Blood Platelet Economy during Moderate and Intensive Heparin Therapy

By J. F. Mustard and Edmond A. Murphy

The effect of heparin on the physiology of platelets in vitro has been extensively studied. We share the misgivings of O'Brien that results of in vitro studies on coagulation cannot uncritically be applied to effects in vivo. Previous studies in vitro and in extracorporeal circulations showed that heparin decreases platelet adhesiveness and inhibits the formation of platelet thrombi, and that this effect is sensitive to the level of dosage. Berman has reported that the inhibition by heparin therapy of platelet thrombus formation at points of injury in the microcirculation of the hamster is also related to the dosage of heparin used.

We have found in experimental studies on thrombus formation that heparin must be given in large doses if it is to be efficacious. Recently, however, McDonald and Edgill have published some interesting results showing that if 1,500 units of heparin be given twice a week subcutaneously over a period of several months, platelet adhesiveness is greatly reduced. It might be inferred from this that thrombus formation also would be inhibited, because there is good correlation between the platelet adhesive index and the amount of thrombus formed in extracorporeal circulations. It may be that McDonald and Edgill's unexpected results are related to the duration of treatment: most other investigators have confined their attention to the acute effects of heparin therapy.

We have attempted to obtain more direct evidence of the effect of heparin on platelets undisturbed by removal from the body. The only satisfactory quantitative method we have of studying platelet economy in vivo is the measurement of platelet survival. It seemed to be reasonable to study the effect of varying doses of heparin over a prolonged period of time.

Patients and Methods

Paired studies were done on 22 subjects, the results during the control period being compared with results after various courses of heparin treatment.

Patients

In age they varied between 31 and 85 years (table 1); all were Canadian veterans, and all but two of them were male. Thirteen of them suffered from complications of atheroscler-
Table 1.—The Subjects Studied

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) With Manifest Vascular Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>44</td>
<td>F</td>
<td>Angina pectoris</td>
</tr>
<tr>
<td>Ch</td>
<td>76</td>
<td>M</td>
<td>Myocardial infarction; macular degeneration</td>
</tr>
<tr>
<td>Co</td>
<td>31</td>
<td>M</td>
<td>Recurrent thrombophlebitis</td>
</tr>
<tr>
<td>Ev</td>
<td>75</td>
<td>M</td>
<td>Intermittent claudication</td>
</tr>
<tr>
<td>Gu</td>
<td>65</td>
<td>M</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>H</td>
<td>70</td>
<td>M</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>Ke</td>
<td>72</td>
<td>M</td>
<td>Cerebrovascular accident</td>
</tr>
<tr>
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<td>60</td>
<td>F</td>
<td>Angina pectoris; myocardial infarction</td>
</tr>
<tr>
<td>P</td>
<td>74</td>
<td>M</td>
<td>Intermittent claudication</td>
</tr>
<tr>
<td>R</td>
<td>67</td>
<td>M</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>Sh</td>
<td>43</td>
<td>M</td>
<td>Angina pectoris; myocardial infarction</td>
</tr>
<tr>
<td>Wh</td>
<td>80</td>
<td>M</td>
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</tr>
<tr>
<td>Wi</td>
<td>45</td>
<td>M</td>
<td>Intermittent claudication</td>
</tr>
<tr>
<td>(b) Without Manifest Vascular Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>79</td>
<td>M</td>
<td>Macular degeneration</td>
</tr>
<tr>
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<td>67</td>
<td>M</td>
<td>Chronic bronchitis</td>
</tr>
<tr>
<td>Gw</td>
<td>76</td>
<td>M</td>
<td>Macular degeneration</td>
</tr>
<tr>
<td>Kn</td>
<td>77</td>
<td>M</td>
<td>Macular degeneration</td>
</tr>
<tr>
<td>Sc</td>
<td>70</td>
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<td>Macular degeneration</td>
</tr>
<tr>
<td>Si</td>
<td>70</td>
<td>M</td>
<td>Chronic bronchitis</td>
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<tr>
<td>Su</td>
<td>78</td>
<td>M</td>
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</tr>
<tr>
<td>Wa</td>
<td>75</td>
<td>M</td>
<td>Macular degeneration</td>
</tr>
</tbody>
</table>

otic vascular disease (myocardial infarction, intermittent claudication or cerebral ischemia),
the remaining nine from chronic bronchitis and emphysema, or macular degeneration.

