

ROLE OF PROTHROMBIN AND FACTOR IX IN THE INITIATION OF THROMBUS FORMATION. F. Kennedy, H.I. Hassouna, J.A. Penner, and J. Schultz. Dept. of Medicine, College of Human Medicine, Michigan State University, East Lansing, Michigan, U.S.A. and Dept. of Chemical Engineering, University of Michigan, Ann Arbor, Michigan, U.S.A.

This study was designed to investigate the role of plasma clotting factors in the initiation of thrombus formation on materials intended for prolonged blood contact. Prothrombin was isolated from dog plasma using  $\text{BaCO}_3$  adsorption, citrate elution and chromatography on dextran sulfate. Antibodies against various fractions were purified and tested for their ability to inhibit clot formation in normal dog plasma. Prothrombin time was not significantly affected by antibodies, but recalcification time was more than doubled. This recalcification time was not corrected by adding  $\text{BaCO}_3$  adsorbed plasma back to normal dog plasma incubated with antibody. These results led to the conclusion that these antibodies were specifically inhibiting prothrombin and factor IX.

These purified antibodies were tested in preconditioned male dogs to specifically block prothrombin from taking part in coagulation. Dogs had previously been surgically fitted with arteriovenous shunts between the carotid artery and the internal jugular vein, and were given autologous platelets labelled with  $^{51}\text{Cr}$  and  $^{125}\text{I}$ -labelled human fibrinogen prior to testing. The arteriovenous shunt was opened into the flow chamber for one hour, during which time a dual channel scintillation counter was positioned over the test chamber to monitor fibrinogen and platelet deposition at 5 minute intervals. After one hour the chamber was removed and the size of the resulting thrombus was measured by total weight, fibrinogen, red cell, and platelet content. In dogs previously given antibodies against prothrombin, thrombus was not found on the various biomaterials applied to the rod within the flow chamber. We conclude that prothrombin is of major importance in the initiation of clot formation on artificial surfaces.

## 0989

INTERACTION OF PLASMA PROTEINS WITH ARTIFICIAL SURFACES WITH REFERENCE TO PLATELET ADHESION. O.P. Malhotra, M.N. Helmus and D.F. Gibbons. Medical Research Service, Veterans Administration Medical Center and Departments of Pathology and Biomedical Engineering, Case Western Reserve University, Cleveland, Ohio 44106 U.S.A.

On occasion, fewer platelets from platelet rich plasma (PRP), adhered to hydrophilic (glass) surfaces exposed to platelet poor plasma (PPP) for 3 min than areas exposed for 3 s. The decrease was dramatic and consistent when platelet suspension (gel-PLS, obtained from PRP by 2B Sepharose gel filtration) was used instead of PRP. To further explore the factors which influence platelet adhesion, we used the following: for surfaces, a) sparkleen-cleaned glass (hydrophilic), b) acid-washed (somewhat hydrophobic), and c) siliconized (hydrophobic); for proteins, a) PPP, b) fibrinogen (96% clottable), c) defibrinogenated (defib.) plasma, and d) defib. plasma plus fibrinogen; for platelet suspension, a) PRP, b) gel-PLS, and c) platelets in defib. plasma (defib. PLS).

From gel-PLS, non-siliconized surfaces exposed to fibrinogen for 3 s attached more platelets ( $P < 0.05$ ) than those exposed to PPP or defib. plasma plus fibrinogen. The latter two attracted more platelets ( $P < 0.01$ ) than defib. plasma. Hydrophilic sparkleen-cleaned glass previously exposed to PPP (or defib. plasma plus fibrinogen) attached a minimum of 10-fold as many platelets from gel-PLS than from PRP. Under similar conditions acid-cleaned surfaces attached 2-fold from gel-PLS, while hydrophobic glass did not show any change. By exposing the surfaces to PPP followed by gel-PLS, the sparkleen-cleaned glass showed the greatest decrease ( $P < 0.001$ ) in the number of platelets attached to areas exposed to PPP for 3 min as compared to 3 s, while siliconized showed no such decrease. If, however, the surfaces were exposed to defib. plasma, they all showed decreases in platelet attraction at 3 min.

