Elemental Composition of Platelets. Part III. Determination of Ag, Au, Cd, Co, Cr, Cs, Mo, Rb, Sb, and Se in Normal Human Platelets by Neutron Activation Analysis

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The elements Ag, Au, Cd, Co, Cr, Cs, Mo, Rb, Sb, and Se were determined in platelets from seven normal donors. The results, in ng/g wet weight, for plasma-free platelets follow: "Pure" platelets: Ag = 29 ± (18), Au = 0.22 ± (0.22), Cd = 6.2 ± 3.4, Cs = 54.8 ± 19.2, Cr = 6.1 ± 2.5, Co = 7.5 ± (5.0), Mo = 3.4 ± 1.3, Rb = 10400 ± 3000, Sb = 18 ± (26), and Se = 782 ± 127. "Impure" platelets: Au = 0.23 ± (0.28), Cd = 6.4 ± 2.6, Cs = 35.2 ± 13.8, Cr = 8.2 ± 2.9, Co = 2.9 ± (3.0), Mo = 3.2 ± 0.8, Rb = 8700 ± 1700, Sb = 13.2 ± (8.7), and Se = 679 ± 57. To our knowledge, none of these 10 trace elements has been determined in platelets before. The selenium concentration in platelets exceeds that in other tissues (e.g., liver). We suggest that glutathione peroxidase or other unknown selenoenzymes are particularly important in platelet metabolism. Platelets are crucial for triggering thrombosis, and so may be involved as links between selenium deficiency and the concomitant increased death rate from cardiovascular disease.

Additional Keyphrases: selenium and cardiovascular disease • platelet maturity vs. size • comparison with values for other tissues • platelet volume

As mentioned in Parts I and II (this issue), we believe that zinc is the only trace element previously determined in platelets (1, 2). The trace elements copper, iron, and zinc have been determined in normal human platelets and discussed in Part II. In this third paper in the series, data on the trace elements silver, gold, cadmium, cobalt, chromium, cesium, molybdenum, rubidium, antimony, and selenium are reported and discussed.

Materials and Methods

For determining the 10 trace elements mentioned above, the same samples were used as in Part II. The procedural details for obtaining them, and the calculations involved, are discussed in Parts I and II.

The trace elements gold, cadmium, chromium, and molybdenum were determined by neutron activation analysis with use of radiochemical separation (NAARC). The trace elements silver, cobalt, cesium, rubidium, antimony, and selenium were determined by use of instrumental neutron activation analysis (INAA). The irradiation conditions and radioactivity measurements were the same as discussed under NAARC and INAA in Part II.

Results and Discussion

Cell Purity and Contamination

Because we used the same samples as in Part II, details about cell purity and control of contamination can be found in the Discussion in Part II. To control contamination, we determined the elemental concentrations of the original serum, of the first portion of the original platelet-rich plasma (PRP0), and of the supernatant plasma. As can be seen (Table 3, below), there was no danger of contamination with the elements Ag, Co, Cs, Rb, and Se. The elements Au, Cd, Cr, Mo, and Sb were determined only in the supernatant plasma and not in serum or the first portion of PRP0, so that similar assurance for these elements is not possible; however, the results obtained for them lie within the normal ranges reported in the literature (3), so that substantial contamination can also be ruled out for these elements (see also Part I regarding contamination).

Concentrations of Various Trace Elements in Platelets

Silver. We found the silver concentration (wet-weight basis) to be 29 ± (18) ng/g for the pure platelets (Table 1). Because only two values could be obtained for the impure platelets, a mean value was not calculated. Silver was 10-fold greater in platelets than in serum; thus this element could have some importance in platelets. A comparison with the silver concentration of other tissues is not possible, because available data vary widely (3).

Gold. We found the gold concentration (wet-weight basis) to be 0.22 ± (0.22) ng/g for the pure platelets (Table 1) and 0.23 ± (0.28) ng/g for the impure platelets (Table 2). Also for this element a comparison with other tissues is not possible because of insufficient data (3).

