

BACTERIAL HEPARINASE PERMITS QUANTITATIVE IN VITRO ASSAYS OF CLOTTING FACTORS, AND NEUTRALIZES HEPARIN IN VIVO. Henry S. Kingdon. University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, U.S.A.

We have reported previously that preparations of heparinase from the bacterium F. heparinum eliminate heparin prolongation of partial thromboplastin times and prothrombin times (J. Lab. Clin. Med. 79, 1027, 1972). Therefore, when the PTT is prolonged, this deviation from normal can be shown to be due to heparin (or otherwise) before embarking on expensive specific assays to define a possible hemorrhagic disorder. Similarly, the use of heparinase in prothrombin times renders them insensitive to heparin effect and makes them a more accurate measure of the effect of oral anticoagulants. In the current studies, it was demonstrated that quantitative assays of factor VIII in normal human plasma, while uninterpretable after the plasma was heparinized, returned to normal baseline values after the heparinized plasma was treated with heparinase. The quantitative values for factor VIII were identical when heparinized plasma treated with heparinase were compared with the same plasma before heparinization. In other experiments, heparinized rabbits were rendered hemostatically normal within 15 minutes of heparinase infusion, whereas control rabbits exhibited significant heparin inhibition of blood coagulation for 3-4 hours after heparin infusion. Finally, mutants of F. heparinum which produce large quantities of heparinase when grown in the absence of heparin have been isolated and appear to provide an inexpensive and readily available source of heparinase, so that the enzyme might conceivably become commercially available for use in laboratory evaluation of coagulation disorders, and also for definitive studies of its possible therapeutic applications.

DETECTION AND PARTIAL PURIFICATION OF A NATURAL HEPARIN INHIBITOR.

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A naturally occurring heparin inhibitor has been detected in the mucosa of the fresh hog small intestine and has been partially purified. After the homogenized mucosa was extracted with Tris buffer overnight (3^o) and the resulting supernatant was fractionated with ammonium sulfate, a large quantity of antiheparin activity was detected in the ammonium sulfate precipitate. This precipitate contains antiheparin activity with a specific activity of 0.68 unit/mg of protein. Therefore, each hog small intestine contains an amount of this inhibitor enough to inhibit approximately 20,000 units of heparin. Further purification of this heparin inhibitor was carried out by the technique of heparin affinity chromatography (covalently linked the heparin by the cyanogen bromide procedure). Eluted by a controlled NaCl and buffered gradient at 3^o, the chromatogram contains a major peak and a minor peak. Antiheparin activity was located in the minor peak and has a specific activity of 9.7 units/mg of protein. Thus, we have achieved a 14-fold purification of this heparin inhibitor. This partially purified protein inhibits heparin stoichiometrically. Further experiments to purify this heparin inhibitor are in progress. This naturally occurring heparin inhibitor probably has an important biological function in balancing the action of heparin which is an important factor in maintaining blood fluidity.

INTERACTIONS AMONG HEPARIN, COLD-INSOLUBLE GLOBULIN, AND FIBRINOGEN IN FORMATION OF THE HEPARIN PRECIPITABLE FRACTION OF PLASMA. N.E. Stathakis and M.W. Mosesson. State University of New York - Downstate Medical Center, Brooklyn, New York, USA.

Fibrinogen and the cold-insoluble globulin of plasma (CIG) are the main protein components of the heparin cryoprecipitable fraction (HPF) of normal plasma. The interactions between these proteins and heparin were examined. Heparin formed a cold precipitable complex with purified CIG or with mixtures of CIG and fibrinogen ($\tau/2$, 0.2; pH 7.2) but not with fibrinogen alone. Cryoprecipitation could be augmented by addition of Ca⁺⁺ or by selection of optimal heparin levels; it could be reduced or even abolished by raising the ionic strength or pH or both, or by raising the heparin level above that needed for maximum precipitation of CIG. Fibrinogen reduced the threshold level of CIG at which heparin-induced cryoprecipitation occurred and, by co-precipitating with heparin and CIG, increased the total precipitate that formed. In contrast to the HPF from normal plasma which contained both fibrinogen and CIG, that from afibrinogenemic plasma contained CIG but lacked fibrinogen. Normal plasma depleted of CIG failed to form a heparin-induced cryoprecipitate. Thus, CIG is essential for heparin-induced cryoprecipitation to occur. Fibrinogen, as assessed by chromatographic experiments with heparin-Sepharose columns, has a considerably lower heparin-binding affinity than does CIG, indicating that it participates in formation of the HPF mainly, if not entirely, by virtue of its affinity for CIG.