Time

14.30 **0194** PURIFICATION OF A HIGHLY POTENT HEPARIN-LIKE ANTICOAGULANT FROM HEALTHY HUMAN PLASMA

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A highly potent heparin-like anticoagulant (accelerator) has been purified from citrated healthy human plasma. After heat defibrination and $BaSO_4$ treatment on the plasma, the accelerator was adsorbed onto a DEAE-cellulose column and elution was achieved using a high ionic strength linear buffered salt gradient. The eluted accelerator was further purified by polyacrylamide gel electrophoresis. The purified accelerator can accelerate the inhibition of thrombin by antithrombin III and has a specific activity of 226 heparin units/mg of carbohydrate. The accelerator contains a small amount of protein-like material. The amount of accelerator present in healthy human blood is extremely small and can only be first detected in the concentrate after the DEAE-cellulose chromatography. A mere 0.5 mg of the purified accelerator is obtained from 100 ml of human plasma. Concomitant with this investigation, a second heparin-like substance also has been purified but has very low anticoagulant activity in terms of heparin units. The naturally occurring accelerator may function as heparin in the circulating blood and its level in blood may have a clinical significance in thrombotic vascular disease. Further work on its physical and chemical properties is now in progress.

14.45 **0195** ATTEMPTED DETERMINATION OF ENDOGENOUS HEPARIN IN BLOOD

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A method was developed for the micro-scale determination of heparin in blood plasma. After extraction with water-saturated phenol glycosaminoglycans were recovered from the aqueous phase by ion-exchange chromatography on DEAE-cellulose. The heparin content of the product was determined by a colorimetric antithrombin-activation assay. Recoveries of 40-60% of both radioactivity and biological activity were obtained when the method was applied to the reisolation of ^{3}H-labeled heparin, added at a concentration of 20 ng (about 0.003 unit) per ml blood. In the absence of exogenous heparin no antithrombin-activating material could be detected. The amount of endogenous heparin, if any, in human blood was calculated to less than 2 ng (about 0.0003 unit) per ml.

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15.00 **0196** HEPARIN INDUCED RELEASE OF LOW AFFINITY PLATELET FACTOR FOUR

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Heparin has been reported to effect platelet function. We studied the effect of commercial beef lung heparin on the release of a platelet specific protein, the low affinity platelet factor 4(LA-PF4) in vitro and vivo. LA-PF4 shares common antigenic determinants with B thromboglobulin. LA-PF4 was measured in platelet poor plasma (PPP) by radioimmunoassay. The mean levels of LA-PF4 in PPP from citrated blood and citrated blood with added heparin (final concentration 1.5 unit/ml) were 78.3±18.3 and 242.5±117.1 ng/ml respectively. (Mean ± SD, $p<0.01$). Addition of heparin to blood collected in a mixture of EDTA, prostaglandin E_1, and theophylline did not result in an increase in LA-PF4 in PPP. A single intravenous bolus of heparin was administered to two normal individuals and the levels of LA-PF4 measured before and at 15,30 and 180 minutes later. An increase in the levels of LA-PF4 in the PPP from a baseline of 66.7 and 30.2 ng/ml/10^6 platelets to 166.7 and 167.4 ng/ml/10^6 platelets respectively were noted in 30 minute sample. There was no increase in LA-PF4 at 15 minutes. At 180 minutes levels were at baseline. It is concluded that heparin stimulates release of LA-PF4 from platelets both in vitro and in vivo. LA-PF4 has antiheparin action and is being investigated as a marker for thrombosis. Its release by heparin may, therefore, have an important implication.