Platelet Turnover and Kinetics in Immune Thrombocytopenic Purpura: Results With Autologous $^{111}$In-Labeled Platelets and Homologous $^{51}$Cr-Labeled Platelets Differ

By Anthon du P. Heyns, Philip N. Badenhorst, Mattheus G. Lötter, Henry Pieters, Paula Wessels, and Harry F. Kotzé

Mean platelet survival and turnover were simultaneously determined with autologous $^{111}$In-labeled platelets ($^{111}$In-AP) and homologous $^{51}$Cr-labeled platelets ($^{51}$Cr-HP) in ten patients with chronic immune thrombocytopenic purpura (ITP). In vivo redistribution of the $^{111}$In-AP was quantitated with a scintillation camera and computer-assisted image analysis. The patients were divided into two groups: those with splenic platelet sequestration (spleen-liver $^{111}$In activity ratio > 1.4), and those with diffuse sequestration in the reticuloendothelial system. The latter patients had more severe ITP reflected by pronounced thrombocytopenia, decreased platelet turnover, and prominent early hepatic platelet sequestration. Mean platelet life span estimated with $^{51}$Cr-HP was consistently shorter than that of $^{111}$In-AP. Platelet turnover determined with $^{51}$Cr-HP was thus overestimated. The difference in results with the two isotope labels was apparently due to greater in vivo elution of $^{51}$Cr. Although the limitations of the techniques should be taken into account, these findings indicate that platelet turnover is not always normal or increased in ITP, but is low in severe disease. We suggest that this may be ascribed to damage to megakaryocytes by antiplatelet antibody. The physical characteristics in $^{111}$In clearly make this radionuclide superior to $^{51}$Cr for the study of platelet kinetics in ITP.

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MATERIALS AND METHODS

Patients

Ten patients (patients 1 to 10) with ITP, and not on any treatment, gave informed, written consent to participate in a study approved by the Ethical Committee of the University of the Orange Free State. ITP was diagnosed on the basis of conventional clinical criteria: adult-onset thrombocytopenia of undetermined etiology, normal or increased numbers of bone marrow megakaryocytes, and the absence of a demonstrable cause of platelet destruction or utilization such as systemic lupus erythematosus, drug ingestion, or hypersplenism. Relevant patient details are given in Table 1.

Platelet Studies

Autologous platelets were labeled with $^{111}$In-oxine (Radiochemical Centre, Amersham, England), fully described elsewhere. In brief, 200 to 300 mL of blood was collected in syringes containing acid-citrate dextrose NIH formula A (ACD). Platelet-rich plasma was prepared by centrifugation and was acidified with ACD to pH 6.2–6.5. The maximum number of platelets was harvested from the blood by washing platelets from the red cell layer four times. Platelets were labeled with $^{111}$In-oxine in a saline medium, resuspended in autologous platelet-poor plasma, and reinjected only if they aggregated normally with ADP. Homologous platelets were labeled with $^{51}$Cr-disodiumchromate as recommended by the International Committee for Standardization in Hematology. $^{111}$In-AP and $^{51}$Cr-HP were injected simultaneously. Contamination of the platelet product with $^{111}$In or $^{51}$Cr-labeled plasma proteins and red cells was measured.

In vivo distribution of $^{111}$In-AP was quantitated with a scintillation camera and a computer-assisted imaging system as previously described. Whole body and region of interest (ROI) $^{111}$In-radioactivity was corrected for attenuation by the geometrical mean method, and ROI activity was expressed as a percentage of whole body activity. The initial (equilibrium) radioactivity was derived by back extrapolation of linear least-squares regression analysis of data, and the final organ radioactivity was determined at the patient’s own platelet survival time.

