852 A SENSITIVE MICROTITER ELISA TO STUDY PLATELET MEMBRANE SPECIFIC IgG ANTIBODIES (Ab) IN ISOIMMUNE AND AUTOIMMUNE THROMBOCYTOPENIA. Nai-Kong V. Cheung, Bonnie Landmeier, Case Western Reserve University, Rainbow Babies and Childrens Hospital, Department of Pediatrics, Cleveland, Ohio 44106 (sponsored by Peter F. Coccia, M.D.)

Platelet membranes were prepared from platelet sonicates by discontinuous sucrose gradient ultracentrifugation, solubilized and coated onto microtiter plates for ELISA on sera or ACD-A plasma from patients with suspected immune thrombocytopenia. Samples from 27 normal volunteers had a mean titer of 1:3 (range 2-14). Sera from 9 $P1^{A1}$ negative mothers with infants born with isoimmune neonatal thrombocytopenia had geometric mean titer of 1:3640 (range 100-102400) which was 25 to 10^4 times more sensitive than the standard immunofluorescence assay. Specificity of the Ab was established by inhibition with $P1^{A1}$(+) and $P1^{A1}$(-) platelets in the ELISA. 91 patients with immune thrombocytopenia were also studied. Titer of ≥20 was regarded as positive.

Disease	Total #	#Ab Positive	Range of Titer
Adult ITP	45	25	20-20480
Child. Chronic ITP	26	13	20-640
Child. Acute ITP	20	10	20-160

Ten untransfused children (5 Ab+, 5 Ab-) with acute ITP were studied after complete recovery of platelet counts. The five Ab positive patients had persistent Ab despite clinical remission. The role of Ab titer was analyzed in patients receiving high doses of γ-globulin infusions. Positive Ab titer was associated with poor (<60K) platelet count rise, rapid fall of platelets to <20K or resistance to booster infusions in 5/12 patients.

853 ESTIMATED IRON BALANCE AND PLASMA FERRITIN LEVELS IN VLBW INFANTS. Raul F. Cifuentes, Patricia A. Miller, and Amos S. Deinard. Dept. of Pediatrics, Hennepin County Medical Center and University of Minnesota, Minneapolis.

25 VLBW infants (BW 1.3 kg) had plasma ferritin levels (PF), Hgb, Hct, and MCV measured when they reached about 2 kg in weight. 10 of them had bronchopulmonary dysplasia (BPD). None of these infants were supplemented with medicinal iron or received iron-fortified formula. Estimated iron balance (EIB) was determined by calculating iron intake from milk ingested, whole blood and packed red cells, and losses from blood drawing. Plasma ferritin was measured by RIA. Their gestational age was 29 ±2.4 wks. Results are expressed in the table as x ± SD.

Age	Weight	Hgb	Hct	MCV	PF	EIB
days	gm	gm/dl	%	fl	ug/l	mg
Birth	1054 ± 77	16.4 ± 1.6	47.1 ± 5	118 ± 5.8	--	--
66 ± 26	2096 ± 187	12.0 ± 1.4	34.4 ± 4	90 ± 5.2	136 ± 98	65 ± 40

Plasma ferritin levels ranged from 34 to 430 ug/l. There was no significant correlation between oral intake of iron and plasma ferritin levels. However, there was correlation between iron balance and plasma ferritin levels (y=43+1.43x, r:0.58, SEE:18.3, p 0.01). Infants with BPD had higher plasma ferritin levels than those without BPD, which may reflect the more frequent transfusions. These data suggest that in VLBW infants, iron supplementation is not indicated prior to their reaching 2 kg in weight.

● **854** REVERSIBLY SICKLED CELLS: A CORRELATE OF THE FREQUENCY OF PAINFUL CRISES IN HbSS SICKLE CELL ANEMIA. Robert R. Chilcote and Dianne Gallagher (Spon. Marc O. Beem), The University of Chicago, Wyler Children's Hospital, Chicago, IL

Despite intensive investigation, no prognostic factor has yet been identified for predicting the frequency of painful crises in HbSS. Direct microscopic observation of a transfused animal model suggested that reversibly sickling cells (RSC), rahter than ISC, were more likely to obstruct vessels (Lacelle, Blood Cells, 1977). We examined the significance of this observation by following 65 children with HbSS for the frequency of painful crises. Without knowledge of the clinical course, hematologic parameters and proportion of ISC (morphologic criteria) were used to estimate the absolute number of cells capable of being RSC (100% -%ISC -%F) X RBC. The patients were followed for a mean of 27 months (144 prospective patient-years). A median of 1 event occurred per 12 months (range 0-10). The frequency of painful events was correlated with RSC (r=0.32, p <0.01), those patients with the highest red cell levels and fewest ISC having higher RSC levels and more frequent crises. RSC was for any given patient stable over time and inversely correlated to MCH (r= -0.64, p <0.01). These results indicate that the proportion of RSC is a prognostic factor and imply that strategies which decrease the number of ISC or increase the hemoglobin level without decreasing the number of cells capable of reversible sickling may increase the frequency of painful crises.

