Separation of Platelets from Whole Blood by the Use of Silicone Liquids

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A QUANTITATIVE method for the separation of morphologically intact functional platelets from whole blood is not available at the present time. Methods have been described which use differential centrifugation or sedimentation, with and without washing procedures. The prolonged manipulations required with these procedures to insure total recovery leads to platelet damage. Shortening of procedures in order to preserve platelet function results in inconsistent or poor recovery. In the course of investigation of survival of platelets isotopically tagged in vitro, the necessity for quantitative recovery of platelets from small (1 to 10 ml.) blood samples became apparent. Previous work by Vanamee indicated that separation by the use of silicones might prove useful. The method described here depends on differences in the specific gravity of silicones and blood elements. It is rapid and simple to perform, requires little manipulation of platelets and yields quantitative recovery.

MATERIALS AND METHODS

Initial studies were performed using rabbit blood obtained by cardiac puncture and dog blood obtained usually by jugular venipuncture. Human blood was obtained by antecubital venipuncture. All syringes, needles and glassware were silicone-coated (General Electric Co., SC 87 and 99). Anticoagulation was obtained with 0.5 ml. of a 10 per cent solution in water of disodium ethylene diamine tetraacetic acid for each 10 ml. of blood. In later studies of human blood a 2 per cent solution was used with equally satisfactory results. One ml. of a 2 per cent solution in isotonic saline of oxyethylated tertiary octyl phenol formaldehyde polymer (Winthrop-Stearns Superinone WR-1339) was used for each 10 ml. of blood to assure resuspension of platelets.

Silicone liquids were obtained from Dow Corning Corporation and blended by mixing silicone fluids #555 with viscosity of 10-30 centistokes and #200 with viscosity of either 20 or 50 centistokes. The specific gravities of the two silicone liquids were reported by the manufacturer as 1.06 and 0.96 respectively. Approximately 15 ml. of #200 was mixed with 85 ml. of #555. The resultant specific gravity was determined for each batch by standard gravimetric methods. When the mixture aged over a long period, specific gravity tended to increase and readjustment was necessary by addition of small amounts of #200. The first specific studied was 1.056, using rabbit blood which yielded red cell contamination of the platelet fraction. Later studies with rabbit, dog, and human blood were done with specific gravities of 1.034 to 1.040. Values lower than 1.034 yielded inconsistent results.

One ml. of blood was quantitatively added to the anticoagulant mixture in a 10 x 75 mm. pyrex tube and mixed by gentle swirling motion. One ml. of the mixture was layered by volumetric pipette over 0.5 ml. of silicone in a micro-centrifuge tube made from borosilicate glass tubing (5 mm. I.D. x 12 cm. long) and centrifuged at 8 C at 3,000 rpm for 15 minutes. On examination of the microtubes, sharp separation of layers of plasma, huffy coat, silicone and red cells was seen (fig. 1). The microtube was scratched with a file and broken just below the huffy coat-silicone interface over a recipient tube, allowing collection of the plas-
Fig. 1.—Appearance of microtube after 15 minutes centrifugation: red cell, silicone, buffy coat and plasma layers are sharply delineated. The buffy coat (see text) contains the platelet fraction of whole blood.

The red cell-silicone remnant usually was discarded. The buffy coat was broken up and resuspended in the plasma by vigorous hand shaking.

When 10 ml. samples were studied the procedure was similar except that usually 2 ml. of silicone was introduced over the whole blood in a 15 ml. graduated centrifuge tube. On completion of centrifugation, it was necessary to withdraw plasma and buffy coat by Wintrobe pipette* in stages. A few milliliters of clear plasma were withdrawn first and set aside for later rinsing of the pipette. Additional small portions of plasma were then removed, carefully approaching the buffy coat which was removed last with a minimum amount of silicone carried along. The pipette was then rinsed with the plasma previously set aside. Although the metal pipette and glass attachment have an obvious disadvantage in quantitative transfer due to the square design at the base of the glass portion, the opaque character of the tip allows careful visual approach to the suspended buffy coat atop the silicone layer. Attempts to use glass pipettes were uniformly unsuccessful because of inability to see the fine clear tip in the clear media at all times; as a consequence, the suspended buffy layer was easily lost into the silicone layer by mechanical disturbance.

Recovery of platelets by this method of separation was assessed by performing platelet counts on the anticoagulated blood and on the plasma fraction containing the resuspended platelets. Phase microscopy was utilized in conjunction with standard platelet counting techniques. The volume of suspension was used in the final calculation of platelet recovery.

