Elemental Composition of Platelets. Part I.
Sampling and Sample Preparation of Platelets for Trace-Element Analysis

Govindaraja Venkatesh Iyengar, Helmut Borberg, Karl Kasperek, Josef Kiem, Manfred Siegers, Ludwig Emil Feinendegen, and Rudolf Gross

Sampling of platelets for trace-element analysis poses special problems: obtaining adequate sample material, achieving a sufficient cell purity, preserving viability (integrity), correcting for trapped plasma, and controlling contamination. We used a blood-cell separator for the primary isolation of platelets from blood, and differential centrifugation in natural plasma to further isolate them. The pyrimidopyrimidine RA233 was used as a stabilizer to maintain viability. 131I-labeled human serum albumin was used to estimate trapped plasma. Contamination was controlled by using five-times-distilled water to simulate donor’s blood in the system and by comparing three fractions: the serum, the first portion of the platelet-rich plasma, and the supernatant plasma after the final centrifugation. Neutron activation analysis was used for the elemental analysis. A single differential centrifugation of the platelet-rich plasma from the blood-cell separator at 400 × g for 8 min was optimum (mean mass fractions: erythrocytes/platelets < 5 mg/g and leukocytes/platelets < 20 mg/g). The trapped plasma in the wet platelet samples amounted to about 0.40 g/g. No appreciable contamination from the sampling system was found for the elements Ag, Cd, Co, Cr, Cs, Cu, Fe, Mo, Rb, Sb, Se, and Zn.

Additional Keyphrases: wet- and dry-weight bases - neutron-activation analysis - variation, sources of

Many aspects of platelets—production, function, morphology, metabolism, enzymes and other proteins, transfusion, and storage—have already been investigated, whereas the elemental composition of platelets has been neglected, compared with other tissues and body fluids. Some investigations have been reported on the bulk elements Ca, Mg, K, Na, and P (1–12) and the trace element Zn (13, 14), but to our knowledge no information is available on other trace elements. We report in this series of papers our data on the concentration of 14 elements in human platelets, some of known biological importance—e.g., Cd, Co, Cr, Cu, Fe, K, Mo, Se, and Zn. But sampling and sample preparation of platelets involve several problems, as described below.

Sample size: The relative amount of platelets in the blood is small, so that a large blood sample is necessary to obtain enough that the elemental data can be expressed on a weight basis rather than only on the number of platelets. The main disadvantage in relating element content to platelet number is the difficulty in obtaining a standardized mean volume of the single platelet. Hence intercomparison of values from different investigations is difficult.

Viability: “Viability” of platelets means the retention of their functional integrity. Platelets are very sensitive and easily damaged, even by washing with isotonic solutions (15–17), so it is difficult to preserve their viability. Loss of viability results in significant changes of certain components, such as a decrease in potassium concentration (5, 18). It is reasonable to suppose that trace elements also will probably be affected by any loss of viability. Hence it is necessary, insofar as possible, to preserve the viability of platelets during sampling and the preparation steps by, for example, using a stabilizer that has been shown to preserve optimum viability (19).

Cell purity: Complete isolation of platelets from other blood components such as plasma, erythrocytes, and leukocytes is not possible. Attempting to do so invariably results in a loss of much of the platelet mass; thus it is necessary to start the isolation of platelets with as large a blood volume as possible.

Trapped plasma: If information on the wet weight of the platelet sample is required, the trapped fluid (plasma or washing medium) has to be estimated and corrected for. The trapped plasma in samples of platelets and leukocytes amounts to about 40% of the total sample weight on centrifuging at about 3000 × g for 20 min (4, 5), whereas trapped plasma in samples of erythrocytes is only about a tenth that in platelets (20).

Contamination: From the primary isolation of platelets from blood until their final purification, several sample-handling steps are necessary, depending upon the method chosen. The following methods are commonly used to isolate platelets: differential centrifugation in natural plasma (4, 5), differential centrifugation in combination with washing (6, 15, 16), gel filtration (1, 2, 21, 22) and density-gradient centrifugation (23–25). The various steps from these methods are potential sources of trace-element contamination and need to be checked.