**Platelet Survival**

This was measured by the DFP32 method of Leeksma and Cohen11 with some modifications which we have previously described.10 We have introduced the following further modifications. The red cells in the blood samples were first sedimented by addition of one part of dextran (Glaxo Laboratories, Intraderm) and 2.5 parts of 1 per cent EDTA solution to 20 parts of blood. The supernatant plasma was then centrifuged at 1,000 rpm (ref 225) for 12 minutes. The new supernatant was then transferred to another tube and centrifuged at 2,600 rpm (ref 1540) for 15 minutes. The resulting platelet button was then washed twice and prepared as described in previous papers. The top half of the final washed platelet button was suspended in 1 ml. of distilled water and transferred to a planchette which was then dried at 48 C. The weight of the dried material was determined and radioactivity was determined and expressed as counts per minute per mg. of dried platelet material. Readings were taken every day (except Sunday) over a period of exactly 10 days. Platelet survival was computed both on an exponential and Gaussian ("linear") model, and from these and from a knowledge of the mean platelet count for each subject it was possible to make an estimate of mean platelet turnover per mm.3 per day. Details of these methods of calculation are given elsewhere.10 It should be pointed out that estimates of turnover suppose stable platelet counts, and that what differences there are between arterial and venous platelet counts can be ignored with little loss of accuracy.

**In Vitro Clotting Tests**

Clotting tests were done on each occasion just before the time for the morning dose of heparin. The following coagulation tests were done: the whole blood clotting time (WBCT),
EFFECTS OF HEPARIN ON PLATELET SURVIVAL

the one-stage prothrombin time (OSPT), the platelet clumping time (PCT), the platelet adhesive index (PAI), the plasma thromboplastin time (PTT). These methods have been previously described. The values reported were determined during the 10-day period of each platelet survival study. The mean values for the results for each test were used in the calculations. The tests were carried out at intervals over the 10-day period. The statistical methods used have been previously described.

Dosage of Heparin

The following schemes of dosage were used. Eighteen patients were given 5000 units of heparin subcutaneously twice a day at 9:00 a.m. and 9:00 p.m. for 4 weeks. Clotting tests and platelet survival studies were done (1) before and (2) at the end of this 4-week period, the heparin treatment being continued until the end of the second platelet survival study. (3) Nine of these 18 patients were continued on the same regimen for a further 7 weeks (i.e., 11 weeks in all) and the studies again repeated. (4) Fourteen of the original 22 patients were studied before and after receiving 8000 units of heparin three times a day for 4 weeks. (See tables 2 and 4.)

RESULTS

In Vitro Tests

The results for these are shown in tables 2 and 3. From the t tests on the paried differences it is seen that the control figures are little changed by 5000 units of heparin twice daily, either for 4 or for 11 weeks. On the larger dosage, however, the platelet clumping time, the whole blood clotting time and the prothrombin time were prolonged significantly (table 3).

In Vivo Tests

The results for the platelet survival studies are shown in tables 4 and 5. They show little effect from the low dosage at either 4 or 11 weeks. Considerable prolongation of platelet survival with a drop in platelet count and a corresponding decrease in turnover was seen during the more intensive regimen. The changes produced by heparin in the in vitro and in vivo tests are similar in subjects with vascular disease and those without; if anything, heparin had less effect in the vascular group.

DISCUSSION

These results show that heparin in higher doses usually prolongs platelet survival and diminishes platelet turnover, and that these are associated with prolongation of clotting as judged by in vitro tests. It is of interest that the mean platelet adhesive index was increased (albeit not significantly) under all three regimens of treatment. Since all specimens were taken 7 or more hours after the last injection of heparin, it is possible that as the effect of heparin wears off, it produces a period of hypercoagulability. Evidence for the existence of a rebound effect has recently been obtained in experimental animals.

