Reversible Decrease in Platelet Retention by Glass Bead Columns (Adhesiveness) Induced by Disturbing the Blood[1] (35361)

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When blood is passed through a column of glass beads, a large percentage of the platelets is retained if conditions such as the number of beads and flow rate are appropriate (1, 2). We used the technique of Bowie *et al.* (3) which entails pumping heparinized blood through a column at a standard flow rate, and comparing the initial platelet count with the count in the combined 4th and 5th ml of blood emerging from the column. The degree of platelet retention was found to be markedly affected by the manner of handling the blood.

Materials and Methods. Columns were made with medical grade polyvinyl tubing (Becton-Dickinson B-D No. 6238, i.d. 0.118 in.) filled with 2.6 g of 3 *M* Superbrite 070-5005 glass beads (Minnesota Mining and Manufacturing Co.) and packed by applying the end of the tubing to a vibrator (Vortex Jr. Mixer). The ends were sealed with B-D No. 3113 male–male adaptors with nylon mesh attached to the inner end with Duco cement. Dr. Bowie also kindly supplied us with columns made in his laboratory. Blood was drawn through an 18-gauge needle into a 20- or 50-ml syringe (B-D Plastipak) containing enough heparin for a final concentration of 4 units/ml (Panheprin, 4000 units/ml, diluted with isotonic saline to 1000 units/ml and kept frozen). Care was taken to avoid mixing the blood with air. To mix the heparin with the blood in the 20-ml syringe, the syringe was held between the palms and rotated back and forth on its long axis 30 times in about 9 sec—a maneuver which we called twirling. When a 50-ml syringe was used, the syringe was attached nozzle-to-nozzle with an empty 50-ml syringe by a polyvinyl collar 0.5 in. long. After transferring a small volume of blood and expelling the air bubble in the dead space, we slowly transferred all but 5 ml of the blood into the other syringe and back again (0.6 ml/sec; speed 1 on a Harvard Pump, Model 975), and then 10 ml samples were distributed into 20-ml syringes at the same speed. Blood collected and mixed by these methods was considered "undisturbed."

Blood mixed in a 50-ml syringe as described above was disturbed by transferring it rapidly to and from another 50-ml syringe five times (about 3 ml/sec). This step, too rapid for the pump, was done by hand. The blood was then distributed into 20-ml syringes. In some experiments, the blood was disturbed by Bowie's orginal blood collection technique. Ten ml of blood was drawn into an empty syringe and placed in a polycarbonate tube containing 40 units of heparin. The tube, capped with Parafilm, was inverted six times, and the disturbed blood was then drawn into a 20-ml syringe.

To perform the platelet retention test, a 20-ml syringe containing 10 ml blood was twirled to ensure uniform suspension of the cells and connected to a column by 0.5 in. of polyvinyl tubing. Blood was then pumped through at 5.7 ml/min (speed 5). The first 3 ml was discarded. The pooled 4th and 5th ml as well as a control sample were collected in plastic tubes (Falcon No. 2005, 12 × 75 mm sterile-disposable) containing 0.013 ml 15% K_3EDTA/ml. Platelet counts were done in duplicate with a Coulter Counter Model B, and the percentage retained by the column was calculated. Since even low counts were diluted 1:3000, the lowest counts were re-

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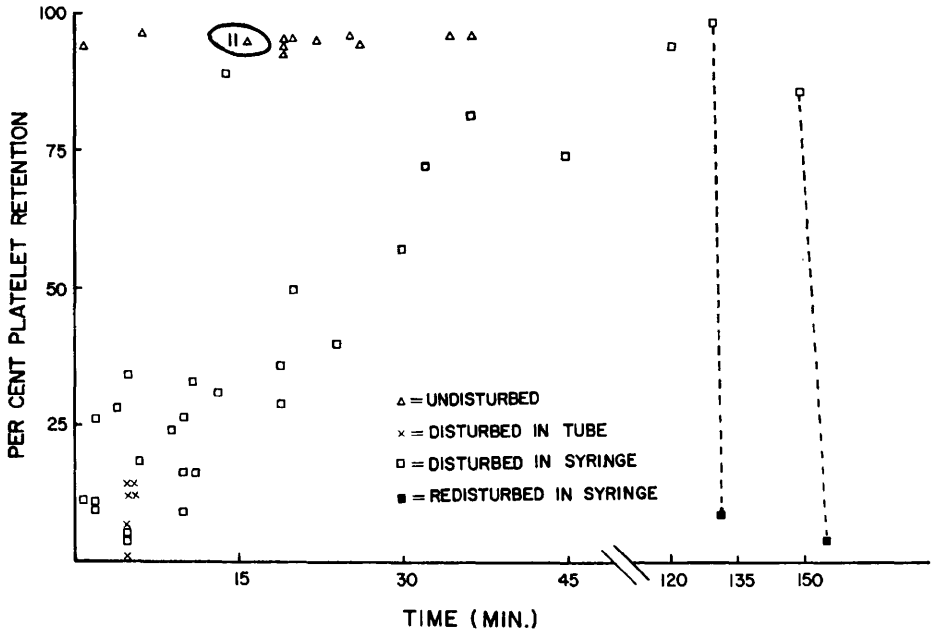

