A SIMPLE, QUANTITATIVE, OBJECTIVE MEASUREMENT OF PLATELET SHAPE. S. Holme and S. Murphy. Carderza Foundation, Thomas Jefferson University, Philadelphia, Pa., U.S.A.

Previous studies of platelet aggregation have shown that platelet disc-to-sphere transformation induced by ADP is accompanied by a decrease in light transmission. Our studies were directed towards an understanding of this effect; they also resulted in the development of a method which quantitatively reflected platelet shape. A Payton aggregometer with a 0.5 ml cuvette was used. Electronic settings were standardized to give a read out of 100% transmission (10 mV) on the recorder with water in the cuvette, and 0% transmission (0 mV) with background current alone. For fresh, discoid platelet suspensions there was a fall in light transmission to a minimum at 200 r.p.m. with an increase in transmission to a maximum at 700 r.p.m. with increasing stirring speed. After disc-to-sphere transformation by ADP, EDTA, or chilling, a constant light transmission, unaffected by stirring rate, was observed. When the changes in light transmission due to the disc-to-sphere transformation were measured, only a small decrease took place at 0 r.p.m. At 200 r.p.m. there was actually an increase due to the shape change, while maximum decrease was obtained at 700 r.p.m. This shows that the platelet shape change observed in the aggregometer is mainly determined by the difference of discoid and spherical platelets in their effect on light transmission in presence of stirring. When the ratio $O.D._{700}/O.D._{200}$ was determined for 38 suspensions of fresh normal PRP kept at 37°C, with platelet count ranging from $0.05 - 0.4 \times 10^6$ per mm^3 , there was little variation (0.66 ± 0.04 S.D.). The ratio approached unity with spherical platelets. With spherical and discoid platelets mixed in various ratios $O.D._{700}/O.D._{200}$ could detect as little as 10% change in discoid platelets, demonstrating that this ratio gives a sensitive, objective and quantitative reflection of platelet shape.

MORPHOLOGICAL SEQUENCE OF SPONTANEOUSLY FORMING PARIETAL THROMBI AS OBSERVED BY SCANNING ELECTRON MICROSCOPY (SEM). H.J. Genz, H. Metzger, P.F. Tauber and H. Ludwig. University of Essen School of Medicine, University Clinics, Essen, West Germany.

Spontaneous thrombus formation in human mesenteric veins was studied with the SEM. Tissue specimens were prepared according to Ludwig et al., *Acta anatomica*, 96, 469-477 (1976). Platelet shape change, thrombus formation and organization and the morphological interactions between the various corpuscular elements of blood are demonstrated. The following morphological criteria of these processes are observed: (1) Platelets adhere to distinctly altered endothelial surfaces and exhibit pores in the membrane and pseudopodia. (2) Platelet aggregation and thrombus formation occur next to each other along the endothelial surface. Thrombi contain red blood cells and also a larger number of lymphocytes, but only a few platelets are held prisoners within the fibrin network. Once caught in the mesh, such platelets do not show shape change compared to those being in contact with the endothelium. (3) Red blood cells between the thrombus fibers undergo form changes. Lymphocytes remain unaltered, but vice versa destroy adjacent fibrin fibers leading to partial loss of thrombus stability. This destruction occurs to a much lesser degree when platelets are near to the lymphocytes. It seems conceivable that platelets exert an inhibitory effect towards lymphocyte-induced fibrin proteolysis. The data suggest that both platelets and lymphocytes possibly represent a cellular control system that is responsible for the physiological clearance of spontaneously formed thrombi.

PLATELET WATER CONTENT IS DECREASED BY GEL-FILTRATION. J.S. Wiley, G. Wray and I.A. Cooper, Cancer Institute, Melbourne, Australia.

One approach to platelet sizing is to measure the intracellular water space of platelets with $^3H-H_2O$ since the % water content of platelets remains constant in states with different platelet sizes. Fresh citrated blood was centrifuged for 10 min at 150 'g' to obtain PRP. Aliquots of PRP were briefly incubated with either $^3H-H_2O$ or ^{14}C -sucrose then layered over 0.3 ml dibutylphthalate and spun 4 min at 8000 'g'. The cell pellet was solubilized and counted to enable spaces to be calculated. The extracellular (sucrose) space was subtracted from the total water space of the pellet to give a mean intracellular water space of $0.56 \pm 0.12 \mu l/10^8$ platelets ($n = 19$). Assuming a water content of 75% and a density of 1.04, the mean platelet volume for normal subjects is 7.2 fl. Gel-filtration of platelets (GFP) on Sepharose-2B reduced their mean water space by $0.12 \mu l/10^8$ platelets. However the amount of shrinkage on gel-filtration depended on the initial water space of the platelets in PRP and there was a linear relation between these two variables ($r = 0.82$). Shrinkage was 40% for an initial platelet water space of $0.70 \mu l/10^8$ platelets but there was almost no shrinkage below a water space of $0.40 \mu l/10^8$ platelets. Recovery of platelets from each column averaged 80% and showed no relation to the reduction in the mean cell water space. The lower water space of GFP may indicate a reduction in mean cell volume due to gel-filtration.