Platelet survival was estimated from whole blood specimens collected at 5, 15, 30, 45, 60, 90, and 180 minutes after reinjection of
labeled platelets and thereafter as necessitated by the rate of disappearance of platelets from the circulation.6 Blood volume was estimated,12 and platelets were counted by phase microscopy, or, if more than 30 × 109/L, with an electronic particle counter. Mean platelet survival was calculated by a nonlinear, least-squares curve-fitting technique. A computer program fitted the data to the following mathematical models: weighted mean,6 multiple hit,13 Dornhorst,14 Meuleman,15 polynomial,16 and icaid order.17 All data points lying between equilibrium or maximum blood radioactivity, and 20% of this activity, were included. Curve fitting was visually inspected with a graphics display monitor. Goodness of fit of each of the mathematical models to the data was evaluated by calculating the SD of the data points around the regression curve. There was no significant difference in the goodness of fit with these models.18 Therefore, only the results of the mean platelet survival time estimated with the multiple hit model are presented. In all patients, the clearance of platelets from the circulation was exponential, reflected by removal of platelets after a single "hit."

Platelet recovery in the circulation at equilibrium was derived by back extrapolation of the platelet survival curve to zero time. Platelet turnover was calculated from the blood platelet count and the estimate of the splenic platelet pool size, with others in this group, had relatively long platelet survival of the latter group were segregated as a subgroup because spleen-liver ratio I.4 were classified as having "splenic sequestration," and those with a lower ratio were classified as diffuse RES sequestration. Two patients (9 and 10) had exceptionally low recovery values and, as compared having "diffuse RES sequestration." Five with a splenic sequestration pattern, and five with a diffuse pattern of platelet destruction in the RES. The distinction was based on the percentage of 111In-AP in the spleen at the end of platelet life span are given in Table 2.

Platelets from four normal subjects were labeled with 111In and 51Cr as described above. Platelet subpopulations in the platelet-rich plasma were separated by density on discontinuous arabinogalactan (stractan) gradients as described by Corash et al,19 and the radioactivity was measured. Radioactivity in the four buoyant density subpopulations was expressed as a percentage of that of the total. Mean platelet volume was measured electronically with a multichannel analyzer.19

**Elution of Radionuclide From Labeled Platelets**

The in vivo release of 51Cr and 111In from platelets was measured by determining the radioactivity present in platelet-poor plasma of all the specimens collected for the estimation of mean platelet life span. This radioactivity, not associated with platelets or other cells, was expressed as a percentage of 111In or 51Cr whole blood radioactivity at equilibrium, normalized to 100%.

**Table 1. Patient Data and In Vivo Organ 111In Radioactivity at Zero Time**

<table>
<thead>
<tr>
<th>Patients and Pattern of Platelet Sequestration</th>
<th>Number of Pregancies</th>
<th>Blood Platelet Count (x 109/L)</th>
<th>111In Activity In Spleen (% of Whole Body)</th>
<th>111In Activity In Liver (% of Whole Body)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splenic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>F/16</td>
<td>0</td>
<td>18</td>
<td>32.1</td>
</tr>
<tr>
<td>2</td>
<td>F/34</td>
<td>1</td>
<td>95</td>
<td>29.8</td>
</tr>
<tr>
<td>3</td>
<td>F/68</td>
<td>9</td>
<td>88</td>
<td>22.1</td>
</tr>
<tr>
<td>4</td>
<td>F/16</td>
<td>0</td>
<td>119</td>
<td>46.9</td>
</tr>
<tr>
<td>5</td>
<td>F/25</td>
<td>0</td>
<td>60</td>
<td>33.5</td>
</tr>
<tr>
<td>Mean ± 1 SD</td>
<td></td>
<td></td>
<td>76 ± 39</td>
<td>32.8 ± 9.8</td>
</tr>
<tr>
<td>Diffuse Res</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal recovery</td>
<td></td>
<td></td>
<td>6</td>
<td>20.6</td>
</tr>
<tr>
<td>7</td>
<td>F/19</td>
<td>0</td>
<td>4</td>
<td>8.8</td>
</tr>
<tr>
<td>8</td>
<td>M/20</td>
<td>—</td>
<td>5</td>
<td>22.2</td>
</tr>
<tr>
<td>Low recovery</td>
<td></td>
<td></td>
<td>9</td>
<td>22.4</td>
</tr>
<tr>
<td>10</td>
<td>F/16</td>
<td>0</td>
<td>7</td>
<td>32.9</td>
</tr>
<tr>
<td>Mean ± 1 SD</td>
<td></td>
<td></td>
<td>6 ± 2</td>
<td>21.4 ± 8.6</td>
</tr>
<tr>
<td>Reference values</td>
<td></td>
<td></td>
<td>150-400</td>
<td>31.1 ± 6.1</td>
</tr>
</tbody>
</table>

