783 PRESENCE OF A SERINE PROTEASE IN UNACTIVATED PLASMA OF CHILDREN WITH COOLEY'S ANEMIA. M. Andrew, M. Manno, M. Karpatkin (Spon.by Saul Krugman) NYU Med.Ctr.,Dept. of Pediatrics, NYC.

Biologic activity of factors Xl, Xll and prekallikrein (PK) (chromogenic assay) was low in 10 patients aged 3-17 with Cooley's anemia; mean ± S.E., Xl: 56%±6; Xll: 51%±7; PK 59%±6. These levels were not due to generally impaired liver function as other clotting factors (1,ll,V,Vll,Vlll,lX & X) were in the normal range. The patients were studied to determine whether Xl, Xll and PK were depressed due to 1) synthesis of biologically inactive factors or 2) in vivo factor activation followed by removal. Factor Xll measured by Laurell technique equalled biologic activity. Non-activated plasma cleaved the chromogenic substrate for thrombin S2238 (31 different samples from the 10 patients); mean nM p-nitroanaline (pNa) released/ml/min was 33±5 compared to 3±.4 in 20 plasma samples from 10 healthy controls ($p < .001$). Comparisons between 3 substrates showed greatest activity for 2302 (plasma kallikrein substrate) and least for 2222 (Xa) (mean nM pNa/ml/min: 2302: 185±27; 2238: 43±11, 2222: 11±4; $p < 0.001$). Plasma activity was stable at -70° and was destroyed at 56°. It was not inhibited by the thrombin inhibitor Hirudin but was inhibited by DFP (serine protease inhibitor) and Trasylol (kallikrein inhibitor). This is the first demonstration of protease activity in unactivated plasma of patients who do not have DIC. We postulate that due to iron overload a zymogen (? prekallikrein) is activated to a protease (? kallikrein) which activates Xl&Xll with subsequent removal of Xla and Xlla.

● **784** BONE MARROW TRANSPLANTATION (BMT) IN CHILDREN. Edward B. Arenson, Stephan K. Ladisch, Robert P. Gale, and Stephen A. Feig. UCLA School of Medicine, Departments of Pediatrics and Medicine, Los Angeles.

BMT is used with increased frequency to treat aplastic anemia (AA) and acute leukemia (AL). We report the results of 90 BMT performed on children at UCLA through June, 1980. Thirty-three BMT were done on previously transfused AA patients; 18 recipients survive (55%). In 17 of these 33 patients, low-dose (3Gy) total body irradiation (TBI) was added to the cyclophosphamide conditioning regimen (CY) to decrease the risk of graft rejection. Thirteen of these patients survive (76%, median = 791 + d). Failure to use TBI was associated with a 43% rejection rate, while use of 10Gy TBI was associated with an 83% risk of fatal graft versus host disease (GVHD). Fifty-seven patients had BMT for AL. Three of 34 patients transplanted in relapse survive (>3.5 y). There were 6 early deaths; 17 of the remaining 28 patients developed GVHD (16 fatal), and 9 of the remaining 12 patients relapsed. Twenty-three had BMT during remission: there were 2 early deaths, 6 cases of fatal GVHD and 6 patients relapsed. Nine patients survive (39%, median = 264 + d). No relapses occurred among 6 patients who had BMT for AML in first remission, using a matched sibling donor.

We conclude that BMT is the treatment of choice for children with severe AA, if a compatible sibling donor is available. Addition of low-dose TBI to CY improves the survival of previously transfused AA patients. BMT offers the possibility of prolonged disease-free survival to patients with ultra-high-risk AL.

● **785** PREMATURE TERMINATION OF OXIDATIVE BURST IN GLUTATHIONE PEROXIDASE DEFICIENT RAT NEUTROPHILS. Susan S. Baker, Harvey J. Cohen. (Spon. by Stuart H. Orkin). Children's Hospital, Division of Hematology, Boston, MA.

In order to determine the role of the glutathione cycle in granulocyte (PMN) oxidative metabolism, rats were made glutathione peroxidase (GSH-Px) deficient(def), by feeding them a selenium def diet. After 12-15 weeks def rat PMN GSH-Px was 23 U/mg protein compared to 155 U/mg protein for controls. We studied the effect of GSH-Px deficiency on phorbol myristate acetate (PMA) stimulated PMN hexose monophosphate shunt (HMPS) activity and O_2^- production. HMPS activity was similar for def and control rats for the first 5 min. of incubation with PMA. However, over 20 min.,def PMN produce 480 cpm/2.5x10^6 cells and control 920 cpm/2.5x10^6 cells. When stimulated with L-amino acid oxidase and L-leucine HMPS was 220 in def and 570 cpm/20 min/2.5x10^6 cells in control PMN. Initial rates of PMA stimulated O_2^- production were the same in def and control (2.8, 2.78 nmol O_2^-/min/10^6 PMN). However, at 5, 10, and 20 min. the rates of O_2^- production in the def were 44%, 14%, and 3% of O_2^- produced at 2 min. This is compared to 52%, 36%, and 23% of O_2^- produced at 2 min. in the control. Incubation of PMN with L-amino acid oxidase and L-leucine for 20 min. resulted in a loss of 85% of PMA stimulatable O_2^- production in def compared with 43% in controls. This effect was abolished with the concomittant addition of catalase. We conclude that H_2O_2 in the rat PMN is metabolized through the glutathione cycle and GSH-Px protects PMN against H_2O_2 mediated destruction of their O_2^- generating system.