● **855** SHAPE CHANGE (ECHINOCYTOSIS) IN PYRUVATE KINASE DEFICIENT RED CELLS IS MEDIATED BY THE COUPLED BILAYER HYPOTHESIS. Robert R. Chilcote and Barbara Jones (Spon. Marc O. Beem), University of Chicago, Wyler Children's Hospital, Chicago, IL

Patients with pyruvate kinase (PK) deficiency have spiculated red cells termed echinocytes, but little is known about the basis for this characteristic morphologic abnormality. PK deficient RBC were incubated at 0°C for 15' with the membrane active agent chlorpromazine (CPZ) which reverses echinocytosis and lysolecithin which causes echinocytosis. Samples were fixed in 1% glutaraldehyde (pH 7.4) and using phase contrast microscopy the proportions of echinocytes quantitated using criteria of Bessis. In PK deficient RBC echinocytosis was exacerbated by lysolecithin and completely reversed by CPZ. Since recent experiments have suggested that echinocytic shape reversal depends on the hexose monophosphate shunt (HMPS), we incubated PK RBC with hydrogen peroxide and 1 mM methylene blue (MB) at both 20°C and 0°C. Peroxide was minimally effective at either temperature. MB, a tertiary amine similar in structure to CPZ, was highly effective instantaneously reversing echinocytosis at both 20°C and 0°C. These results indicate that echinocytic shape change in PK deficiency can be completely reversed by membrane active agents, a process which is independent of HMPS activity and can in fact occur independent of cell metabolism. These results also imply that the metabolic abnormality of PK deficient RBCs which leads to shape change is mediated by an effect on the relative surface areas of the bilayer leaflets.

† **856** DANAZOL-INDUCED FACTOR IX AND VIII BYPASSING ACTIVITY James J. Corrigan, Jr, Harinder S. Garewal, Brian G. M. Durie, Monette Jeter, Mary Lou Damiano. Depts of Peds and Intern Med, Univ of Ariz Health Sciences Center, Tucson

Danazol, a synthetic androgen reported to increase factor VIII and IX activity levels, was given to 5 hemophiliacs who were >17 years old (3 with severe hemophilia A, one moderate hemophilia A and one moderate hemophilia B). With danazol therapy (600 mg/da) the PTTs shortened 20-30% of pre-treatment times. The prothrombin times and activity levels of factors VIII, IX, and XII did not change during the study period. Addition of plasma from danazol-treated patients to plasma with a known factor VIII inhibitor and to plasma from an untreated severe hemophiliac A patient caused a similar shortening of the respective PTTs. A typical mixing experiment is shown in the Table (IP=inhibitor plasma; NP=normal plasma; HP=hemophilia A control; HP-D =danazol plasma in hemophilia A. The PTTs, in secs, are shown using 1 vol to 1 vol mixing, incubating for 4 min @ 37°C).

	IP	NP	HP	HP-D
IP(121)*	-	67	120	68
NP(35)*	67	-	39	36
HP(95)*	120	39	-	75
HP-D(66)*	68	36	75	-

*Initial PTT (in secs) before mixing.

Absorption of the danazol plasma with precipitating antibody against factor VIII and IX did not remove the PTT correcting principal. The data suggest that danazol may cause the de novo appearance of an intrinsic coagulation pathway activator having factor VIII and IX bypassing activity.

857 PENICILLAMINE TREATMENT IN HEMATHROSIS-INDUCED CHRONIC ARTHRITIS. James J. Corrigan, Jr, Karen Kolba, Eric P. Gall, Joyce Trombley, Keith Meredith, and Mary Lou Damiano. Univ of Ariz Health Sciences Center, Tucson.

Current medical management for established hemophilic arthritis is unsatisfactory and does not modify the eventual outcome. D-Penicillamine (PEN), an anti-inflammatory drug effective in rheumatoid arthritis, has not been evaluated in hemarthroses-induced joint disease. 83 NZ white rabbits had weekly intra-articular injections of citrated autologous blood (right knee) and citrated saline (left knee) for 6 months. PEN 15 mg or 50 mg/kg/da, IM, was begun early (day 1) or late (at 8 wks). Controls were saline treated rabbits. The animals were killed at 6 mo. Analysis included joint fluid WBC counts, gross and histologic examination of the synovia (acute and chronic inflammatory cells, synovial hyperplasia, and iron deposition). The saline-injected knees showed no inflammatory change and the blood-injected knees had iron deposition in all animal groups. The early high and low dose, and late low dose PEN treated groups showed no difference from untreated animals. Late high dose PEN treatment showed marked suppression of the synovitis. Four hemophiliacs with synovitis were given PEN, 5-10 mg/kg/da, p.o. for>2 months. All have had significant reduction in the synovial thickening with concurrent increased range-of-motion and decreased number of bleeds into the affected joint. These studies suggest that PEN is beneficial in the chronic arthritis induced by hemarthroses. Its anti-inflammatory mechanism is not known but may be inhibiting free radical formation, not by removing iron by its chelating ability.