Materials and Methods

Five apparently healthy male volunteers participated in this experiment. These samples were used for standardizing the
sampling method and scrutinizing the associated problems such as elimination of leukocytes and erythrocytes, estimating the trapped plasma, and checking of contamination from various elements.

**Sampling System with Use of the Blood-Cell Separator**

In the present experiment a differential centrifugation method was used to isolate the platelets with the help of a blood-cell separator of intermittent-flow type (Blood Processor, Model 30, Haemonetics; Fresenius, 6380 Bad-Homburg, F.R.G.), which processes blood in liter quantities. Figure 1 shows, schematically, the sampling circuit with the blood-cell separator connected to the donor. With this set-up, under special conditions, platelets of considerable purity could be obtained during this first stage of isolation (26, 27), as shown below. The blood-cell separator operates as follows: Blood is drawn intermittently, about 500 mL at a time, from a vein, mixed simultaneously with isotonic saline and citrate (18 parts of blood, one part of saline, and one part of citrate) and goes to the centrifuge. The citrate was used in the form of “ACD-A” (Biostet, 6000 Frankfurt, F.R.G.). Because the different blood components (erythrocytes, plasma) are visibly separated during centrifugation, it is possible to choose and collect a specific layer of the plasma that is rich in platelets (“platelet-rich plasma,” PRP) and relatively free from leucocytes and erythrocytes (see below). The remaining part of the blood is recirculated to the donor. This procedure was repeated eight times in this experiment, and enough PRP (about 400 mL) was collected by centrifuging about 4 L of blood from each donor. The PRP was placed in precleaned polyethylene bottles, and immediately 2 mL of a platelet stabilizer (R98a-RA233, 50 mg/2 mL; Karl Thomae GmbH, 7950 Biberach, F.R.G.) was added to the PRP (19; page 288 in ref. 28).

The PRPs obtained from the five donors by this sampling procedure were treated in different ways, as described below.

The sampling system consists of the following parts: several polyethylene accessories, such as tubes and sample-collection bags, needles for connecting the donor to the sampling system, a centrifuge made of a plastic (polycarbonate) housing with some parts made of aluminum alloy coated with a layer of lacquer, and infusion solutions such as polyethylene saline and citrate. To avoid contact with rubber from the stopper of the bottles containing the infusion solutions, we bottled these solutions in polyethylene containers. To assess contamination from the sampling system, we circulated five-times-distilled water through the sampling circuit in place of donor’s blood, simulating the experimental conditions. These procedural details are shown schematically in Figure 2. Various fractions, as shown in the first two columns of Table 1, were collected and analyzed for several elements.

**Neutron Activation Analysis**

We used neutron activation analysis to determine certain elements, as described in Part II (Kiem et al., this issue).

**Various Treatments of Platelet-Rich Plasma Samples**

All the sampling aids used in the experiments were pre-cleaned with five-times-distilled water.

For counting platelets, leucocytes, and erythrocytes, the following counting systems were applied: counting chamber (system Neubauer; Brand, 6980 Wertheim, F.R.G.), Coulter Counter (Model S), Thrombocounter C, and Coulter Counter (Model ZP2; Coulter Electronics GmbH, 4150 Krefeld, F.R.G.).

The blood cells in the blood of the donors were counted before connecting the blood-cell separator, in the different PRPs (see below, depending upon the isolation procedure chosen for the different donors), and in the supernatant plasma of the final PRPs. However, not all these fluids were counted for all the blood cells in all the donors, and the different counting systems were applied partly simultaneously, partly alternatively. Occasionally, differential cell counting was also performed in the PRPs by using “Testsimples” (Boehringer, 6800 Mannheim, F.R.G.), which showed that the leucocytes were mostly lymphocytes. Therefore, for calculating the mass fractions of the total leucocytes in relation to total platelets in the PRPs, we assumed that the mean mass ratio of the single leucocyte to the single platelet was less than 50 (29). With respect to erythrocytes this ratio was assumed to be less than 10.

