Determination of Antithrombin, Heparin Co-Factor and Heparin in Plasma Using the Chromogenic Substrate S2238. M.F. Gall 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-Pro-Arg-p-nitroanilide-2HCl (S2238, Ealing, 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-Pro-Val-Arg-p-nitroanilide 5HCl (S2160, KabiVitrum, London). Using this procedure antithrombin levels in up to 10 μl of plasma were measured without preliminary deribinization.

The method has, but are rendered unacceptable to its dispersing action by prior incubation in normal undiluted plasma and serum or 3% ammonium sulphate fractions. The serum fraction was dissolved in and dialyzed against pH 7.4 phosphate-buffer 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.

Absorption of Different Salts of Heparin Following Subcutaneous Injection. G. Cella, D.A. Lapp, J. 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 SN 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 0.1% 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.