STUDY OF THE NORMAL SERUM ACTIVITY SENSITIZING STABILIZED FIBRIN TO UREA DISPERSION BY SEPHADEX G. 200 CHROMATOGRAPHY. F. de Cataldo, F. Baudo and E. Mussini*. Department of Hematology, Ospedale Maggiore Niguarda, Istituto Ricerche Farmacologiche Mario Negri*, Milano, Italia.

Clots from human normal native platelet-poor plasma are dispersed by the addition of urea at a final concentration of 2.5 M. The same clots, washed in buffered saline (pH 7.4), are not dispersed by urea, but are rendered susceptible to its dispersing action by prior incubation in normal undiluted plasma and serum or 33% ammonium sulphate fractions. The serum fraction was dissolved in and dialyzed against pH. 7.4 phosphate-buffered saline and analyzed by Sephadex G. 200 chromatography. Two peaks were obtained; the relative materials were dialyzed against pH. 7.4 phosphate-buffered distilled water, lyophilized and dissolved in saline. The activity sensitizing the stabilized fibrin to urea dispersion was recovered in the second peak material.

DETERMINATION OF ANTITHROMBIN, HEPARIN CO-FACTOR AND HEPARIN IN PLASMA USING THE CHROMOGENIC SUBSTRATE S2238. M.F. Scully and V.V. Kakkar. King's College Hospital Medical School, London.

Antithrombin, the thrombin inhibiting capacity of human plasma, was measured in normal plasma and also as a partially purified protein with the synthetic substrate, H-D-Phe-Pip-Arg-p-nitroanilide-2HCl. (S2238, KabiVitrum, London). A method was developed which was sensitive in the range 0.05 to 0.25 NIH units of thrombin in a total volume of 0.5 ml. Under these conditions a change in optical density of 0.36 per minute per 0.1 units of thrombin was observed. The substrate is, therefore, 5 to 6 times more sensitive than the previously used B2-L-Phe-Val-Arg-p-nitroanilide (S2160, KabiVitrum, London). Using this procedure antithrombin levels in up to 10 μ ls of plasma were measured without preliminary defibrination.

The method has been adapted for the automated determination of thrombin (0.025 to 0.4 units/incubate and also antithrombin levels in 5-50 μ ls of plasma using an LKB 2086 reaction rate analyzer. Triplicate measurements of 25 μ l aliquots of 15 random samples of normal human plasma agreed within each sample set to $\pm$ 3% (1 standard deviation). A correlation was shown with antithrombin III levels measured by a clotting method. A modification of the method produced a more sensitive assay measuring in the range 80-100% plasma antithrombin. Heparin cofactor was measured in 10 μ l aliquots of heparinized plasma (NIH/ml). An adaption allowed automated measurement of heparin levels in the range 0.2 to 1.0 units/ml of plasma.

Preliminary experience indicates that with the use of this substrate various clotting tests may be substituted with a method which is automated and readily reproducible.

ABSORPTION OF DIFFERENT SALTS OF HEPARIN FOLLOWING SUBCUTANEOUS INJECTION. G. Cella, D.A. Lane, I. MacGregor and V.V. Kakkar. Thrombosis Research Unit, King's College Hospital Medical School, London, England.

Two recent studies (1,2) have demonstrated that the MW of heparin and nature of the inorganic ion associated with it influence the heparin level in blood following subcutaneous injection. In the present study we have studied the absorption of heparins containing different amounts of Na and Ca and yet all having similar MW's when characterized by gel filtration upon a polyacrylamide-agarose matrix (3). Four heparins from the same source material containing approximately 11% Na, 2.5 % Ca, 7%, 10.5% Ca together with two Na salts and two Ca salts that are commercially available have been studied in a double-blind crossover trial in six normal volunteers. It was found that mean heparin levels circulating 1, 3, 5 and 7 hours post injection fell as the % of Ca associated with the heparins increased. The differences between the mean levels obtained following injection of the Na salts and the 10-11% Ca salts were highly significant at 1, 3 and 5 hours, while differences between Na salt and 7.3% Ca were significant only at 1 hour post injection. In one individual with small amounts of subcutaneous tissue inconsistent results were obtained, with two Ca salts (7.3% and 11%) producing twice the levels obtained following subcutaneous injection of the Na salts and another Ca salt (10.5%) producing characteristically low levels.

1. Johnson, E.A. et al. *Thrombosis and Haemostasis* 35: 586, 1976.

2. Thomas, D.P. et al. *Thrombosis Research* 9: 241, 1976.

3. Johnson, E.A. and Mulloy, B. *Carbohydrate Research* 51: 119, 1976.