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EFFECTS OF VITAMIN K ON VITAMIN K DEPENDENT PROTEINS IN NEWBORN INFANTS. K. YAMADA (1), T. MEGURO (1), A. SHIRAHATA (2), T. NAKAMURA (2) and A. ASAKURA (2). Dept. of Pediatrics, School of Medicine, St. Marianna Univ. (1) and Dept. of Pediatrics, School of Medicine, Univ. of Occupational and Environmental Health, Japan (2).

Plasma levels of vitamin K (VK) and VK dependent proteins (factor II, factor VII, factor X, protein C and osteocalcin) were determined before and after VK administration to 22 newborn infants. Vitamin K_2 syrup (2 mg/kg of body weight) was orally administered to 9 healthy premature, 11 high risk and 2 VK deficient infants under 3 days of age. VK families extracted from plasma were separated by high performance liquid chromatography using a Cosmosil 5 C_{18} column, and separated VK families were detected by a fluorometry after their reaction with ethanolic sodium borohydride in a reaction coil connected by one-line to a chromatographic column. Total activity of factor II, factor VII and factor X was assayed by a Normotest (Nyegaard), and protein C was measured by protac/APTT and protac/chromogenic substrate (S-2366) functional assay system (American Diagnostica). Osteocalcin levels were assayed by using of a RIA method before and after the absorption of plasma by hydroxyapatite.

After VK administration, plasma VK_2 (menaquinone-4) content increased from levels less than 0.012µg/ml to levels between 15.9 and 70.9µg/ml, excluding one case in whom plasma VK was not detected after VK administration. Compared with Normotest values and osteocalcin levels of age-matched healthy newborn infants treated without VK, premature, high risk and VK deficient infant levels significantly increased after 24 hrs and after 7 days of VK administration. No correlation was seen between the increase of plasma VK contents and the increase of Normotest values after VK administration. On the other hand, no significant increase of protein C assayed by both methods was observed in healthy premature and high risk infants after VK administration.

These results indicate that the change of protein C after VK treatment is different from that of factor II, VII, X and osteocalcin.

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LOW PLASMA CONCENTRATIONS OF C4b-BINDING PROTEIN AND VITAMIN K-DEPENDENT PROTEIN S IN PRETERM INFANTS WITH DECREASED FORMATION OF PROTEIN S-C4b-BINDING PROTEIN COMPLEXES. J. Malm (1), R. Bennhagen (2), L. Holmberg (2) and B. Dahlbäck (1). Department of Clinical Chemistry, University of Lund, Malmö General Hospital, 214 01 Malmö, Sweden (1) and Department of Paediatrics, University Hospital, 221 85 Lund, Sweden (2).

Protein S is a vitamin K-dependent plasmaprotein functioning as a non-enzymatic cofactor to the activated form of protein C in the degradation of coagulation factors V_a and $VIII_a$. In the circulation approximately 60% of protein S is complexed to the complement protein C4b-binding protein (C4BP). Only the remaining, free fraction exhibits protein C_a cofactor activity.

The plasma concentrations of protein S and C4BP were determined in 25 term and 26 preterm infants. Both proteins were quantified with radioimmunoassays. The free, functionally active form of protein S and the total protein S concentration were determined separately. The level of C4BP in preterm infants was found to be very low (mean 6% of the adult level). In term infants the level had increased to a mean of 18%. Also the total concentration of protein S was decreased in preterm as well as in term infants; 18% and 31% of the adult level, respectively. Free protein S was the predominant form in plasma representing 83 % of total protein S in preterm and 68 % in term infants. This was probably due to the very low C4BP levels. In adult controls the corresponding value was 34%. The plasma concentration of free protein S in preterm and term infants, when compared to the adult level, was 44% and 66%, respectively. These results demonstrate that although the total protein S concentration in preterm and term infants was very low when compared to adult levels, the difference in the concentration of free, functionally active protein S between infants and adults was less pronounced.

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LEVELS AND DISTRIBUTION OF FREE AND C4b-BP-BOUND-PROTEIN S IN HUMAN FETUSES AND FULL-TERM NEWBORNS. P. MOALIC (1), Y.GRUEL (1), P. FOLOPPE (1), B. DELAHOUSSE (1), G. BODY (2), J. LEROY (1). (1): Lab. Hématol. CHU Trousseau, TOURS FRANCE. (2): DPT. Gynecol. Obstet. CHU Bretonneau, TOURS, FRANCE.

