

**FUROSEMIDE AND PLATELET FUNCTIONS.** I.S.Chohan\*, I.Singh\*, J.Vermeylen\*\* and M.Verstraete\*\*. \*Directorate General Armed Forces Medical Services, New Delhi, India and \*\* Laboratory of Blood Coagulation, University of Leuven, Leuven, Belgium.

Furosemide inhibits primary platelet aggregation by adenosine-5'-diphosphate and prolongs the latent period before Thrombofax- or collagen-induced platelet aggregation, both in vitro and ex vivo. Furosemide also inhibits the release of platelet factor 4 and  $^{14}\text{C}$ -serotonin. The inhibitory concentrations of furosemide in vitro range between 0.5 and 2.5 mM. The ex vivo effects were obtained after an intravenous injection of 40 mg furosemide.

The furosemide concentrations required for ex vivo inhibition are hundred fold lower than those required for in vitro effect. This suggests in vivo metabolic effects of this drug, such as, calcium ions shifts, or action of other metabolic products of furosemide. In support of this concept platelet aggregation was found more disturbed six hours than 10 minutes after injection of the drug.

**BLOOD COAGULATION, FIBRINOLYTIC ACTIVITY, PLATELET FUNCTION AND IMMUNOGLOBULINS IN FROSTBITE.** I.S.Chohan and I.Singh. Directorate General Armed Forces Medical Services, New Delhi, India.

Fifteen males, 19-45 years old, stationed between altitudes 3690 and 5540 m in the Western Himalayas who were frostbitten were studied within 24 hours of the injury and then 4 weeks and 1 year after for blood coagulation defects. The following disturbances were found: fibrinogen degradation products and factor VIII-related antigen were increased; fibrinogen, platelet counts and haematocrit were decreased; platelet adhesiveness was increased; euglobulin lysis time was prolonged; antithrombin III,  $\alpha_1$ -antitrypsin and  $\alpha_2$ -macroglobulin were markedly decreased; IgG and IgA immunoglobulins and cryoglobulins were increased; serum albumin was decreased and IgM immunoglobulin consumption was increased.

These abnormalities increase platelet adhesiveness and diminish fibrinolytic activity and promote intravascular thrombosis.

Furosemide increases fibrinolytic activity and suppresses platelet adhesiveness in vivo (I.Singh and I.S.Chohan, Int.J.Biometeor.17,73,1973). Its use in the prevention of frostbite is under investigation.

**MOLECULAR BINDING RATIO OF ANTI-FACTOR VIII TO PURIFIED FACTOR VIII.** C.G.Cockburn, Diana M.L.Reay and R.M.Hardisty. Department of Haematology, Institute of Child Health, London, England.

A simple method has been developed for cutting washed FVIII:anti-FVIII immunoprecipitates from agarose gels and applying the SDS-denatured, reduced protein to SDS-polyacrylamide gels. The relative amounts of IgG heavy and light chains and FVIII have been quantitated by recording the areas under the densitometer traces of the Coomassie brilliant blue-stained gels. The results show that approximately 20 molecules of anti-FVIII bind per 200,000 daltons of native FVIII. We conclude from this that the factor VIII subunits have a high surface-area-to-volume ratio.