Preparation of lyophilized partial thromboplastin time reagent composed of synthetic phospholipids: usefulness for monitoring heparin therapy

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To contribute to the development of a reference reagent for monitoring heparin therapy, a lyophilized partial thromboplastin time (PTT) reagent was prepared from synthetic dioleoylphosphatidylcholine, dioleoylphosphatidylserine, and dioleoylphosphatidylethanolamine, with colloidal silica as activator. The reagent, coded 91/558, was contained in sealed glass ampoules; it deteriorated in a heat degradation experiment, but its activity remained constant for at least 4 years when stored at −70 °C. Within- and between-run precision with this reagent complied with the requirements proposed by the International Committee for Standardization in Haematology (ICSH) Panel on PTT. The response of this reagent and of two other reagents to heparin added to pooled normal plasma was nonlinear. Citrated samples from 58 patients receiving intravenous heparin and from 24 apparently healthy volunteers were tested with reagent 91/558, with Automated APTT (Organon Teknika), with Manchester APTT reagent, with an anti-factor Xa assay, and with an anti-factor IIa assay. The correlation of APTT with anti-Xa and anti-IIa activity was poor. The best correlation was observed between reagent 91/558 and the Organon Teknika reagent. Correlations were improved when individual patients’ samples were replaced by pooled plasmas from heparinized patients, in whom the effect of oral anticoagulation was minimal. These results suggest that preparation of a lyophilized synthetic phospholipid reagent is feasible for use in monitoring heparin therapy.

INDEXING TERMS: anticoagulants • standardization

The first report on the usefulness of the partial thromboplastin time for monitoring anticoagulant therapy appeared in 1962 [1]. Ever since, the activated partial thromboplastin time (APTT)1 has become the most popular laboratory test for monitoring full-dose heparin therapy. Many different APTT reagents are available and show considerable variation in their responses to heparin. Consequently, a therapeutic ratio of 1.5 to 2.5 times the value for a healthy (“normal”) control may be appropriate for some reagents, but not for all [2–5]. The need for progress in standardization of the APTT monitoring of heparin administration is urgent [6–10]. Standardization of the APTT may be made feasible by establishing a reference reagent; however, no international reference preparation for the APTT is available.

Most APTT reagents are prepared from biological lipid sources, e.g., animal brain or soya bean extracts. The phospholipid class and fatty acid composition of these reagents are highly variable and cannot be controlled in a simple way. In recent years, the possibility of replacing natural phospholipids with synthetic preparations has been investigated [11]. Indeed, the use of a synthetic phospholipid material may be preferable because its chemical composition is well defined. Synthetic phospholipids have also been used to prepare a recombinant tissue factor reagent [12].

Here, we describe the preparation of a lyophilized APTT reagent comprising synthetic phospholipids and colloidal silica. This reagent was characterized with respect to precision of clotting times and response to in vitro and ex vivo heparin. For comparison, the response of two other widely used APTT reagents was assessed— one prepared from rabbit brain phospholipids with silica as

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1 Nonstandard abbreviations: (A)PTT, (activated) partial thromboplastin time; DOPC, dioleoylphosphatidylcholine; DOPS, dioleoylphosphatidylserine; DOPE, dioleoylphosphatidylethanolamine; ICSH, International Committee for Standardization in Haematology; and INR, International Normalized Ratio.
activator (Automated APTT; Organon Teknika), the other from human brain phospholipids with kaolin as activator (“Manchester reagent,” which has been evaluated in international collaborative trials [13]).

This synthetic reagent should be regarded as a first step towards a reference material for standardization of the control of heparin therapy.

Materials and Methods

Materials

Chemicals. 1,2-Dioleoyl-sn-glycero-3-phospho-L-serine sodium (DOPS); 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC); and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) were obtained from the Biochemical Laboratory, State University of Utrecht, The Netherlands. Cholesterol, D-mannitol, and butylated hydroxyanisole were from Sigma Chemical Co., St. Louis, MO. Hepes was from Calbiochem, La Jolla, CA. Silica powder (Aerosil OX50, 40-nm particle diameter) was kindly provided by Degussa Nederland, Amsterdam, The Netherlands. Porcine mucosal heparin (Thromboliquine) was from Organon Teknika, Boxtel, The Netherlands. Light kaolin was obtained from British Drug House (Poole, UK).

Reagents. Automated APTT was kindly provided by Organon Teknika, Turnhout, Belgium. Manchester APTT reagent (cephalin batch 343) was kindly provided by J.M. Thomson (UK Reference Laboratory for Anticoagulant Reagents and Control, Manchester, UK). These reagents were used as recommended by their respective suppliers. The incubation times were 5 and 10 min, respectively.

