655 A SIMPLE TECHNIQUE FOR ELECTRONIC COUNTING AND SIZING PLATELETS. Paulette Smedresman and Sergio Piomelli, New York University School of Medicine, Bellevue Hospital, Division of Pediatric Hematology, N.Y.

Platelet sizing is a newly explored method for distinguishing thrombocytopenia due to increased peripheral destruction from that due to decreased production. We have devised a method for simultaneously counting and sizing platelets on a fingerstick specimen using the Electrozone electronic counter. 0.02 ml of blood is collected into a capillary tube. A layer of Stractan II is added and the tube is spun in a micro-capillary centrifuge. A distinct platelet layer then lies above the Stractan. The tube is cut and the platelet layer is diluted and passed through the electronic counter using a 48μ aperture. Platelet number is determined by the counter, while size distributions are performed by a multichannel particle analyser interfaced with a logarithmic amplifier. Platelet yields are 93% by comparison to manual counts, thus electronic counts/0.93 show a very close relationship to manual counts (r=0.99). Platelet volumes in normal adults range from 1.18μ^3 to 49.6μ^3 with a mean volume of 7.03μ^3. Female subjects had significantly larger platelets than male subjects($p<0.05$). A random relationship existed between platelet size and number. Mean platelet volume in full term infants was 5.90μ^3 and in premature infants 6.34μ^3. No relationship was found between gestational age or birth weight and platelet size. This method represents a simple and accurate method for simultaneous platelet counting and sizing and the entire procedure can be done in less than 6 minutes.

656 RED BLOOD CELL (RBC) ABNORMALITIES IN CHRONIC GRANULOMATOUS DISEASE (CGD) WITH MCLEOD PHENOTYPE. Clark M. Smith II*, Peter F. Coccia*, John W. Eaton†, Kenneth L. Dreher†, Jane L. Swanson°, Paul G. Quie*, James G. White* and William Krivit*. University of Minnesota School of Medicine and Hospitals, Departments of *Pediatrics, †Medicine, †Biomedical Engineering and °Laboratory Medicine, Minneapolis.

CGD patients lack the Kell group precursor substance, Kx, on their leukocytes (CGD-1, x^3K allele). Some CGD patients also lack Kx substance on their RBC's and have variable degrees of echinoacanthocytic changes associated with mild compensated hemolysis (CGD-2, x^2K allele, Mcleod Phenotype). In one patient available to us with documented CGD-2, scanning electron microscopy revealed ∿ 25% echino-acanthocytic RBC. Several other parameters were normal: red cell cholesterol and fractionated phospholipids, osmotic fragility, RBC Na^+ and K^+, and membrane Ca^{++}. However, the cells were less filterable through 3 micron pores than control. Maternal RBC's (dimorphic population Kx^+, Kx^-) were slightly less filterable. Furthermore, micropipette membrane aspiration of both morphologically normal and abnormal cells showed membranes more resistant to fragmentation. Finally, membrane SDS polyacrylamide gel electrophoresis (PAGE) stained for protein showed absence of a low molecular weight band and the presence of a unique higher molecular weight diffuse band. The abnormalities of filterability, micropipette aspiration, and PAGE suggest an intrinsic membrane defect as the etiology for the compensated hemolysis in these patients.

657 ADRIAMYCIN (ADR), VINCRISTINE (VCR), AND PREDNISONE (PRED) FOR REMISSION INDUCTION IN ACUTE MYELOGENOUS LEUKEMIA (AML). Kenneth A. Starling, Baylor College of Medicine, Department of Pediatrics, Houston; Jeanette Pullen, Jackson; G. Bennett Humphrey, Oklahoma City; William A. Crist, Birmingham; Tribhawan Vats, Kansas City; and Hernan Sabio, Charlottesville, for the Pediatric Division, Southwest Oncology Group (SWOG).

SWOG studies using ADR alone and ADR in combination with VCR and PRED for induction of remission in children with AML and acute monomyelogenous leukemia (AMML) were encouraging: 16 of 32 children treated with ADR alone achieved marrow remission. Fifteen of 31 children treated with VCR, PRED, and ADR achieved marrow remission. The present study utilized ADR, 30 mg/M^2 IV on days 1, 2, and 3; VCR, 2 mg/M^2 IV on days 1, 8, and 15, and PRED, 60 mg/M^2 PO daily for 21 days. Bone marrow aspirations were obtained at 21 days. Thirty-eight children with AML or AMML were treated with this regimen. Five children died before day 12 and were not considered evaluable for drug response. Twenty-five of the remaining 33 children achieved marrow remission--1 child achieved M_2 marrow status (7% blasts), and the remaining 7 children failed to respond. Seventy-six percent of the children with non-acute lymphocytic leukemia achieved complete marrow remission. If the early deaths are included as nonresponders, this figure falls to 66%. The 3-drug combination--ADR, VCR, and PRED--is promising for remission induction in AML and AMML.

