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PROTEIN C AND OTHER CLOTTING STUDIES IN MEMBRANOUS AND NON-MEMBRANOUS GLOMERULONEPHRITIS. M. Myśliwiec (1), D. Alderson (1), L. Poller (1) and P. Ackrill (2). Haematology Department (1) and Renal Unit (2), Withington Hospital, Manchester, U.K.

The occurrence of thrombosis in the nephrotic syndrome has long been known. Thrombotic complications are predominantly associated with membranous glomerulonephritis (MG). The aim of the present work was to study whether the tendency of nephrotic patients with MG to thrombotic episodes could be attributed to a hypercoagulable state. Thirty consecutive patierts with the nephrotic syndrome were studied. Of these 17 suffered from MG and 13 had other forms of glomerulonephritis. The control group consisted of 10 healthy volunteers. In addition to standard coagulation assays, we studied: soluble fibrin monomer complexes (FM test, Boehringer), fibrin monomer polymerization, factor VIII:C, factor VIII:vWF, antithrombin III (AT III) and alpha$_2$ antiplasmin (alpha$_2$AP) using chromogenic substrates; the levels of AT III and alpha$_2$AP were measured immunologically; beta thromboglobulin (BTG), platelet factor 4 and fibrinopeptide A (FPA) using radioimmunoassay kits; protein C was studied functionally and immunologically. There was a significant shortening of the prothrombin time and activated partial thromboplastin time, increase in alpha$_2$ AP, factor VIII:vWF, FPA and BTG in nephrotic patients associated with increases in both functional and immunological protein C levels and impairment of fibrin polymerization. FM test was negative in all but one of the patients. None of the coagulation tests showed a significant difference in the two nephrotic groups. High protein C and impaired polymerization may be considered as mechanisms counteracting disclosed hypercoagulability in the nephrotic syndrome.

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THROMBO-HEMORRHAGIC SKIN NECROSIS DUE TO RAPID DEVELOPMENT OF SEVERE VITAMIN K DEFICIENCY ASSOCIATED WITH CHOLESTASIS. J.J. Michiels, R.M. Bertina. Department of Hematology, University Hospital Dijkzigt Rotterdam and Hemostasis and Thrombosis Research Unit, Department of Hematology, University of Leiden, The Netherlands.

A female patient is presented, who developed thrombotic and hemorrhagic skin necrosis of the feet and toes during an acute episode of severe vitamin K deficiency due to cholestasis in the absence of coumarin treatment. Painful blue toes and feet progressed to erythematous swelling and bluish discoloration with blister formations and immanent gangrene. The histopathology of skin excisions from the erythematous skin lesions showed extravasation of erythrocytes and extensive fibrin thrombus formations in capillaries and venules as has been described in patients with coumarin skin necrosis. At the time of painful acrocyanosis the results of coagulation investigations (platelets 109 × 10^9/l, APTT 56/35 sec., PT 61/15 sec., Thrombotest^R (TT) less than 3%, Normotest^R (NT) less than 10%, fibrinogen 1,6 g/l, absence of fibrin monomers and degradation products, factor V 1.00 u/ml, antithrombin III 1.03 u/ml and alfa$_2$ antiplasmin 0.97 u/ml were consistent with severe vitamin K deficiency. Measurements of vitamin K dependent factors revealed very low levels for procoagulant factor VII (16%) and protein C (22%) antigen concentration and normal levels for procoagulant factor II (97%) and procoagulant factor X (87%) antigen concentration. After substitution of 5 mg vitamin K1 both the TT and NT normalized.These data confirm the hypothesis that an imbalance within procoagulant and anticoagulant vitamin K dependent factors (severe protein C and factor VII deficiency as compared to the procoagulant factors IX, X and II due to rapid development of vitamin K deficiency) contributes to the pathogenesis of thrombo-hemorrhagic skin necrosis and that the so-called coumarin necrosis is not due to drug toxicity.