Cadmium. We found the cadmium concentration (wet-weight basis) to be 6.2 ± 3.4 ng/g for the pure platelets (Table 1) and 6.4 ± 2.6 ng/g for the impure platelets (Table 2). In Figure 1 these values are compared with the range of reported mean values for selected tissues, taken from a recent compilation (3). As was the case of copper (see Part II) the concen-

1 A more detailed account of this work is available from either the editorial office of this journal or one of the authors (L.E.F.).
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Received Nov. 17, 1978; accepted Feb. 8, 1979.
Table 1. Elemental Concentrations in Plasma-Free Pure Platelets from Seven Donors (Wet-Weight Basis)\(^a\)

<table>
<thead>
<tr>
<th>Donor</th>
<th>Ag</th>
<th>Au</th>
<th>Cd</th>
<th>Co</th>
<th>Cr</th>
<th>Cs</th>
<th>Mo</th>
<th>Rb (^b)</th>
<th>Sb</th>
<th>Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.9</td>
<td>12.2</td>
<td>11.6</td>
<td>8.3</td>
<td>35.8</td>
<td>4.7</td>
<td>8.8</td>
<td>3.8</td>
<td>11919</td>
<td></td>
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<tr>
<td>2</td>
<td>31.7</td>
<td>0.50</td>
<td>9.1</td>
<td>9.9</td>
<td>4.3</td>
<td>54.5</td>
<td>1.8</td>
<td>12.2</td>
<td>10.0</td>
<td>633</td>
</tr>
<tr>
<td>3</td>
<td>12.5</td>
<td>0.14</td>
<td>3.3</td>
<td>4.2</td>
<td>8.1</td>
<td>75.9</td>
<td>4.3</td>
<td>11.8</td>
<td>624</td>
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<td>4</td>
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<td>3.1</td>
<td>2.0</td>
<td>4.0</td>
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<td>2.1</td>
<td>744</td>
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<tr>
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<td>0.00</td>
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<td>1.8</td>
<td>4.8</td>
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<td>1.8</td>
<td>10.3</td>
<td>937</td>
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<tr>
<td>6</td>
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<td>0.14</td>
<td>6.9</td>
<td>14.7</td>
<td>3.6</td>
<td>84.9</td>
<td>4.8</td>
<td>15.4</td>
<td>56.5</td>
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</tr>
<tr>
<td>7</td>
<td>44.4</td>
<td>0.48</td>
<td>3.7</td>
<td>8.0</td>
<td>9.9</td>
<td>41.2</td>
<td>2.8</td>
<td>7.5</td>
<td>765</td>
<td></td>
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<tr>
<td>Mean</td>
<td>29</td>
<td>0.22</td>
<td>6.2</td>
<td>7.5</td>
<td>6.1</td>
<td>54.8</td>
<td>3.4</td>
<td>10.4</td>
<td>18</td>
<td>782</td>
</tr>
<tr>
<td>SD</td>
<td>(18)</td>
<td>(0.22)</td>
<td>3.4</td>
<td>(5.0)</td>
<td>2.5</td>
<td>19.2</td>
<td>1.3</td>
<td>3.0</td>
<td>(26)</td>
<td>127</td>
</tr>
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</table>

\(^a\) For calculations see Parts I and II, this issue. \(^b\) μg/g.

Table 2. Elemental Concentrations in Plasma-Free Impure Platelets from Seven Donors (Wet-Weight Basis)\(^a\)

