

THE EFFECTS OF DEFIBRATION PROCEDURES IN PLASMA AT III ASSAYS. R.D. Philo and P.J. Gaffney. National Institute for Biological Standards and Control, London, NW3 6RB, U.K.

Defibrination procedures using heat or anicrod (a coagulant fraction from *Agkistrodon rhodostoma* venom) are commonly used in heparin co-factor AT III assays which use a clotting time endpoint to measure residual thrombin. The test plasma is compared to a freeze-dried standard plasma which has been defined to contain a particular number of AT III units. This study examines the effects of both heat and anicrod defibrination of fresh and freeze-dried plasmas using a chromogenic-based AT III assay system in which no defibrination is necessary.

The mean potency of the heat (56°C x 15 min) defibrinated freeze-dried standard plasma compared to the untreated standard was 0.51 (mean of 9 assays using a multidose bioassay design) whereas the corresponding figure for the frozen test plasmas was 0.72. Thus a test plasma would give falsely elevated readings for AT III compared to a freeze-dried plasma standard. Assays of anicrod (0.55 iu/ml) defibrinated plasma against untreated plasma gave mean potencies not significantly different from 1.0 for both the freeze-dried and frozen plasmas. Thus this form of defibrination allows a more valid comparison of fresh and freeze-dried plasmas.

Analysis of these samples by crossed immunoelectrophoresis in the presence of heparin confirms that the anicrod defibrinated samples are essentially identical to the untreated ones whereas the heat defibrinated samples are altered, the freeze-dried plasmas markedly so.

The data suggest that, should a defibrination step be used in the assay of AT III, the use of anicrod is preferred to heat. The heparin co-factor AT III assay using the chromogenic substrate, S-2238, to measure residual thrombin would seem the preferred method of assay in order to avoid any defibrination step.

## 1172

ENDOGENOUS HEPARIN ACTIVITY IN NORMAL HUMAN PLASMA. Hyman Engelberg and Stephen Lee. Cedars-Sinai Medical Center, Los Angeles, California.

The scientific literature is contradictory as to the normal presence of heparin activity in human blood. The question has physiologic and clinical significance. The purpose of this study was to investigate whether biologic heparin activity was demonstrable in extracts of normal human plasma. The method involved initial precipitation of the plasma proteins by methanol-acetone, proteolysis of the precipitated proteins by papase or trypsin, dialysis of the supernatant, lyophilization, and then assay. The final extract showed heparin activity using the Kabi chromogenic substrate, the activated partial thromboplastin time test, and the anti factor Xa procedure. The level of heparin activity was 10-25 units % (app. 1-2 mg/L of plasma). We conclude that endogenous heparin activity is present in normal human plasma at physiologically significant levels, and that it is protein bound.

## 1171

PURIFICATION OF ANTITHROMBIN III USING CHINESE YELLOW ( OCHER ORIGINATED FROM CHINA ) AS A NEW ADSORBENT AND HEPARIN - SEPHAROSE AFFINITY CHROMATOGRAPHY

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In recent years, we have presented several reports with respect to Chinese Yellow ( ocher originated from China ) as a new adsorbent for blood coagulation factors. Adsorbability of this ocher is strong for many coagulation factors in plasma including fibrinogen, while quite weak for antithrombins. After ocher adsorption treatment, AT III activity of plasma in comparison with the value before adsorption is 86 - 96 % in clotting assay ( Biggs thrombin time methods ) and 96 - 100 % in immunologic activity ( detected by SRID ). In this study, purification of plasma AT III, we have used this ocher as the adsorbent in pretreatment of plasma together with heparin - sepharose affinity chromatography. As the actual procedure, 5 g of ocher was added to 100 ml of ACD plasma, and the mixture was subjected to adsorption with stirring at room temperature to obtain the ocher adsorbed plasma, crude AT III fraction, in the supernatant after centrifugal separation. Highly purified AT III with a molecular weight of approximately 67,000 ( determined by SDS-PAGE ) was obtained from the crude AT III fraction by means of heparin - sepharose affinity chromatography. The antiserum from rabbits immunized with the purified AT III showed a single fuse precipitin line against the starting plasma, ocher adsorbed plasma and AT III fraction. However, it did not exhibit reaction with  $\alpha_2$ -macroglobulin and  $\alpha_1$ -antitrypsin. From the above results, it has been found that the ocher is an effective adsorbent in pretreatment of plasma for AT III purification.

## 1173

DIFFERENT AFFINITIES OF HEPARIN PREPARATIONS WITH IDENTICAL MOLECULAR WEIGHT TO PLASMA PROTEINS AND FACTOR Xa. J. Harenberg, F. Fussi, M. Grün, A. Schlegel, K. Mattes, R. Zimmermann and E. Weber. Department of Clinical Pharmacology and Internal Medicine, University of Heidelberg, GFR and University of Padua, Italy

The study intends to get information on the in vivo comparability of different heparin preparations with the same molecular weight (mean MW 15000). Four heparins (150-154 USP/mg, sodium salt) were applied s.c. to each of six volunteers randomly at weekly intervals.

The pharmacodynamic effects were controlled on the factor IIA activity (thrombin clotting time), aPTT and factor Xa activity (chromogenic substrate S2222) over 10 hrs by 12 blood samples. On the factor IIA activity no differences were seen between the heparins. The coagulation times of the aPTT first prolonged and then shortened below the initial value after 4-6 hrs indicating an activating of the intrinsic coagulation system. One of the heparins had a higher anticoagulation effect than the other preparations ( $p < 0.05$ ). For this heparin a more effective anti-Xa-activity was measured ( $p < 0.05$ ). In vitro no differences of the heparins were registered on the test systems in a range of 0.01-2.0 USP/ml plasma.

The data indicate, that heparin preparations with the same MW and the same in vitro activities may have different effects in vivo. They let suppose, that these differences may be mediated by different affinities of the heparins to plasma proteins as AT III or lipoprotein lipase, which might have caused the activation of the intrinsic coagulation system by a release of phospholipids, and by different affinities of the heparin AT III complexes of each heparin to factor Xa.