## 0988

KINETICS OF ADSORPTION OF PROTEINS FROM HUMAN PLASMA ONTO FOREIGN SURFACES. J.L. Brash and S. Uniyal. Depts. of Chemical Engineering and Pathology, McMaster University, Hamilton, Ontario, Canada.

It is believed that adsorption of proteins is the first occurrence after blood/foreign surface contact. The composition of the protein layer, how it depends on surface properties, and how it changes with time are essentially unknown. The objective of this work was to develop data relevant to these questions. To this end, the quantities of albumin, fibrinogen and IgG adsorbed on seven surfaces from human plasma as a function of time were measured. Human plasma (ACD anticoagulant) was diluted 1:4 with tris buffer. Purified proteins were labelled with iodine isotopes using the ICI method and added to the plasma as tracers. Materials studied include several segmented polyether-urethanes, both hydrophilic and hydrophobic, glass, siliconized glass (SG), polystyrene (PS) and polyethylene (PE).

The results may be summarized as follows: **Fibrinogen:** Within the 2 min to 3 h range of contact times, fibrinogen was not detected on any of the hydrophilic surfaces. On PE and SG the quantity adsorbed passed through a maximum between zero time and 2 min, then declined to near zero. Only on PS was adsorption substantial ( $0.4 \mu\text{g cm}^{-2}$ ) and constant with time, similar to that from a solution of fibrinogen. **Albumin:** Albumin was also not detected on the hydrophilic materials. In general its surface concentration when it was adsorbed (hydrophobic surfaces) was similar to that observed for solutions of albumin. **IgG:** IgG was detected on all surfaces. The surface concentrations were low (about  $0.1 \mu\text{g cm}^{-2}$ ) compared to solution values but were generally constant with time.

The following conclusions are drawn: (1) The plasma itself modifies adsorption. Therefore solution adsorption data cannot be used to predict plasma adsorption. (2) Contrary to popular belief, fibrinogen is absent or transient on most surfaces. (3) IgG appears to be ubiquitous as a component of protein layers adsorbed from plasma.

## 0990

PLATELET DEPOSITION IN EXTRACARDIAC CONDUITS IN HUMANS: A NONINVASIVE QUANTIFICATION. K.C. Agarwal, M.K. Dewanjee, V. Fuster, R.H. Feldt, H.W. Wahner, J.S. Robertson, M.L. Brown, J.H. Chesebro, M. Kaye, F.J. Puga, G.K. Danielson. Mayo Clinic and Mayo Foundation, Rochester, MN, U.S.A.

In right-sided extracardiac conduits inserted for surgical correction of certain congenital heart defects, obstruction is a significant late complication requiring conduit replacement in about 5% of the patients. Our histopathologic study indicates a significant role of platelets in the pathogenesis. This study was designed to evaluate the feasibility of imaging procedure with  $^{111}\text{In}$ -indium-labeled platelets to localize and later quantify platelet accumulation in the conduits. Four patients with pulmonary atresia and 1 with truncus arteriosus underwent conduit placement, 2 with and 3 without porcine valve. Autologous platelets collected from 43 ml of blood drawn prior to surgery were labeled with  $^{111}\text{In}$ -indium-oxine in ACD-saline medium (labeling efficiency 50-60%). 400-500  $\mu\text{Ci}$  of autologous  $^{111}\text{In}$ -indium-labeled platelets were administered intravenously immediately after surgery in 4, and five days after surgery in 1 patient. Imaging was performed at 6 hours and daily for 1-4 days using a portable gamma camera interfaced with a computer and fitted with medium-energy parallel hole collimator. Platelet deposition was noted at the site of the conduit in all 5 patients. Maximum accumulation (0.1-0.3% of dose) of platelets was seen within the first 24 hours. Activity over blood pool and lungs declined and over liver and spleen increased in a predictable manner over the imaging period. This technique has potential use as a noninvasive quantitative tool to study the effect of platelet inhibitor drugs in preventing conduit obstruction.