Determination of platelet and fibrinogen half-life with $[^{75}\text{Se}]$selenomethionine: studies in normal and in diabetic subjects

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Summary

1. Simultaneous platelet and fibrinogen survival studies were carried out on a group of seven normal persons and eleven diabetic patients.

2. Survival time was calculated: (a) by measuring the interval between 50% of the maximum radioactivity in the anabolic phase and 50% of the maximum radioactivity in the catabolic phase, and (b) by fitting an exponential function to the decay phase of the curve.

3. With the first method, the normal group had a mean platelet survival which did not differ significantly from that in the diabetic group. With the second method, however, the platelet half-life in the diabetic patients was significantly shorter than in the normal subjects.

4. Fibrinogen survival was significantly shorter in the diabetic group with either method.

5. It is concluded that there is an increased utilization of platelets and fibrinogen in diabetic subjects.

Key words: fibrinogen, platelet, $[^{75}\text{Se}]$selenomethionine.

Introduction

Occlusive vascular disease is a major complication of diabetes mellitus. This may reflect intrinsic disease in the vessel walls, an increased tendency to intravascular thrombosis, diminished lysis or to a combination of these factors. The simultaneous measurement of platelet and fibrinogen survival offers a potentially useful assessment of the dynamic state of the haemostatic mechanism and this study was carried out to observe if there was any difference between diabetic and non-diabetic subjects with respect to these indices.

Subjects

Seven normal, healthy subjects, six male and one female, aged from 26 to 46 years, were studied and compared with eleven diabetic patients (Table 1). All had given informed consent; the normal subjects were members of the staff of the Materia Medica Department. In three of the diabetic subjects, the platelet survival was not calculated as inadequate numbers of blood samples were obtained. None of the diabetic subjects had overt peripheral vascular complications although one had a moderate diabetic retinopathy. This was thought to be important as the existence of major arterial disease might have in itself influenced the results; for example, the peripheral utilization of platelets may have been increased by the diseased vessels. None of the subjects had proteinuria or a raised blood urea. The diabetic subjects were all insulin-dependent and the approximate duration of their diabetes is indicated in Table 1. There is an obvious difference in age distribution between the two groups but the mean values were not significantly different.
TABLE 1. Age of subjects and duration of diabetes

<table>
<thead>
<tr>
<th>Subject</th>
<th>Diabetic Age (years)</th>
<th>Duration of diabetes (years)</th>
<th>Normal Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58</td>
<td>20</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>1</td>
<td>34</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>68 (1)</td>
<td>30</td>
<td>46</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>&lt;1</td>
<td>28</td>
</tr>
<tr>
<td>7</td>
<td>60 (1)</td>
<td>30</td>
<td>31</td>
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<td>8</td>
<td>67</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>55</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>22 (1)</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>35</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>43 ± 18</td>
<td></td>
<td>33 ± 6</td>
</tr>
</tbody>
</table>

(1) Not included in platelet survival.

Methods

A dose (50 µCi) of $[^{75}\text{Se}]$selenomethionine (radiation dose 0.4 rad) was injected intravenously and blood removed by venepuncture (19 gauge needle into a plastic syringe) at 1 h, 5 h, and daily for 1 week and on alternate days thereafter. The sample (18 ml) of whole blood was placed in 2 ml of 3.8% sodium citrate and a whole-blood platelet count carried out. In seven subjects (three diabetic patients and four normal subjects) the platelet counts were done visually (Dacie & Lewis, 1968); in the remaining subjects a Coulter Thrombocounter was used. The blood was centrifuged at 1000 r.p.m. (250 g) for 10 min and 5 ml of platelet-rich plasma removed. A platelet button was obtained from this which, after washing, was counted in a Wallac automatic gamma counter. In order to correct for changes in the total numbers of platelets sampled each time, the c.p.m. were corrected by using the equation:

\[
\text{Corrected platelet button activity (\%)} = \frac{\text{platelet button activity (c.p.m.)}}{\text{platelet count}} \times \left(\frac{\text{maximum platelet count}}{\text{maximum platelet button activity}}\right) \times 100
\]

Platelet-poor plasma was obtained by further centrifugation of the whole blood and fibrinogen assayed by the method of Ratnoff & Menzies (1951). The fibrinogen clots were washed and the radioactivity was counted as for platelets. The counts were again corrected to the maximum fibrinogen concentration observed and expressed as a percentage of the highest clot radioactivity.

