A Method for Separating and Concentrating Platelets from Normal Human Blood

By Allen H. Minor, M.D. and Lee Burnett, M.S.

The effects of transfusions of platelet-rich blood from polycythemic donors given to patients with thrombocytopathic purpura have been studied by Hirsch, Favre-Gilly and Dameshek, Hirsch and Gardner, Stefanini and Chatterjea, and Stefanini et al. Such transfusions have been followed by changes in prothrombin consumption, clot retraction, bleeding time and capillary fragility toward normal, and in many instances to normal, for varying periods of time; and, in the presence of active bleeding, by a more or less pronounced beneficial effect on the hemorrhagic diathesis, lasting well beyond the time during which the platelet count had been temporarily normalized. Duke had previously described the effect of direct transfusions of whole blood given to 3 thrombocytopathic patients; he noted a rise in the platelet count, lasting about two days, and complete relief from spontaneous hemorrhages for three days.

These studies have suggested the desirability of a method for obtaining large numbers of functionally intact platelets from normal blood. Two procedures have recently been described for this purpose: that of Freeman, in which columns of ion-exchange resin are used, and that of Dillard, Brecher and Cronkite, which depends on the use of Sequesterene Na₂ as an anticoagulant, and differential centrifugation. The present report describes a method which has been used for the preparation of platelet concentrates from ACD blood, and which thus involves no significant change in the procedure for blood collection now widely used.

Preliminary clinical studies have indicated that transfusions of these platelet concentrates have effects similar to those described following the transfusion of platelet-rich polycythemic blood.

A plasma suspension of leukocytes and platelets may be obtained from fresh citrated blood by the addition of an agent which accelerates erythrocyte sedimentation. This suspension will contain most of the platelets present in the whole blood, providing platelet agglutination does not occur in the presence of this agent. Furthermore, the addition of a surface-active agent will permit complete resuspension of the platelets following prolonged centrifugation. These observations are the basis of the following method for obtaining platelets.

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METHOD

Apparatus

The apparatus consists of the following: blood plasma centrifuge; centrifuge bottles, round bottom, 500 ml.; sedimenting bottles, 700 ml. (e.g. Cutter Saftiflasks); serum stoppers to fit all bottles; tubing, 8” lengths, fitted with needle adapters; aspirating needles, 8” long.

All glassware and needles are siliconed before use. The application of Dow Corning silicone, DC 200, 350 cs., is simple and effective. A 2 per cent solution of the liquid silicone is made up in carbon tetrachloride. The bottles are filled with the solution and the needles immersed in it; they are then drained and the solvent is evaporated by heating for 30 minutes at 100 C. or by air drying at room temperature for 2 to 4 hours. The silicone film is then fixed on the surface of the apparatus by baking for 30 minutes at 300 C. (572 F.) in a kitchen oven. This coating will last through 25 to 30 washings and sterilizations.

Reagents*

Dextran solution, 6 per cent. Any dextran fraction having an average molecular weight of 240,000† or above (intrinsic viscosity 0.37 or higher) may be used. The solution is prepared by dissolving 6 Gm. of dextran substance per 100 ml. of pyrogen-free saline with the aid of heat. It is then passed through a “Seitz” filter, and 60 ml. of the filtrate are added to each sedimenting bottle. These units are autoclaved, and may be stored at room temperature.

This reagent is a polysaccharide built up from glucose molecules. It is used to accelerate erythrocyte sedimentation. The rate of sedimentation is proportional to the molecular weight of the dextran; for this reason, a high molecular weight fraction is selected.

Triton solution, 2 per cent. This is prepared by diluting 2 parts of Triton WR-1339 with 98 parts of pyrogen-free saline at a temperature between 50 and 60 C. Twenty ml. of this solution are added to each centrifuge bottle. These units are autoclaved, and may be stored at room temperature.

This reagent is a non-ionic surface-active agent, an alkyl aryl polyether alcohol. It is used to prevent the agglutination of platelets brought together by centrifugation, and thereby permits them to be resuspended as discrete elements. It was selected because of its low toxicity and because it is nonhemolytic.

Procedure

Sterile precautions are observed throughout.

Four or more pints of blood have been used in the preparation of platelet concentrates for transfusion. The donors may be of any blood group, since the final concentrate is essentially free of erythrocytes.

The blood is collected by gravity in standard ACD bottles. These need not be siliconized if the blood is processed within three hours. Care should be taken to avoid the formation of clots, for these invariably lower the final yield of platelets. Venepuncture should be performed with a very sharp wide-bore needle which is passed directly into the lumen of the vein; the flow of blood into the bottle should be maintained at a uniform rate, using gentle suction if necessary; and the blood kept mixed with the ACD solution by swirling at frequent intervals.

