ASSAY FOR PREKALLIKREIN LEVELS AND PREKALLIKREIN ACTIVATION IN HUMAN PLASMA USING DEXTRAN SULPHATE AS A SOLUBLE ACTIVATOR. C. Kluft. Gaubius Institute, Health Research Organization TNO, Herenstraat 5d, Leiden, The Netherlands.

Incubation of human citrated plasma with dextran sulphate at 0°C was found to generate kallikrein activity as determined with the chromogenic synthetic tripeptide α -N-Bz-Pro-Phe-Arg-p-nitroanilide (0.16 mM). The possible use of the synthetic soluble dextran sulphate (MW 500,000) instead of the commonly used insoluble kaolin for activating the plasma kallikrein system was explored. The rate of activation of prekallikrein to kallikrein was dependent on: a) the concentration of dextran sulphate until a plateau was reached at 12.5 μ g/ml; and b) the concentration of factor XII in the entire range up to 100%, as varied by mixing deficient and normal plasma. Activation was only slightly influenced by increased levels of C1-inactivator. In comparing dextran sulphate with kaolin, it was noticed that activation by means of dextran sulphate was slower (optimum at 7 and 2 min, respectively) and could be stopped by dilution. With kaolin (or glass), the activation continued even at high dilution (1:162) in the cuvet, preventing a proper study of the activation process. Dextran sulphate also did not interfere with the spectrophotometric assay, in contrast to the light-scattering properties of the insoluble kaolin. Optimum conversion of prekallikrein to kallikrein is reached in normal pooled plasma in 7 min at 0°C with 25 μ g/ml dextran sulphate. The same is true for other plasma samples, if not liable to unwanted preactivation or incomplete activation, as revealed by control experiments. In 15 apparently healthy individuals, a mean activity of 476 ± 58 (SD) mU/ml was found. It is concluded that dextran sulphate can be used advantageously in the assay of prekallikrein activation and prekallikrein levels.

TURNOVER OF PLASMA KININOGENS IN RHESUS MONKEYS. T. Yamada, D. A. Wing and J. V. Pierce. U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD and National Institutes of Health, Bethesda, MD, U.S.A.

Radiolabelled purified plasma kininogens were injected into healthy rhesus monkeys for the purpose of studying their normal metabolic turnover. Human kininogen was purified using affinity chromatography and further fractionated into high molecular weight (HMW) and low molecular weight (LMW) proteins as described by Pierce and Guimaraes (Fogarty International Center Proceedings No. 27, pp. 113-119, 1976). The kininogens were labelled with 125 I using either the choramine-T or microdiffusion techniques. Labelled mixed kininogen, LMW kininogen, and HMW kininogen preparations were injected into 6, 4, and 6 rhesus monkeys respectively. Blood samples were drawn at various time intervals, and assayed in a gamma counter. The disappearance of injected labelled kininogen from the intravascular compartment could be expressed as a double exponential equation. The difference between the half-life of HMW kininogen (26.0 ± 1.6 h) ($\pm$ SE) and that of LMW kininogen (20.2 ± 2.1 h) was only marginal ($P > 0.05$). In contrast there was a highly significant ($P < 0.001$) difference in their fractional catabolic rates (1.12 ± 0.09 day $^{-1}$ for HMW kininogen and 2.05 ± 0.12 day $^{-1}$ for LMW kininogen). These observations indicate that although there is a minimal difference in the metabolic rates of intravascular HMW and LMW kininogens, a considerable difference exists in their rates of flux between the intravascular and extravascular spaces.

TURNOVER STUDIES OF DES-A FIBRIN AND FIBRINOGEN IN HEALTHY HUMAN BEINGS.

G. Müller-Berghaus and I. Mahn. Justus-Liebig-Universität, Giessen, W. Germany.

Soluble fibrin was injected into man. Two methods were used to prepare soluble des-A fibrin. Purified human I-125-fibrinogen was transformed into soluble des-A fibrin (des-A FM): (1) by thrombin in the presence of 2.0 M urea (method according to Hogg and Blombäck, 1975), (2) by anrod. Eight male volunteers were injected either with thrombin-induced soluble I-125-fibrin (FM-T) or anrod-induced soluble I-125-fibrin (FM-A). The control consisted of I-131-fibrinogen injected simultaneously with soluble I-125-fibrin.

The mean distribution volume of des-A FM-T was 48.9 ml/kg and that of des-A FM-A 50.3 ml/kg which is similar to that of the injected fibrinogen (43.0 ml/kg) indicating a homogenous distribution of the injected FM in the circulating blood. The injected des-A FM disappeared faster from the circulating blood than des-AB fibrin monomer. Six hours after injection 99.5 % of the injected FM-A and 95 % of the injected FM-T were removed from the circulating blood. The FM were catabolized since free I-125 were quantitatively recovered from the urine. The elimination curve of des-A FM-A as well as des-A FM-T does not represent a monophasic exponential decay.

The experiments demonstrate that des-A fibrin can be traced in man and is cleared from the circulating blood at a high elimination rate.

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