McDonald and Edgill in their study found that heparin given in small doses (1500 units) twice a week decreased platelet adhesiveness. If it is assumed that the methods used in this and the present study are measuring essentially the same aspect of platelet adhesiveness, it would seem reasonable to
<table>
<thead>
<tr>
<th>Subject</th>
<th>Prothrombin Time (seconds)</th>
<th>Whole Blood Clotting Time (seconds)</th>
<th>Platelet Clumping Time (seconds)</th>
<th>Plasma Thromboplatin Time (seconds)</th>
<th>Adhesive Index</th>
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</thead>
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<tr>
<td></td>
<td>I</td>
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<td>15.8</td>
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</tr>
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</tr>
<tr>
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</tr>
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For explanation of the significance of the roman numerals, see text.
The number in parentheses indicates in each case the number of tests from which the mean value given is computed.
Table 3.—The Effect of Heparin in Varying Doses on Blood Coagulation in Man as Judged by in Vitro Tests

<table>
<thead>
<tr>
<th>Period</th>
<th>Number of Pairs</th>
<th>Prothrombin Time (seconds)</th>
<th>Whole Blood Clotting Time (minutes)</th>
<th>Platelet Clumping Time (seconds)</th>
<th>Plasma Thromboplastin Time (seconds)</th>
<th>Platelet Adhesive Index</th>
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<tr>
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<td>14.3</td>
<td>295</td>
<td>10.4</td>
<td>1.13</td>
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<tr>
<td>t</td>
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<td>p</td>
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<td>&lt; 0.8</td>
<td></td>
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<td>&lt; 0.5</td>
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<tr>
<td>I</td>
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<td>12.6</td>
<td>297</td>
<td>10.5</td>
<td>1.08</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>9</td>
<td>14.8</td>
<td>13.3</td>
<td>293</td>
<td>10.3</td>
<td>1.13</td>
</tr>
<tr>
<td>t</td>
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<td>1.22</td>
<td>0.19</td>
<td></td>
<td></td>
<td>0.71</td>
</tr>
<tr>
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<td>&lt; 0.9</td>
<td></td>
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<td>&lt; 0.5</td>
</tr>
<tr>
<td>I</td>
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<td>12.5</td>
<td>306</td>
<td>10.3</td>
<td>1.12</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>14</td>
<td>15.8</td>
<td>17.4</td>
<td>460</td>
<td>10.9</td>
<td>1.25</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>2.76</td>
<td>5.33</td>
<td>3.90</td>
<td>1.94</td>
<td>1.72</td>
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<tr>
<td>p</td>
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<td>&lt; 0.001</td>
<td>&lt; 0.005</td>
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</table>

For explanation of the significance of the roman numerals, see text.

expect that small doses of heparin will prolong platelet survival in view of the good correlation between platelet adhesiveness and platelet survival. If this is so, then the action of heparin on platelet survival is complex since it would mean that very small doses and moderately large doses of heparin prolong platelet survival, while intermediate doses do not. In view of the potential therapeutic usefulness of small doses of heparin, this problem should be resolved by repeating McDonald and Edgill’s study and determining whether the decreased platelet adhesiveness which they found is associated with prolonged platelet survival and decreased platelet turnover. However, within the range of heparin used in our study, the effects on blood coagulation and platelet economy increase as the dosage is increased. This is in accord with the findings of most other investigators using in vitro tests and animal experiments.

Although the fate of the circulating platelet is not completely known, it is of interest that high doses of heparin, like high doses of Dicumarol, prolong platelet survival. Since Dicumarol and heparin have different pharmacologic effects on blood coagulation, it seems unlikely that this effect on platelet survival is attributable to changes in the internal economy of the platelet. It seems more likely that these changes are due to effects on its external economy. This suggests that platelet survival is not solely determined by senescence or aging as is implied by some investigators.