To estimate trapped plasma, we used $^{131}$I-labeled human serum albumin ($^{131}$I-HSA; Amersham-Buchler, 3300 Braunschweig, F.R.G.) for all the donors. $^{131}$I-HSA was controlled for radiocuhermic purity and stability by dialysis (the free iodine fraction was <0.05) and also by a radioactive isotope uptake experiment comparing the uptake of 10 different radioisotopes (51Cr chloride, 134Cs chloride, 59Fe chloride, 131-I-HSA, 42K chloride, 54Mn chloride, 24Na chloride, 86Rb chloride, 75Se selenite, and 65Zn chloride) by platelets and erythrocytes at different incubation times of platelets and erythrocytes in natural plasma (data to be published).

The $^{131}$I radioactivity counting was done with a 7.7-cm diameter well-type Na(Tl)-detector (Clinimat 200; Picker, 4992 Espekamp, F.R.G.) for the wet platelet samples to determine the trapped plasma on wet-weight basis, and with a 96 cm$^3$ well-type Ge(Li)-detector (see neutron activation analysis, Part II, Kiem et al., this issue) for the dried platelet samples.
Table 1. Various Solutions Analyzed to Check for Contamination from the Sampling System (Values in μg/L)

<table>
<thead>
<tr>
<th>Fractions collected</th>
<th>Solutions</th>
<th>Ag</th>
<th>Cd</th>
<th>Co</th>
<th>Cr</th>
<th>Cs</th>
<th>Cu</th>
<th>Fe</th>
<th>Mo</th>
<th>Rb</th>
<th>Sb</th>
<th>Se</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Five-times-distilled water</td>
<td>&lt;0.2</td>
<td>&lt;0.1</td>
<td>0.2</td>
<td>&lt;0.1</td>
<td>&lt;0.002</td>
<td>&lt;0.25</td>
<td>&lt;59</td>
<td>&lt;1</td>
<td>&lt;0.1</td>
<td>&lt;1</td>
<td>&lt;0.6</td>
<td>&lt;2</td>
</tr>
<tr>
<td>2.</td>
<td>Saline</td>
<td>&lt;0.9</td>
<td>&lt;1</td>
<td>0.5</td>
<td>&lt;1</td>
<td>&lt;0.08</td>
<td>35</td>
<td>&lt;40</td>
<td>&lt;0.2</td>
<td>&lt;2.8</td>
<td>&lt;0.4</td>
<td>&lt;1</td>
<td>40</td>
</tr>
<tr>
<td>3.</td>
<td>Citrate (ACD-A)</td>
<td>&lt;0.6</td>
<td>&lt;1</td>
<td>0.2</td>
<td>&lt;1</td>
<td>&lt;0.20</td>
<td>50</td>
<td>&lt;212</td>
<td>&lt;0.2</td>
<td>&lt;8.4</td>
<td>&lt;1.3</td>
<td>&lt;3</td>
<td>1700</td>
</tr>
<tr>
<td>4.</td>
<td>Five-times-distilled water containing saline run through the sampling system</td>
<td>—</td>
<td>&lt;0.1</td>
<td>0.2</td>
<td>&lt;0.2</td>
<td>&lt;0.03</td>
<td>3</td>
<td>88</td>
<td>&lt;1.9</td>
<td>&lt;0.2</td>
<td>&lt;2</td>
<td>&lt;11</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Five-times-distilled water containing saline and citrate run through the sampling system</td>
<td>&lt;1.0</td>
<td>&lt;0.1</td>
<td>0.6</td>
<td>&lt;0.2</td>
<td>&lt;0.10</td>
<td>5</td>
<td>16</td>
<td>&lt;3.4</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>235</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>About 4 L of five-times-distilled water run through the sampling system simulating the donor</td>
<td>&lt;0.5</td>
<td>&lt;0.1</td>
<td>0.08</td>
<td>&lt;0.2</td>
<td>&lt;0.06</td>
<td>—</td>
<td>&lt;20</td>
<td>&lt;2.7</td>
<td>&lt;0.6</td>
<td>&lt;0.3</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Stabilizer</td>
<td>80.8</td>
<td>—</td>
<td>0.90</td>
<td>&lt;0.3</td>
<td>465</td>
<td>—</td>
<td>&lt;29</td>
<td>1.1</td>
<td>&lt;27</td>
<td>&lt;946</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>121I-HSA</td>
<td>&lt;0.04</td>
<td>2.90</td>
<td>&lt;0.5</td>
<td>144</td>
<td>&lt;23</td>
<td>4.4</td>
<td>&lt;8</td>
<td>2450</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

