Preserved Platelets: Their Preparation, Storage and Clinical Use

By James L. Tullis, Douglas M. Surgenor and Philippa Baudanza

The isolation of platelets from fresh, human blood in an apparatus that permits immediate washing in order to free the cells of entrained plasma proteins has led to sufficient stability to permit preservation in vitro. Preliminary reports on the characteristics of such preserved platelets dealt chiefly with retention of their thromboplastic activity. The apparatus used to concentrate the cells was a bacteriologically "open" system that precluded large scale transfusion studies. Subsequent modification of the equipment has made possible a semiautomatic biomechanical device which incorporates all the original features inside a sterile "closed" system. Details of this instrument, the Cohn-ADL Centrifuge, are described in a separate communication. It is believed that its unique feature of immediate separation of the cells from their plasma substrate in a controlled gas atmosphere is largely responsible for the excellent stability of platelets during preservation.

The present report deals with 320 units of platelets preserved for periods of up to three years. Two hundred and forty units have been transfused into thrombocytopenic recipients after storage between 1 and 24 months. Eighty units have been used in studies concerning the physiologic requirements of platelets maintained in the extracorporeal state and the morphologic and physiologic integrity of the stored cells. The practical advantages in the use of preserved platelets are discussed in relation to fresh, platelet-rich plasma and plastic bag concentrates.

Methods

Blood Collection and Separation of Erythrocytes

One pint of blood is collected by gravity through sharp needles and plastic tubing into a reservoir containing ACD solution NIH Formula A. Special care is taken to avoid traumatic venipuncture. "Half-bleedings" or collections requiring more than one venipuncture are not used for platelet isolation. The blood is then passed through a 2 ft. coil of plastic tubing immersed in a coolant bath at 4 C, and thence flows at a rate of 50 ml. per minute to the top of an inverted bowl revolving around its longitudinal axis.

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*A unit is defined as the platelet yield from 1 pint of whole blood. It contains, on the average, 50 ml. of suspended platelets with a count of 1,000,000/mm. 3
†Pretreated with argiall 1/1 (Armour Chemical Division, Chicago, Ill.).
‡All metal parts and connections are previously treated with non-wettable surfaces.
at a centrifugal force of 300 x gravity. Within this compartment, the red cells and white
cells are removed and platelet-rich plasma overflows into an individual container. These
separations take place during blood collection and are completed synchronously with a
completion of the phlebotomy.*

Separation of Platelets

The platelet-rich plasma effluent is passed at a flow rate of 10 ml./min. into a cylindrical
falling-film bowl where the platelets are concentrated at 250 x gravity. In this bowl,
similarly cooled to 4 C., the flowing plasma forms a thin vertical film. The platelets need
only to traverse a short distance to escape the flowing stream and thus be retained within
the bowl. At the end of the separation, the bowl is decelerated slowly to allow the super-
natant plasma to drain off without disturbing the retained cells. The bowl is then
accelerated and a solution comprised of sodium acetate, 0.2 Gm. %; sodium chloride, 0.8 Gm. %
and dextrose, 5 Gm. % (pH 6.3) is introduced to wash the platelets free
of entrained plasma proteins. Following this, 50 ml. of the same solution is introduced
as a resuspension medium and the bowl is decelerated abruptly. The platelets flow out
into an attached bag containing sufficient sterile, pyrogen-free gelatin to give a final
concentration of 1.4%. (The original gelatin is available as an 8.5% solution in .15 M
Sodium Chloride.) For increased yields, the bowl may be removed, placed at room
temperature for 30 minutes, and then gently rocked to recover the platelets which remain
on the walls of the liner. The tubing leading to the bag of platelets is sealed with a
dielectric sealer after which the sterile bag is severed from the apparatus. The bag is
then warmed slightly to liquify the contents. The platelets are homogeneously dispersed
and the bag is transferred to a standard 4 C. refrigerator where regelling occurs. The
platelets remain suspended discretely in the gel throughout the period of preservation.
A small diverticulum remains attached to the bag for subsequent culture, typing, platelet
counting or other desired sampling (fig. 1).

Sterility and Bacteriologic Safety

Despite the number of operations involved in obtaining washed platelets, the risk of
bacterial contamination is completely eliminated by use of the Cohn-ADL Centrifuge.
By making all connections to the equipment prior to autoclaving, the only connections
which need to be made thereafter are to the vein of the donor and to the vein of the
recipient. With these precautions, 320 consecutive platelet experiments have been carried
out without a single instance of bacteriologic contamination.† Moreover, an additional
series of over 1000 cell separations of other types have been carried out in the Cohn-
ADL Centrifuge with completely sterile products.

Other Methods

Platelet counting: All platelet counts were done by the method of Brecker and Cronk-
hite utilizing direct phase microscopy. The reproducibility of this method is reported
as within 7.8% for counts done of free flowing capillary blood. The error of the method
in our hands has approximated 6%. As a further check on accuracy the counts in many

*Platelet-rich plasma may alternately be prepared by spontaneous overnight sedimentation. This had the advantage of a simpler plastic assembly, but platelets so prepared have shown decreased survival on subsequent long-term storage.

†Average molecular size: 36,000 to 40,000. Prepared through the collaboration of Dr. D. Tourtellotte, Knox Gelatin Co., Inc., 4th and Erie Street, Camden, New Jersey, whose kind cooperation is gratefully acknowledged.

