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PLASMA AND LIVER LEVELS OF VITAMIN K IN THE NEWBORN. P.McCarthy (1), G.Gau (2) and M.Shearer (1). Dept. of Haematology, Guy's Hospital, London SE1 9RT, U.K. (1) and Dept. of Histopathology, Queen Charlotte's Maternity Hospital, London W6, U.K. (2).

Few measurements have been made of vitamin K in neonatal tissues. Using an assay based on HPLC with dual-electrode electrochemical detection we have investigated the vitamin K status of the newborn from analyses of paired cord-maternal plasma samples and liver samples obtained at post-mortem. For vitamin K_1 (K_1) the median value in cord plasma (16 pg/ml, range 4-45 pg/ml) in 20 babies was some 30 fold lower than that in maternal plasma (median 0.47 ng/ml , range 0.14-2.42 ng/ml). This is the highest maternal-cord gradient of all the fat-soluble vitamins and together with the lack of correlation between cord and maternal values suggests that K_1 does not rapidly equilibrate across the placenta. Hepatic neonatal-adult differences in K_1 levels were less marked being about 5 fold lower at birth (median 1.2 ng/g, range 0.1-8.8 ng/g, n = 22) than in adults (median 5.4 ng/g, range 1.1-21.3 ng/g, n = 32). No relationship was found between hepatic K_1 and gestational age and relatively high levels (1-2 ng/g) were detected at 10-12 weeks gestation. Post mortem livers obtained after intramuscular K_1 prophylaxis at birth (0.5-1.0 mg) had K_1 levels which were raised dramatically (1000 to 5000 fold after 24-48 h) and which remained raised for at least one week. A preliminary assessment of the contribution of vitamins K_2 (menaquinones, MKs) to vitamin K status revealed undetectable levels in fetal or neonatal livers until about 14 days post-partum. This was in marked contrast to adults in whom MKs 7-10 accounted for the majority of liver vitamin K (75-97% on a molar basis). In adult plasma MKs were present at much lower levels than K_1; the low circulating levels and poor placental transport would explain our inability to detect MKs in newborn livers. When expressed as total vitamin K (K_1 and MKs) we concluded that the newborn may have only about 2% of adult hepatic concentrations; this relative deficit of MKs may be responsible for the high susceptibility of the newborn to vitamin K deficiency.

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ANTITHROMBOTIC PROPERTIES OF HEPARIN IN A NEONATAL MODEL OF THROMBIN INDUCED VENOUS STASIS THROMBOSIS. B. Schmidt (1), M.R. Buchanan (1), F. Ofosu (1,2), L.A. Brooker (1), M. Andrew (1). McMaster Univ.(1), Canadian Red Cross Society, Blood Transfusion Service (2), Hamilton, Ontario, Canada.

Anecdotal clinical experience suggests that more heparin is required in newborn infants that in adult patients to effectively treat thrombotic disease. We compared the ability of heparin to inhibit thrombus formation induced by a pathological bolus of thrombin and stasis in newborn piglets and 3 week old pigs. The coagulation system of the newborn piglet closely resembles that of the human neonate including low antithrombin III (AT-III) activity (0.5U/ml). By 3 weeks, adult porcine values for coagulation factors and inhibitors are reached, while blood volume/kg body weight still approximates that of the newborn piglet. Piglets and pigs were pretreated with saline, 10 or 25U/kg heparin (n≥16/group/dose. Following an injection of 100U/kg thrombin, systemic ^{125}I-fibrinogen consumption and local ^{125}I-fibrinogen incorporation into jugular venous stasis thrombi were measured. Peak heparin levels were identical in both age groups (Anti-factor Xa assay and protamine sulphate titration). Heparin was less effective in preventing thrombus formation in piglets than in pigs (Table). Heparin was also less effective in preventing systemic neonatal ^{125}I-fibrinogen consumption ($p<0.0001$ at both heparin doses).

Table: Percent ^{125}I-fibrinogen incorporation into thrombi ($\bar{x}\pm SD$)

Heparin	Pigs	Piglets	2-tail p
None	62±18	66±20	0.559
10U/kg	48±26	64±23	0.081
25U/kg	24±19	41±21	0.032

Raising AT-III levels to adult values significantly improved the antithrombotic properties of heparin in neonatal piglets. Thrombus formation was completely abolished in 19 or 22 piglets who received a combination of human or porcine AT-III concentrate and 25U/kg heparin. Raising the heparin dose to 50U/kg had the same effect. We conclude that the efficacy of heparin in neutralizing thrombin is decreased in newborn piglets. Treatment with AT-III concentrate overcomes this relative heparin resistance, and may help reduce high heparin doses otherwise required in neonatal thrombotic disease.

