Haemodilution induces a hypercoagulable state

T. G. RUTTMANN, M. F. M. JAMES AND J. F. VILJOEN

Summary
It has been suggested that haemodilution with saline may increase whole blood coagulation. This study was conducted in two parts. First, we investigated the effect of in vitro dilution of blood with saline on whole blood coagulation as measured by the thrombelastogram (TEG). Blood (4 ml) was diluted with 0.9 % saline 1 ml and coagulation compared with that of an undiluted control specimen obtained concurrently from the same subject. In the second part, the study was repeated using a modified gelatin colloidal solution (Haemaccel) as the diluent. The $r$ time, $k$ time and $r+k$ time were decreased relative to control in both diluent groups. The alpha angles were increased compared with control in both groups while maximum amplitude was unchanged in the Haemaccel diluted group. We conclude that haemodilution per se increases the coagulability of whole blood in vitro, but that saline haemodilution has a more marked effect on final clot strength. (Br. J. Anaesth. 1996; 76: 412–414)

Key words
Blood, coagulation. Fluids, i.v. Measurement techniques, thrombelastography.

Haemodilution with i.v. fluids decreases the concentration of clotting factors in blood and, intuitively, it would be anticipated that this should induce some degree of impairment of coagulation. There is, however, some evidence that moderate haemodilution with crystalloid solutions may induce a hypercoagulable state [1]. Thrombelastography (TEG) is a technique first developed by Hartert in 1948 [2]. It had little clinical use for many years until it was re-evaluated in 1974 [3]. Subsequently, it was developed further [4] and applied and validated in liver transplantation [5] and cardiac surgery [6].

TEG is essentially a graphic method of displaying the stages in the formation of a whole blood clot (thrombosis) and as such provides a visual pattern of functional clotting status [7]. It has been used to measure coagulation in clinical anaesthesia [8] and we therefore investigated whether or not haemodilution with crystalloid and colloidal solutions in vitro altered TEG measurements.

Methods
The TEG involves two mechanical parts, a cuvette and a piston. Freshly drawn blood (0.36 ml) is placed in the cuvette which oscillates through 4°45′ at 37 °C. The piston is suspended in the blood sample by a torsion wire which is chart recorded. When no clot exists, the motion of the cuvette does not affect the piston and the chart records a straight line. As strands of fibrin form they attach to the piston and it becomes coupled to the motion of the cuvette, and hence the shearing elasticity of the evolving blood clot is transmitted to the thermal paper.

The TEG pattern is divided into component variables. Reaction time ($r$) is the interval between the start of recording and the time at which the amplitude of the tracing is 2 mm. It reflects the function of the intrinsic clotting pathway. Coagulation time ($r+k$) is the time required for the amplitude to reach 20 mm and provides information on not only intrinsic factors but also platelets and fibrinogen, which are also represented by the clot formation rate ($k_r$). Maximum amplitude (MA) is the greatest amplitude achieved on the thrombelastogram and is a measure of clot strength and elasticity, again reflecting the properties of platelets and fibrinogen in addition to factor XIII.

The experiment was conducted in two parts. In the first part, blood samples were obtained from 20 healthy, conscious volunteers. Blood was obtained from a free-flowing forearm vein in a 10-ml syringe and separated into two aliquots of 4 ml each in polypropylene plastic tubes. Isotonic saline 1 ml was added to one sample while the other was left undiluted and served as a control. Specimen tubes containing each sample were inverted several times to ensure thorough and similar mixing of blood in each tube. The control samples were inverted in a similar way to those of the diluted group to ensure that, as far as possible, each sample was treated in a similar manner. Specimens from each sample tube (0.360 ml) were then added to the thrombelastograph and simultaneous traces recorded of the normal blood sample and the saline diluted sample. The TEG trace was recorded for 1 h and $r$ and $k$ times, alpha angle ($\alpha^\circ$), and MA were measured for each sample.

In the second part of the study, the method described above was repeated, except that the test samples were diluted with 1 ml of a modified gelatin colloidal solution (Haemaccel). Again, one sample...
Coagulation and haemodilution

Table 1  TEG values in control and saline diluted samples (mean (SD))

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Saline</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>r (min)</td>
<td>8.3 (2.2)</td>
<td>6.5 (1.7)</td>
<td>0.007</td>
</tr>
<tr>
<td>k (min)</td>
<td>3.7 (1.1)</td>
<td>2.2 (0.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>r + k (min)</td>
<td>12.0 (3.2)</td>
<td>8.8 (2.4)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>α (°)</td>
<td>38.7 (9.6)</td>
<td>51.0 (10.1)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MA (mm)</td>
<td>50.7 (6.1)</td>
<td>55.0 (6.4)</td>
<td>0.033</td>
</tr>
</tbody>
</table>

Table 2  TEG values in control and Haemaccel diluted samples (mean (SD))

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Haemaccel</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>r (min)</td>
<td>9.1 (1.6)</td>
<td>7.1 (1.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>k (min)</td>
<td>4.3 (1.0)</td>
<td>2.4 (0.8)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>r + k (min)</td>
<td>13.4 (2.6)</td>
<td>9.5 (2.2)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>α (°)</td>
<td>33.15 (6.4)</td>
<td>49.7 (9.3)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MA (mm)</td>
<td>46.5 (6.4)</td>
<td>47.2 (6.9)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

was used for testing the effect of haemodilution while the other served as a control.