‡Since preparation of this report, an aerial shipment containing 15 units of plasma and 18 units of preserved platelets was reported to be contaminated with Pseudomonas sp. Re-check of original aliquots from these preparations show that the preserved platelets and plasma were sterile at the time of preparation, thus suggesting that contamination occurred during shipment or subsequent storage.
of the experiments were tested as unknowns and the pipette samples were drawn by a
different observer from the individual doing the counts.

Platelet morphology: Differential platelet counts were done by a method reported pre-
viously with subdivision of the cells into forms with fibrillar processes greater in length
than the cell from which they arose; forms without such fibrillar processes but otherwise
appearing normal; blebbed forms; ballooned forms showing partial disruption. This sub-
division is wholly arbitrary, but on the basis of countless observations it is believed to
represent the series of degradative morphologic changes which isolated platelets undergo
during preservation.

Residual serum prothrombin was tested by the 2-stage method of Ware and Seegers,
except where noted. Initially, a 15-minute incubation of serum was used. This later was
extended to 60 minutes for convenience in transporting specimens. No difference in pro-
thrombin content was found in control tubes incubated for the two periods of time. Normal
serum levels varied between 0 and 6 units/ml.

Recalcification time: Individual plasmas were centrifuged at full speed for 40 minutes
in a clinical centrifuge at room temperature, in a bacteroid centrifuge tube. Recalcification
and timing of clotting was done by standard technics.

Anticoagulants were assayed for with the use of a modified one-stage procedure in
which fresh normal plasma was used as the substrate. Commercial thromboplastin (Acu-
plastin, Ortho) was diluted 1:10 with the test serum or plasma. This was used as a
thromboplastin source in the one-stage procedure before and after incubation for 30
minutes at 37 C. The serum was separated one hour after collection and was not de-
calcified; however, when plasma was used, the source was the patient’s resin plasma
without calcium. Determinations were made in duplicate and results are expressed as
the average.

Thromboplastin generation was assayed by the standard method of Biggs and MacFar-
lane; except where noted, the platelets were assayed by the thrombin generation test
of Surgenor et al. Fresh platelets collected the same day as the test were used as the
control mixture for thromboplastin assay.
Clot Retraction was assayed by the method of Budtz-Olsen, using a gradient column of trichloroethylene and mineral oil. In contrast to the use of a solid surface, the gradient column permitted quantitative measurements to be made. Results are expressed as % decrease in the original volume of the clot.

**EXPERIMENTAL BASIS FOR METHODS OF SEPARATION AND PRESERVATION**

*Effect of Plasma Proteins*

Blood coagulation is known to involve a sequence of chemical reactions, of which the visible end point is the formation of a fibrin clot. The intermediate reactions are only partially understood. The initial event is believed to involve interactions between platelets, anti-hemophilic factor and plasma-thromboplastin component (PTC) in the presence of calcium ions. The interactions are initiated by physical changes which occur when platelets are exposed to a nonphysiological surface and by chemical changes which occur when the partial pressure of carbon dioxide suddenly decreases as the blood leaves the vascular network and is exposed to atmospheric air. Anticoagulants interrupt the clotting process by inhibiting certain specific levels; calcium, against whose reactivity citrate, oxalate and cation exchange resins are directed, is thought to enter the coagulation reactions at more than one place. The problem of preventing even the earliest chemical reaction of clotting is, therefore, considerably more subtle than that of inhibiting fibrin formation. Since no single anticoagulant now at hand is known completely to inhibit all chemical changes leading to clotting, and since any activation of coagulation, no matter how slight, may cause irreversible changes in platelets, prompt separation of the cells and plasma should result in optimal survival during storage.

The importance of interaction between platelets and plasma proteins was readily demonstrated experimentally. In one study, the survival of platelets in their native plasma was compared with the survival of platelets from the same blood sample freed of plasma by centrifugation and resuspended in dextrose-gelatin medium. Survival was assessed by simple counting of the intact platelets at intervals during the preservation period. The results are shown in table 1. Whereas 80 per cent of the platelets that had been promptly separated in the Cohn Centrifuge remained intact after 60 days in the dextrose gelatin medium, only 1 per cent remained when stored in their own citrated plasma. In a similar experiment the effect of delayed removal of platelets from their native plasma was studied. A single unit of blood was divided into two aliquots. Both halves were subjected to similar experimental handling except that the erythrocytes were removed in a Cohn Centrifuge in one and by spontaneous sedimentation at +4 C. in the other. This latter method involved 20 hours delay before the platelets were subsequently removed from the platelet-rich plasma. Subsequent resuspension and storage of the platelets was similar in both aliquots. Results were assessed in terms of the thromboplastin generating ability of the platelets when incubated in a Biggs-MacFarlane system (fig. 2). The platelets which had been separated promptly in a Cohn Centrifuge did not generate thromboplastin as rapidly as the control platelets, possibly reflecting their greater freedom from interacting plasma proteins. Moreover, this behavior was remarkably constant.
TABLE 1.—Comparison of Platelets in Native Plasma and Platelets Preserved in Media Free of Clotting Proteins