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FREQUENCY AND IMPLICATIONS OF SEVERE NEONATAL PROTEIN C DEFICIENCY. M.J. Manco-Johnson, T.C. Abshire and L.J. Jacobson. Dept of Pediatrics, Univ Colorado School of Med, Denver, CO, USA.

The newborn infant has a physiologically low level of protein C which rises very slowly in postnatal life. The frequency and significance of severe neonatal protein C deficiency has not been reported. In this study, protein C levels were measured in 110 newborn infants at the time of birth using functional (amidolytic, Cact) and immunologic (Laurell rocket, Cag) assays. The protein C levels were compared with a marker of thrombin activation (D-dimer fragment of fibrin, +D-D) and infants were subsequently followed for signs and symptoms of thrombosis. Results are summarized below (protein C levels are expressed as U/ml).

Group	N	$\bar{x}$Cact	$\bar{x}$Cag	C<0.20	C<0.10	+D-D	Thrombosis
Term, well	36	0.40	0.39	0%	0%	10%	0%
Preterm, well	18	0.30	0.32	23%	0%	10%	0%
Fetal distress	20	0.33	0.28	20%	10%	45%	0%
Sick preterm	14	0.18	0.18	57%	7%	71%	30%
Twins, all	21	0.13	0.16	62%	48%	24%	43%
≥35 weeks	7	0.18	0.18	57%	57%	-	43%
<35 weeks	14	0.11	0.15	64%	43%	-	43%

Thirteen infants had protein C levels compatible with the homozygous deficiency state. Extremely low levels of protein C (<0.20 U/ml) were not found in well term infants and were rarely noted in stable preterm infants. D-D were infrequently present and no thrombosis occurred. Near term infants born with fetal distress frequently showed +D-D but rarely demonstrated extremely low levels of protein C. None of these infants required indwelling arterial catheters and no thromboses occurred. Preterm infants with severe respiratory distress showed lower protein C levels at birth ($p<0.01$). Although 71% had +D-D, thromboses in these infants were all related to invasive catheterizations. In contrast, the study population of twins demonstrated a high frequency of severe protein C deficiency with negative D-D and frequent thromboses, three of which occurred in the absence of instrumentation. In summary, severe protein C deficiency and thrombin activation are common in sick preterm infants with the risk of thrombosis increased by intravascular catheterization. In contrast, twins with severe protein C deficiency may manifest a thrombotic risk which is independent of thrombin activation or catheterization.

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LOW TOTAL PROTEIN S ANTIGEN BUT HIGH PROTEIN S ACTIVITY DUE TO DECREASED C4b-BINDING PROTEIN (C4b-BP) LEVELS IN NEWBORNS. H.P.Schwarz (1),W.Muntean (2).II.Med.Univ. Klinik, Vienna (1), Univ.Kinderklinik Graz (2), AUSTRIA

Vitamin K-dependent coagulation proteins are known to be decreased in the neonatal period. So far no data have been published on protein S (PS), the vitamin K-dependent cofactor for the antithrombotic enzyme, activated protein C (APC) in this period. We determined, therefore, PS antigen, PS activity and C4b-BP,a regulatory protein of the classical complement pathway to which PS is complexed, in 36 neonates. Total PS antigen in newborns was below the range associated with thromboembolism in patients congenitally deficient in this protein (22±9.6%, mean±SD). None of these infants had clinical or laboratory evidence of thromboembolism or DIC. In contrast to the PS antigen level PS activity measured by the ability of APC to prolong the clotting time of a modified APTT assay using PS-immunodepleted plasma was significantly higher (77.6±14%, mean±SD, p< 0,001), suggesting a shift in PS to the free form. In fact two dimensional immunoelectrophoresis studies revealed the absence of protein S-C4b-BP complexes and only one precipitation indicating free PS was seen in 15 out of the 36 infants. In these 15 neonates C4b-BP was below the limit of detection by sensitive quantitative immunoblotting techniques using monoclonal or polyclonal antibodies. In the remaining 21 infants PS-C4b-BP complexes were detected, but in contrast to adult normal plasma approximately 80% of PS was found in the free form. Mixing experiments with normal human plasma and newborn's plasma indicate that PS in neonate deficient of C4b-BP can bind normally to C4bp. Absence of C4b-BP did not correlate to gestational age. If an equilibrium distribution of PS between bound and free form regulates the cofactor activity of PS for the anticoagulant and profibrinolytic properties of APC in normal adults, our study demonstrates that the absence of PS-C4b-BP complexes in newborns and the presence of free PS only may contribute to the increased bleeding risk of premature infants.