MECHANISM OF PLATELET DYSFUNCTION IN NEWBORN INFANTS. D. G. Corby (Intr. by William E. Hathaway). Clinical Res. Svc., Fitzsimons Army Med. Ctr. & Univ. Colo. Med. Ctr, Denver, CO.

Although platelet defects have been documented in newborn (NB) infants, the mechanism of this abnormality is not clear. In this study we have obtained data showing adenosine diphosphate (ADP) release in NB platelets is impaired because of a lack of sensitivity to external stimuli. % aggregation to strong collagen (SC), weak collagen (WC), and epinephrine (E) was less in NB platelet-rich plasma than paired maternal (M) samples (57 ± 5% vs. 74 ± 4%, $p < 0.02$; 36 ± 7% vs. 59 ± 7%, $p < 0.008$; and 9 ± 2% vs. 69 ± 3%, $p < 0.0004$ respectively). ADP release from NB platelets with SC, WC, and E was significantly decreased ($p < 0.01$). Platelet ATP and ADP content in NB were less than M ($p < 0.01$). A strong correlation ($r = 0.96$) existed between % aggregation and ADP release to WC in NB. The % available ADP released was similar in NB and M in response to SC ($p > 0.4$). Whereas M responded equally well to WC; NB did not ($p < 0.004$). Nonmetabolic ATP release was similar in both groups. Specific activity of ADP (labeled with adenine-8-^{14}C) was similar in platelets from both groups, indicating the nucleotide deficit in NB platelets is equally divided between the metabolic and nonmetabolic pools. NB and M platelet cAMP levels were equal. Therefore, since metabolic ATP is essential for the release reaction and these data show that storage pool deficiency does not exist in NB platelets, it may be postulated that the functional abnormality most probably results from a lack of metabolic ATP.

EXPERIMENTAL GRAM-NEGATIVE SEPSIS: EFFECT OF HEPARIN. James J. Corrigan, Jr., James F. Kiernat, Cheryl J. Pagel, Dept. of Ped., Univ. of Arizona Medical Center, Tucson, Arizona.

The beneficial effect of heparin therapy in children with bacterial septicemia and associated disseminated intravascular coagulation (DIC) is controversial. Although endotoxin treated animals have been used as the model for DIC recent evidence suggests that experimentally induced infection approximates more closely the human disease state. The purpose of this study was to evaluate the effect of heparin on mortality and DIC in rabbits with gram-negative septicemia. Rabbits were given live Pasteurella multocida (2×10^9/Kg body wt.) organisms intraperitoneally. The results in 200 animals showed: rabbits given live organisms developed positive blood cultures and endotoxin levels (lysate test) at 2 hours which persisted to death; thrombocytopenia by 8 hours; DIC (reduced fibrinogen factors II, V and VIII) after 8 hours; and 100% mortality by 24 hours. Rabbits given live organisms and heparin (1000 units/Kg IV q 4 H) showed: thrombocytopenia by 8 hours; elevated fibrinogen after 8 hours; and 100% mortality between 16-18 hours. Mean fibrinogen level in animals not given heparin was 256 mg% (± 23) as compared to 403 mg% (± 23) in those given heparin. The data indicates that survival rate was not improved with heparin in experimental septicemia although fibrinogen consumption (DIC) was abolished. The results suggest that successful therapy for DIC in the clinical setting of septicemia should not be equated with reduced mortality.

THE DISPLACEMENT OF ALBUMIN-BOUND BILIRUBIN BY GENTAMICIN by Julio O. Cukier, Shatchai Seungdamrong, John L. Odell and Gerard B. Odell, Johns Hopkins Univ., Dept. of Pediatrics, Baltimore.

Gentamicin is extensively used in the neonatal period but preliminary evidence indicates it can cause the displacement of bilirubin from albumin (Odell, Ann. N.Y. Acad. Sci. 226: 225, 1973). Addition of gentamicin at therapeutic concentrations to model sera and human serum from jaundiced infants caused dissociation of bilirubin from albumin similar to salicylate. This was reflected by shifts in the absorption curves to lower wave lengths and by adsorption to red cells and mitochondria. There was also a very strong correlation between the amount of displacement by salicylate and gentamicin in pre-exchange blood samples, ($r = .94$, $p < .001$ $n = 18$). On a molar basis gentamicin is more effective than salicylate and sulfonamides in displacing bilirubin from albumin.