As soon as possible after collection, each pint of blood is transferred to a 700 ml. sedimenting bottle which contains 60 ml. of dextran solution. This is accomplished by gentle suction, with the tip of the needle in the solution, thus avoiding the formation of froth, which tends to trap erythrocytes. The blood is then allowed to sediment at room temperature for about two hours.

* The authors are grateful to Pharmacia Laboratories, Inc., 270 Park Avenue, New York, for supplies of several dextran fractions; and to Rohm and Haas Co., Philadelphia, for supplies of Triton WR-1339.
† As determined by ultracentrifugation method.
The supernatant fluid in each sedimenting bottle is aspirated into a 500 ml. centrifuge bottle which contains 20 ml. of Triton solution. The contents of the bottle are mixed by inversion several times and then centrifuged at 2000 r.p.m. for 30 minutes. The formed elements initially present in suspension are now packed in the sediment. The cell-free fluid is aspirated until only about 10 ml. remain. Forty ml. of non-pyrogenic physiologic saline are added to this residual material, and the sediment resuspended by vigorous shaking. The concentrated suspensions obtained in this manner are then combined.

The pooled suspension is centrifuged briefly at low speed, e.g., for 7 to 10 minutes at 800 r.p.m., so as to bring down the erythrocytes and leukocytes while leaving nearly all the platelets in suspension. The supernatant suspension is then aspirated as completely as possible and the platelet concentrate is ready for transfusion.

Should further concentration of the platelets be desired, a second centrifugation at 2000 r.p.m. for 30 minutes may be followed by resuspension of the sediment in a small volume of residual fluid; a volume of Triton solution equivalent to 10 per cent of the volume of the platelet suspension should be mixed with the latter prior to centrifugation so as to prevent platelet agglutination. Concentrations of discrete platelets exceeding 20 million per cu. mm. have been obtained in this way.*

Similarly, the platelets may be subjected to repeated washings with saline to remove

* All platelet counts have been made by the direct method, using Rees-Eckers diluting fluid.
residual plasma, and negligible losses will occur providing a small volume of Triton solution is added prior to each centrifugation.

Based on an analysis of 40 concentrates, the composition of a platelet transfusion prepared from four pints of blood by this procedure is as follows (within a range of ± 25 per cent):

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (ml)</th>
</tr>
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<tbody>
<tr>
<td>Total platelets</td>
<td>350 billion</td>
</tr>
<tr>
<td>Total volume</td>
<td>200</td>
</tr>
<tr>
<td>Physiologic saline</td>
<td>160</td>
</tr>
<tr>
<td>Plasma</td>
<td>21</td>
</tr>
<tr>
<td>ACD solution</td>
<td>11</td>
</tr>
<tr>
<td>Dextran solution, 6%</td>
<td>6</td>
</tr>
<tr>
<td>Triton solution, 2%</td>
<td>2</td>
</tr>
</tbody>
</table>

About 80 per cent of the total number of platelets initially present in the ACD blood are recovered, concentrated eight to ten times. They appear morphologically intact, they are discrete, and they stain normally with Wright’s stain (fig. 1).

Studies on Platelet Concentrates: Effect on Clot Retraction and Prothrombin Consumption in Platelet-Poor Recalcified Plasma and in Thrombocytopenic Blood.

Platelet concentrates were prepared according to the procedure described; the final centrifugation was prolonged in some instances in order to lower the platelet count.

Platelet-poor plasma was prepared from the blood of normal individuals; chilled, siliconized equipment and a two-syringe technic were used. A small quantity of blood was drawn into the first syringe; a second syringe was then substituted and 9 ml. of blood drawn without stasis or frothing and placed in a tube containing 1 ml. 0.2 molar sodium citrate. The blood was centrifuged at 2000 r.p.m. for 2 hours, and the plasma removed.

Eight-tenths ml. of this plasma was placed into each of three glass tubes. To the first was added 0.1 ml. additional plasma, to the second, 0.1 ml. saline, and to the third, 0.1 ml. platelet concentrate. One-tenth ml. 0.2 molar calcium chloride was added to each, and the tubes mixed by lateral shaking. They were then incubated at 37°C.

One hour after clotting had occurred the tubes were inspected, and the degree of clot retraction recorded. The degree ranged from 4+, which represented retraction comparable to that found in control studies on platelet-rich normal plasma, to 0, which represented absence of retraction.

The clots were then removed with a wooden applicator, and the sera incubated at 37°C for 15 minutes. Prothrombin times were determined on the sera and plasmas by an adaptation of the prothrombin method of Quick, using rabbit lung thromboplastin. Prothrombin-free plasma, obtained by adsorption with barium sulfate, was used as the source of fibrinogen in the determinations on serum, and also as a diluent in the preparation of plasma containing 10 per cent prothrombin.