<table>
<thead>
<tr>
<th>Donor</th>
<th>Ag</th>
<th>Au</th>
<th>Cd</th>
<th>Co</th>
<th>Cr</th>
<th>Cs</th>
<th>Mo</th>
<th>Rb (^b)</th>
<th>Sb</th>
<th>Se</th>
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</thead>
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<td>0.07</td>
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<td>0.3</td>
<td>6.1</td>
<td>15.6</td>
<td>2.3</td>
<td>6.7</td>
<td>632</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>22.2</td>
<td>0.22</td>
<td>9.2</td>
<td>8.9</td>
<td>9.5</td>
<td>45.7</td>
<td>2.5</td>
<td>9.4</td>
<td>7.3</td>
<td>636</td>
</tr>
<tr>
<td>3</td>
<td>0.05</td>
<td>2.6</td>
<td>1.3</td>
<td>5.0</td>
<td>48.0</td>
<td>3.8</td>
<td>9.8</td>
<td>3.2</td>
<td>715</td>
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<tr>
<td>4</td>
<td>0.06</td>
<td>3.4</td>
<td>0.9</td>
<td>4.5</td>
<td>31.1</td>
<td>4.3</td>
<td>6.7</td>
<td>6.1</td>
<td>689</td>
<td></td>
</tr>
<tr>
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<td>0.11</td>
<td>6.8</td>
<td>3.1</td>
<td>10.3</td>
<td>51.2</td>
<td>3.1</td>
<td>8.2</td>
<td>23.8</td>
<td>782</td>
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<tr>
<td>6</td>
<td>0.22</td>
<td>9.0</td>
<td>1.7</td>
<td>11.1</td>
<td>33.9</td>
<td>2.5</td>
<td>11.5</td>
<td>20.0</td>
<td>678</td>
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<tr>
<td>7</td>
<td>48.9</td>
<td>0.85</td>
<td>6.5</td>
<td>4.2</td>
<td>10.9</td>
<td>21.0</td>
<td>4.0</td>
<td>8.3</td>
<td>19.0</td>
<td>619</td>
</tr>
<tr>
<td>Mean</td>
<td>0.23</td>
<td>6.4</td>
<td>2.9</td>
<td>8.2</td>
<td>35.2</td>
<td>3.2</td>
<td>8.7</td>
<td>13.2</td>
<td>679</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>(0.28)</td>
<td>2.6</td>
<td>(3.0)</td>
<td>2.9</td>
<td>13.8</td>
<td>0.8</td>
<td>1.7</td>
<td>(8.7)</td>
<td>57</td>
<td></td>
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</tbody>
</table>

\(^a\) For calculations see Parts I and II, this issue. \(^b\) μg/g.

Concentrations of cadmium were the same in both pure and impure platelets. As compared with other tissues such as liver, kidney, and muscle, the concentrations found in platelets are low and more resemble those of whole blood.

**Cesium.** We found the cesium concentration (wet-weight basis) to be 54.8 ± 19.2 ng/g for the pure platelets (Table 1) and 35.2 ± 13.8 ng/g for the impure platelets (Table 2). In Figure 2 these values are compared with the ranges of reported mean values for selected tissues (3). The ratio between the mean intracellular and extracellular cesium concentration is about 50–60 for the pure platelets (Tables 1 and 3), and about 30–40 for the impure platelets (Tables 2 and 3). Because of the high ratio observed, the exchangeability of cesium in platelets as compared with other elements such as potassium and rubidium was checked in the radioisotope uptake study mentioned in Parts I and II. The results of that study showed that the uptake of cesium into platelets increases with time, similar to potassium and rubidium, but at a much lower rate. The uptake of cesium was slower in the impure than in the pure platelets. Both the lower cesium concentration and the slower cesium uptake in the impure compared with the pure platelets support the hypothesis mentioned in Part II, that the bigger platelets, which appear preferentially in the impure platelet samples, are metabolically weaker than the smaller ones, which appear preferentially in the pure platelet samples. The cesium concentration found in the pure platelets (Figure 2) exceeds that in any other tissue except muscle and bone. However, a valid comparison with muscle and bone is complicated by the wide variations among the reported data. Nevertheless, the high concentration of cesium suggests a special role of this element in platelets.

**Chromium.** We found the chromium concentration (wet weight basis) to be 6.1 ± 2.5 ng/g for the pure platelets (Table 1) and 8.2 ± 2.9 ng/g for the impure platelets (Table 2). Chromium is another element for which the available data are rather inadequate and extremely variable (3), mainly because of the analytical difficulties that still exist for determination of chromium in biological samples (4–7). Therefore we did not attempt to compare the chromium concentrations of platelets with those of other tissues. However, the chromium concentrations we found are in the same range for both pure and impure platelets.

**Cobalt.** We found the cobalt concentration (wet-weight basis) to be 7.5 ± (5.0) ng/g for the pure platelets (Table 1) and 2.9 ± (3.0) ng/g for the impure platelets (Table 2). Figure 3...
shows these values, comparing them with the ranges of reported mean values from selected tissues (3). As an intralaboratory comparison, results for cobalt concentration in heart muscle from an earlier investigation (8) have also been included on the left side of Figure 3, along with the results for platelets. The mean cobalt concentration was greater in the pure than in the impure platelets. However, a reliable comparison is not possible, because the values are widely dispersed, which may be mainly a result of the analytical difficulties in determining such low concentrations of cobalt (7). Nevertheless, the relatively low cobalt concentration in platelets as compared with other tissues such as liver, kidney, and muscle, may reflect a diminished biological role of this element in platelets.