**Statistics**

The normal distribution of the data was assessed with the Shapiro-Wilk test. Paired or unpaired data were evaluated with either the t-test for different means or the nonparametric Wilcoxon's rank sum test where appropriate. The relationship between two variables was expressed as the correlation coefficient, r, and its significance was evaluated with the t test.

The percentage SD of the platelet turnover owing to the propagation of errors introduced by the measurements of the platelet count, the platelet life span, and the estimate of the splenic platelet pool size was computed by addition in quadrature of the fractional SDs.20

**RESULTS**

**Pattern of 111In-Labeled Platelet Destruction**

The results of quantification of organ 111In radioactivity at the end of platelet life span are given in Table 2. The patients were separated into two groups on the basis of major organ or regional 111In-AP radioactivity accumulation: five with a splenic sequestration pattern, and five with a diffuse pattern of platelet destruction in the RES. The distinction was based on the percentage of 111In-AP in the spleen and the liver at the end of the platelet life span. The normal spleen-liver ratio is 1.4 ± 0.6. Those patients with a spleen-liver ratio ≥ 1.4 were classified as having "splenic sequestration," and those with a lower ratio were classified as having "diffuse RES sequestration." Two patients (9 and 10) of the latter group were segregated as a subgroup because they had exceptionally low recovery values and, as compared with others in this group, had relatively long platelet survival times (Table 3).

In the group with a splenic sequestration pattern, the mean spleen 111In activity was 50.8% ± 13.6%, and that of the liver was 19.4% ± 7.1%. Splenic activity was significantly higher (P < .025), and that of the liver was significantly lower (P < .025), than that of controls (t test). In the group with diffuse RES sequestration, the mean hepatic 111In activity of
42.5% ± 3.6% was significantly higher ($P < .0005$), and the mean splenic activity of 22.1% ± 9.1% was significantly lower ($P < .025$), than that of normal subjects ($t$ test).

The $^{111}$In radioactivity other than in the liver or the spleen, designated "remainder" (Table 2), varied from patient to patient and did not reflect the pattern of platelet sequestration. Although the spleen-remainder ratio was lower in the diffuse RES sequestration group, this ratio was too variable to distinguish patterns of platelet destruction.

Distribution of the In Vivo $^{111}$In Platelet Activity at Equilibrium

In the group with diffuse RES destruction, mean hepatic $^{111}$In activity was four times higher ($P < .005$, $t$ test) and that of the spleen was significantly lower ($P < .025$, $t$ test) than normal (Table 1).

Platelet Recovery, Life Span and Platelet Turnover

Blood platelet counts, mean platelet survival, recovery in the circulation, and platelet turnover measurements as determined with $^{111}$In-labeled and $^{51}$Cr-labeled platelets are given in Table 3.

$^{111}$In-labeled platelets. Patients were reinjected with a mean of 11.8 MBq ± 5.3 (319 μCi ± 143) $^{111}$In, labeling $20.7 \times 10^9 \pm 22.1$ platelets. The blood platelet counts were much higher in the splenic sequestration group than in the diffuse RES sequestration group. A greater mean number of labeled platelets and more $^{111}$In was thus reinjected per patient in the splenic sequestration group. Mean recovery of platelets in the circulation of all patients was 55% ± 25%, which was somewhat lower, but not significantly so ($P > .2$), than that of normal. In patients 6, 7, and 8, with diffuse sequestration of platelets, recovery was about normal. Patients 9 and 10 had very low recoveries.

Platelet size distribution in whole blood, platelet-rich plasma, and the labeled platelet-rich saline eventually injected was determined in three patients. Although there was a loss of approximately one-half of the platelets during the isolation and labeling procedures, mean platelet volume did not change significantly: whole blood 16 ± 2 fL, platelet-rich plasma 17 ± 2 fL, and platelet-rich saline 15.5 ± 2 fL.