<table>
<thead>
<tr>
<th></th>
<th>DAYS OF STORAGE</th>
<th>Left in Native Plasma</th>
<th>Resuspended in Standard Preservation Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Platelet Count</td>
<td>368,000</td>
<td>102,000</td>
<td>64,000</td>
</tr>
<tr>
<td>% of Surviving Platelets</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With Spicules</td>
<td>70</td>
<td>40</td>
<td>23</td>
</tr>
<tr>
<td>Without Spicules</td>
<td>11</td>
<td>30</td>
<td>36</td>
</tr>
<tr>
<td>Blebbed</td>
<td>18</td>
<td>18</td>
<td>33</td>
</tr>
<tr>
<td>Ballooned</td>
<td>1</td>
<td>10</td>
<td>8</td>
</tr>
</tbody>
</table>

Fig. 2.—Thromboplastic generation of preserved platelets.

throughout the 10-month preservation period. In contrast, platelets separated from plasma after gravity sedimentation of erythrocytes reacted initially at the same rate as fresh platelets. With time, however, this reactivity diminished, and at the end of 42 weeks these platelets showed less thromboplastic activity than the control platelets or the rapidly separated group. The platelet concentrations were comparable in the controls and in the test mixtures. However, experiments to be reported subsequently have shown that thromboplastic generation is independent of platelet concentration in the test mixture.
Fig. 3.—Activity of preserved platelets in a thrombin-forming reaction mixture consisting of highly purified human clotting factors: prothrombin, factor V, PTC, AHF and calcium. Between 18 and 36 months there is a decline in activity, as indicated by the curve for 26-month-old platelets. The control curve represents the activity in the absence of platelets.

over a 10-fold concentration between 2,000 and 20,000/mm. The use of thromboplastin generation as an assay in the present experiments is thus not an index of platelet numbers alone. Rather, it is used to show that the platelets which remain in the preservation medium retain clot-promoting activity. Independent evidence that preserved platelets can generate thromboplastin was obtained in a thrombin evolution derived by Surgenor et al. This is shown by the data in figure 3; platelets preserved for periods up to 18 months showed good activity in a system comprised of highly purified clotting factors.

Since gross removal of plasma had such a beneficial effect upon platelet survival, the effect of further processing to remove all but traces of plasma proteins was investigated. In this experiment, 500 ml. of blood was processed to yield platelets, and an aliquot of approximately one third of the platelets was removed and suspended in dextrose-gelatin medium after a single wash step. A second aliquot was removed after two wash steps. Finally, the remaining platelets were washed once more and then suspended in the preservation medium. The samples were studied over a three-year period both with respect to numerical survival and retention of thromboplastin in in vitro coagulation systems. The data for numerical survival are shown in figure 4. The singly washed cells deteriorated rapidly in numbers. In six months only 20 per cent remained intact. The decline was less rapid if the platelets had been washed twice. The triply washed cells showed striking stability. In vitro coagulation assay of these same platelets showed an essentially undiminished thromboplastic activity in any specimen during the first year.
of storage, as indicated by a reduction in the clotting time and residual serum prothrombin following addition to fresh platelet-poor plasma and recalcification (table 2A). For these studies the platelets were not separated from the substrate media. Data to show the thromboplastic activity of the platelets as opposed to the surrounding media have been published previously. Detailed study of the apparent decrease in in vitro thromboplastic activity between 12 and 18 months is now in progress. The finding of a diminished effect of platelets stored 1½ years on the clotting of platelet-poor plasma in the same samples which showed the best retention of morphology is considered confirmatory rather than contradictory evidence. The very fact that triply washed platelets last longest may result from their freedom from coagulation factors of plasma. The retention of an intact cell structure, moreover, is believed to be one of the singular advantages which preserved platelets have over lyophilized platelet material17 and other thromboplastin agents such as soy bean extract.18 As discussed below, the in vivo effectiveness of preserved platelets is often far greater than would be expected from the simple correction of thromboplastic deficit per se.

The Effect of the Original Anticoagulants

This was assessed in terms of morphologic survival. The results are shown in figure 5. Despite the very short time of contact with the anticoagulant (the interval between collection of the blood and separation of the platelets from the plasma being only a few minutes), the survival during subsequent preservation was affected significantly by the nature of the anticoagulant used. The effect may have been as much the result of variations in hydrogen ion concentration as the nature of the anticoagulant. Thus, sodium citrate gave the poorest preservation while standard ACD gave the best. Decalcification with exchange resin has, per se, no significant effect on pH, but the uncontrolled loss of CO₂ subsequent to resin treatment results in resin plasma having an alkaline pH. These data implicate surfaces and plasma interaction
### TABLE 2.—In Vitro Thromboplastic Activity Preserved Platelets

#### A. Varying Freedom From Entrained Plasma

<table>
<thead>
<tr>
<th>Days</th>
<th>Wash 1</th>
<th>Wash 2</th>
<th>Wash 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count</td>
<td>110,000</td>
<td>430,000</td>
<td>404,000</td>
</tr>
<tr>
<td>Exp.</td>
<td>3 1/2</td>
<td>4 0</td>
<td>4 1/2</td>
</tr>
<tr>
<td>Control (Saline)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Residual Prothrombin</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Incubation Time (min.)</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