White cell contamination of the buffy coat was assessed by the performance of standard cell counts and the examination of smears stained with Wright’s stain. The ability of separated platelets to promote clot retraction in platelet poor plasma was tested using

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The standard error of the mean of platelet counts in whole blood was ±1500 where the mean count was 167,700 and N was 22.
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previously described methods.4 The calcium chloride used was 0.04 M. The method was adapted by diluting separated platelets with platelet poor plasma so that tubes containing 25,000/mm.² to 200,000/mm.² would give graded results from 0 to 4 plus, thereby giving information on the function of the majority of platelets. The controls consisted of platelet rich plasma, whole blood and platelet poor plasma.

Human blood was obtained in 100 ml. samples from 26 donors. These individuals had the following diagnoses: 7 normal, 1 inactive pulmonary tuberculosis, 5 chronic obstructive pulmonary emphysema, 2 bronchitis, 1 aortic stenosis, 4 diabetes mellitus, 2 peptic ulcer, 1 myasthenia gravis, 1 rheumatoid spondylitis, 1 serum sickness due to penicillin sensitivities, 1 thrombocytopenia and anemia of unknown origin. Triplicate 1 and 10 ml. samples were studied for platelet and white cell recoveries with each of three specific gravities (1.034, 1.037 and 1.040) in the majority of donors.

Clot retraction studies were performed with platelets separated from duplicate 10 ml. and 1 ml. samples, using a single specific gravity of 1.037 and 1.040 in 10 patients. The diagnoses were as follows: 3 peptic ulcer, 1 essential hypertension, 1 asthma, 1 emphysema, 1 active pulmonary tuberculosis, 1 exogenous obesity, 1 hypothyroidism, and 1 diabetes mellitus.

RESULTS

Initial studies in 5 rabbits, using a silicone liquid with specific gravity of 1.040, revealed average platelet recovery from 10 ml. whole blood duplicates of 94 per cent (range of 66 to 119 per cent). White cell determinations were not performed. These encouraging results led to the study of 10 ml. and 1 ml. whole blood duplicates from 2 dogs, using specific gravities of 1.030, 1.032, 1.034 and 1.036. In these studies no advantage was demonstrated for any of the silicone gravities. the overall recovery for the group being 103 per cent with either volume of blood. White cell recoveries in the platelet fraction were 22 and 13 per cent for 10 ml. and 1 ml., respectively, for all gravities.

The results of 137 triplicate determinations from blood obtained from 26 human subjects using silicone mixtures with specific gravities of 1.034, 1.037 and 1.040 are shown in table 1. The data show that good recovery of platelets is obtained with any of these liquids which vary in specific gravity range of 0.006. White cell contamination was variable and occurred to the same degree despite prolonged centrifugation up to 45 minutes. Because we did not wish to prolong centrifugation further (to avoid platelet damage) and no advantage was gained by 30 or 45 minutes centrifugation, the majority of the determinations listed were performed at 15 minutes. Smears of the platelet fraction stained with Wright’s stain revealed that the majority of the white cells were lymphocytes. When the tube containing separated platelets was allowed to stand at

| Table 1.—Platelet and White Cell Recovery from Whole Blood Using Silicone Liquids for Separation |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 ml. blood | | | | 1 ml. blood | | | |
| | 1.034 | 1.037 | 1.040 | | 1.034 | 1.037 | 1.040 | | |
| Platelet | 106.82 ± 3.06 | 112.24 ± 3.13 | 110.52 ± 3.11 | | 110.53 ± 3.17 | 115.13 ± 3.33 | 109.14 ± 2.83 | | |
| (24) | (23) | (22) | | (24) | (21) | (24) | | |
| White cell | 26.77 ± 1.26 | 29.38 ± 1.51 | 31.13 ± 1.13 | | 26.79 ± 1.71 | 25.64 ± 1.69 | 30.96 ± 1.69 | | |
| (22) | (22) | (22) | | (21) | (21) | (21) | | |

*Containing anticoagulant and Superinone.

1 Mean per cent recovery from triplicate samples in the indicated number of donors.

1 Standard error of mean.
room temperature for approximately 5 minutes, the white cells and the small amounts of silicone present almost completely settled to the bottom leaving the platelets in supernatant plasma. Thus, separation of white cells from platelets is possible with little loss or damage to platelets.