The mean platelet life span, range 4.3 to 65.9 hours, of $^{111}$In-AP was decreased in all patients and correlated with the platelet count ($r = 0.58, P < .05$). The mean platelet life span of the diffuse RES sequestration group was shorter than that of patients with splenic sequestration. The results, however, were variable, and patients 9 and 10 had platelet survival times that were much longer than those of the others in this group.

Mean platelet turnover of all patients was $53 \times 10^9$ platelets per liter per day ± 49 (range 4 to 114). The mean platelet turnover of the splenic sequestration group was significantly higher ($P < .005$, Wilcoxon test) than normal. Platelet turnover in patients with a diffuse RES sequestration pattern was variable and was markedly low in patients 9 and 10.

$^{51}$Cr-labeled platelets. Patients were reinjected with a mean of 2.8 MBq ± 0.8 (84 μCi ± 15) $^{51}$Cr, labeling $135.2 \times 10^9 \pm 57.3$ platelets. Mean platelet recovery in the circulation in all patients was 51% ± 21% and, although lower than normal, was not significant ($P > .5$). The mean recovery in the circulation of the diffuse RES sequestration group was much lower than normal in patients 9 and 10.

The mean life span of $^{51}$Cr-HP was shortened to varying degrees (range 1.8 to 39.6 hours), and in the patients with diffuse RES sequestration it was shorter than in patients with splenic sequestration. Patients 9 and 10 were noteworthy exceptions. Mean platelet turnover of the whole patient group was normal, $93 \times 10^9$ platelets per liter per day ± 80 ($P > .1$). There was, however, a significant ($P < .005$, Wil-
Table 3. Isotope Data: Estimates of Platelet Recovery, Life Span, and Turnover

<table>
<thead>
<tr>
<th>Patient and Sequestration Pattern</th>
<th>Blood Platelet Count (x 10^4/L)</th>
<th>111In-Labeled Autologous Platelets</th>
<th>51Cr-Labeled Homologous Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Platelets Injected (x 10^4)</td>
<td>Recovery in Circulation (%)</td>
<td>Life Span (h)</td>
</tr>
<tr>
<td></td>
<td>Administered MBq(μCi)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Splenic</td>
<td>1</td>
<td>18</td>
<td>40.0</td>
</tr>
<tr>
<td>2</td>
<td>95</td>
<td>52.0</td>
<td>18.5(500)</td>
</tr>
<tr>
<td>3</td>
<td>88</td>
<td>40.0</td>
<td>22.2(600)</td>
</tr>
<tr>
<td>4</td>
<td>119</td>
<td>52.0</td>
<td>14.1(380)</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>3.4</td>
<td>13.3(360)</td>
</tr>
<tr>
<td>Mean ± 1 SD</td>
<td>76 ± 39</td>
<td>30.3 ± 24.8</td>
<td>(408 ± 151)</td>
</tr>
<tr>
<td>Diffuse RES</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal recovery</td>
<td>6</td>
<td>8</td>
<td>3.5</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>2.7</td>
<td>6.7(180)</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>4.0</td>
<td>9.3(250)</td>
</tr>
<tr>
<td>Low recovery</td>
<td>9</td>
<td>5</td>
<td>4.5</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>5.2</td>
<td>11.1(300)</td>
</tr>
<tr>
<td>Mean ± 1 SD</td>
<td>5.8 ± 1.6</td>
<td>8.4 ± 2.2</td>
<td>44 ± 31</td>
</tr>
<tr>
<td>Reference values</td>
<td>(± 1 SD)</td>
<td>150–400</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviation: pl, platelets.

*The percentage SD of platelet turnover introduced by the fractional SD of the platelet count (0.5), the platelet life span (0.1), and the estimate of the splenic pool size (0.06) was 26%.
coxon test) difference in the mean platelet turnover of the patients with different sequestration patterns.

