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MIXED POSTERS VII

Platelets

PLATELET ADHERENCE TO COLLAGEN: A SIMPLE REPRODUCIBLE METHOD. M.J. Mant. Departments of Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada.

An optimal method for measuring platelet adherence to collagen should be simple, quantitative, reproducible, adaptable to varying experimental conditions and have no interfering platelet aggregation nor use reagents potentially influencing the results. An adaptation of previously described techniques has been developed which satisfies most of these requirements. Platelets were stirred with a stable collagen suspension in 0.2% EDTA at 37°C. Platelet counts were performed with a Coulter counter before and after stirring and % adherent platelets calculated. Platelet aggregation was excluded by phase contrast microscopy and by unchanged adherence after addition of aggregating agents (ADP and adrenalin) and an inhibitor of aggregation (prostaglandin E1). The counting technique was validated by absence of particle counts with collagen alone and by simultaneous "manual" post-adherence platelet counts. Adherence % was found to plateau at a maximum value with time, increasing collagen concentration and increasing stirring speed. It fell exponentially with increasing platelet count and linearly when pH was reduced. Utilizing platelet rich plasma (PRP) at a platelet count of 300,000/ μ l, a final collagen concentration approximating 0.05 mg/ml, a stir speed of 900 rpm and a pH of 7 ± 0.5 adherence % on 30 normal donors varied from 43.0 to 59.3%. A normal range of $51.6 \pm 8.2\%$ (± 2 SD) was established. Replicate single sample testing gave a mean ± 2 SD of $51.9 \pm 2.8\%$. Serial adherences in 1 individual showed only minimal weekly variation. This simple, reproducible, quantitative method can be adapted to a wide variety of experimental conditions. It should therefore be useful for investigating many aspects of platelet adherence to collagen.

SEPARATION OF THE BINDING STEP FROM THE COLLAGEN PLATELET REACTION. P. L. Kronick and S. A. Jimenez. Franklin Institute and University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, U.S.A.

Determination of activity of most agents in stimulating platelets to aggregate or release adenine nucleotides is conveniently done by titrating the platelet reaction with the agent. Platelets have previously been titrated with different types of collagen (types I, II, and III) in this way to compare the activities of the collagens. It has been concluded that the order of activity is type III > I > II. Whether this order is due to differences in binding was not obvious from these experiments because the binding was not determined directly. We have developed a method of comparing activities by measuring the targeted dose for each point in the titration - the amount of collagen which actually binds to platelets. The collagens used in these experiments were prepared *in vitro* from embryonic chick tissue to give labelled products of extremely high specific activity without structural alteration. We find that type I collagen is at least 20 times as active as previously reported, and that the activity of Type III collagen is not significantly higher when the amounts bound are taken into account. The fraction of the labelled tendon collagen which was bound to platelets was identified as type I by its hydroxyproline/proline ratio. Direct measurement of the bound fraction in dose-response studies is required for valid comparison of collagen activities.