FIG. 1. Platelet retention or its change with time in blood that is undisturbed (△); disturbed by inversion in tube (X); or by rapid transfer between two syringes (□); and subsequently redisturbed (■). For undisturbed blood, abscissa represents time after drawing; for disturbed blood, time after disturbing. Eleven observations on undisturbed blood fall within the circled area.

corded simply as less than 12,000/mm³.

Results. Platelet retention of disturbed and undisturbed blood is shown in Fig. 1. Over 91% of the platelets were retained in 22 expts. on undisturbed blood samples from 15 normal subjects. Samples inadvertently mixed with air often had lower values and were not included in the data. In contrast, blood disturbed in the syringe and tested promptly showed very low platelet retention (except in 1 expt.). Retention was gradually restored, however, when the blood was left in the syringe at room temperature before being passed through the column. Disturbed blood which regained adhesiveness in this way lost it again if redisturbed. Blood collected and disturbed in glass syringes showed the same retention and loss of retention as that in plastic syringes.

Blood samples prepared by inversion in a tube also had very low platelet retention when tested on our columns (Fig. 1), whereas Bowie *et al.* (3) reported high retention. To determine whether this discrepancy was caused by the columns themselves or other technical differences, four experiments were carried out in Bowie's laboratory and three in ours, using the same blood sample for both types of column. The blood was disturbed by inversion in all of Bowie's experiments and in one of ours, and by the syringe method in our other two experiments. Retention was al-

TABLE I. Platelet Retention in Heparinized Blood Passed Through Various Columns.

Expt.	Method of disturbing	Platelet retention (%) in various columns			
		Our tubing, our beads	Bowie's tubing, Bowie's beads	Our tubing, Bowie's beads	Bowie's tubing, our beads
1	Transfer between syringes	4	38	7	54
2	Inversion in tube	37	63	66	86

ways higher with his columns than with ours, averaging 80.5 ± SD 22.7 and 39.5 ± 27.8, respectively, with a significant difference ($p<0.01$) when the data were analyzed by the paired t test. Table I shows the results of the experiments carried out in our laboratory with hybrid columns made by combining our tubing with Bowie's beads and vice versa. (For technical reasons the hybrid columns in Expt. 1 had one of our adaptors and one of Bowie's and the polyvinyl collars received with his columns. In Expt. 2, we used our adaptors and polyvinyl collars.) The results suggest that tubing and beads are both significant factors and may act synergistically.

Undisturbed blood was centrifuged at 630g for 10 min at room temperature in a 20-ml syringe held vertically with the nozzle sealed after the air had been expelled. When the platelet-rich plasma and red cells were remixed by unusually vigorous twirling, retention was only —4, —4, 18, and 30% in four experiments. Retention in uncentrifuged control samples subjected to equally vigorous twirling was 77–80%. The increase in twirling probably explains why these control values are less than those shown in Fig. 1.

Discussion. Our studies indicate that platelet retention is exquisitely sensitive to the manner of blood handling. Bowie had observed that handling reduces platelet retention (personal communication); in our experience, retention may be markedly reduced when air bubbles move through blood in the syringe or a tube. Retention is decreased in the absence of air when blood is transferred rapidly between two syringes, or slowly centrifuged and remixed. Fortunately, mixing by twirling does not appear to affect retention. Since centrifuging decreases platelet retention, experiments using platelet-rich plasma must be interpreted cautiously. Platelet retention decreased by disturbing the blood in a syringe was gradually restored by leaving the blood undisturbed at room temperature and could again be decreased by disturbance.

The plastic tubing and glass beads also exert a marked effect on retention, explaining the difference in results between our columns and those of Bowie's group (3). These investigators demonstrated that platelet retention is significantly reduced in patients with von Willebrand's disease, and our findings were similar with undisturbed blood (unpublished observations).

Summary. When human blood was drawn into a syringe containing heparin and pumped through a column of glass beads, over 90% of the platelets were retained. Platelet retention was markedly reduced if the blood was first disturbed by inversion in a tube, slow centrifugation and remixing, or rapid back-and-forth transfer between two plastic or glass syringes. Retention was restored within 1 hr after the last-named maneuver but could be reduced again by redisturbing the blood. The high platelet retention noted when blood inverted in a tube was passed through columns prepared in Bowie's laboratory indicates the marked effect of the plastic tubing and beads.

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