Synthetic APTT reagent. Synthetic phospholipids (16 μmol of DOPS, 32 μmol of DOPC, and 32 μmol of DOPE) were mixed with 80 μmol of cholesterol in chloroform. The chloroform was evaporated under a stream of nitrogen in a waterbath at 37 °C. The dried lipid mixture was suspended in 50 mL of an aqueous solution of 25 mmol/L Hepes (pH 7.5) containing 18 mg/L butylated hydroxyanisole [14], by mechanical agitation with glass beads. Next, Hepes buffer containing mannitol and silica powder was added. The final concentrations were, per liter: 0.067 mmol of phospholipid, 0.067 mmol of cholesterol, 0.75 g of butylated hydroxyanisole, 50 g of D-mannitol, and 3 g of silica. The mixture was stored at 4 °C and was shipped to the National Institute for Biological Standards and Control at Potters Bar, Herts, UK. At the Institute, the preparation was dispensed in ampoules (mean ± SD mass of suspension per ampoule, 1.0165 ± 0.0007 g) and lyophilized. After secondary desiccation of the material over phosphorus pentoxide, the ampoules were sealed under nitrogen. The mean (SD) dry mass per ampoule was 54.63 (0.19) mg, and the residual moisture was 0.0629% (0.0156%). The batch size was 900 ampoules. The lyophilized material was stored at −70 °C. The reagent was coded 91/558. The contents of each ampoule was reconstituted with 1.0 mL of water and was used between 20 and 90 min after reconstitution.

Samples

Plasma samples. Plasma samples were prepared from blood collected from 24 apparently healthy volunteers and from 58 patients receiving a continuous infusion of sodium heparin (Thromboliquine). The 58 patients were also treated with oral anticoagulants, starting on the first day of heparin infusion. From each subject 4.5 mL of blood was added to 0.5 mL of 0.11 mol/L citrate (in a Vacutainer Tube; Becton Dickinson, Franklin Lakes, NJ) and centrifuged at 2200g for 10 min, and the decanted plasma was further centrifuged at 27 000g for 30 min. The processed plasma was frozen and stored at −70 °C. Before assay, the samples were thawed in a waterbath at 37 °C.

Plasma pools. Plasmas from patients receiving a continuous infusion of sodium heparin, collected as described above, were pooled. Three pools of patients’ plasmas were prepared, each with a different amount of heparin activity. Labeled H1, H2, and H3, the three pools comprised 86, 84, and 84 individual plasmas, respectively. To minimize the effect of oral anticoagulation, only plasmas with International Normalized Ratio (INR) <2.5 were included. INR values of the pooled plasmas were determined with the Thrombotest reagent after heparinase treatment [15].

A fourth pooled plasma was prepared from patients on long-term coumarin treatment who did not receive heparin therapy. The INR of this pooled plasma (labeled C1) was ~5. All pooled plasmas were stored at −70 °C.

Procedures

Coagulation time determinations. Reconstituted reagent 91/558 was used as follows: 0.1 mL of reagent was mixed with 0.1 mL of plasma in a polystyrene tube at 37 °C; the mixture was incubated for 5 min at 37 °C, unless indicated otherwise; and 0.1 mL of prewarmed calcium chloride (0.025 mol/L) was added and mixed, at which time the timer was started. Coagulation times were determined with a coagulometer according to Schnitger & Gross (Amelung, Lemgo, Germany), with a KC10 (also Amelung), with an ACL-300 (Instrumentation Laboratory SpA, Milan, Italy), with an Elecra-900 (Medical Laboratory Automation, Pleasantville, NY), or with a Sysmex CA 5000 (Toa Medical Electronic Co., Kobe, Japan).

Heat degradation study. Ampoules of reagent 91/558 were stored at 4 °C, 37 °C, and 44 °C for a total of 12 weeks. At 1-week intervals, ampoules were tested with two plasma samples: pooled normal plasma, and pooled normal plasma containing heparin, 0.2 IU/mL. Clotting times were determined with the coagulometer according to Schnitger & Gross. APTT ratios were calculated by dividing the APTT of abnormal plasma by the APTT of the normal plasma determined with the same reagent sample.
**Heparin assay.** Heparin activity was measured by factor Xa inhibition with the chromogenic peptide substrate S-2222 (Chromogenix AB, Mölndal, Sweden), and by factor IIa inhibition with use of a chromogenic substrate (Instrumentation Laboratory). The assays were performed with the ACL-300. Thromboliquine was used to construct calibration curves.

