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PROTEIN C LEVELS DURING THE FIRST MONTH OF LIFE.

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Protein C (PC) is a unique vitamin K dependent plasma protein which acts as an anticoagulant. Thromboembolism has been reported in kindreds with PC levels which are congenitally 38-60% of normal. PC was measured by electro-immunoassay in 47 neonates ranging in gestational age (GA) from 28-40 weeks. None had evidence of DIC; all had received vitamin K. On day 1 of life mean PC was $27\% \pm 2.9$ SEM (range <10 to 67%) of the normal adult mean. PC correlated with GA in the 22 infants who were healthy ($r=.65$ $p<.01$) but did not correlate in the 25 sick infants. Postnatally PC was 32% on day 7 and 31% on day 28. None of the infants had evidence of thromboembolism. Ca^{++} reduced the electrophoretic mobility of infants' PC indicating that it was γ -carboxylated. It is concluded that 1) For at least the first 28 days of life, PC was below the range reported in kindreds with congenital deficiency and thromboembolism, yet thromboembolism did not occur in these neonates. 2) PC in the newborn is γ -carboxylated indicating that the low levels are due to decreased synthesis and/or short half life. 3) PC levels in healthy newborns correlates with GA.

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NORMAL CHEMOTAXIS AND OTHER GRANULOCYTE FUNCTIONS IN HEMOPHILIACS WITH ABNORMAL T HELPER/T SUPPRESSOR CELL RATIOS. P. Kempert, J. Katz, C. Mallett, C. VanDenVen, B. Walter, G. Bennetts, M. Cairo, UCI/CHOC, Orange, CA 92668. (Sponsored by Donald Sperling).

Last year, Tannous (Ped Research #1183) reported plasma chemotactic inhibitors in hemophiliacs receiving Factor VIII concentrates. To confirm this finding, and to assess total granulocyte function we tested 11 severe hemophiliacs 21-64 years old (median 25) (8 on Factor VIII and 3 on cryo). All had normal T and B cell numbers but significantly decreased T_4/T_8 ratios $0.3 \pm .6$ cryo, $p<.01$ FVIII $0.8 \pm .2$ $p<.001$ vs control $1.5 \pm .3$. Cells were normal when tested for bactericidal killing against *S. aureus*, FMLP (N-Formyl-L-Methionyl-L-Leucyl-L-Phenylalanine) stimulated aggregation and cyto B/FMLP stimulated superoxide production.

	TOTAL	CONTROL	P-VALUE
Superoxide (n moles/10 ⁷ cells)	227.9-71.1	182.6-49.8	NS
Aggregation (cm ²)	31.4-10.9	32.4-9.90	NS
Bacterial Killing (%kill)	49.69-3.68	49.48-1.93	NS

	P-VALUE
Human PMNs were tested for chemotactic activity using the Gallin modification of the Boyden Chamber technique with prelabelled Cr^{51} PMNs stimulated by E.Coli Endotoxin with and without patient serum. In contrast to previous findings, no abnormality was noted.	
P cells P serum	21.71-9.88% NS
C cells C serum	24.56-8.72% NS
P cells P serum	21.82-10.4% NS
C cells C serum	21.96-13.9% NS
C cells P serum	23.49-12.2% NS
C cells C serum	25.3-8.32% NS
C cells C serum	25.3-8.32% NS
P cells C serum	21.96-13.95% NS

In addition further initial studies show no change in chemotactic activity when normal donor cells were incubated with commercial Factor VIII preparations. Thus in hemophiliacs with abnormal cellular immunity we were unable to demonstrate any cellular or serum defects in chemotaxis or in other granulocyte functions. A larger series of patients will be studied to confirm this.

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HEMOLYTIC TRANSFUSION REACTION (HTR) ASSOCIATED WITH INTRAVENOUS IMMUNE GLOBULIN (IV Ig). Haewon C. Kim, C. Lucy Park, James H. Cowan, III, Francis Fattori, Charles S. August, Univ. of Penn. and Children's Hosp. of Phila. Depts. of Pediatr. and Clin. Labs.

High dose IV Ig has been tried in immune cytopenia(s) with few complications. We report a severe HTR due to IV Ig. A 10 yr-old B Rho(D) pos. boy who received a bone marrow transplant (BMT) from his O Rho(D) neg. sister was given IV Ig (CS1911, GAMIMUNE, 5%, Cutter Lab.) for platelet alloimmunization. On day 9 post-BMT he developed a chill and hypotension after 120 ml of IV Ig and hemoglobinuria later. On day 10 he had a severe shaking chill after 100 ml of IV Ig and symptoms recurred after 2 ml of infusion. On days 10 to 12 he had hyperbilirubinemia (4.5 mg/dl), hemoglobinemia (98 mg/dl), hemoglobinuria (75 mg/dl) and no haptoglobin. Hb remained between 6-7 g/dl with 8 units of RBC. On day 13 urine became clear and Hb rose without transfusions (Tx). No Tx were given 48 hrs. pre-IV Ig. After BMT he was given 0 neg. deglycerolized-RBC and group B plasma and platelets. Pre-IV Ig he was B pos. and had a negative direct antiglobulin test (AGT) and antibody screen. Post-IV Ig direct and indirect AGT were positive (days 10-13); anti-B (1:4) was detected in his sera from day 12. Sera and eluate demonstrated antiglobulin reactivity with group B RBC. The IV Ig he received had 1:64 for anti-B and Anti-A. On day 16, patient was typed as 0 neg. without reticulocytosis, indicating massive intravascular hemolysis of autologous RBC, not a response to BMT. Since blood group antibodies are present in human immune serum globulin preparations, compatibility test should be done when using IV Ig to prevent HTR.