Molybdenum. We found the molybdenum concentration (wet-weight basis) to be 3.4 ± 1.3 ng/g for the pure platelets (Table 1) and 3.2 ± 0.8 ng/g for the impure platelets (Table 2). We have not tried to compare these results with those for other tissues because of the inadequacy and discrepancies in published data (3). However, note that the molybdenum concentrations in platelets show a rather narrow spread. Evidently molybdenum is biologically well controlled in platelets.

Rubidium. We found the rubidium concentration (wet-weight basis) to be 10.4 ± 3.0 μg/g for the pure platelets (Table 1) and 8.7 ± 1.7 μg/g for the impure platelets (Table 2). In Figure 4 these values are compared with the ranges of reported mean values from selected tissues (3). The ratio between the mean intracellular and extracellular rubidium concentration is about 50–60 for the pure platelets (Tables 1 and 3), and about 40–50 for the impure platelets (Tables 2 and 3). As we observed in the case of cesium, rubidium was also taken up faster by the pure than by the impure platelets in the mentioned radioisotope uptake study (data to be published). Both the higher rubidium concentration and the faster rubidium uptake in the pure than in the impure platelets are in agreement with the hypothesis mentioned for cesium. As compared with values for other tissues such as liver and muscle, the rubidium concentration in platelets is slightly higher. Rubidium is like potassium in this respect and also with respect to its fast uptake by the cells.

Antimony. We found the antimony concentration (wet-weight basis) to be 18 ± 26 ng/g for the pure platelets (Table 1) and 13.2 ± 8.7 ng/g for the impure platelets (Table 2). The concentration of antimony in different tissues and fluids seems hardly to have been investigated; available results do not permit a meaningful comparison with those of platelets (3). Also a comparison between pure and impure platelets is ruled out because of the wide dispersion in the values.

Selenium. We found the selenium concentration (wet-weight basis) to be 782 ± 127 ng/g for the pure platelets (Table 1) and 679 ± 57 ng/g for the impure platelets (Table 2). In Figure 5 these values are compared with the ranges of reported mean values from selected tissues (3). As an intralaboratory comparison, results from an earlier investigation (8) for the selenium concentration in liver have also been included on the left side of Figure 5 along with the results for platelets. The mean selenium concentration was slightly greater in the pure than in the impure platelets.

Compared with tissues that are known to have a high concentration of selenium, the values we found for platelets are

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**Fig. 2. Cesium in platelets as compared with selected tissues**

**Fig. 3. Cobalt in platelets as compared with selected tissues**

**Fig. 4. Rubidium in platelets as compared with selected tissues**

**Fig. 5. Selenium in platelets as compared with selected tissues**
striking and interesting. Selenium, discovered to be an essential element in 1957 (9), is the active site of glutathione peroxidase (EC 1.11.1.9) (10), an enzyme discovered in 1957 (11). It also has been known since 1973 (12) that glutathione peroxidase contains four atoms of selenium. Since then, various aspects of the metabolic functions of selenium have been comprehensively reviewed (13–16). There appears to be only one report discussing this enzyme in platelets; it compared the activity in normal platelets with that of platelets from patients with thombasthenia. The latter had a markedly lower activity in their platelets than the control group (17). However, the absolute activity in platelets (compared with, e.g., that of other cells such as erythrocytes) seems to be still unknown, because the report mentioned (17) was based on relative activities of normal and pathologic platelets.