**Comparison of Mean Platelet Survival and Turnover Measurements With \(^{111}\)In-AP and \(^{51}\)Cr-HP**

A mean of 8.0% ± 3.1% \(^{51}\)Cr and 7.5% ± 6.5% \(^{111}\)In was present in the plasma in which the labeled platelets were suspended. Contamination of labeled platelets with labeled red cells was low; 1.3% ± 1.1% and 1.1% ± 1.6% for \(^{51}\)Cr and \(^{111}\)In, respectively.

Mean survival of \(^{111}\)In-AP was 26.3 hours ± 19.7, and that of \(^{51}\)Cr-HP was 16.8 ± 13 hours. This difference was significant (\(P < .005\), Wilcoxon test). This difference was evident whether women patients had had previous pregnancies or not. The difference influenced the estimate of mean platelet turnover: with \(^{111}\)In-AP it was 53 ± 10\(^9\) platelets per liter per day ± 49, which was significantly (\(P < .005\), Wilcoxon test) lower than the 93 ± 10\(^9\) platelets per liter per day ± 80 as estimated with \(^{51}\)Cr-HP.

**Labeling of Platelet Subpopulations**

Labeling efficiencies with \(^{111}\)In and \(^{51}\)Cr of density-dependent platelet subpopulations separated on a stractan gradient, were similar (\(P > .2\); \(t\)-test) (Table 4).

**Plasma Radioactivity**

The \(^{111}\)In and \(^{51}\)Cr radioactivity in platelet-poor plasma was measured for the duration of the platelet life span. The changes in radioactivity in plasma during the first nine hours after reinfusion of labeled platelets are illustrated in Fig 1. \(^{51}\)Cr activity was higher than that of \(^{111}\)In (\(P < .005\), \(t\)-test) throughout the life span of the platelets, but the difference was more striking in the first few hours after reinfusion of labeled platelets.

**DISCUSSION**

It has generally been stated that platelet turnover is increased in patients with ITP, and that this increase is especially evident when the blood platelet count is < 50 \(\times 10^9/L\).\(^4\)\(^-\)\(^5\) but not all authors agree.\(^9\)\(^-\)\(^2\)\(^4\) A major difficulty thwarting the clarification of the problem is the inaccuracy of the measurement of platelet turnover in ITP. In ITP, it is difficult to measure three of the variables used in calculating platelet turnover: mean platelet life span, recovery of platelets in the circulation, and the blood platelet count. We have exploited the superior physical characteristics of \(^{111}\)In and its high platelet labeling efficiency to minimize these difficulties. This radionuclide makes it possible to determine platelet survival and to image the in vivo distribution of autologous platelets quantitatively even in the presence of severe thrombocytopenia.\(^9\)\(^,\)\(^10\) In addition, the size of the splenic pool may be determined accurately with quantitative imaging.\(^11\)\(^,\)\(^27\)

It is convenient to consider our findings in the context of the in vivo distribution of the labeled platelets. In our previous study, we divided patients with ITP into three groups on the basis of the sites of platelet destruction: splenic, hepatic, and diffuse RES sequestration.\(^5\) Because this grouping was somewhat arbitrary and there was considerable overlap, we have now grouped the hepatic and diffuse RES sequestration patients together. The division between the two major groups was based on the spleen-liver ratio and was clearcut. Although they had low spleen-liver ratios, patients 9 and 10 had low recovery values and relatively long mean platelet survival times; we have therefore separated them as a subgroup. Most of the platelets not destroyed in the liver or spleen, ie, the remainder, are sequestrated in the bone marrow.\(^4\)\(^,\)\(^9\)\(^,\)\(^2\)\(^5\) Although the spleen-remainder ratio was lower in patients with diffuse RES platelet sequestration, this ratio neither identified another pattern of sequestration nor improved on the discriminatory value of the spleen-liver ratio. We observed a relationship between the blood platelet count and the sequestration pattern. Splenic sequestration was seen in patients with a relatively high platelet count, whereas diffuse RES sequestration occurred in those with low platelet counts. We therefore agree that the pattern of platelet sequestration is related to the severity of the disease.\(^4\)

**Table 4. Percentage of Distribution of Normal Platelets of Different Densities Labeled With \(^{111}\)In and \(^{51}\)Cr**