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EFFECTS OF FETAL ACIDOSIS ON FETAL & MATERNAL BLOOD COAGULATION (A FETAL LAMB MODEL). C. Thomas Kisker, William R. Clarke, David P. Bohlken, University of Iowa College of Medicine, Iowa City, Iowa.

The effects of two hours of fetal acidosis (mean pH 6.93) on fetal and maternal blood coagulation were measured. Coagulation test results from 10 fetal lambs and mother ewes (127 ± 2 days mean gestation) before and after fetal lactic acid infusion were compared with test results from 8 control fetal lambs and mother ewes (127 ± 3 days mean gestation) before and after control glucose infusion. Tests included Hb, Hct, WBC, platelet count, pH, pCO_2 , pO_2 , PT, PTT, thrombin time, fibrinogen, factors II, V, VII, VIII, IX, X, XI, XII activities, fibrin monomer (FM), AT III, and FDP levels. Significant changes in acidotic fetal lambs not seen in controls included increased WBC (mean $2800/mm^3$ to $3600/mm^3$; $p=.0009$), shortened thrombin time (mean 17.8 sec to 11.2 sec; $p=.0001$), decreased factor V (mean 57% to 37%; $p=.0014$), factor IX (mean 35% to 29%; $p=.0128$), and fibrinogen (mean 147 mg % to 125 mg %; $p=.0492$). There were no increases in FM or FDP and no decreases in platelet counts or AT III levels. Although there was no change in pH or lactate, a decrease in factor V was found in ewes with acidotic fetuses (mean 141% to 113%; $p=.006$) and a decrease in factor IX (mean 119% to 102%) approached significance ($p=.0564$). Fetal acidosis thus induces a hypercoagulable state in the fetus by shortening the thrombin time. Decreases in the levels of factor V and IX are also observed in both fetus and mother, suggesting the liberation of a mediator capable of crossing the placenta.

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HUMAN T-CELL LEUKEMIA VIRUS (HTLV) STUDIES IN SUBJECTS WITH HEMOPHILIA. B. Kloster, R. Tomar, J. Stockman, H. Lamberson, S. Merl, P. John, D. Groth, B. Poiesz. Depts. Pediatrics/Pathology/Medicine, SUNY, Syracuse, New York.

Subjects with hemophilia may be at risk for developing AIDS. HTLV has been linked to AIDS in several reports. Because of this possible association, sera from 46 asymptomatic F-VIII and F-IX deficient hemophiliacs, ages 16 mos-19 yrs, and 51 adult non-hemophilic AIDS patients were examined for antibodies to membrane antigens (MA) on HTLV infected lymphocytes (HUT 102-B2 cell line) using flow cytometry. Fluorescence histograms of serum samples were computer analyzed for comparison to cells exposed to buffer. Lymphocyte subsets were determined using commercially available monoclonal antibodies. The anti-HTLV-MA titers were as follows:

	n	$\leq 1:20$	1:40	$\geq 1:75$ (titers)
Controls	38	90%	10%	0%
Hemophiliacs	46	50%	35%	15%
AIDS	51	25%	16%	59%

Among hemophiliacs, titers $\geq 1:75$ were found only in VIII deficient subjects using concentrates. Those using cryoprecipitate and F-IX subjects had titers comparable to controls. In subjects receiving concentrate, the titers did not correlate with factor usage (u/kg/yr). Inverted T_4/T_8 ratios occurred in 0% of cryo users, 28% of IX conc and 61% of VIII conc users. T_4/T_8 were noted in 28% of hemophiliacs with anti-HTLV-MA titers $<1:20$, 44% with titers $\geq 1:40$, and in 100% with titers $\geq 1:75$ suggesting an association. No correlation was found between T_4/T_8 ratios and antibody positivity for CMV or HBV.

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CEREBROSPINAL FLUID (CSF) B2 MICROGLOBULIN (B2M): A NON-SPECIFIC INDICATOR OF CNS LEUKEMIA, SOMNOLENCE SYNDROME, AND THERAPY-RELATED CNS INJURY.

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To evaluate CSF B2M as a marker of CNS leukemia, B2M was measured in the CSF (N=485) and serum (N=178) of 58 children with ALL over a 34 month period. CSF B2M and/or the CSF/serum B2M ratio were increased in patients with blast cells on cytospin, previous CNS disease in remission, overt CNS involvement, the somnolence syndrome and recent intrathecal methotrexate ($p<.005$). CSF B2M increased with, but not prior to, the development of overt CNS disease and peaked 3-4 weeks thereafter (N=4 patients). To evaluate CSF B2M as a marker of therapy-related CNS injury, 15 patients without CNS involvement were studied serially for 20 to 80 weeks from diagnosis. CSF B2M increased following intrathecal methotrexate alone (weeks 0 to 4) from $0.58 \pm .44$ to $0.77 \pm .54$ mg/l ($p<.01$). Following cranial irradiation (weeks 4 to 6), CSF B2M increased further to $1.00 \pm .73$ mg/l ($p<.001$), and peaked after six weeks. A second peak (2.14 ± 1.47 mg/l) was observed after 26 weeks. CSF B2M returned towards pre-therapy levels by the end of the first year. It is concluded that CSF B2M increases in response to overt but not occult CNS leukemia and to therapy-related CNS injury. The latter follows a predictable bi-modal curve following cranial irradiation and intrathecal methotrexate during the first year of treatment. Studies demonstrating production of B2M by endothelial cells in culture suggest that the source of the elevated CSF B2M seen following CNS therapy, in the absence of overt CNS leukemia, may be vascular.