285 RHEOLOGICAL PROPERTIES OF BLOOD IN NEONATAL SEPTICAEMIA: AN OVERIEW.

R.P.A. Rivers, I. Wright, O. Linderkamp*: Dept. Pediatrics, St. Mary's Hospital, Med. School, Praed Street, London W2, UK, *Children's Hospital University of Heidelberg, FRG

This review focusses on alterations of rheological properties of blood which might contribute to the development of circularoty deterioration in neonatal septicaemia. The following alterations in rheological properties have been shown in septicaemia: 1. Decrease in RBC deformability. RBC deformability is determined by the shape and geometry (in particular, by the excess surface area) and by membrane and cytoplasma properties. We have shown that in severe neonatal septicaemia RBC may lose membrane as well as cell water. This results in decreased excess membrane surface and increased internal viscosity. Moreover, endotoxin may markedly decrease RBC deformability in vitro and in vivo. This appears to be due to a loss in membrane elasticity. A decrease in RBC deformability may also occur in necrotizing enterocolitis as a result of either endotoxin-mediated membrane destruction or neuraminidase-induced loss of membrane charge. 2. Increase in RBC aggregation. RBC aggregation is principally caused by plasma macromolecules (e.g., fibrinogen). In neonatal septicaemia, plasma fibrinogen may increase. Moreover, in neonates with septicaemia RBC aggregation is more enhanced than expected from the rise in plasma fibrinogen. We have shown that fibrin-monomer- fibrinogen complexes induce a marked rise in RBC aggregation in both neonatal and adult blood. Since the complexes are formed as a result of endotoxinaemia, they might contribute to the increase in RBC aggregation in neonatal septicaemia. These data indicate that altered rheological blood properties may contribute to circulatory compromise in septicaemia.

286 RHEOLOGICAL PROPERTIES OF ERYTHROCYTES IN PATIENTS WITH CONGENITAL DYSERYTHROPOETIC ANEMIA II.

M. Koehler, W.E. Brandeis, L. Schmidt-Riese, D.E. Müller-Wiefel, O. Linderkamp. Children's Hospital University of Heidelberg

The aetiology of congenital dyserythropoetic anemia (CDA) II is unknown. The diagnosis is based on morphologic and immunologic criteria. We present three girls with well docomented CDA II who were followed for 5 to 8 years. The anemia was always mild and the body iron status (assessed by serum ferritin, serum iron and transferrin) was normal. In none of the girls was splenectomy indicated. Morphologic features of erythrocytes and bone marrow erythoid cells were studied by means of light and electron microscopy. 15-3o% of the erythrocytes showed invaginations with endocytic cysterns and shape abnormalities (echinocytosis, anisocytosis, microcytosis). Erythrocyte deformation and aggregation were studied in the three patients by means of rheoscope techniques. Erythrocytes with abnormal shape showed significantly decrease deformability, particularly at low shear stress. At low shear stress, up to 15% of erythrocytes did not deform at all. At high shear stress, both shape abnormalities and erythrocyte rigidity were less pronounced. Erythrocyte aggregation studied under standard conditions was slightly increased. This agrees with previous findings of decreased neraminic acid in erythrocyte membrane of these patients. Our studies indicate that both decreased erythrocyte deformability and increased erythrocyte aggregation may contribute to the shortened life span of erythrocytes in patients with dyserythropoetic anemia type II.

287 ASSOCIATION OF INCREASED Na^+-INFLU AND LACK OF AN INTEGRAL ERYTHROCYTE MEMBRANE PROTEIN .N HEREDITARY STOMATOCYTOSIS.

S.W. Eber[1], C.R. Bartram[2], P. Höhn[1], W. Schröter[1], Departments of Pediatrics, Universities of Göttingen[1] and Ulm[2], FRG.

Hereditary stomatocytosis is a hemolytic anemia caused by an increased Na^+ influx and consecutive hydrocytosis of the erythrocytes. In search of the pathogenesis, the erythrocyte membranes of two unrelated splenectomized patients suffering from severe stomatocytosis were analyzed by an analytical SDS polyacrylamid gradient gel electrophoresis. A diminution of the erythrocyte membrane protein band 7.2 (M_r ~ 28000) was detected. By separating the integral proteins in the outer plasma membrane (Low ionic strength extraction) from the peripheral proteins in the inner cytoskeleton (Triton X-100-extraction) we found that band 7.2 normally consists of two fractions. 50% represent a peripheral membrane protein (7.2a), while the other half is embedded in the lipid bilayer (7.2b). The investigation of the red cell cation fluxes showed, that the passive influx of Na^+ was disproportionately increased (50-fold) compared to only a slightly enhanced passive efflux of K^+ (5-fold). Inspite of an elevated total Na^+ efflux, there is a net Na^+ influx of 17.8 mval/l cells x hour. The heterozygous parents, who are clinically unaffected, compensate their 3-fold increase of passive Na^+ influx by an increased total Na^+ efflux. It is suggested that by the absence of band 7.2b an oligomeric complex of transmembrane proteins, responsible for the passive Na^+ influx, may desintegrate and thereby allow an enhanced Na^+ influx into the stomatocytic erythrocyte. The lack of band 7.2b, proven by SDS-polyacrylamidgel electrophoresis, should be a valuable diagnostic for hereditary stomatocytosis.