#### B. Various Types of Original Anticoagulants

<table>
<thead>
<tr>
<th>Anticoagulant</th>
<th>ACD</th>
<th>Resin</th>
<th>Citrate</th>
<th>EDTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp.</td>
<td>23</td>
<td>3 1/2</td>
<td>2 1/2</td>
<td>2</td>
</tr>
<tr>
<td>Prothrombin Control (%)</td>
<td>0</td>
<td>9%</td>
<td>4%</td>
<td>7%</td>
</tr>
<tr>
<td>EDTA</td>
<td>35</td>
<td>12</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>Incubation Time (min.)</td>
<td>15</td>
<td>15</td>
<td>60</td>
<td>60</td>
</tr>
</tbody>
</table>

rather than the citrate ion for the poor survival of the platelets in ordinary "banked" blood. All platelets used in this experiment were washed once prior to preservation. The reason for the unusually good early survival of the platelets in this experiment is not known but may represent individual variation in donor platelets in the original phlebotomy, since all aliquots were derived from the same source. Data which show the thromboplastic activity of these same platelets in terms of their effect upon the recalcification time and prothrombin consumption of fresh, platelet-poor plasma are presented in section B of table 2. ACD (with prompt separation) and each of the other three types of original anticoagulant were studied. No difference
in clot-promoting activity was seen in the platelets derived from the various types of anticoagulant. These studies were continued for one year only, however, due to exhaustion of the platelet material.

**Effect of the Composition of the Gas Phase**

The importance of carbon dioxide in the maintenance of the in vivo fluidity of the blood was suggested by Widenbauer and Reichel\(^4\) who demonstrated that blood collected under constant partial pressure of CO\(_2\) (40 mm.) showed markedly delayed coagulation, whether in glass or paraffin containers. Similarly, Colldahl\(^5\) observed a prolonged clotting time in animals subjected to high partial pressures of CO and low partial pressures of oxygen. More recently, in experiments from this laboratory,\(^6\) isolated platelets were exposed to controlled atmosphere of oxygen and carbon dioxide, and striking effects were observed upon the morphology as well as on the clot-retracting ability of platelets. Thus, in an atmosphere with a partial pressure of 46 mm. Hg of carbon dioxide, platelets retained optimal clot-retracting activity. It was of interest, therefore, to determine whether any long-term benefits could be attained by controlling the gas phase during preservation. Platelets collected under routine conditions in an atmosphere of air were compared with platelets collected and separated under a 5 per cent CO\(_2\) 95 per cent N\(_2\) atmosphere. The results of two experiments are shown in table 3. In one experiment, platelets collected and stored in 5 per cent CO\(_2\) 95 per cent N\(_2\) showed clot retraction during the first week of storage which was only equal to the control level. After one week, however, there was a steady increase in clot-retraction ability in the experimental samples which, by the end of three months, equaled the maximal activity found in fresh whole blood containing its full quota of normal platelets. Representative data for platelets collected and preserved in air atmosphere are shown in the lower half of table 3. It can be seen that the number of forms with spicules is sig-
TABLE 3.—Effect of Gas Phase upon Platelet Morphology and Clot Retraction

<table>
<thead>
<tr>
<th>Age-days</th>
<th>Platelet Count x 10^9</th>
<th>% Spicule Forms</th>
<th>% Clot Retraction*</th>
<th>Experimental</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment No. MAO 4375</strong> (5% CO₂; 95% N₂)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>10.50</td>
<td>84</td>
<td>23</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>10.60</td>
<td>70</td>
<td>50</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>11.40</td>
<td>60</td>
<td>60</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>135</td>
<td>10.60</td>
<td>66</td>
<td>28</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>10.50</td>
<td>61</td>
<td>24</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>223</td>
<td>9.96</td>
<td>51</td>
<td>20</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td><strong>Experiment No. MAO 4669</strong> (Air)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.38</td>
<td>63</td>
<td>16</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7.72</td>
<td>48</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>6.02</td>
<td>30</td>
<td>9</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>4.82</td>
<td>38</td>
<td>15</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

*Expressed as per cent decrease in the original volume of a clot formed from fresh, platelet-poor resin blood.

The methods of gas phase preservation appear to be significantly less, and the clot-retracting ability of the platelets is not nearly as well preserved. Further evidence is being sought regarding the effect of CO₂ on clot retraction, before incorporating this advance into the routine system of platelet preservation. However, the unique feature of a closed-system type of centrifugation is already a standard feature of the Cohn-ADL equipment used in the present platelet work. Indeed, any desired type of gas mixture may be added without increasing significantly the technical complexity of the system.

**Retention of Antigenicity**

Preserved platelets appear to retain their full antigenicity in in vivo systems used for determining the presence of platelet agglutinins and lysins. They have been used routinely in the performance of platelet antibody tests and for periods up to two years in storage have shown no measurable loss of antigenic activity. No studies have been conducted on the in vivo isoantigenicity of preserved platelets. As noted below, transfusions were given only within proper red cell grouping, and no evidence of sensitization was found.

