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STUDY OF A SPANISH FAMILY WITH INHERITED PROTEIN S DEFICIENCY.
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Protein S (PS) is a plasma glycoprotein that serves as a cofactor for activated protein C (PC) anticoagulant activity. Inherited PS deficiency has been found to be associated to thrombotic disease in several families. In the present study, we report on a Spanish family with type II PS deficiency.

The propositus is a 40 year-old male that was referred to our center for study after having suffered from multiples thrombotic events since he was 20 year-old. After his first episode of deep vein thrombosis (DVT) he had 4 recurrences, three of them complicated with pulmonary embolism. It should be remarked that one of the episodes occured while the patient was under oral anticoagulant treatment. The basic screening of haemostasis and hepatic function were normal for a patient that was being treated with oral anticoagulants. Functional and antigenic levels of antithrombin III, protein C and plasminogen were also normal. When total and free protein S levels (method of Comp et al.) were measured using both an electroimmunoassay and an ELISA assay ,almost indetectable levels of free protein S (between 0 and 10%) and very low levels (20%) of total plasma PS, were found. These results were also confirmed by crossed-electrimmunophoretic studies.

When the family of this patient was studied it was found that his two sons, aged 15 and 8 years, as well as one of his sisters, aged 35 years, and her daughter of 4 years, were also affected (free PS levels between 38-60% and total PS between 35 and 39%). All these members had been assymptomatic up to now and are not under oral anticoagulants.

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HEMATOLOGICAL CHARACTERISTICS OF PREGNANT BLOOD AND LOCALIZATION OF THROMBOMODULIN IN HUMAN PLACENTAL VILLOUS TISSUE.
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Thrombomodulin(TM) is a cell surface protein found on endothelial cell that binds thrombin and increases thrombin's ability to activate protein C(PC). In the present study, we examined hematological characteristics and behavior of plasma PC level in pregnant women and localization of TM in the placental villous tissue. The results obtained are reported here. Cubital venous blood of 20 normal pregnants, 8 purperants and 60 non-pregnants. PC antigen(PC:Ag) was measured by the Laurell's technique using Assera plate-proteinC. Localization of TM was determined in such a way that the villous tissue was fixed in formalin, cut into paraffin sections, and stained by ABC method using anti-TM antibody. Coagulation-related factors of the pregnant blood, i.e. fibrinogen, FV, FVIII, FX, FXII and prekallikren showed statistically higher values compared with the control group, and AT-III showed almost simillar value to the control group or tended to decrease to some extent compared therewith. As for fibrinolysis-related factors, on the other hand, plasminogen, α_1-antitripsin showed higher values with the progress of pregnancy, and α_2-macroglobulin showed slightly lower values in both 3rd trimester and puerperal stage. PC:Ag increased in the 2nd and 3rd trimester ($p<0.01$). Comparative examinations made of PC:Ag level between pre- and post-taking oral centraceptives revealed a significant ($p<0.05$) decrease from 131.0%(pre-taking) to 117.0%(post-taking). At the 11th week of pregnancy, TM was confirmed to be highly localized in syncytiotrophoblast in the villous tissue, especially in microvilli. At the 40th week, TM was also confirmed in the same site, but with weaker stainability. It was suggested that thrombosis- and hemostasis-related factors were in a state of co-existing overproduction and consumption. Moreover we supposed that the existence of TM and the increase in PC might be just appropriate for the maintenance of anti-thrombogenesis in the uteroplacental circulation.

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DETECTION AND EFFECTS OF THROMBOMODULIN ACTIVITY IN CRUDE THROMBOPLASTIN PREPARATIONS FROM PLACENTA AND LUNG.
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The activation of protein C (PC) by thrombin requires the presence of an endothelial membrane cofactor, thrombomodulin (TM). Activated PC (APC) exerts its anticoagulant activity by degrading factors (F) Va and VIIIa in the presence of phospholipids and of a vitamin K-dependent cofactor, protein S. Tissue factor (TF) is the essential cofactor of factor VII/VIIa in the activation of factor X. TF is synthetized by several cell lines including endothelial cells. Using a specific TM assay, up to 0.85 units of TM activity could be detected in commercial thromboplastin (TP) preparations from human placenta or rabbit or porcine lung, when the amount of TP was adjusted to contain 1 unit of TF activity. Preparations from brain contained very low amounts, if any, of this activity (< 0.02 TM units). In order to evaluate the effects of the presence of TM activity in some TP preparations, the stability of F V and VIII activities was examined after activation of the coagulation system by these TP in various plasmas. PC deficient plasmas, plasmas lacking F V, VIII or IX and immunoadsorbed PC deficient plasma supplemented with purified human PC (5 µg/ml) were used. After activation with placenta or lung TP, F V and VIII activities were markedly reduced (~ 90 % reduction) in normal and hemophiliac plasmas, whereas they remained high after activation with brain TP. F V and VIII activities were preserved in protein C deficient plasma after activation by all TP preparations. The same decrease of F V and VIII activities was observed after activation of immunoadsorbed PC deficient plasma supplemented with purified PC with placenta or lung TP only. Preincubation of TP from human placenta with antibodies to human TM raised in laying hens abolished the capacity of this preparation to destroy F V activity of PC containing plasmas. These results establish the presence of TM activity in crude thromboplastin preparations from placenta or from lung. Surprisingly, this anti-coagulant activity seems to be absent from brain. TM from placenta or lung extracts is responsible for the degradation of F V and VIII.

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CONCENTRATION DEPENDENCE OF ACTIVATION OF ACARBOXYPROTEIN C BY THE CONTORTRIX ACTIVATOR.
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The protein C activator in Southern Copperhead (Agkistrodon Contortrix Contortrix) venom was isolated by sequential chromatographies on SP-Sephadex, Con A Sepharose and hydroxylapatite. It was found to be a single chain glycoprotein with an apparent molecular weight of 36,000 and an enzymatic specificity on chromogenic substrates resembling kallikein.

This "contortrix activator" was used in a solid-phase immunochromometric assay (ICMA) for functional protein C in which heterologous antibody against protein C was passively coated onto microtitre wells and used to immoblize protein C. This was then activated, easily freed of excess activator by washing and assessed by its subsequent overnight cleavage of chromogenic substrates sensitive to activated protein C.

Correlation between protein C results obtained by ICMA and immunoradiometric assay (IRMA) on a variety of patient samples was excellent when relatively high concentrations of the venom activator was used. However with lower concentration of activator plasmas from patients deficient in vitamin K gave lower protein C values by ICMA then obtained by IRMA.

Normal protein C and "acarboxy" protein C from a patient on oral anticoagulant therapy were immuno-immobilized and studied by the ICMA technique using varying concentrations of the venom activator. The acarboxy-protein C, although completely activatable by high concentrationa of activator, was found to activate much more slowly than normal protein C at low concentrations of the contortrix activator. Thus by reducing the intensity of the activation step, the ICMA protein C results were increased in their sensitivity for functional protein C.