288 ERYTHROCYTE DEFORMABILITY AND PLATELET FUNCTION IN CHILDREN WITH CYANOTIC CONGENITAL HEART DISEASES.

A. Menichelli, V. Bastianon, V. Colloridi, G. Bosco, P.M. Strappini, and D. Del Principe. Dept. of Pediatrics, University of Rome, Rome, Italy.

Children with erythrocytosis due to cyanotic congenital heart diseases (CCHD) show an increased tendency to thrombotic disorders. We studied red cell deformability and 2,3-DPG concentration, platelet aggregation and platelet release in 18 children with CCHD (PaO_2 <7o mm Hg). Erythrocytosis (Ht>55%) was present in 3 patients. Red cell deformability was measured by a filtration technique. Platelet aggregation was assessed by the change in light transmission of platelet-rich plasma after addition of ADP (1-3 µM). Plasma β-TG level was determined by a radioimmunoassay, and 2,3-DPG by enzymatic analysis. Filtration time was 8o±1o sec/ml (mean±SD) vs 3o±8 sec/ml ($p<o.o1$). 2,3-DPG was not significantly increased: 4.83±o.15 mmol/l erythrocytes (mean±SD) vs 4.18±o.1. In all samples platelet aggregation was impaired, while plasma β-TG was increased: 55±5µg/ml (mean± SD) vs 2o±2, $p<o.oo1$. No correlation was demonstrated between red cell deformability and erythrocytosis or 2,3-DPG content. Our data show that rigid red cells and "exhausted" platelets (as suggested by impaired in vitro aggregation and increased plasma β-TG level) may occur in patients with CCHD also in absence of severe hypoxia.

289 DEFORMABILITY AND IN VITRO FLOW PROPERTIES OF IRON DEFICIENT RED CELLS

O. Linderkamp, H.C. Sengespeik, H.J. Klose, K. Betke. Children's Hospital, University of Heidelberg, Children's Hospital University of Munich, FRG

Previous studies have shown that iron deficient red blood cells (RBC) are less deformable than normal cells (Yip et al. Blood 62:99, 1983). RBC from 2o children with severe iron deficiency and from 2o healthy adults were used to study deformability (rheoscope), geometry (micropipette), membrane viscoelasticity (micropipette) and the Fahraeus-Lindqvist effect (decrease in blood viscosity as tube diameters decrease from 5oo to 5oµm). RBC deformability decreased as the MCV decreased ($p<o.o5$). The excess surface area of iron-deficient RBC was up to 2o% larger than that of normal RBC ($p<o.o5$). The membrane elastic shear modulus of iron-deficient RBC was by 2o% increased (i.e., elasticity was impaired) whereas the time constant for recovery from extensional deformation was shortened by about 2o% when compared to normal RBC. Membrane viscosity (computed as product of the elastic shear modulus and the time constant) was similar for both cell types. Viscosity of normal blood decreased by 31% when the tube diameter was decreased from 5oo to 5oµm, whereas the decrease in blood viscosity was only 25% for iron deficient cells ($p<o.o5$). Thus,the Fahraeus-Lindqvist effect was less for iron-deficiency.We conclude that iron-deficient RBC show several peculiar properties which may impair blood flow particularly when the hematocrit is increased (e.g., in patients with chronic hypoxemia or primary polycythemia).

290 RISK FACTORS FOR ERYTHROCYTES DURING FIRST DAYS OF LIFE.

B Talluri, G Buonocore, G Martini, S Berni, M Strambi, M Longini, R Bracci (Introduced by O Linderkamp).Division of Neonatology, Inst. Puericultura,University of Siena.Dept. Chemistry,University of Florence. Italy.

In an attempt to clarify the risk factors involved in the decreased survival of the red cells of the neonate,a comparison of erythrocytes in cord blood and those in 4 day olds was performed.The following determinations were carried out:Hb,Htc,MCV,electron spin resonance spectroscopy of nitroxide radical Mal-6,age dependent and oxygen scavenger erythrocyte enzyme activities G-6-PD,PK,GPI,GSH-Px,SOD and CAT;F and A_1 Hb percentages.The findings were as follows:1)lower reduction kinetics of the specific -SH reagent Mal-6 in the cord blood.This finding suggests limited availability of reducing agent in cord blood or decreased membrane permeability there to.2)Normalization of Mal-6 kinetics after resuspending cord blood red cells in the plasma of 4 day old infants. It is evident that significant modifications of the newborn's erythrocytes are induced by plasma factors.3)Increase in the mean oxygen scavenger activity GSH-Px from birth to 4th day of life.This is most likely due to the disappearance from the circulation of the red cells less resistant to oxidative stress.4)Increase in F Hb during the first 4 days of life.

The results of the present investigation demonstrate that a certain number of erythrocytes viable uder intrauterine conditions,are removed from circulation after birth.