● **786** IMMUNO-IDENTIFICATION OF HUMAN FETAL FIBRINOGEN. Ronald D. Barr, Mary M. Storbeck, Marilyn A.Johnston, Peter Horsewood, Jack Gauldie. (Spon. by Alvin Zipursky) McMaster University, Department of Pediatrics, Hamilton, Ontario.

Samples of plasma were obtained from the umbilical cords (UP) of uncomplicated pregnancies at term, from neonates with 'reactive' hyperfibrinogenemia (NP) and from normal adults (AP). Fibrinogen was measured as thrombin-clottable protein (TCF) by the methods of Clauss and Astrup, and as immunoassayable material (IAF) by radial diffusion and nephelometry. Functional and structural characterization were pursued respectively by studies of fibrin monomer polymerization (FMP) and by cross-over immunoelectrophoresis (CIE). IAF-UP exceeded TCF-UP ($n = 40$, $p < 0.001$). No such difference existed in NP or AP and the observation could not be explained by partial proteolysis nor by inhibition of thrombin. Prolonged incubation (24 hours) with thrombin resulted in higher values for TCF-UP but not for TCF-AP. FMP was delayed in UP - median 12 minutes - by comparison with AP - median 9 minutes ($n = 17$, $p < 0.005$). CIE revealed disproportionate anodal migration in UP fibrinogen in all samples investigated ($n = 13$) with mobility ratios of 1.10 - 1.86 to pooled AP fibrinogen. The TCF-IAF difference in UP may represent fetal fibrinogen. Furthermore, the lack of an inverse relationship between 'fetal fibrinogen' levels and gestational age, and the previously reported rise in the concentration of TCF within a few days of delivery, suggest that the stimulus to change from the synthesis of fetal to that of adult fibrinogen is provided by the event of birth or its immediate sequelae.

787 ANTI-BOVINE SERUM ALBUMIN (BSA) ANTIBODIES ARE A COMPONENT OF CIRCULATING IMMUNE COMPLEXES (IC) IN CHILDREN WITH NEUROBLASTOMA (NB). Daniel A. Beck and Roger D. Rossen (Spon. by Jan vanEys). U. of Tex., M.D. Anderson Hosp. Tumor Inst., Baylor Col. Med. and VA Med. Ctr., Houston.

The Raji cell assay (RA) and C1q binding test (C1qBT) were used to monitor changes in IC levels over 1 to 15 mos. in 17 patients with NB, 3 of whom were stage IVs. During the period of observation 19 tumor recurrences and 7 deaths were observed. IC were found in 10 of 62 samples (16.1%) by C1qBT and in 29 of 62 samples (46%) by RA. Changes in serum C1qBT or RA did not correlate with changes in disease activity or tumor burden. Sera from NB patients but not from age-matched controls precipitated a protein in ^{125}I extrinsically labeled extracts of the LA-N1 NB cell line which had the molecular weight and antigenic characteristics of BSA. Using a modified Farr assay, 11 of 17 patients had anti-BSA antibodies; 23% of the initial samples and 14% of the subsequent 61 sera contained more anti-BSA activity than was found in 95% of sera from 13 age-matched controls. Incubation at pH 3.0 overnight in the presence of ^{125}I-BSA, followed by neutralization, substantially increased the anti-BSA activity of 3 sera with high levels of IC but did not influence the anti-BSA activity of 3 sera with moderate to low IC. This demonstration of abnormally increased anti-BSA activity as well as "blocked" anti-BSA activity in IC suggests an altered reactivity to milk antigens or abnormal regulatory control of humoral immune responses in NB.

788 EOSINOPHILIA AFTER BONE MARROW TRANSPLANTATION. John W. Bender and Rein Saral (Spon. by Elias Schwartz), Univ. of Pa. Sch. of Med. and The Children's Hospital of Phila., Dept. of Pediatrics, Phila., and the Johns Hopkins Univ. Sch. of Med., Bone Marrow Transplant Unit, Baltimore, Md.

Eosinophilia (EOS) is seen after bone marrow transplantation (BMT) and has been described in several case reports of graft-versus-host disease (GVHD). Increased numbers of eosinophils (Eo) are also seen in skin, liver and bone marrow biopsies from patients with GVHD. EOS is often associated with in vivo neutropenia and inhibits in vitro granulopoiesis. EOS was examined in 74 BMT patients and correlated with the incidence of GVHD and polymorphonuclear granulocyte (PMN) recovery. EOS (weekly average > 10%) was seen in 6/33 syngeneic patients (S), 10/27 syngeneic patients with GVHD (S/GVH) and 2/14 autologous/allogeneic patients (A/A). These incidences are not significantly different. Of the 16 patients with EOS, 9/16 had GVHD ($P<.01$). When the weekly average was >20%, 6/8 patients had GVHD ($P<.025$). The averages of the maximum weekly Eo percentages (mean±SD) for each group were 7.9± 8.6% (S), 13.8±15.9% (S/GVH) and 6.0±9.2% (A/A) ($P>.05$). EOS did not correlate with the severity of GVHD. In 8 patients where the weekly Eo percentage was >20%, there were significant negative correlations between weekly PMN and Eo percentages ($P<.02$), as well as weekly changes in PMN and Eo counts ($P<.01$) and percentages ($P<.001$). PMN and Eo recovery after BMT may be interrelated. EOS after BMT is suggestive of GVHD and may be a helpful criterion in diagnosing the presence, but not the severity of GVHD.