658 NEWBORN RED BLOOD CELLS (RBC's), THE NATURE OF OXIDANT INJURY. James A. Stockman III, Dept. of Ped., SUNY, Syracuse, N.Y. (Spon. by Frank A. Oski).

Unlike adult cells, newborn RBC's readily undergo oxidant damage. The nature of the defect causing this is unclear. In order to determine those factors related to newborn RBC oxidant susceptability, cord blood was obtained from 88 term infants. As expected, acetylphenylhydrazine (APH) induced Heinz Body (HB) formation was greater in newborn vs adult RBC's (3.24 HB/RBC vs 0.27). Although newborn RBC superoxide dismutase (SOD) activity, at 91% of adult levels, was slightly but significantly lower than adult RBC's (paired $T=4.0$ $p<0.001$), higher levels of SOD were associated with greater APH induced HB and H_2O_2 formation (as determined by the aminotriazole - catalase inhibition assay). The addition of SOD to newborn RBC's produced additional significant increases in HB and H_2O_2. No correlation existed between %HbF, amount of HbF oxidized and HB or H_2O_2 formed although H_2O_2 production was 82% >in newborn RBC's. With equivalent H_2O_2 production, HB formed readily in newborn cells only. Glutathione peroxidase (GSH Px) levels were equivalent in newborn and adult RBC's. Despite the> H_2O_2 formed, the fall in newborn RBC reduced glutathione (GSH) levels induced by menadione failed to exceed that of adult RBC's when incubated in the absence of glucose.

These data demonstrate that SOD deficiency is not a cause of the vulnerability of newborn RBC's to oxidant damage; in fact > SOD is associated with > oxidant effect. It appears that > H_2O_2 generation and ineffective detoxification by GSH (inspite of "normal" levels of GSH Px) are the major determinants of oxidative damage.

659 STORAGE POOL DEFICIENCY IN NEONATAL PLATELETS. Marie J. Stuart. Dept. of Peds., SUNY, Upstate Med. Ctr., Syracuse, N.Y.

It is recognized that newborns exhibit a transient thrombocytopathy. Some investigators have described this as an "aspirin-like" defect, while others have ascribed it to a "storage-pool" abnormality. In an attempt to resolve this discrepancy platelets (plts.) from 10 normal newborns, 10 normal adults and 10 aspirin treated adults were studied. None of the mothers of the newborn infants had ingested drugs known to interfere with plt. function. Plt. aggregations with ADP (3uM), epinephrine (5uM) and collagen demonstrated abnormal 2nd phase aggregation in both the newborns and in the aspirin treated adults. When mixtures of equal volumes of plts. from newborn and aspirin treated controls were tested, normal, irreversible aggregation was observed. Mutual correction was not observed on preincubation of newborn plts. with aspirin prior to mixing. When plt. malonaldehyde production was measured, as an index of prostaglandin synthesis, in the presence of thrombin or HEM, no differences were observed between normal adults and newborn plts. Malonaldehyde production was markedly reduced in the aspirin treated controls. Storage-pool deficient plts., when combined with aspirinized plts., prove mutually corrective in aggregation studies since storage pool deficient plts. overcome the abnormality in prostaglandin synthesis of the aspirin treated cells. These latter cells in turn provide the necessary storage-pool nucleotides to cause mutual correction and irreversible aggregation of the mixture. Our findings indicate that newborn plts. have a "storage-pool" defect.

660 IMMUNOLOGICAL ABNORMALITIES IN CHILDHOOD CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP). Marie J. Stuart, Russell H. Tomar, Merrill L. Miller and Fredrick R. Davey. Depts. of Ped. and Path., SUNY, Upstate Med. Ctr., Syracuse, N.Y.

Chronic ITP, unlike acute ITP, may be the result of an underlying immunologic disorder. To examine this possibility 3 children, and their families, were studied in an attempt to identify underlying abnormalities in cellular and humoral immunity. All 3 patients had their disease for more than 1 year. All had shortened platelet life spans. None were receiving drugs, and none had had their spleens removed. In these patients, 2 of 3 were found to have decreased numbers of T lymphocytes and PHA reactivity; 2 of 3 had dysgammaglobulinemias; 1 of 3 had decreased isohemagglutinins, and all 3 had abnormal reactivity to a variety of intradermal antigens. In the family members these same abnormalities were found with increased frequency. In addition, autoantibodies such as a biologically false positive serological test for syphilis, and anti-thyroid antibodies were also found. One parent and 2 asymptomatic siblings of one of the proposita were also documented to have shortened platelet life spans in the presence of normal platelet counts. The HLA antigens, A3 and B7 were found in all 3 families. These 2 HLA antigens have previously been documented to occur with increased frequency in a variety of "auto-immune" disorders. These studies demonstrate that chronic ITP occurs in families with immunologic defects, and suggests that this disease has a genetic rather than an exclusively environmental basis.