To determine the trapped plasma on dry-weight basis. All the samples were oven-dried for one day at 75 °C, then for one more day at 100 °C (type T-5042F; Heraeus, 6450 Hanau, F.R.G.).

Donor 1: PRP (225 g) was mixed with 20 μL of 121I-HSA (about 20 μCi), transferred into a 500-mL plastic tube, and centrifuged (type 2KS; Heraeus Christ, 3360 Osterode, F.R.G.) for 40 min at 2000 rpm (radius: 24 cm from the bottom of the tube). The supernatant plasma was transferred into a polyethylene bottle, and the centrifuge tube containing the cell sediment was kept inverted for about 15 min, to let excess plasma seep down the walls. The sediment in the centrifuge tube contained leukocytes and erythrocytes at the very bottom in the form of a small, distinctly reddish ring (lower part), covered by a big pellet of platelets (upper part), which was yellow because of the stabilizer. With the help of specially prepared Suprasil quartz implements, the upper part (pure platelets) and the lower part (impure platelets) were separately transferred into Suprasil quartz tubes; 2 mL of the supernatant plasma was also transferred to a quartz tube. All these samples were weighed to determine their wet weights, and after the 121I-counting they were oven-dried. The quartz tubes containing the dry samples were sealed and used for neutron activation analysis. Because for this donor the dry weights were not determined, the elemental concentrations were obtained only on a dry-weight basis without correction for trapped plasma. To determine the residual sediment in the 500-mL tube, we washed the tube with distilled water, and this washing water (about 5 mL) was counted for 121I radioactivity. This was necessary for calculation of mean platelet weight.

Donor 2: From 227 g of PRP, 162 g was taken and divided into two parts. One part was treated as described for donor 1; the other part was washed with isotonic glucose. The purpose of this experiment was to check whether the washing procedure changed the elemental concentration in platelets. Because the unwashed and washed platelet samples were critically small for an exact determination of the elemental concentrations, neutron activation analysis was omitted, and the results from this procedure will not be reported here. The rest of the PRP (65 g) was used for estimating the uptake of Na131I by platelets, to get a correction factor with respect to the free-iodine fraction in 121I-HSA, which is used for estimating the trapped plasma in all the platelet samples from the following donors. This PRP was mixed with 8 μCi of Na131I (Amersham-Buchler, 3300 Braunschweig, F.R.G.), transferred into a 500-mL centrifuge tube, and centrifuged for 30 min at 2600 rpm, in the same centrifuge as used for donor 1. Applying a similar procedure for the 131I measurements as described for donor 1, we compared the 131I radioactivity (cpm/g) of the plasma-containing platelet samples (those containing Na131I and those containing 121I-HSA) with corresponding supernatant plasma samples, thereby providing an estimate of the uptake of free iodine by plasma-free platelets, because the centrifugation conditions were identical for both the Na131I-containing and the 121I-HSA-containing PRP from this donor.

Donors 3-5: The PRPs from these donors were treated similarly. As an additional proof that there was no contamination from various elements during the connection of the blood-cell separator, the following portions were collected for comparison:

(a) About 10 mL of blood was collected before connecting the blood-cell separator, and the serum was obtained from it.

(b) The first portion of the PRP (35, 69, and 61 g, respectively, from these donors) was collected in a separate polyethylene bottle without adding the stabilizer.

(c) The supernatant plasma of the finally purified PRP (used to obtain the "pure" platelet samples, see below) was collected in a polyethylene bottle.

From each of these three portions (serum, first portion of PRP, and supernatant plasma) a defined volume (1 mL, 1 mL, and two 2-mL samples, respectively) was pipetted into quartz tubes, oven-dried, and used for neutron activation analysis, after we determined the dry weight.