Platelet and fibrinogen survival times were estimated by two methods, that of Brodsky, Siegel, Kahn, Ross & Petkov (1970), who took the interval between 50% of the maximum radioactivity on the anabolic phase of the survival curve and 50% of the maximum radioactivity on the catabolic phase, and also by calculating a half-life by fitting the decay phase of the curve to an exponential function. This was done by plotting the points after the maximum on log linear paper and fitting them to a straight line by a least-squares fitting programme on a Hewlett Packard 9100B Programmable Calculator. In general the decay phases of both platelet and fibrinogen survival curves were good fits to single exponential functions and there was a high correlation between log activity and time during this phase. The mean correlation coefficient was 0.93, with a range from 0.8 to 0.99.

Results

A group of typical curves for normal platelet activity are shown in Fig. 1 and a group of those obtained from diabetic subjects in Fig. 2.

![Fig. 1. Platelet survival curves for three normal subjects.](image)
The shape of the fibrinogen curve differs from that of the platelets as the anabolic phase is so rapid. A group of curves for normal fibrinogen activity are shown in Fig. 3 and those obtained from diabetic subjects in Fig. 4.

Platelet survival for the normal group by the Brodsky et al. (1970) method was 12.4 ± 2.5 days (mean ± 1 SD), which was comparable with the normal values obtained by Brodsky of 10.6 ± 3.3 days. This did not differ significantly from the values for the diabetic group, which were 10.2 ± 2.0 days ($t_{13} = 1.91, P > 0.05$) (Fig. 5).

The time taken to reach maximum platelet button radioactivity was also not significantly different between normal subjects (7.3 ± 1.7 days) and diabetic patients (8.9 ± 3.2 days, $t_{13} = 1.2, P > 0.2$) (Fig. 6). The platelet half-life was, however, significantly shorter in diabetic patients, being 5.2 ± 2.3 days compared with 11.7 ± 5.4 days in normal subjects ($t_{13} = 3.1, P < 0.01$). This is illustrated in Fig. 7. In the diabetic group the fibrinogen survival (4.0 ± 2.4 days) was significantly shorter than that of the normal group (6.9 ± 2.1 days, $t_{16} = 3.6, P < 0.005$), and this difference was also reflected in the fibrinogen half-life, which for diabetic patients was 6.7 ± 2.5 days and for normal subjects was...
9.9 ± 2.3 days ($t_{1/2}=2.8, P<0.02$). This is illustrated in Fig. 8 and Fig. 9. The mean platelet counts and mean fibrinogen concentrations of all samples showed no significant difference between the two groups (Table 2).

![Fig. 6. Time taken to reach maximum platelet button activity. Results for diabetic and normal subjects are shown.](image)

![Fig. 7. Platelet half-life in diabetic and normal subjects.](image)

![Fig. 8. Fibrinogen survival obtained with the method of Brodsky et al. (1970).](image)

![Fig. 9. Fibrinogen half-life in diabetic and normal subjects.](image)

**Table 2. Mean daily platelet counts and fibrinogen concentrations in normal and diabetic subjects**

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Diabetics</th>
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<tbody>
<tr>
<td>Mean daily platelet count (number/mm$^3$)</td>
<td>216 000 ± 65 000</td>
<td>230 000 ± 65 000</td>
<td>0.4</td>
</tr>
<tr>
<td>Mean daily fibrinogen (mg/100 ml)</td>
<td>403 ± 51</td>
<td>415 ± 89</td>
<td>0.3</td>
</tr>
</tbody>
</table>