Clot retraction in platelet poor rabbit plasma promoted by rabbit platelets separated from 0.15 to 0.25 ml. whole blood was contrasted with that seen in equal amounts of non-separated whole blood. Platelets separated from as little as 0.15 ml. whole blood promoted clot retraction to the same extent as an equivalent volume of non-separated whole blood. Retraction studies done in human blood yielded the results shown in figure 2. It is apparent that platelets separated by this method do not suffer sufficient damage to impair the ability to promote clot retraction in platelet poor plasma even when diluted to 30,000-40,000/mm.³

DISCUSSION

Previously described methods of platelet separation preparatory for biological, biochemical and physiological studies, using differential centrifugation technics or resin-elution technics, in general yield 30-90 per cent of the platelets calculated to be present in the initial whole blood. In order to obtain more than the platelets contained in the platelet rich plasma, washing procedures with platelet poor plasma, saline or citrate have been applied to the red cell fraction remaining after removal of platelet rich plasma. Recentrifugation and repeat washing will ultimately provide 90-100 per cent of the platelets. However, it has been known that platelet function is diminished with exposure to simple electrolyte solutions and with repeated manipulation and centrifugation. In addition, these maneuvers are time-consuming and tedious to perform. Other means of sedimenting red cells from plasma containing the platelets involve trapping of varying numbers of platelets in the

Fig. 2.—Clot retraction in platelet poor plasma promoted by platelets recovered from human blood and diluted prior to test to contain platelets as follows: (1) whole blood (194,000 mm.³); (2) platelet rich plasma (390,000/mm.³); (3) platelet poor plasma; (4) 1 ml. of extracted platelets (297,000 mm.³); (5) 0.5 ml. of extracted platelets and 0.5 ml. of platelet poor plasma (148,000/mm.³); (6) 0.25 ml. of extracted platelets and 0.75 ml. of platelet poor plasma (74,000/mm.³); (7) 0.15 ml. of extracted platelets and 0.85 ml. of platelet poor plasma (45,000/mm.³); (8) 1 ml. of extracted platelets (238,000 mm.³); (9) 0.5 ml. of extracted platelets and 0.5 ml. of platelet poor plasma (119,000 mm.³); (10) 0.25 ml. of extracted platelets and 0.75 ml. of platelet poor plasma (59,000/mm.³).
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red cell fraction causing lesser recoveries. Platelets obtained by these procedures by themselves might not be suitable for reinfusion studies because of the presence of such contaminating materials.

This silicone method offers real advantages over those methods previously described in that it involves one short centrifugation and one simple pipette transfer to attain quantitative recovery. The platelets are functional even when diluted in numbers to as low as 40,000/mm³. No special equipment is needed to perform the separation and the technic is readily mastered by any individual proficient in quantitative transfers. Several interesting observations evolved from use of this technic: (1) more than 100 per cent of platelets counted in whole blood are recovered, (2) variability of platelet "specific gravity" occurs in normal humans.

In experiments in which no technical problem arose, more than 100 per cent recoveries were seen consistently. We believe the basis for this observation is the obscurring of platelets by the presence of red cells in the counting chamber when whole blood samples are examined. Counting of platelets in plasma removes red cells and allows visualization of all the platelets.

Variability in ability to extract platelets in the range of 1.034–1.040 specific gravity silicones did occur, several individual's samples yielding better results at the higher gravity. Indeed, a few required as high as 1.044 and 1.048 for maximum yields. This would indicate that variability in platelet "specific gravity" does exist and may correlate with Rebuck's¹³ findings of multiple platelet sizes and forms in normals and disease states. We are not convinced that specific gravity of the platelet is the sole factor in successful isolation of platelets since surface tension changes in 1 ml., and 10 ml. procedures appeared to play a role in the results.

Although clot retraction studies indicate unimpaired function of the separated platelets, total preservation of function can be assumed only after reinfusion studies of such separated platelets. Survival of the majority of platelets in the intact animal indicates normalcy of function. Preliminary reinfusion studies with C¹⁴-labeled separated platelets indicates good early recovery and a normal life span. More detailed investigation of survival is underway at the present time.

**SUMMARY**

A method of separating platelets from whole blood has been developed. The method involves simple centrifugation with blended silicone fluids. Quantitative recovery of platelets is achieved. Platelets appear to have no loss of ability to promote clot retraction in platelet poor plasma.

**SUMMARIO IN INTERLINGUA**

Esseva elaborate un metodo pro le separation de plachettas ab sanguine total. Le metodo utilisa un simple centrifugation con un mixtura de liquidos siliconas. Le recuperation quantitative del plachettas es effectuate. Le plachettas, assi obtenite, pare sufer nulle perdita in lor capacitate de promover le retraction del coagulo in plasma a deficientia plachettal.
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REFERENCES


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