The successive portions (after the first portion) of the PRP were pooled in a polyethylene bottle for each donor (250, 335, and 520 g, respectively). After immediately adding 2 mL of stabilizer, we mixed them with 20 μL of 121I-HSA (about 50
Table 2. Elemental Concentrations in the Wet Samples of Serum, First Portion of Platelet-Rich Plasma, and Supernatant Plasma from Donors 3–5, To Check Contamination from the Sampling System

<table>
<thead>
<tr>
<th>Donor no.</th>
<th>Sample description</th>
<th>Ag, µg/L</th>
<th>Co, µg/L</th>
<th>Ca, µg/L</th>
<th>Fe, mg/L</th>
<th>Rb, mg/L</th>
<th>Sb, µg/L</th>
<th>Se, µg/L</th>
<th>Zn, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Serum</td>
<td>7.4</td>
<td>0.2</td>
<td>0.6</td>
<td>1.1</td>
<td>0.1</td>
<td>1.7</td>
<td>95</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>First portion</td>
<td>7.4</td>
<td>0.1</td>
<td>0.7</td>
<td>0.8</td>
<td>0.2</td>
<td>0.1</td>
<td>69</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Supernate</td>
<td>9.5</td>
<td>0.2</td>
<td>0.6</td>
<td>0.5</td>
<td>0.1</td>
<td>0.6</td>
<td>56</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>Serum</td>
<td>1.7</td>
<td>0.2</td>
<td>1.5</td>
<td>1.7</td>
<td>0.3</td>
<td>&lt;1.3</td>
<td>109</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>First portion</td>
<td>&lt;5.1</td>
<td>0.3</td>
<td>1.4</td>
<td>0.9</td>
<td>0.3</td>
<td>1.6</td>
<td>78</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Supernate</td>
<td>&lt;0.2</td>
<td>0.4</td>
<td>1.5</td>
<td>1.0</td>
<td>0.3</td>
<td>0.9</td>
<td>74</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>Serum</td>
<td>2.8</td>
<td>0.2</td>
<td>0.9</td>
<td>2.0</td>
<td>0.2</td>
<td>&lt;1.7</td>
<td>104</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>First portion</td>
<td>3.4</td>
<td>0.1</td>
<td>1.0</td>
<td>1.5</td>
<td>0.2</td>
<td>&lt;0.7</td>
<td>80</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Supernate</td>
<td>0.3</td>
<td>0.1</td>
<td>0.8</td>
<td>0.9</td>
<td>0.2</td>
<td>0.6</td>
<td>49</td>
<td>0.5</td>
</tr>
</tbody>
</table>

| µCi), distributed them into 100-mL plastic tubes, and centrifuged (Rotixa K with special accessories; Hettich, 7200 Tuttlingen, F.R.G.) for 8 min at 1400 rpm (radius, 17.8 cm from the bottom of the tube). The upper parts (donor 3: 50/80, 52/80, 60/90 mL/mL; donor 4: 45/85, 45/85, 45/80, 47/77 mL/mL; donor 5: 45/84, 45/79, 45/80, 37/82, 37/84, 42/82 mL/mL, the volumes being determined by comparison with a graduated 100-mL plastic tube) were pipetted out and treated differently for these three donors. For donor 3 the upper parts were distributed into two 100-mL plastic tubes and centrifuged once more for 8 min at 1400 rpm (two-step differential centrifugation of the PRP). The upper parts (50 and 50 mL, respectively) were aspirated and handled further again as were the upper parts for donor 4 and 5 (single-step differential centrifugation of the PRP). All these upper parts were transferred into one polyethylene bottle for each donor, mixed, and, after 5 mL was removed for cell counting, distributed again into 100-mL plastic tubes (90 mL for donor 3; 90 and 89 mL, respectively, for donor 4; and 82, 82, and 83 mL, respectively, for donor 5) and centrifuged (20 min at 4000 rpm, corresponding to 3200 X g at the bottom of the tube). The supernatant plasma was transferred into a polyethylene bottle. The platelet sediments from donors 3–5 were transferred, insofar as possible, from the bottoms of the 100-mL tubes into one quartz tube for each donor, with use of the same implements as mentioned for donor 1. The platelet samples in the quartz tubes were called “pure” platelet samples.