**Transfusion Results**

In vivo studies were made in connection with 240 transfusions of preserved platelets. In group I, 180 units were administered in cases of emergent medical and surgical thrombopenia in which other supportive patient care made detailed study difficult or of questionable value. In these cases, the results were assessed solely in objective clinical terms. In group II, the transfusions were done under carefully controlled conditions during which time the recipients received no other intravenous therapy. These experiments were followed by means of multiple laboratory and clinical determinations. We have searched for laboratory findings that would fully document the in vivo effectiveness of preserved platelets. Changes in serum prothrombin and rises in the plate-
let count proved useful, but in the final analysis the most important criterion was of necessity the evaluation of the patient as a whole: cessation of bleeding, improvement in capillary fragility, and shortening of bleeding time.

**Group I—Recipients observed clinically:** In this group of thrombopenic patients, the only pretreatment screening consisted of blood typing and demonstration of a negative test for platelet antibodies. The latter test was occasionally omitted if the thrombopenia stemmed from a known source (leukemia, excessive blood replacement with banked blood during surgery, etc.). Whenever possible, platelets were used from donors of the same ABO, Rh red cell group as the recipient. Platelets derived from Group O Rh negative red cell donors were used when type specific material was not available in the platelet bank. These 180 transfusions were largely administered at the New England Deaconess Hospital, Boston, Massachusetts. A few were administered by observers in 12 other cities.\(^*\) No fever, chills, pyrogenicity, local discomfort or systemic toxicity were noted in any transfusions.\(^1\) The only reaction reported was transient syncope in an epileptic patient who had many similar petite and grand mal episodes prior to and subsequent to the platelet transfusions. The platelets were administered directly from their plastic storage bag; the average elapsed time for administration was two minutes per unit. On the average, between three and four units were given to each patient. The maximal number of units given to a single individual was 28. The maximum time interval between a first and last transfusion was 25 months. The average number of platelets given was \(49.5 \times 10^9\) platelets per unit (range \(14.8 \times 10^9\) to \(198 \times 10^9\)).

The clinical improvement was sometimes dramatic, with immediate cessation of hemorrhage. This was particularly evident in patients whose thrombopenia was secondary to nitrogen mustard, triethylene melamine, thio-triethylene phosphoramide and other chemotherapeutic drugs. In leukemia and in cases where the thrombopenia was associated with multiple coagulation defects, the thromboplastic deficit appeared to be corrected by transfusion with preserved platelets, but the control of hemorrhage, improvement in capillary fragility and lessening of mucous membrane ooze was less dramatic. Platelets preserved longer than 24 months have thus far not been administered in vivo. Within this period, no apparent decrease in therapeutic effectiveness has been noted. If many units are used in any single individual within a 24-hour period of time, the patient's plasma fibrinogen level should be followed using a method such as that of Schneider.\(^2\) The correction of a thromboplastic deficit by a sudden load of platelets may improve over-all “clotting” so effectively that a transient hypofibrinogenemia results. This condition is easily corrected by the intravenous infusion of 2 Gm. or more of fibrinogen. A case illustrating such a reaction is cited in detail below.

An important feature of the preserved platelet bank has been the speed.

\(^*\)Platelets dispatched for use elsewhere were shipped by standard air express without special handling other than the use of a thermos container for maintenance of a temperature near +4 C.

\(^1\)See \(*\) footnote on page 457.
Thrombopenic hemorrhage often is an emergent condition; the time required for preparation of fresh platelet concentrates can mean the difference between death and survival. With preserved platelets, about 10 minutes are required to liquify the gelatin prior to infusion. The time may be shortened in emergencies by immersion of the bag in +37 C. water in gentle agitation. After removal of the sterile sheath from the administration port, a sterile arquad-coated needle is attached, a venipuncture is made (fig. 6) and a small amount of blood is allowed to reflux into a bag to free the tubing of air. The contents of the bag are then manually expressed. Separate administration sets possessing a Y tube were sometimes used in multiple administration.

**Group II—Recipients studied in detail:** Sixty units of preserved platelets were administered to selected recipients in whom detailed study of the in vivo effectiveness could be made. These recipients were chosen on the basis of availability for long-term follow-up, freedom from other types of intravenous therapy and the absence of any expected change in general supportive management. The only feature common to all cases was a profound thrombopenia of a non-autoimmune type.

**Effect on bleeding time:** Platelets produced a decreased bleeding time in each case tested. The data in table 4 show the results in eight cases selected at random from the group. The duration of effect was approximately 24 hours. The age of the platelet units varied from 1 week to 13 months.

**Effect on capillary fragility:** The reversal of capillary fragility following the transfusion of preserved platelets may be seen in figure 7. A blood pressure cuff, inflated to the mid-point between systole and diastole, caused the shower of petechiae seen on the right arm. A transfusion of preserved platelets was then given and the tourniquet test repeated by a similar technic on the left arm. No petechiae occurred. The improvement noted in both capillary fragility and in bleeding time appeared to be unrelated to the changes in prothrombin consumption which occurred. This was believed to indicate that the preserved platelets retain cellular and vascular effects independent of thromboplastin formation per se. Wildebrandt, Luscher and Asper have shown that the increased capillary permeability which follows perfusion of rat leg muscle with calcium chelating agents can only be reversed if both
TABLE 4.—Effect of Platelet Transfusions on Bleeding Time