Summary

A study was made of the effects of heparin on platelet survival. The same subjects were studied during control periods and during heparin treatment at two levels of dosage. In most subjects, platelet survival was considerably prolonged and platelet turnover correspondingly diminished when 8000 units of heparin were given 8-hourly, but not with smaller dosage. The evidence in no
Table 4.—The Individual Responses of Platelet Economy to Heparin

<table>
<thead>
<tr>
<th>Subject</th>
<th>Platelet Count (thousands/mm³)</th>
<th>Platelet Exponential Half-life (days)</th>
<th>Platelet Turnover (exponential) (thousands/mm³/day)</th>
<th>Platelet Mean Survival (gaussian) (days)</th>
<th>Platelet Turnover (gaussian) (thousands/mm³/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>IV</td>
<td>I</td>
</tr>
<tr>
<td>Co</td>
<td>272 (5)</td>
<td>—</td>
<td>—</td>
<td>245 (4)</td>
<td>1.865</td>
</tr>
<tr>
<td>H</td>
<td>185 (4)</td>
<td>146 (5)</td>
<td>—</td>
<td>146 (5)</td>
<td>2.832</td>
</tr>
<tr>
<td>O</td>
<td>264 (5)</td>
<td>—</td>
<td>—</td>
<td>220 (4)</td>
<td>2.403</td>
</tr>
</tbody>
</table>

For explanation of the significance of the roman numerals, see text.
The number in parentheses indicates in each case the number of tests from which the mean value given is computed.
EFFECTS OF HEPARIN ON PLATELET SURVIVAL

Table 5.—The Effect of Heparin in Varied Doses on in Vivo Platelet Economy in Man

<table>
<thead>
<tr>
<th>Period</th>
<th>Number of Pairs</th>
<th>Platelet Count (thousands per mm.(^3))</th>
<th>Exponential Treatment of Results</th>
<th>Gaussian Treatment of Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Half-life (days)</td>
<td>Turnover (thousands/mm.(^3)/day)</td>
</tr>
<tr>
<td>I</td>
<td>18</td>
<td>195.8</td>
<td>3.23</td>
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<td>II</td>
<td>203.1</td>
<td>3.98</td>
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<td>1.88</td>
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<td>1.44</td>
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<tr>
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<td>&lt; 0.7</td>
<td>&lt; 0.1</td>
<td>&lt; 0.4</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>III</td>
<td>9</td>
<td>201.3</td>
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<td>41.91</td>
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<tr>
<td>t</td>
<td>0.62</td>
<td>0.05</td>
<td>0.21</td>
<td>0.46</td>
</tr>
<tr>
<td>p</td>
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<td>&lt; 1.0</td>
<td>&lt; 0.9</td>
<td>&lt; 0.7</td>
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<tr>
<td>IV</td>
<td>14</td>
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<td>3.11</td>
<td>50.46</td>
</tr>
<tr>
<td>t</td>
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<td>12.31</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.1</td>
<td>&lt; 0.005</td>
<td>&lt; 0.005</td>
<td>&lt; 0.02</td>
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</table>

For explanation of the significance of the roman numerals, see text.

way implies that such a dosage is optimal—several patients showed little or no response to it. Comparable results were obtained with various in vitro measures of blood coagulability. There was some evidence suggesting that platelet adhesiveness was paradoxically increased when the effect of heparin was wearing off.

These findings raise questions of clinical importance. Moreover, they provide yet further evidence that the survival of the blood platelet is considerably influenced by factors in its external environment.

SUMMARIO IN INTERLINGUA

Esseva interpretante un studio del effectos de heparina super le longevitate del placchatas. Le mesme subjectos esseva studiate durante periodos de controlo e durante tractamento con heparina a duo nivellos de dosage. In le majoritate del subjectos, le longevitate del placchatas esseva considerablemente prolongate e le metabolisation del placchatas esseva correspondentemente reducita quando 8000 unitates de heparina esseva administrate a intervallos de 8 horas, sed plus basse doses non produceva iste effecto. Le evidentia non indica in ulle maniera que le dosage usate esseva optimal. De facto, plure patientes monstrava pauc o nulle responsa a illo. Comparabile resultatos esseva obtenite con varie mesuras in vitro del coagulabilitate de sanguine. Certe observationes pareva indicar paradoxemente que le adhesivitate del placchatas cresceva quando le effecto del heparina subsideva.

Iste constatationes subleva questiones de importantia clinic. In plus, illos provide evidentia additional que le longevitate del placchatas de sanguine es influentiate considerablemente per factores in le ambiente externe.
REFERENCES


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