For donor 5, the lower parts of the once-centrifuged PRP were also centrifuged further for 20 min at 4000 rpm, the supernate was discarded, and the platelet sediments were transferred into one quartz tube. This platelet sample was called “impure” platelet sample, because it contained more erythrocytes and leukocytes than did the corresponding “pure” platelet sample. We counted the three “pure” platelet samples from donors 3–5, the “impure” platelet sample from donor 5, and the corresponding supernatant plasma samples for 131I on a wet-weight basis; after drying, they were also counted on a dry-weight basis. Thus, we assessed the trapped-plasma fraction on both bases. Because the sample shapes and sizes were different, we obtained from a separate experiment geometric correction factors for both the NaI(Tl)- and the Ge(Li)-detector. The trapped plasma was also corrected for free iodine in 131I-HSA by multiplying with a factor of 0.99 (see results for donor 2).

Then all the dried samples in the quartz tubes were sealed and used for neutron activation analysis.

The elemental composition of plasma-free platelets on a wet-weight basis was calculated by use of the following formulas:

\[ A = (B - C - D)/(1 - C - E), \]

where

A is the concentration of a given element in plasma-free platelets on a wet-weight basis

B is the concentration of a given element in the dried plasma-containing platelet sample

C is the fraction of trapped plasma in the sample on a dry-weight basis

D is the concentration of a given element in the corresponding supernatant plasma on a dry-weight basis

E is the ratio of wet to dry weight of plasma-free platelets

\[ E = F \cdot (1 - G)/[H \cdot (1 - C)], \]

where

F is the wet weight of the platelet sample

G is the fraction of trapped plasma in the sample on a wet-weight basis

H is the dry weight of the platelet sample

C is the same as above.

The water content of plasma-free platelets (I) was calculated as follows: I = (E - 1)/E

Results and Discussion

Control of Contamination

Table 1 shows our results from the various fractions we analyzed to check for any contamination from the sampling system. In addition, we also checked contamination by comparing three different fractions from donors 3–5 (see Materials and Methods); these results are shown in Table 2. As can be seen from these data, the sampling system was found to be satisfactory with respect to lack of contamination from the elements we measured. The citrate solution contained as much as 1.7 mg of Zn per liter, but, because this solution is used in a 20-fold dilution during the sampling procedure, the resulting contamination was not serious, as can also be seen from the comparison of the different fractions (Table 2). Moreover, platelets are very rich in Zn, and the radioisotope uptake experiment mentioned under Materials and Methods shows that Zn is taken up relatively slowly by platelets. Because this radioisotope-uptake experiment showed that only potassium and rubidium are taken up to a considerable extent within a few hours, contamination from other elements such as Zn, Cr, Se, Cs, and Fe is not a serious problem.

Trapped Plasma

As can be seen from Table 3, trapped plasma was found to amount to about 0.40 g/g for donor 1, where there was plenty of sample. This value agrees with the literature (see Introduction). For donors 3–5, especially for donor 3, trapped plasma was found to be clearly higher. Also, related data, such as the ratio of wet to dry weight and the water content of plasma-free platelets (see Table 3), are not in agreement with the literature (see Part II, Kiem et al., this issue). We do not know exactly the reason for these high values for trapped plasma and low values for water content, but it is probable that critically small sample weights, as was the case for donors...
3–5, are a problem for such measurements and calculations. The following reasons may be considered: an evaporative loss of sample water during transfer of the sediments into the quartz tubes, an uptake of water between the end of drying and the determination of the dry weights, and a rather constant amount of $^{131}\text{I}$-HSA sedimenting without representing trapped plasma.