Data from each diluent group were compared with those obtained from the matched controls in each limb of the experiment and any differences noted were analysed for statistical significance using a paired Student’s t test.

Results

The results are shown in tables 1 and 2. In the first part of the study there were statistically significant differences between the saline-treated and untreated samples in all variables measured by the thrombelastograph. There was a highly significant decrease in r and k times, a highly significant increase in the α angle and a more moderate, although still significant, increase in MA.

In the second part of the study, there were statistically significant differences between the Haemaccel-treated and untreated samples for all variables measured by the thrombelastograph, except for MA, which was not affected by dilution with Haemaccel. There was a highly significant decrease in r and k times, and a highly significant increase in the α angle.

Discussion

In 1950, Tocantins, Carroll and Holburn [1] suggested that saline dilution of blood may render it hypercoagulable. This was supported by an in vitro study in rats [9]. In 1980, Janvrin, Davies and Greenhalgh [10] demonstrated an increase in the coagulability of whole blood and an increased incidence of postoperative deep vein thrombosis (DVT) in patients treated with i.v. crystalloid during surgery compared with a group who received no i.v. fluid. An in vitro study noted that patients receiving crystalloids demonstrated an increase in coagulability, as measured by the TEG [11]. This was not attributed directly to the crystalloid infusions. Indeed, some authors have used saline dilution as a control when investigating the effects of other substances on coagulation [12].

We performed an in vitro study on human blood using the TEG to compare the dynamic coagulation profile of 20 % haemodilution with either crystalloid or colloid with that of an undiluted control. We have shown that haemodilution with both solutions causes a decrease in r and k times, reflecting increased intrinsic coagulability and platelet and fibrinogen activity. The α angle, reflecting speed of clot formation, was increased significantly in both test groups. Maximum amplitude showed a moderate increase in the saline group, reflecting an increase in clot strength, again indicating increased fibrin and platelet activity, but this effect was not apparent in the colloid diluted group. Mean k time and r + k time for both test groups were shorter than the minimum values quoted by Mallet and Cox [8], although the other measurements were within their quoted ranges.

The changes demonstrated in our study represent an increase of 20–40 % in the initiation of coagulation and the speed of clot formation induced by haemodilution, with a lesser effect on the development of clot strength. This implies that moderate haemodilution with either crystalloid or colloid could cause blood to clot more readily, with minimal effects on the strength of the final clot formed. What the effect of greater or lesser degrees of haemodilution might be has not been examined in this study, but Tocantins, Carroll and Holburn [1] suggested that the effect increases with whole blood dilutions of up to 40 % and that the changes in the speed and character of thrombus formation were as a result of haemodilution rather than the properties of saline itself. By repeating the study using a colloid solution as diluent, we have shown similar decreases in r and k times and an increase in α angles, thereby offering further evidence that the documented changes are brought about by dilution rather than by the nature of the actual diluent itself.

Although the intuitive assumption is that haemodilution would increase bleeding tendencies through dilution of coagulation factors, in reality the reserve capacity of the coagulation system under normal conditions is probably so great that dilution is of no relevance unless large volumes are used. Smaller quantities of infused fluids, however, may well activate the coagulation process in several ways, the mechanisms for which would need to be examined if this in vitro observation were to be confirmed in vivo.

The mechanism by which haemodilution causes this increase in coagulation is far from clear. Tocantins, Carroll and Holburn [1] showed that the nature of the crystalloid diluent was not important, and that all crystalloid solutions produced similar effects; our own unpublished data confirm this impression. They suggested also that haemodilution disturbed the relationship of thrombin and antithrombin resulting in a relative decrease in the concentration of antithrombin, but Monkhouse [9] considered this explanation insufficient to account for his own observations in rats. By performing an in vitro study, we have eliminated extraneous factors such as heparinization, stress response, tissue damage and endothelial injury, all of which could affect coagulation in an in vivo study. All of the changes we have been able to demonstrate have thus been a...
direct consequence of the addition of diluent to normal blood.

Our results confirm that the addition of either saline or Haemaccel to a 20 % dilution (equivalent to 1000 ml of an i.v. fluid in an average 70-kg person) in vitro renders blood hypercoagulable, affecting aspects of the intrinsic coagulation cascade from initial fibrin formation (decreased r time), rapidity of fibrin build-up and cross-linkage (decreased k time), and speed of clot formation (increased α angle). Final absolute clot strength (MA) was increased only with saline haemodilution and was unchanged with Haemaccel haemodilution. As the TEG measures only the function of the intrinsic pathway, great care was taken not to initiate coagulation via the extrinsic pathway.

An in vivo study using the TEG supported by other estimations of coagulation status is necessary to establish coagulation profiles for a range of surgical patients treated with perioperative i.v. fluid solutions.

References