<table>
<thead>
<tr>
<th>Patient</th>
<th>Platelets Transfused Number x 10^6</th>
<th>Age in Months</th>
<th>Pretransfusion</th>
<th>1 hr.</th>
<th>2 hr.</th>
<th>3 hr.</th>
<th>4 hr.</th>
<th>7 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>H.R.</td>
<td>10.6</td>
<td>4.5</td>
<td>5.5</td>
<td>3.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J.B.</td>
<td>11.7</td>
<td>5.0</td>
<td>7.0</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.McC.</td>
<td>8.2</td>
<td>1.0</td>
<td>7.5</td>
<td>3.5</td>
<td>2.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L.L.</td>
<td>9.3</td>
<td>4.0</td>
<td>15.5</td>
<td>7.5</td>
<td>5.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E.S.</td>
<td>25.8</td>
<td>5.0</td>
<td>8.0</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F.S.</td>
<td>22.2</td>
<td>0.2</td>
<td>5.5</td>
<td>3.0</td>
<td>4.0</td>
<td>3.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J.K.</td>
<td>9.4</td>
<td>13.0</td>
<td>7.5</td>
<td>5.0</td>
<td>5.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.L.</td>
<td>18.4</td>
<td>2.0</td>
<td>9.5</td>
<td>6.0</td>
<td>2.5</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 7—(Left) Right forearm after tourniquet test. Thrombocytopenic patient. (Right) Tourniquet test, left arm. Same patient after two units of preserved platelets.

calcium and platelet protein extract are infused. This platelet substance, previously named Protein "S" by Luscher, is believed to be a platelet component with singular effect on maintaining capillary integrity. The unique benefit of preserved platelets, as opposed to various agents which have only thromboplastic activity, may, in part, be due to the presence of this protein.

Improvement in capillary fragility was generally observed in patients in whom thrombopenia was the major hemostatic defect. Patients with multiple hemostatic defects or with generalized disease such as acute leukemia showed less alteration in capillary fragility despite a satisfactory rise in platelet count and improvement in prothrombin consumption.

Effect on platelet count: In all 60 cases studied in detail, the transfusion of preserved platelets was accompanied by an increase in circulating platelet count. The average recipient platelet count prior to transfusion was 24,000/mm³. The average 15-minute post-transfusion count after the first unit was given was 40,000/mm³. Administration of more than one unit resulted in
corresponding increases to from 55,000/mm$^3$ to 70,000/mm$^3$. In many cases, this was followed by a secondary, and more pronounced rise, which reached its maximum within three to seven hours after the transfusion. The immediate post-transfused rise was attributable to the numbers of transfused platelets. The secondary rise in many cases exceeded by as much as 200 per cent the calculated number of platelets administered. Three illustrative cases are shown in figure 8. These findings can only be explained on the basis of a production of new, endogenous platelets as a result of the temporary correction of a thromboplastic deficit. This would suggest that the normal clotting is, in itself, stimulatory to the production of new platelets. Support for this concept was found when the recipients were divided into two groups: those with marrow aplasia and sparse megakaryocytes and those with hyperactive marrow and poor platelet production. The former group, mostly patients undergoing cancer chemotherapy, showed only the calculated rise in count from the mass administered. The latter group, in all cases, showed higher levels than the theoretical number of platelets given, suggesting the production of new platelets a few hours after correction of the clotting defect. Proof of this hypothesis must await experiments now in progress with Cr$^{51}$-labelled, preserved platelets.

Results of clotting tests: Many of the common laboratory tests of coagulation function remain normal in thrombocytopenia. Tests such as the prothrombin time, which do not depend upon the early stages of coagulation, are generally unaltered. The most frequent laboratory finding in thrombocytopenia
TABLE 5.—Results of Clotting Tests

<table>
<thead>
<tr>
<th>Patient</th>
<th>Type of Thrombocytopenia</th>
<th>E.S.</th>
<th>J.K.</th>
<th>M.McC.</th>
<th>J.B.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Platelets Transfused</td>
<td>25.9 x 10^10</td>
<td>9.4 x 10^10</td>
<td>8.2 x 10^10</td>
<td>11.7 x 10^10</td>
<td></td>
</tr>
<tr>
<td>Preservation Time (mos.)</td>
<td>6</td>
<td>13</td>
<td>1</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Serum

<table>
<thead>
<tr>
<th>Two-Stage Prothrombin Determination u/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretransfusion 24</td>
</tr>
<tr>
<td>Post-transfusion 0.1 hr. 3</td>
</tr>
<tr>
<td>1.0 hr. 7</td>
</tr>
<tr>
<td>4.0 hr. 33</td>
</tr>
<tr>
<td>7.0 hr. 26</td>
</tr>
<tr>
<td>24.0 hr.</td>
</tr>
<tr>
<td>48.0 hr.</td>
</tr>
</tbody>
</table>

Plasma

<table>
<thead>
<tr>
<th>Quick One-Stage Prothrombin Time (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretransfusion 0.1 hr.</td>
</tr>
<tr>
<td>1.0 hr.</td>
</tr>
<tr>
<td>4.0 hr.</td>
</tr>
<tr>
<td>7.0 hr.</td>
</tr>
<tr>
<td>24.0 hr.</td>
</tr>
<tr>
<td>48.0 hr.</td>
</tr>
</tbody>
</table>

*Average of 2 determinations.
†Average of 3 determinations.
‡Normal control for this data 16.2 sec.
§Normal control for this data 12.8.