The results obtained in these studies are recorded in table 1. In all instances good clot retraction was observed in the recalcified plasmas to which the platelet concentrates had been added; no retraction occurred in the control tubes. In all instances the serum prothrombin times of the recalcified plasmas to which platelet concentrates had been added were greater than the prothrombin times of plasma containing 10 per cent prothrombin. The prothrombin consumption in
these tubes was therefore greater than 90 per cent. The serum times in the control tubes, however, were only slightly greater than the plasma prothrombin times, indicating the abnormally low prothrombin consumption caused by the virtual absence of platelets in the specially prepared plasmas.

Similar results were obtained when freshly drawn blood from thrombocytopenic patients was added to tubes containing small volumes of platelet concentrates. Definite clot retraction was observed after one hour, and prothrombin consumption was consistently over 90 per cent. No clot retraction occurred in control tubes, and the prothrombin consumption was abnormally low.

**Table 1.**—Effect of Platelet Concentrates on Clot Retraction and Prothrombin Consumption in Platelet-Poor Recalcified Plasma

<table>
<thead>
<tr>
<th>Platelet Concentrate</th>
<th>Clot Retraction with added</th>
<th>Prothrombin Consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Platelets</td>
<td>10%* prothrombin time (seconds)</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>Saline</td>
</tr>
<tr>
<td>No.</td>
<td>Count X 10^9/cu.mm.</td>
<td>Plasma</td>
</tr>
<tr>
<td>1</td>
<td>445</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>785</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>830</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>925</td>
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<tr>
<td>5</td>
<td>935</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>940</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>1335</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>1210</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>1305</td>
<td>0</td>
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<tr>
<td>10</td>
<td>1340</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>1460</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>1580</td>
<td>0</td>
</tr>
</tbody>
</table>

**Transfusions of Platelet Concentrates**

Twenty-five transfusions of platelet concentrates have been given to 11 patients who were thrombocytopenic and bleeding when first transfused. There were 5 cases of thrombocytopenic purpura, of which 4 were idiopathic and 1 secondary to a drug, Gantrisin®; 4 cases of aplastic anemia and 2 cases of acute leukemia. In 9 of the patients there was definite clinical evidence of a hemostatic effect lasting from 1 to 5 days following a single platelet transfusion; in the other 2 patients this effect followed subsequent transfusions. Concomitantly, there was a return to normal, or near normal, of bleeding time, capillary fragility and prothrombin consumption. One pyrogenic reaction occurred in the series of 25 transfusions. Thromboembolic phenomena have not been observed, nor have other adverse reactions.

**Discussion**

By means of the procedure presented above, normal blood can be processed to yield large numbers of platelets concentrated in a small volume of physiologic fluid. It is thus possible to transfuse the equivalent of the total platelet complement of three or more pints of blood without significant effect on the circulating
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fluid volume of the recipient. The platelets so obtained are functionally active, as indicated by their effect on prothrombin consumption and clot retraction in vitro, and by their hemostatic effect when transfused into thrombocytopenic patients.

The procedure is sufficiently flexible to permit modification according to convenience. It can be adapted to use with small volumes of blood for the preparation of platelet concentrates for laboratory studies. Satisfactory recoveries of platelets can be obtained with high molecular weight polyvinylpyrrolidone as the sedimenting agent; Tween 80 has been used in laboratory studies to replace Triton WR-1339. With these agents, about 12 per cent of the platelets initially present in the ACD blood remain with the red cell concentrate following sedimentation, essentially no loss occurs during concentration of the platelets, and about 8 per cent are centrifuged out with the residual erythrocytes and leukocytes in the final step. The latter loss may be avoided by omitting this centrifugation, as may be done when the erythrocytes in a platelet concentrate prepared for transfusion are compatible with those of the recipient. Similarly, the time of sedimentation may be reduced to one hour, if the presence of small numbers of erythrocytes in the final concentrate is of no consequence.

It is probable that somewhat higher recoveries of platelets might be obtained by collecting the blood directly into siliconed or plastic containers. Until such containers become widely available, however, it would seem more convenient in most instances to leave unchanged the standard technic of blood collection.

SUMMARY

A method is described for the preparation of platelet concentrates from pint volumes of normal human blood collected in standard ACD bottles. Erythrocytes are removed by sedimentation with high molecular weight dextran, and platelets are concentrated by centrifugation in the presence of a surface-active agent, which permits their resuspension in a small volume of physiological fluid following prolonged centrifugation. The procedure results in the recovery of about 80 per cent of the total platelet complement of the blood. The platelets obtained are discrete and morphologically intact, and induce normal prothrombin consumption and clot retraction when added to platelet-poor plasma or thrombocytopenic blood. Transfusions of platelet concentrates given to thrombocytopenic patients have been followed by hemostatic effects lasting from 1 to 5 days.

REFERENCES

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