Tuesday, July 14, 1981

Poster Presentations

Platelets – XI

Methods, Constituents, Function

11:00–12:30 h

Grand Ballroom Lobby Boards 213–230

0397

THE TEFLON TUBING BLEEDING TIME IN MAN. P. Didisheim, D.A. Claypool, J.Q. Stropp and R.W. Baker. Thrombosis Research Laboratory and Department of Laboratory Medicine, Mayo Clinic, Rochester, MN 55901, and Payton Associates, Buffalo, NY 14202, USA.

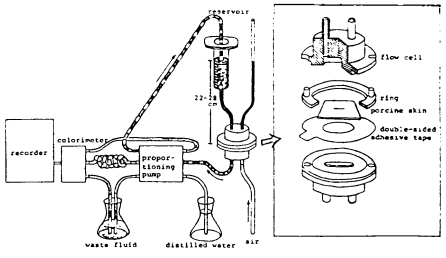
The skin bleeding time (BT)(Mielke, Ivy, Duke) is an important part of the diagnostic workup of bleeding disorders. Because of variable vascularity of the skin from one location to another, results may vary widely with the site and type of incision and the thickness of the skin. In some patients bleeding is difficult to stop. The discomfort of the test and the possibility of inducing scarring limit the frequency with which it can be repeated. A standardized BT that could be performed on the patient's blood in the laboratory could avoid these problems. We have developed such a test, basing it on our previously described method in which a hole is made in a Teflon arteriovenous shunt in rats, and modified from the method of Blakely et al. A cylindrical hole of 75 μ diameter is made in a 50 cm length of 457 μ I.D. Teflon tubing with the aid of a needle, micromanipulator and dissecting microscope. Blood (2 U heparin/ml) from normal human subjects displaces buffer from the tubing at a rate (.6 ml/min) determined by a syringe pump. Pressure is controlled by raising the distal end of the tubing 5 cm above the hole. BT is recorded by an electronic drop rate counter (Payton BTR 400). Under these conditions, BT is 150 ± 47 sec, and bleeding rate from the hole, shear rate and shear stress on the wall of the hole are .11 ± .02 ml/min, 40,700 ± 8,100 sec^{-1} and 1,830 ± 360 dynes/cm^2 respectively. The BT can be varied from 0 to >15 min by lowering or raising pressure or flow in the system or by varying hole diameter. By phase microscopy the hole is seen to be occluded by a mass of aggregated platelets and occasional PMN. These observations show that hemostasis at small incisions is controlled by rheologic factors and platelets and does not require physical or chemical properties of a blood vessel. This new method may be useful in the diagnosis of bleeding disorders and in studying the effects of rheologic factors and drugs in hemostasis.

0398

IN VITRO BLEEDING TIME. S.SAWADA, A.KIMURA, Y.YOSHIDA. First Department of Internal Medicine,Hirosaki University School of Medicine,Hirosaki,Japan.

One of the„authors, A.Kimura,et al. reported the technique called "in vitro bleeding time" using fresh pig skin and the heparinized animal blood (BLOOD 54:6 1347-1357). The system was used to measure both the normal hemostasis of swine and to investigate what causes abnormal hemostasis in the von Willebrand animals. Author modified this system to use dried pig skin and heparinized normal(n=62) and abnormal hemostatic human blood(n=20). Heparinized human whole blood at constant hydrostatic pressure was allowed to flow through a 5mm incision in the dried pig skin (LYODERM,ARMOUR PHERMACEUTICAL CO.) in the flow cell(HELLIGE CO.), shown in the figure. Exuded blood was drawn by the proportionning pump and hemolized with distilled water. The hemoglobin concentration was measured and recorded by the automatic electrophotometer. The time elapsed from the start of flow to the cessation was measured as in vitro bleeding time. In vitro bleeding time was 7.6±3.6(mean±SD) minutes in normal, and in the subjects of which platelets counts were under 100,000/μl, it was

$28.7-\frac{\text{platelet counts}/\mu l}{3800}$ ($p<0.05$). In von Willebrand's disease and Hermansky-Pudlak syndrome in vitro bleeding time was prolonged.



0399

A NON-INVASIVE METHOD FOR MEASURING PLATELET AGGREGATION IN VIVO. J. Morley, P.M. Hoyle, J. Leyton & O.J. Davies*. Department of Clinical Pharmacology, Cardiothoracic Institute & Department of Electronic & Electrical Engineering*, University College, London, England.

In experimental animals, intravenous injection of ADP produces platelet accumulation in the lungs, together with thrombocytopenia. Since platelets can be isotopically labelled without gross functional disturbance, we have sought to develop a method for measurement of aggregation and disaggregation of labelled platelets in vivo.

Guinea-pig platelets are labelled with Indium-111 and injected into narcotised animals (Sagital, 37 mg/kg). Two collimated crystal scintillation probes are used to monitor the thoracic region (heart and lung) (C1) and the vascular compartment (hind limbs) (C2). An indwelling cannula (25 SWG) is inserted into a foot vein and kept patent by small volumes of heparin (100 u/ml). Intravenous ADP (10 mg/kg) causes transient accumulation of platelets within the lung, as well as thrombocytopenia, within a two minute period. During a response, counts are monitored from both probes and retained in a dedicated microcomputer, so as to permit rapid display of results in tabular and graphical form. Usually, ninety consecutive 4 sec. counts are recorded, results being expressed both as a paired difference (C1-C2) and as a ratio (C1/C2). Repeated challenge can be made with ADP and a dose-related response is obtained over the range 10-30 mg/kg. Treatment with sulphinpyrazone or prostacyclin inhibits aggregation.