Selenium deficiency appears to influence the death rate from cardiovascular disease (18–20). We propose that platelets are the hitherto unknown link between selenium deficiency and thrombosis, as already communicated previously (21). Conceivably, selenium deficiency lowers glutathione peroxidase activity or that of other unknown selenoenzymes in platelets, so that the aggregability of platelets will be increased, thereby promoting the formation of thrombi. Glutathione peroxidase appears to play a decisive role in the biosynthesis of prostaglandins (13), which may therefore be involved in the chain from selenium deficiency to thrombus formation. The simultaneous occurrence of arteriosclerosis as a long-term disease and damaged platelet function as a short-term factor may induce formation of a thrombus that may be responsible for the death of a cardiovascular disease patient. Because the lifetime of the platelet is about 10 days (22–25), the actual selenium status of the body should promptly influence the selenium content and function of the platelets. Plantin (19) observed that the selenium concentration in various organs (although only significant in the case of the kidney cortex) was slightly lower in subjects who died from cardiovascular disease than in control subjects (who died from accidents). It is conceivable that at least some of the subjects who died from cardiovascular disease had lower concentrations of selenium also in their platelets at the time of death from thrombosis. The arteriosclerotic plaques might therefore have acted only as critical targets for the formation of platelet aggregation, resulting in thrombosis, because platelets are very sensitive to any rough surface.

An additional clue to the above-mentioned concept may be the fact that selenium deficiency lowers the glutathione peroxidase activity in erythrocytes (26–29) and leads to an increased sensitivity to oxidative damage of membranes and oxidant-sensitive proteins such as hemoglobin (28).

Influence of Platelets on Elemental Concentrations in Plasma and Serum

Although the mass fraction of platelets in whole blood is only about 3 mg/g (if it is assumed that the platelet number per microliter of whole blood is 300,000 and the mean weight of the single platelet 10 pg), they can influence the elemental concentrations in plasma or serum of such elements as cesium, potassium, rubidium, and zinc, which are higher in platelets than in plasma by a factor of about 30 to 70. Platelets disintegrate and dissolve to a great extent during the clotting process or if anticoagulated blood is allowed to stand for a long time. Therefore, obtaining the plasma soon after the collection of anticoagulated blood and using a relatively high centrifugation speed and a relatively long centrifugation time should help obviate this influence of the platelets. On the other hand, the elemental concentrations of serum should be influenced to a great extent by the platelets that disintegrate during the clotting process. Therefore, differences of as much as 20% between plasma and serum concentrations of elements such as cesium and rubidium can be expected. We recommend that, whenever these elements are determined in serum or plasma, the platelet number in the blood and the centrifugation conditions should be taken into account.

**Table 3. Elemental Concentrations in the Serum, First Portion of Platelet-Rich Plasma, and Supernatant Plasma**

<table>
<thead>
<tr>
<th></th>
<th>Ag mean (µg/L)</th>
<th>Au mean (µg/L)</th>
<th>Cd mean (µg/L)</th>
<th>Co mean (µg/L)</th>
<th>Cr mean (µg/L)</th>
<th>Cs mean (µg/L)</th>
<th>Mo mean (µg/L)</th>
<th>Rb mean (µg/L)</th>
<th>Sb mean (µg/L)</th>
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</thead>
<tbody>
<tr>
<td>Serum</td>
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<td>0.10</td>
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<td>109</td>
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</tr>
<tr>
<td></td>
<td>(1.52)</td>
<td>(0.28)</td>
<td>(0.35)</td>
<td>(60)</td>
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<td></td>
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</tr>
<tr>
<td>First portion of PRP&lt;sub&gt;0&lt;/sub&gt;</td>
<td>1.46</td>
<td>0.29</td>
<td>1.01</td>
<td>0.33</td>
<td>0.45</td>
<td>0.85</td>
<td>0.59</td>
<td>150</td>
<td>0.52</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>(1.04)</td>
<td>(0.25)</td>
<td>(0.37)</td>
<td>(60)</td>
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<tr>
<td>Supernatant plasma</td>
<td>0.68</td>
<td>0.048</td>
<td>1.01</td>
<td>0.33</td>
<td>0.45</td>
<td>0.85</td>
<td>0.59</td>
<td>150</td>
<td>0.52</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>(0.63)</td>
<td>(0.051)</td>
<td>(0.44)</td>
<td>(0.22)</td>
<td>(0.15)</td>
<td>(0.22)</td>
<td>(0.23)</td>
<td>(20)</td>
<td>(0.19)</td>
<td>(8)</td>
</tr>
</tbody>
</table>

<sup>2</sup>n = 7.

References