is an impaired consumption of prothrombin, as evidenced by the prothrombin content of the patients’ serum after allowing ample time for clotting. Some representative data for recipients of transfused preserved platelets are shown in table 5. Here, as with the platelet survival, capillary fragility and bleeding time data, the cases tended to fall within two categories. Cases E. S., J. K. and M. Mcc. had thrombocytopenia secondary to malignant disease. Their average platelet counts before transfusion were 32,000, 32,000 and 12,000/mm³, respectively. They exhibited serum prothrombin levels significantly elevated over the normal range (in our hands 0–6 units/ml). The response to platelet transfusions as measured by this index varied in these three patients. In E. S., there was a prompt but temporary improvement; J. K. exhibited a delayed but steadfast improvement in prothrombin consumption; while M. Mcc. failed to show any significant change. On the other hand, in all three of these cases, the bedside tests of bleeding time and capillary fragility indicated favorable systemic alterations in the direction of cessation of bleeding tendency. Finally, this group, representing thrombocytopenia associated with acute leukemia and hypercellular marrow, showed a marked

*The thromboplastin generation test run on platelet samples obtained from the recipient might have yielded illuminating data. However, it was not attempted both in the interest of minimizing trauma to the patient and because of the difficulty of obtaining adequate numbers of platelets from such thrombocytic donors, free of other formed elements and plasma proteins.
secondary rise in circulating platelets over and above the level expected on the basis of the number of platelets given.

In contrast to the above, the fourth recipient, J. B., had a normal prothrombin consumption which remained unchanged after the infusion of the platelets. He exhibited a marked thrombopenia (platelet count 14,000/mm³ before transfusion), but despite this he had no abnormalities in clotting tests. This pattern was common in drug-induced thrombocytopenia induced by TEPA, TEM and other chemotherapeutic agents. This group served, therefore, as a kind of control group. It provided an excellent opportunity to study in vivo survival of preserved platelets, inasmuch as it showed a good quantitative response in platelet numbers and lacked side effects such as hemorrhagic manifestations.

The one-stage prothrombin times shown in the lower half of table 5 add little information. In each case there was a slight prolongation of the prothrombin time in the immediate post-transfusion period. This may have been the result of a transient deficit of the plasma factors involved, possibly stemming from increased post-transfusion coagulation activity. The elevated prothrombin time of M. McC. suggests an associated prothrombin deficit of some magnitude, which may indeed be related to the failure of this patient to exhibit an improved prothrombin consumption after the transfusion. The effect of platelet transfusions on residual serum prothrombin as measured by one-stage prothrombin times in two additional patients is presented in figures 9 and 10. In each case, prolongation of the Quick prothrombin time occurred, the change being minimal in one and marked in the other. Both patients had thrombocytopenia associated with acute leukemia, and the pattern of platelet response was again that of a rise greater than the calculated number infused.

In another patient who received several units of platelets over a period of a few hours, the correction of the thromboplastic deficit was so striking that hypofibrinogenemia resulted. The patient, a 68-year-old male with thrombocytopenia and malignant pulmonary adenomatosis, had a marked hemorrhagic diathesis with gastrointestinal bleeding, widespread ecchymoses of the skin and mucous membrane ooze. The bone marrow showed adequate numbers of normal-appearing megakaryocytes. The peripheral platelet counts varied between 8000/mm³ and 29,000/mm³. Platelet agglutinin tests were negative. Over a period of several weeks, the acute bleeding episodes were controlled on four separate occasions by transfusions of preserved platelets. On the day in question, a series of infusions was given to study the effect of fresh serum on production of autogenous platelets after prior correction of the thromboplastic deficit with exogenous platelets. A mass of $14.2 \times 10^{10}$ platelets was infused between 9:15 and 9:25 A.M. The platelet count in the patient rose from 27,000/mm³ to 55,000/mm³ in 15 minutes, and all visible evidence of bleeding ceased; 100 ml. of fresh, normal serum was then given. The platelet count, instead of continuing to rise, abruptly

*Dr. Clarence Merskey, Capetown, South Africa, suggested that this finding might result from the presence of platelet fragments in the vascular bed of the recipient.
Fig. 9.—Preserved platelets. In vivo effect on platelet count and prothrombin consumption.

Fig. 10.—Preserved platelets. In vivo effect on platelet count and prothrombin consumption.
PRESERVED PLATELETS: PREPARATION, STORAGE AND CLINICAL USE

Table 6.—Tests for Anticoagulants: Modified Prothrombin Times Before and After Incubation with Thromboplastin

<table>
<thead>
<tr>
<th>Patient Sample</th>
<th>Pretransfusion</th>
<th>Post-transfusion 0.1 hr.</th>
<th>Post-transfusion 1 hr.</th>
<th>Post-transfusion 4 hr.</th>
<th>Post-transfusion 7 hr.</th>
<th>Post-transfusion 24 hr.</th>
<th>Post-transfusion 48 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.S. Plasma</td>
<td>28.1</td>
<td>26.3</td>
<td>28.4</td>
<td>27.3</td>
<td>26.8</td>
<td>26.5</td>
<td>26.8</td>
</tr>
<tr>
<td>Serum</td>
<td>8.8</td>
<td>13.7</td>
<td>8.6</td>
<td>8.7</td>
<td>13.6</td>
<td>17.2</td>
<td>13.9</td>
</tr>
<tr>
<td>M.McC. Serum</td>
<td>13.6</td>
<td>21.1</td>
<td>18.5</td>
<td>21.9</td>
<td>22.1</td>
<td>20.9</td>
<td>21.9</td>
</tr>
<tr>
<td>J.B. Plasma</td>
<td>11.3</td>
<td>21.1</td>
<td>18.5</td>
<td>21.9</td>
<td>22.1</td>
<td>20.9</td>
<td>21.9</td>
</tr>
<tr>
<td>J.B. Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Average One Stage Time (sec.) Before Incubation