Although the last reason could easily explain the discrepancies mentioned, there are also some arguments against it: (a) Values for trapped plasma and water content for all the platelet samples of the standardized experiment (see Part II, Kiem et al., this issue) agreed with the literature. (b) The radioisotope-uptake experiment mentioned under Materials and Methods showed that, on extrapolation of the time–uptake curves (based on a similar correction for trapped plasma with the use of $^{131}\text{I}$-HSA) to zero incubation time, they intersec the ordinate approximately at zero-uptake for those radioisotopes that are taken up increasingly with time. Overestimated values for trapped plasma (because of sedimentation of $^{131}\text{I}$-HSA) would have led to negative uptake values on extrapolating to zero incubation time in this experiment. (c) A separate experiment, in which we centrifuged serum that was mixed well with $^{131}\text{I}$-HSA, for 30 min at 3000 $\times$ g, showed that there was no gradient in $^{131}\text{I}$ radioactivity from the top to the bottom of the centrifuge tube.

It still remains striking that the smallest samples seem the most susceptible to such discrepancies. We therefore suggest that platelet samples should have a wet weight of at least 100 mg. A distribution of the PRP into too many centrifuge tubes, as in the case of donor 5, also seems to influence these problems negatively, because transferring one sediment into one quartz tube results in a bigger platelet sample than transferring more sediments of an altogether identical weight into one quartz tube. Therefore for the standardized experiment (see Part II, this issue) we avoided such an excessive distribution of the PRPs by obtaining a smaller volume of original PRP from the blood-cell separator with a higher platelet number per volume. The use of only one centrifuge tube for each differential centrifugation step of a single donor would have been optimum (see sample weight for donor 1 in Table 3), but for practical reasons (e.g., availability and quality of the centrifuge and its accessories) we preferred to standardize the method with the device used for donors 3–5.

From the PRP of donor 2 (see Materials and Methods) the fraction of $^{131}\text{I}$ radioactivity ascribable to free iodine in calculating the trapped plasma in platelet samples with the use of $^{131}\text{I}$-HSA was found to be about 0.01, if the free iodine fraction in $^{131}\text{I}$-HSA is about 0.03. Therefore in the following experiments the trapped plasma was multiplied by a correction factor of 0.99.

Because the measurements of trapped plasma on a wet- and dry-weight basis are interconnected, measurement of only one of the two would suffice. Nevertheless, it is advisable to determine both directly for greater accuracy, and, besides, the correspondence of the measured with the calculated values proves that all the measurements involved are reliable (see Part II, Kiem et al., this issue).

As shown at the end of Materials and Methods, a reliable estimate of the trapped plasma on both wet- and dry-weight bases is of utmost importance for calculating the water content of the platelets, correcting the trace-element content for trapped plasma, and converting from dry- to wet-weight basis. However, correcting the element content for trapped plasma is reliable only for those cases where the concentration of a given element in platelets exceeds that in plasma. Therefore, the elements Na and Cl in platelets cannot be determined by this method.

**Cell Purity**

Iron concentration is a sensitive indicator of the presence of erythrocytes in the platelet sample, especially when a method such as neutron activation analysis is used, which determines both hemoglobin iron and transferrin iron, thereby resulting in higher serum iron values (see also donors 4 and 5 in Table 1) than found by the chemical methods used in clinics (30), which measure almost exclusively the transferrin iron (31, 32).

It can be seen from Table 3 that the purification of platelets from erythrocytes and leukocytes was best for donor 3, where the PRP was differentially centrifuged twice. But it can also be seen that the yield of sample material was much less after this procedure. Any step of differential centrifugation as used for donors 3–5 results in a loss of about 70% of the platelets; thus the loss of platelets in the case of donor 3 totalled about 90%. Furthermore, the loss of platelet material by transferring the sediment from the bottom of the centrifuge tube into the quarts tube is drastically increased if the sediment is below a certain critical weight (about 200 mg). In this respect, the procedure used for donor 1 (no differential centrifugation, and taking out the upper part) was optimum, resulting in a minimum loss of platelet material. In the procedure used for donor 1 the upper part of the sediment was also pure with respect to erythrocytes, and it is likely that there were also not many leukocytes in this part. But there are two disadvantages for this procedure: the leukocyte content in the platelet sample cannot be estimated, and the platelet sample is not well standardized.

