

NEW METHOD FOR AGGREGOMETRY. T.Sano, T.Motomiya and H.Yamazaki. Aoyama Tokyo Metropolitan Hospital and Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan.

Platelet sensitivity to aggregation seems to reflect some different aspect from other parameters of platelet function. A new method to assess platelet sensitivity to ADP-aggregation was devised. It has three advantages: it requires 1) no centrifugation, 2) small amount of blood and 3) inexpensive equipments. Venous blood (0.8 ml) was drawn into a polyethylene syringe containing 0.2 ml of citrate. PRP was separated by standing the syringe on a clay board and was added to ADP, serially diluted from 2^{-5} to 2^{-15} mg/ml in a Microtiter tray. The minimum effective ADP concentration to give aggregation (2^{-n} mg/ml) was detected microscopically and platelet sensitivity was expressed by the absolute value of the exponent (n) of the endpoint ADP concentration. Sensitivity correlated with intensity of aggregation obtained by the optical density (OD) and the screen filtration pressure (SFP) method, but not with velocity of aggregation. Using this method, the following findings were observed. 1) Sensitivity was enhanced i) in acute stage of thrombosis, ii) after isometric exercise in the patients with coronary and cerebral arteriosclerosis and diabetic patients. 2) Sensitivity, but not intensity of aggregation, correlated with age ($r = 0.66$, $P < 0.005$) in healthy males. 3) When human citrated blood was filtrated through glass filter, sensitivity was enhanced, while intensity of aggregation did not change. The degree of the enhancement of sensitivity increased as the contact area of glass increased. These findings suggest that the appearance of hypersensitive platelets may be reflected more on sensitivity than on intensity of platelet aggregation. This method is also applicable to ristocetin-induced platelet aggregation and von Willebrand factor assay.

TEMPLATE BLEEDING TIME: INFLUENCE OF CUFF PRESSURE. H. Mielke, Jr., P. Flavell, and R. Rodvien. Institute of Health Research, Pacific Medical Center, San Francisco, California, U.S.A.

We have studied how specific changes in technique affects the bleeding time (BT). Using a template BT (TBT), we have evaluated the influence of a blood pressure cuff set at 40 mm. Hg. The cuff influenced the TBT, and increased the sensitivity to aspirin (ASA) ($N = 12$, $\bar{X} = 5'00''$ to $10'16''$ versus $2'41''$ to $5'08''$, $p < .05$). A vertical incision produced a shorter TBT than a horizontal incision ($N = 100$, $\bar{X} = 4'21''$ versus $5'34''$, $p < .001$). In 9 normal subjects, the Ivy (I) and the Duke (D) methods were compared to vertical (V) and horizontal (H) TBTs with and without ASA. D was not influenced by ASA, and I showed a mild prolongation which returned to normal in 3 days. The TBT showed the greatest prolongation ($H > V$) returning to normal within 6 days. The TBT was then compared to a Warner-Lambert (WL) spring loaded device which produced a 6×1 mm vertical incision. Eight subjects were studied twice weekly for 5 weeks. The \bar{X} of TBT was shorter than the WL ($N = 80$, $\bar{X} = 4'14'' + 1'14''$ versus $5'28'' + 1'54''$) and the range was smaller. Neither technique was painful and scarring was minimal. The repeatability and sensitivity of the BT to ASA is highly dependent on the technique used. Either the BT measures more than platelet participation in hemostasis or different BT techniques are variably sensitive to different pathways of platelet recruitment.

AUTOMATED DETERMINATION OF PLATELET AGGREGATION BY CONTINUOUS FLOW SYSTEM. M.Yamanaka, S.Naiki, and Y.Kaku. University of Tokyo School of Medicine, Tokyo, Japan.

In general, the determination of aggregation of platelet is performed by aggregometer in which platelet rich plasma (PRP) is agitated mechanically by stirrer in the limited space of tube, and almost all aggregometer has a low ability to perform the tests of many samples as a routine test at the clinical laboratory. To solve the problems of the effect of stirrer and the measurement in the unphysiological condition, we have devised a new method of measurement using the continuous flow system, which is designed finally to determine successively 30 samples per hour. In this system, PRP is passed through only the continuous glass tube partly coiled. One ml. of PRP to be tested is sucked into the tube at a speed of 1 ml. per minute and flowed in the continuous tube line. Solution of adenosine diphosphate (ADP) or physiological saline is simultaneously sucked at a speed of 0.25 ml. per minute and jetted into a flow of PRP at the joint and mixed by coiling tube. The changes in transmittance of PRP during its flow are measured photometrically with the detectors attached to the straight parts of tube at the points arbitrarily settled. An excellent reproducibility of each detector and linearity against the concentrations of platelet in PRP were obtained in this system. Degree of changes in transmittance obtained at the point 20 seconds after an addition of ADP in this system correlated with the maximum aggregation rate of ADP-induced aggregation in aggregometer. Spontaneous aggregation which could not be detected with ordinary aggregometer was obviously observed in this system. These results suggested that this new method might be useful to detect the spontaneous aggregation.