Average One Stage Time (sec.) After Incubation 30 min. at 37°

Normal serum, after incubation, gave an average time of 43 seconds.
Thromboplastin control (incubation with saline) was always unchanged.

fell to 16,000/mm.³ An additional unit of platelets was given and the patient appeared to tolerate the procedure satisfactorily. The residual serum prothrombin which had been 13 per cent originally, decreased to 5 per cent at 4:00 P.M. At approximately 9 P.M., however, while the patient rested in his hospital room, a profuse nasal hemorrhage began. On the possibility that his bleeding on this occasion was secondary to some additional coagulation deficit, a bedside fibrinogen test was performed utilizing 2 units of thrombin to 1 ml. of fresh plasma. A prolonged time of 40 seconds was obtained. Previous fibrinogen tests on the patient had been normal. Four Gm. of human fibrinogen were promptly infused into the patient, with cessation of bleeding. It is of interest that the platelet count then rose to 76,000/mm.³ (135 per cent of the calculated number given that day), suggesting that a definite endogenous platelet production may have taken place on this occasion.

In several cases, the determined two-stage plasma prothrombin content was found to vary with dilution, being greatest at the highest dilution. Closer study showed the presence of antithromboplastic activity as revealed by examination of the one-stage prothrombin time after incubation of the test serum with diluted thromboplastin. Some data are shown in table 6. The inhibitory activity was not found in the plasma* of case E. S. In the case of M. McC., the short times in the unincubated samples undoubtedly reflect the prothrombin content of the serum sample. In this patient the data

*The plasma and serum data of table 6 cannot be directly compared because of the differing contents of calcium ion and of clot-promoting activity in the two solutions.
suggest that the anticoagulant activity was present before the transfusion and was unaffected by the new platelets. The third patient, J.B., showed a different pattern; his plasma appeared to be hyperactive pretransfusion and became prolonged after platelets were administered. There was no added effect following incubation. The serum of J. B. showed changes after the transfusion consistent with the presence of antithromboplastic activity.

Tocantins,23 Craddock and Lawrence24 called attention to the importance of thromboplastin inhibitors in hemorrhagic disorder. Thus, for example, circulating antithromboplastins can sometimes be responsible for a hemophilia-like disease, characterized by poor prothrombin consumption and normal prothrombin time. The relative importance of the present findings is not completely understood; they are reported here together with the incidence of hypofibrinogenemia and the negative findings in other tests in order to present a complete picture of the possible sequelae to platelet transfusion.

**SUMMARY**

A method is described for the isolation and preservation of human blood platelets. Their use in a platelet bank after storage of up to two years is described. The practical advantages to the recipient include small administration volume, freedom from pyrogenicity and immediate availability in the event of thrombocytopenic bleeding. The advantages of this technic to the hospitals include a saving of time, a saving of blood and the elimination of the frantic efforts of blood bank and laboratory personnel to produce large numbers of fresh platelet concentrates at the time of platelet needs.

The remarkable stability of preserved platelets is believed largely due to the method developed for their isolation from blood, wherein the cells are separated from the coagulation proteins with which they normally interact, prior to enzymatic degradation. The importance of a controlled atmosphere of CO₂ for retention of clot retraction is presented. The in vitro and in vivo activities of the preserved cells are discussed. A hypothesis is suggested for the autocatalytic stimulation of new platelet production through temporary correction of a thromboplastic deficit by the transfusion of preserved platelets.

**Summario in Interlingua**

Es describite un metodo pro le isolation e preservation de plachettas ab sanguine human. Lor uso post immagasinage de usque a duo annos in un banca de plachettas es describite. Le avantages practic ab le puncto de vista del recipiente include le micre volumine del administration, le absentia de pyrogenicitate, e le disponibilitate immediate in caso de sanguination thrombocytopenic. Le avantages ab le puncto de vista del hospital include un economia de tempore, un economia de sanguine, e le elimination del effortios phrenetic del parte del personal de banca de sanguine e de laboratorio de producer grande quantitates de fresc concentratos de plachettas al tempore quando le plachettas es requirite.

Es opinate que le remarcabile stabilitate del preservate plachettas resulta in alte mesura ab le metodo disveloppate pro lor isolation ab le sanguine. In iste metodo le cellulas es separate ab le proteinas de coagulation (con
que illos interage normalmente) ante le occurrientia del degradation enzymatic. Es discutite le importantia de tin regulate atmosphera de CO₂ pro le retention del retraction de coagulo. Le activitates del preservate cellulas in vitro e in vivo es discutite. Es presentate un hypothese secundo le qual un nove production de plachettas resulta del stimulation autocatalytic que occurre in le correction transiente de un deficit thromboplastic per le trans-fusion de preservate plachettas.

REFERENCES