Injection (i.p.) of gentamicin in jaundiced Gunn rats at doses of 3, 3.5 and 6 μg/kg was associated with 28, 38 and 52% decreases, respectively, in the serum bilirubin concentrations at 30 min and 19, 28, 35% decreases at 60 min. Animals injected with either saline, or kanamycin (7.5 and 15 μg/kg) exhibited only a 5% decrease in their serum bilirubins at 30 and 60 min.

These results suggest that the use of gentamicin in infants with neonatal hyperbilirubinemia may increase the risk of bilirubin encephalopathy.

GLUCOCORTICOSTEROIDS AND UNBOUND UNCONJUGATED BILIRUBIN. B.H. Doray and L. Choinière, Univ. of Montreal, Dept. Ped., Ste-Justine Hosp, Montreal, Canada. Intr. by J. R. Ducharme.

Since glucocorticosteroids are frequently used in newborn infants, it should be demonstrated that these substances do not interfere with the albumin binding of serum bilirubin. Salicylates have been shown to have such an action on albumin-bound unconjugated bilirubin, and modify the light absorption spectrum of a serum towards the spectrum of unbound bilirubin. 0.04 mg/cc of prednisolone was added in vitro to 26 serums of icteric newborn infants; 5 showed spectrophotometric changes similar to those produced by salicylate and corresponded to a displacement of total bilirubin from 3 to 6% of that produced by salicylate. However no change was produced by adding mepiridine (1.4 mg/cc) to the same 26 serums; in only one case where prednisolone was active, was there a displacement of bilirubin by the addition of 1.4 mg promethazine. Sephadex G-25 chromatography of 18 serums after the addition of prednisolone, mepiridine or promethazine showed no retention of bilirubin. Salicylate, being a more potent bilirubin-albumin unbinder has always produced a retention of bilirubin by Sephadex. The use of glucocorticosteroids in icteric newborns must be considered with caution.

EXPERIENCE WITH THE SEPHADEX GEL FILTRATION ASSAY FOR RESERVE BILIRUBIN BINDING CAPACITY IN NEWBORNS. Claire Dupont, Jeffrey Allen, and Eva Sarkozy, McGill Univ., Montreal Children's Hospital, Dept. of Biochemistry, Montreal, Quebec.

Over a one year period, 44 bilirubin binding assays were requested on premature and sick newborns. Total serum bilirubins ranged from 7.6 to 25.4 mg% with reserve bilirubin binding capacities ranging from -4 to 18 mg%. The saturation points ranged from 7.6 to 29.4 mg%. 85% had a saturation point greater than 15 mg%, with 50% greater than 20 mg%. Only a single baby had a saturation point less than 10 mg%. None of the babies with negative binding capacities showed clinical evidence of kernicterus. Several babies placed on phototherapy showed no change in their saturation points when assayed before, during, and following treatment, thus indicating that the breakdown products of bilirubin or other effects of phototherapy do not interfere with bilirubin binding to albumin.

"SINGLE-POINT" SEPHADEX GEL FILTRATION ASSAY FOR ESTIMATION OF RESERVE BILIRUBIN BINDING CAPACITY. Claire Dupont and Eva Sarkozy, McGill Univ., Montreal Children's Hospital, Dept. of Biochemistry, Montreal, Quebec.

The sephadex G-25 elution method for assay of reserve bilirubin binding capacity has proven to be a valid and useful technique for evaluating the danger level of hyperbilirubinemia in the newborn. A major disadvantage of the method is the relatively large volume of serum required from very small newborns who usually have high hematocrits. A modification of the method has been developed which requires only 0.5 ml total serum. A concentration of bilirubin is chosen to add to half the sample such that the binding capacity will be closely approached but not exceeded, and the optical density of this eluted sample is compared to that of the patient's own serum blank. The bilirubin binding capacity is thus expressed as "greater than" or "less than" the concentration of bilirubin added. Results comparing the same sera using the "single-point" method and the classical 3 or 4 point assay show excellent agreement, thus confirming the "single-point" assay as a valid and useful alternate method when sample size is limited.