Levels and plasmatic distribution of protein S were studied on umbilical cord plasmas from 25 normal full-term newborns (N) and 15 normal fetuses (F) between 20 and 30 weeks of gestation. Samples from fetuses were collected for antenatal diagnosis by direct puncture of the umbilical vein under high resolution real-time ultrasound. Total protein S (PS) level was determined using Laurell rocket immuno-electrophoresis (Diagnostica Stago, Asnières-France). Free PS was measured using this latter method, after precipitation of C4b-BP-bound-PS by polyethylene glycol (PEG). Normal pool plasma, treated as well, was considered as the reference curve. C4b-binding protein (C4b-BP) determinations were conducted by Laurell rocket immunoelectrophoresis. The qualitative distribution of free PS and C4b-BP-bound-PS in plasma was also assessed by crossed-immunoelectrophoresis (CIE). Results (mean ±SD) were expressed in percentage, in relation to healthy adults values (n = 15). Low levels of total PS were obtained in all fetuses (16.4 ±4.2) and newborns (36.4 ±9.5) as compared to adults (91.6 ± 12.2). Free protein S level was also decreased both in fetuses (22.2 ±6.0) and newborns (48.5 ± 12.1 versus 89.4 ± 26.3 in adults). At these stages of development, the ratio Free PS / Total PS (both values were obtained according to a reference curve performed with a normal adult pool plasma untreated by PEG) was significantly higher as compared to normal adults (0.82 ±0.07 in F, 0.64 ±0.17 in N and 0.39 ±0.11 in A, $p<0.001$, Student t test). The predominance of free PS was also visualized in the CIE patterns. These data may be explained by undetectable C4b-BP in 21-week old fetuses (<2% in 10 cases). After the 26th week of gestation C4b-BP level was 7.8 ±7.4 (n=5) and reached a value of 19.2 ±15.6 in newborns (adults = 95.7 ±14.7). In human fetus and newborn, PS essentially circulates under free form and this might compensate the decrease of the total PS level.

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α_2-MACROGLOBULIN IS A MORE IMPORTANT INHIBITOR OF THROMBIN IN INFANT PLASMA THAN IN ADULT PLASMA. F. Fernandez, B. Schmidt, M. Andrew, F.A. Ofosu, CRCS, BTS and the Depts of Path & Peds, McMaster University, Hamilton, Ontario, Canada.

The concentrations of the three major inhibitors of thrombin (IIa) differ significantly in adult and infant plasma. The extent to which these differences contribute to the rates and profiles of IIa inhibition in infant and adult plasma is unknown. We determined this by adding 2 NIH U/mL of ^{125}I-human α-IIa to an equal volume of defibrinated plasma for 30s at 37°C. After SDS-PAGE and autoradiography, free IIa and complexes of IIa with antithrombin III (IIa-ATIII), heparin cofactor II (IIa-HCII) and α_2-macroglobulin (IIa-α_2M) were quantitated in 3 types of pooled plasmas: cord; 6 month old infant and adult. Thrombin was inhibited more slowly in cord plasma than in adult plasma ($p<0.001$). In addition, while ATIII was the major inhibitor of IIa in adult plasma, α_2M was equally as important as ATIII in cord and infant plasmas ($p<0.001$). When cord plasma was supplemented with purified ATIII, the extents and profile of IIa inhibition by cord and adult plasmas were indistinguishable. The addition of heparin (0.2U/mL) accelerated the inhibition of IIa by all 3 plasmas, with ATIII the predominant inhibitor. Thus, 83% of the inactive IIa was bound to ATIII in adult plasma, 77% in each of cord and 6 month old infant plasmas. However, significantly more IIa was inactivated by adult plasma than the other two plasmas. These results suggest that cord plasmas are intrinsically less able to inactivate IIa than adult plasma. While the overall ability of the plasma of 6 month old infants to inactive IIa is comparable to adult plasma, the profile of inhibition of IIa in the former, both in the presence and absence of heparin, is more closely related to neonatal than adult plasma, likely reflecting high α_2M levels.

	IIa Inhib	ATIII U/mL	IIa-ATIII	HCII U/mL	IIa-HCII	α_2M U/mL	IIa-α_2M
Adult	38.1	1.0	59	1.0	11	0.9	31
Cord	30.8	0.6	44	0.4	8	1.4	48
6 Mths	36.9	1.0	46	1.2	9	1.9	45
Cord+ATIII	38.2	1.1	61	0.4	7	1.4	32