With respect to standardization, the method used for donor 5 was optimum, as can be seen from the surprisingly small spread of the mean weights for a single platelet among the donors in the standardized experiment (Part II, this issue). Adopting this procedure also had the advantage that “pure”

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**Table 3. Sample Yield, Trapped Plasma and Water Content, and Purification from Erythrocytes (Fe concns) and Leukocytes on Use of Different Procedures for Donors 1, 3, 4, and 5**

<table>
<thead>
<tr>
<th>Donor</th>
<th>Sample description</th>
<th>Total no. of platelets in original PRP ($10^{10}$)</th>
<th>Wt. of plasma-containing platelet sample, mg</th>
<th>Trapped plasma, g/g</th>
<th>H₂O content in plasma-free platelets, g/g</th>
<th>Fe, µg/g</th>
<th>Estimated mass fractions (leukocytes/platelets, g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>Upper part</td>
<td>24.9</td>
<td>2257.4</td>
<td>0.406</td>
<td>—</td>
<td>—</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>1b</td>
<td>Lower part</td>
<td>320.2</td>
<td>—</td>
<td>0.368</td>
<td>—</td>
<td>—</td>
<td>79</td>
</tr>
<tr>
<td>3</td>
<td>Two-step differential centrifugation</td>
<td>12.2</td>
<td>24.4</td>
<td>0.717</td>
<td>0.241</td>
<td>0.50</td>
<td>10 &lt;0.08 &lt;0.01</td>
</tr>
<tr>
<td>4</td>
<td>One-step differential centrifugation</td>
<td>11.0</td>
<td>132.1</td>
<td>0.473</td>
<td>0.133</td>
<td>0.64</td>
<td>18 —</td>
</tr>
<tr>
<td>5a</td>
<td>“Pure” platelet sample</td>
<td>4.3</td>
<td>68.8</td>
<td>0.804</td>
<td>0.148</td>
<td>0.54</td>
<td>29 &lt;0.36 &lt;0.05</td>
</tr>
<tr>
<td>5b</td>
<td>“Impure” platelet sample</td>
<td>111.2</td>
<td>—</td>
<td>0.449</td>
<td>0.107</td>
<td>0.65</td>
<td>168 —</td>
</tr>
</tbody>
</table>

* In the dried plasma-containing platelet sample.
and "impure" platelets could both be examined and compared with respect to many aspects such as trapped plasma, water content, mean weight of the single platelet, and elemental composition. Some of the differences so observed are very interesting (see Parts II and III, this issue). The platelet sediment from donor 5 was the only one without any reddish spot or ring at the bottom, which derives from erythrocytes and provides a rather sensitive, but not quantitative, check for purity with respect to erythrocytes. As will be discussed in Part II, the mean mass fraction of erythrocytes to platelets was $< 0.005$ g/g, and that of leukocytes to platelets $< 0.02$ g/g when we used a procedure as for donor 5. This means that with respect to cell purity this method was satisfactory. By a single differential centrifugation the leukocyte fraction could be diminished, on the average, by fivefold (see Part II).

**Viability**

Because platelets can be damaged easily, thereby losing viability, we added a stabilizer to the PRP (see *Materials and Methods*). Furthermore, certain critical manipulations such as washing the platelets with isotonic solutions (e.g., glucose, 50 g/L) were avoided in this experiment by correcting for trapped plasma. Retaining the platelets in natural plasma throughout the sample-handling procedure was nearly ideal for preserving the viability. However, the evidence for the preservation of platelet viability in this experiment is all indirect, e.g., high potassium concentrations and a mean weight for the single platelet that corresponds with that expected from the mean volume of the single platelet reported in the literature. The mean weight of the single platelet was found to be 8 pg for donor 1, and about 10 pg for the donors in the standardized experiment (Part II).

**Elemental Concentrations**

Although we also determined the elemental concentrations in the platelet samples from donor 1, 3, 4, and 5, the results will not be presented here, because they are of minor importance in the comparison of different sampling procedures. We refer the reader to Parts II and III, where the concentrations of 14 elements, ascertained from well-controlled and standardized platelet samples, are reported and discussed.

**References**