<table>
<thead>
<tr>
<th>Stractan Density (g/mL)</th>
<th>(^{111})In (g/mL)</th>
<th>(^{51})Cr (g/mL)</th>
<th>(^{111})In (g/mL)</th>
<th>(^{51})Cr (g/mL)</th>
<th>(^{111})In (g/mL)</th>
<th>(^{51})Cr (g/mL)</th>
<th>(^{111})In (g/mL)</th>
<th>(^{51})Cr (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.084</td>
<td>53.0</td>
<td>51.0</td>
<td>32.0</td>
<td>34.0</td>
<td>10.0</td>
<td>10.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>1.071</td>
<td>48.0</td>
<td>48.6</td>
<td>28.6</td>
<td>31.5</td>
<td>17.1</td>
<td>15.8</td>
<td>6.0</td>
<td>4.0</td>
</tr>
<tr>
<td>1.066</td>
<td>50.0</td>
<td>53.0</td>
<td>24.0</td>
<td>25.0</td>
<td>21.0</td>
<td>19.0</td>
<td>5.0</td>
<td>3.0</td>
</tr>
<tr>
<td>1.062</td>
<td>51.0</td>
<td>50.0</td>
<td>23.0</td>
<td>24.0</td>
<td>14.0</td>
<td>16.0</td>
<td>12.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Mean ± 1 SD</td>
<td>50.0 ± 2.1</td>
<td>50.7 ± 1.9</td>
<td>26.9 ± 4.2</td>
<td>28.6 ± 4.9</td>
<td>15.5 ± 4.7</td>
<td>15.2 ± 3.8</td>
<td>7.0 ± 3.4</td>
<td>5.5 ± 3.1</td>
</tr>
</tbody>
</table>
In normal subjects, mean platelet survival estimated with $^{111}$In and $^{51}$Cr as cell labels is similar. We now demonstrate that in ITP the survival of $^{111}$In-AP is significantly longer than that determined simultaneously with $^{51}$Cr-HP. This agrees with our previous view and has recently also been found in moderately thrombocytopenic patients with ITP studied with $^{111}$In- and $^{51}$Cr-labeled autologous platelets. It is unlikely that the discrepancy is due to isoenzymation because previous pregnancies or blood transfusions were not relevant in our patients. Neither is varying use of homologous $^{51}$Cr autologous platelets the explanation.

It has been shown in vitro that small molecules such as $^{51}$Cr are more readily lost from platelets than is $^{111}$In-binding protein. Our results, although not conclusive, suggest that this may occur in vivo. Plasma activity of $^{51}$Cr was significantly higher than that of $^{111}$In, especially during the early period after reinfusion of labeled platelets. The in vitro contamination of labeled platelets with $^{51}$Cr or $^{111}$In in plasma was similar; therefore, the infusion of free radionuclide does not explain the difference in platelet survival times with the two isotopes. However, it is possible that the two radionuclides may be bound to different plasma proteins. This would affect the clearance rates of the isotopes from the circulation and is an alternative explanation for the results in Fig 1.

We also investigated the possibility that $^{51}$Cr and $^{111}$In do not label the circulating platelet population uniformly or to the same extent. Misleading platelet survival information could be obtained if one of the isotopes had a greater affinity for a specific platelet subpopulation. This is unlikely because $^{111}$In and $^{51}$Cr radioactivities were distributed equally in the platelet subpopulations separated on stractan. In addition, a select population of platelets with a different mean volume from that of whole blood platelets was not lost during harvesting and labeling.

Finally, platelets remaining in the circulation in ITP after a steady state has been reached may be relatively resistant to antibody injury. Autologous labeled platelets may thus have a longer mean survival time in the patient than does the heterogeneous platelet population of a normal donor. This has a precedent in autoimmune hemolytic anemia. Our study has not excluded this possibility.