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NORMAL PREGNANCY AND DELIVERY IN A PATIENT WITH SEVERE PROTEIN C DEFICIENCY AND PREVIOUS DEEP-VEIN THROMBOSIS. H. Bounameaux, Ph. de Moerloose, J. Vogel, G. Reber, B. Krahenbuhl and C. Bouvier Angiology and Hemostasis Units, University Hospital of Geneva CH-1211 Geneva 4, Switzerland

Congenital protein C (PC) deficiency is associated with thrombophilia. Heterozygotes with about half-normal plasma PC levels may present with venous thromboembolic events usually beginning during adolescence or young adulthood. A 26-year-old swiss woman had experienced an iliofemoral deep-vein thrombosis without obvious etiology six years ago. In June, 1986 very low levels of PC antigen (25 %) and activity (27 %) were found when she was six-month pregnant. Three other family members (62, 24 and 19-year-old) had PC levels around 50 % but were symptomfree. Because of the post-thrombotic syndrome and the pregnancy, ambulatory heparin therapy was immediately started in the patient (10-16'000 IU twice daily, s.c.) in order to maintain a plasma heparin level between 0.2 and 0.5 U/ml six hs after the morning injection. Delivery was induced at full-term whilst heparin was stopped for a few hours. Four weeks after delivery anticoagulation was discontinued and, so far, the patient remained symptomfree. The newborn showed no perinatal problem and the PC antigen level assayed in the umbilical venous blood was 22 % (normal range in the literature 18-46 %). Antithrombin III and protein S levels as well as fibrinolytic potential were within normal values in all family members.

Conclusions. 1) Only one out of four heterozygote PC deficient family members had presented with venous thromboembolism. 2) The symptomatic subject had the lowest PC level in the family (activity and antigen around 25 %). 3) This woman experienced an event-free pregnancy and delivery although heparin was started only in the sixth month of pregnancy. 4) Thus, penetrance of the thrombotic trait may be quite variable (and low) amongst PC deficient heterozygotes, an observation which raises the question of the indication of long-term anticoagulation in these individuals. 5) Pregnancy did not affect the PC antigen and activity levels in our patient.

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DEVELOPMENT OF A PROTEIN C CONCENTRATE. Prabir Bhattacharya, Carolyn L. Orthner and Dudley K. Strickland. American Red Cross Laboratories, Rockville, MD 20855, U.S.A.

A Protein C (PC) concentrate may be useful in treating patients with congenital or acquired Protein C deficiencies. A method for preparation of a human Protein C concentrate has been developed using a by-product of American Red Cross Factor IX production as the starting material (Menache et. al. Blood, 64, 1220). Levels of other vitamin K dependent proteins in the Protein C concentrate were measured and found to be < 10 units per 100 units of PC, except for Protein S. The level of Protein S as judged by immunological assay was 30 u/100 u PC. Assay of the PC concentrate using chromogenic substrates revealed that levels of thrombin, Factor Xa and Factor IXa were less than 0.006 u/mL. In addition, Antithrombin III and a$_2$-macroglobulin were not detected. The in vivo effects of Protein C concentrate and Protein C activated by thrombin have been tested in anesthetized rabbits. Thrombin was removed from the activated Protein C by ion-exchange chromatography; depletion was verified by S-2238 or by a clotting assay (< 0.006 u/mL). Rabbits were injected with Protein C concentrate (400 ug/kg) or activated Protein C 24 - 48 ug/Kg). The activated partial thromboplastin time (APTT), Factor V (FV) and Factor VIII (FVIII) levels were measured in samples collected over the next three hours. Infusion of PC concentrate elevated the level of PC to 150% of the preinfusion level within 30 min. It did not change the levels of FV, FVIII, fibrinogen or platelet count. In contrast, infusion of activated Protein C produced progressive prolongation of the APTT. Levels of FV and FVIII were decreased to 25% and 50% of preinfusion levels, respectively, three hours after the infusion. Fibrinogen and platelet levels were unchanged during that period. These data demonstrate that activated human Protein C concentrate induces an anticoagulant effect that can be readily measured in rabbits.