**Discussion**

[75Se]Selenomethionine, produced by substituting 75Se for sulphur in methionine, was shown by Cowie & Cohen (1957) to follow the same metabolic pathways as methionine. The first clinical application was by Blau & Manske (1961), who used it to visualize the pancreas by isotope scanning techniques.
Cohen, Cooley & Gardner (1965) first used it to label platelets, initially in dogs and subsequently in humans. The technique was further developed by Brodsky et al. (1970). The technique has the major advantage of the label being incorporated into the platelets during development and this eliminates the risks to platelet function and viability associated with labelling in vitro and re-injection methods. In addition, selenomethionine is incorporated into fibrinogen and survival of this protein can also be measured.

The method of calculating platelet survival times has been widely discussed. How the platelet survival curves obtained in this study are interpreted will depend on what is assumed about the mechanism of platelet destruction. A great deal of conflicting evidence on this point exists in the literature; the two principal models that have been considered are: (1) the platelets are utilized randomly irrespective of age, and (2) 'senescence' is the major factor governing their removal from the circulation (Aas & Gardner, 1958; Adelson, Rheingold & Crosby, 1957; Aster & Jandl, 1964; Cohen, Gardner & Barnett, 1961a, b; Gardner & Cohen, 1966; Ginsburg & Aster, 1969; Heyssel, 1961; Mustard, Rowell & Murphy, 1966). If we consider a 'senescence' model, the platelet survival curves obtained with [75Se]selenomethionine will vary considerably in appearance, depending on whether the platelet life-time is greater or less than the megakaryocyte maturation time, which is essentially the time taken to reach maximum platelet radioactivity and is not necessarily related to the platelet survival time (Harker & Finch, 1969).

If the platelet life-time is less than the megakaryocyte maturation time, at the point of maximum radioactivity the labelled platelets present will have a wide range of ages. Hence if the life-time is fairly well defined the decay phase of the curve will be a straight line; the intercept of this line on the time axis, less the time taken to reach maximum activity, will be equal to the platelet life-time. In this situation the interval used by Brodsky is essentially equal not to the platelet survival time but to the megakaryocyte maturation time.

If the platelet life-time is greater than the megakaryocyte maturation time, the decay phase of the curve will mirror the ascending phase and the platelet life-time will then be given by the interval used by Brodsky et al. (1970). The appearance of the curve will be further modified if the life-time has an appreciable standard deviation. With the present data it is usually not possible to distinguish between the different types of curve referred to above, owing mainly to the small number of points on many of the survival curves and to the experimental errors involved.

If we assume that platelets are utilized randomly, the decay phase of the survival curve will be exponential and it is then appropriate to express platelet survival in terms of a half-life. It is evident that analysis of the data with this model is simpler than with the 'senescence'.

The fibrinogen data present less problems in view of the very rapid anabolic phase, and the half-life has again been estimated by fitting the points on the decay phase to an exponential curve. By using both this and the Brodsky methods of estimating fibrinogen half-life, there is a significant difference between normal subjects and diabetic patients.

Intravascular thrombosis is the consequence of the normal balance between blood coagulation and lysis being altered in favour of fibrin deposition. Results in the current study indicate increased utilization of platelets and fibrinogen in diabetic subjects in comparison with normal subjects. Abrahamssen (1968) demonstrated shortened platelet life-times in diabetic patients with platelets labelled with $^{51}$Cr, and these results are in keeping with our own.

There is no evidence of increased fibrinolytic activity being a prominent feature of diabetes mellitus (MacKay & Hume, 1964), and increased utilization of platelets and fibrinogen demonstrated in the present study cannot be explained on the basis of an overactive fibrinolytic system.

Acknowledgments

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References


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