

RED CELL PROPERTIES IN HAEMOSTASIS. G.M. Housley and G.V.R. Born. Department of Pharmacology, King's College, London, U.K.

Earlier observations of ours have suggested that, under *in vitro* conditions resembling those under which platelets function haemostatically *in vivo*, their activation is promoted by the red cells. Some of the evidence suggested that this is through limited haemolysis with release of ADP. However, newly determined time relationships make this uncertain. Could red cells provide ADP without haemolysis? Or their flow properties affect the process more?

To analyse the problem, we are determining dependence of red cell deformability on membrane constitution; and release of haemoglobin and adenine nucleotides under different conditions. Ten percent human red cell suspensions in physiological saline flow under constant pressures through 2, 3, 4 and 5  $\mu$ m micropore filters, the flow rate measured continuously with an electronic balance. Initial flow rates are increased by fluidising agents, eg. ethanol, and decreased by agents with opposite effect. Our results are consistent with the new hypothesis of S.J. Singer on the mode of action of amphipathic agents, such as chlorpromazine, on red cell membranes.

## 1240

ENZYMIC CLEAVAGE OF *IN VIVO* FORMED MAILLARD-TYPE COMPOUNDS INVOLVED IN HAEMOSTASIS. L. Mester, L. Szabados and M. Mester. Institut de Chimie des Substances Naturelles, Centre National de la Recherche Scientifique, Gif-sur-Yvette-91, France.

Desoxyfructose derivatives of serotonin (Mester et al., 1975), of haemoglobin (Flückiger and Winterhalter, 1976), of poly-L-lysine (Mester et al., 1975) and of lysine rich histones (Kertesz-Crisba, 1977) are easily formed *in vivo* by a simple Maillard-type chemical reaction. Some of these compounds interfere with platelet functions (Mester et al., 1976) or contribute to the thickening of the basal membrane of blood vessels (Cerami et al., 1979).

While the chemical synthesis of Maillard-type compounds proceeds readily even *in vivo*, the chemical cleavage of them needs severe conditions which certainly do not exist *in vivo* (Gottschalk, 1952). However, a slow liberation of serotonin from desoxyfructo-serotonin is observed *in vivo*, suggesting the existence of an enzyme system for the cleavage of Maillard-type sugar-amine derivatives. *In vitro*, using a sheep liver microsomal preparation rich in Cytochrome P<sub>450</sub> enzyme, the liberation of serotonin is in linear correlation with the enzyme concentration. The cleavage of desoxyfructo-serotonin is activated by NADPH having its optimum at pH=7.4, excluding definitely the occurrence of a chemical hydrolysis.

Factors interfering with the enzyme system involved in the cleavage of Maillard-type compounds, may also interfere with haemostasis.

## 1239

FREE ATP IN BLOOD DURING HAEMORRHAGE

M.A.A. Kratzer and G.V.R. Born. Department of Pharmacology, University of London, King's College, Strand, London WC2R2LS

When blood vessels are injured so that they bleed, circulating platelets adhere to the damaged vessel wall and aggregate within the first seconds. The mechanism of the initial platelet aggregation remains uncertain. To investigate the initiation stage of haemostasis, the carotid arteries of rats were punctured with a 100  $\mu$ m needle and free ATP, as an indicator of ADP, was measured in the emerging blood.

This was brought into contact with luciferin-luciferase in a polyethylene tube, internal diameter 0.8 mm. The light produced at the blood/enzyme interface was measured with a sensitive photon-counting device which gave background counts of 1 photoelectron/sec. and could detect  $< 10^{-8}$  M ATP in 2  $\mu$ l blood.

When an artery was injured, the emerging blood contained about  $10^{-7}$  M ATP in a first peak after about 2 sec. After about one min. the ATP concentration rose to a second peak of about  $5 \cdot 10^{-6}$  M. This was decreased by heparin (500 U/kg body weight) or by chlorpromazine (1 mg/kg). The observations suggest that the second peak represents ATP released from platelets. The source of ATP accounting for the first peak remains uncertain; possibly this ATP is released from red cells undergoing high shear stress from the haemodynamic effects of haemorrhage.

## 1241

HEMOSTATIC ACTIVATION DURING TWO-SYRINGE TECHNIQUE. J.A. Caprini, J.P. Vagher, S. Yeager, G. Harvey, J. Mitchell.

These studies were done to compare APTT test results on the first and second aliquots of blood drawn by a 2 syringe technique using a 19 Ga. thinwall needle. Three ml of blood were drawn into a plastic syringe following a clean venapuncture and another 5 ml of blood were drawn into a second plastic syringe. The two specimens were immediately dispensed into polypropylene test tubes for TEG analysis. Polypropylene tubes containing buffered citrate (Exp 1) or pediatric blue-top Vacutainer tubes (Exp 2) were used for APTT analysis. The native whole blood specimens were placed in the TEG cups within 4 min. of venapuncture. The citrated specimens were centrifuged within 5 minutes and plasma APTT assays were done in duplicate. Test results were expressed as the absolute difference between the two samples ( $\Delta$ APTT and  $\Delta$ TEG). Blood from the first syringe showed a TEG pattern visually and statistically different ( $\Delta$ TEG) than the sample from the second syringe 100% of the time.  $\Delta$ TEG mean (SD) in Exp 1 was 3.7 (2.9). The APTT values obtained from the first syringe showed considerable variation when compared to its second syringe counterpart. The variability in APTT ( $\Delta$ APTT) between the first and second aliquots was markedly increased when Vacutainers were used. In Exp 1 with 30 samples  $\Delta$ APTT was 7.6 sec mean (9.5sec SD) which was statistically different from Exp 2 with 15 cases which was 20.6 sec (37.7 sec). In addition, the variability between the two samples increased significantly as the APTT lengthened and this was further augmented by using Vacutainers.

These results emphasize the importance of a 2 syringe technique in obtaining consistent APTT results. The variation in the first syringe may be due to contamination by tissue thromboplastin in the needle and apparently disappears if at least 5 ml of blood are allowed to pass through before samples are taken. Inconsistencies seen with Vacutainers may be due to surface activation of the blood by glass or shear effects from the high velocity of the blood through the needle. These data indicate that TEG can be used to detect samples contaminated by poor drawing