, we measured hemoglobin (Hb), mean corpuscular volume(MCV), serum iron/iron binding capacity (% Sat), serum ferritin (SF), and reticulocyte count (retic) at 0.5, 1, 2, 3, 4 and 5 or 6 mo of age in 117 infants whose birth weights were between 1000 and 2000 g. All received banked breast milk in the hospital and breast milk or cows' milk formula later. A daily vitamin supplement included 5 IU vitamin E as tocopherol polyethylene glycol-1000 succinate. Infants with odd birth dates received 2 mg iron as ferrous sulfate/kg/d starting at 0.5 mo; those with even birth dates received no additional iron unless they developed anemia (Hb<7 g/dl at 2 mo, <9 at 3 mo, <9.5 at 4 mo, and <10.4 at 5 mo). At 2 mo, only the SF was significantly lower in the no-iron group ($p<0.02$); Hb and retic showed no evidence of vitamin E deficiency. At 3 mo, Hb, MCV, % Sat and SF were all lower ($p<0.001$, <0.01, <0.001 and <0.02) in the no-iron group. There was no significant difference between infants who were changed to formula and those who were still exclusively breast fed. The results indicate that low birth weight infants who receive no supplemental iron may develop iron deficiency by 3 mo of age. An iron dose of 2 mg/kg/d started at 2 weeks of age is adequate to prevent iron deficiency.