The estimate of the size of the splenic platelet pool will also influence the calculation of platelet turnover. The size of the splenic pool is considered normal in ITP, but the estimates of the percentage of recovery of labeled platelets in the circulation vary from low to near normal. This discrepancy may be owing to the methods used in determining recovery. It is not clear whether it is more appropriate to estimate recovery by extrapolation of blood radioactivity values to zero time (as in ref 1 and our study) or by measuring blood radioactivity at equilibrium. Our data do not answer this question, but demonstrate that the estimate of recovery may be influenced by the pattern of platelet sequestration. If one assumes that the only significant exchangeable platelet pool is in the spleen, percentage of platelet recovery in the circulation and splenic radioactivity should total ~100%. In normal subjects, mean platelet recovery is 61% ± 12%, and at equilibrium 31.1% ± 6.1% of $^{111}$In-labeled platelets are present in the spleen. This was not so in ITP. In patients with a splenic sequestration pattern, mean recovery was 65.8% ± 11.8%, and the size of the splenic pool was estimated at 32.8% ± 9.8%, resembling that of normal subjects. In contrast, the group of patients with diffuse RES platelet sequestration had a mean $^{111}$In-AP recovery of 44% ± 31% and a mean spleen pool of 21.4% ± 8.6%. This deficit may readily be accounted for by the very high liver $^{111}$In-activity at equilibrium. It seems likely that in patients with diffuse RES sequestration of platelets many platelets are destroyed in the liver before attainment of equilibrium between the blood and splenic platelet pools.

All of the above factors should be taken into account when considering platelet turnover in ITP. We estimate that the propagation of errors introduced by the determination of the parameters used to calculate platelet turnover has a percentage SD of 26% in patients with a blood platelet count of 10 $\times$ 10$^9$/L. There was also marked variation from patient to patient as reflected by the large SDs. Therefore, it is clear that the physiological interpretation of results of kinetic studies in these patients should be guarded.

Notwithstanding our reservations, our results indicate that platelet turnover calculated with $^{111}$In-labeled autologous platelets identifies a subset of patients with low platelet turnover, and that these patients have more severe disease reflected by a low platelet count and diffuse RES sequestration of platelets. Platelet turnover measured with $^{111}$In-AP was high in all patients with a splenic sequestration pattern. The mean increase was 2.2 times that of normal (range 1.2 to 3.0). In contrast, mean platelet turnover was decreased (mean 0.4 times normal) in all patients with diffuse RES sequestration of $^{111}$In-AP, and especially so in patients 9 and 10. Mean platelet turnover estimated with $^{51}$Cr-HP was significantly higher in both patient groups than platelet turnover measured with $^{111}$In-AP. Our results are thus not in accordance with studies in which platelet turnover was found to be either normal or increased in ITP but tend to agree with those of recent studies with $^{51}$Cr-labeled platelets and $^{111}$In-labeled platelets.

These results may be related to other findings difficult to reconcile with the concept that platelet production is always increased in ITP. There is some evidence that antiplatelet antibodies may damage megakaryocytes and impair megakaryocytopoiesis. Rolovic et al have shown that megakaryocytopoiesis in rats with thrombocytopenia induced with heteroimmune antiplatelet serum is markedly altered. They demonstrate coexistent platelet destruction and defective thrombopoiesis in this experimental model of ITP. It has also been shown that the antiplatelet antibody of ITP binds to both platelets and megakaryocytes and may impair platelet production at the level of the mature megakaryocyte. It therefore seems plausible that the low platelet turnover demonstrated in our patients may be owing to antibody-mediated injury to the megakaryocytes. The more severe impairment of thrombocytopoiesis seen in patients with diffuse RES sequestration of platelets may be related to the finding that this destruction pattern typical of severe disease is owing to...
to greater amounts of antibody bound to platelets. It seems plausible that megakaryocytes may be damaged in such patients with high antibody levels.

It is evident that the application of the techniques of labeling autologous platelets with $^{111}$In and the technology of quantification of the in vivo distribution of labeled platelets have provided new insights into the pathogenesis of ITP.

However, they have also exposed new problems reflecting the complexities of the disease and the difficulties associated with the interpretation of the data.

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P Heyns A du, PN Badenhorst, MG Lotter, H Pieters, P Wessels and HF Kotze