**Results**

**Effect of lyophilization.** Before lyophilization of reagent 91/558, we determined a dose–response curve for this reagent, using the coagulometer according to Schnitger & Gross (Fig. 1). The dose–response curve of the lyophilized reagent was shifted to longer coagulation times. Similar results were obtained with the ACL-300 (not shown). Stability of reconstituted reagent at room temperature was tested with pooled normal plasma and with plasma containing heparin (0.4 IU/mL). No significant change of APTT was observed between 20 and 90 min (not shown).

**Effect of incubation time.** Incubation time, i.e., the interval between addition of reagent to the test plasma and recalcification, influenced the coagulation times of pooled normal plasma and pooled patients’ plasmas differently (Fig. 2). The pooled normal plasma showed a monotonic decrease in the coagulation time as the incubation time increased from 1 to 10 min. In contrast, pooled patients’ plasmas H2 and H3 had minimum coagulation times at incubation times of ~3 min. For all other experiments with reagent 91/558, a fixed incubation time of 5 min was used.

**Precision of APTT determinations.** Within-run precision ($CV_p$) for reagent 91/558 was assessed by making 20 APTT determinations with pooled normal plasma and with the same plasma containing heparin, 0.5 IU/mL (Table 1). In a second experiment, between-ampoule variation and precision were assessed as follows. Twenty ampoules of reagent 91/558 were reconstituted and the contents of each ampoule were used for testing pooled normal plasma. The 20 values were used to calculate the between-ampoule-variation ($CV_a$). Then, the contents of 20 ampoules were pooled, and the within-run precision was determined by assaying the same pooled normal plasma 20 times (Table 1).

Between-run variation was calculated from determinations in 20 runs of deep-frozen pooled normal plasma and deep-frozen pooled patients’ plasmas (Table 2).

| Table 1. Within-run precision ($CV_p$) and between-ampoule variation ($CV_a$) of APTT determinations with reagent 91/558 ($n = 20$). |
|---------------------------------|--------|--------|
|                                | APTT/s, mean | $CV_p$ | $CV_a$ |
| **Schnitger & Gross**          |        |        |
| Normal plasma (NP)             | 39.9   | 1.8    | —      |
| NP + heparin, 0.5 IU/mL        | 214    | 2.2    | —      |
| NP (2nd experiment)            | 39.8   | —      | 1.5    |
| NP (2nd experiment)            | 38.8   | 1.0    | —      |
| **ACL-300**                    |        |        |
| NP                              | 35.9   | 0.3    | —      |
| NP + heparin, 0.5 IU/mL        | 246    | 2.1    | —      |
| NP (2nd experiment)            | 35.4   | —      | 1.5    |
| NP (2nd experiment)            | 35.4   | 1.2    | —      |

*A*PTT/s was determined with the coagulometer according to Schnitger & Gross before (○) and after (●) lyophilization of the reagent.
Response to in vitro heparin. The dose–response of reagent 91/558, Manchester reagent, and Automated APTT to heparin in pooled normal plasma is shown in Fig. 3. The dose–response was nonlinear for all three systems. Nonlinearity with Manchester reagent was observed mainly in the concentration range 0–0.1 IU/mL. In the range 0.1–0.75, the response with Manchester reagent was approximately linear.

Response to ex vivo heparin. Plasma samples from patients being treated with intravenous heparin and concomitant oral anticoagulation were used to assess the relations between the three APTT systems. Log-transformed APTT values were plotted (Fig. 4), as was done in previous studies [7–9,16], which yielded a more homogeneous scatter of data points of the original clotting times. The relation between reagent 91/558 and Manchester reagent

![Fig. 3. APTT as a function of heparin added to pooled normal plasma, as determined with three reagent/instrument systems: reagent 91/558-ACL (●); Manchester reagent–Schnitger & Gross (x); and Automated APTT-ACL (△). Determinations were performed on 7 separate days. Between-day SDs are indicated by vertical bars.](image)

![Table 2. Between-run CV of APTT determinations with reagent 91/558 (n = 20).](table)

<table>
<thead>
<tr>
<th>Samples*</th>
<th>Schnitger &amp; Gross</th>
<th>ACL-300</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean APTT/s CV, %</td>
<td>Mean APTT/s CV, %</td>
</tr>
<tr>
<td>Normal plasma</td>
<td>41.2 2.4</td>
<td>37.8 2.4</td>
</tr>
<tr>
<td>Coumarin plasma C1</td>
<td>75 4.9</td>
<td>74 4.0</td>
</tr>
<tr>
<td>Heparin plasma H1</td>
<td>63 2.8</td>
<td>53 3.4</td>
</tr>
<tr>
<td>Heparin plasma H2</td>
<td>107 4.8</td>
<td>92 6.1</td>
</tr>
</tbody>
</table>

* Deep-frozen pooled normal plasma, pooled coumarin plasma (INR ~5, no heparin), and two pooled patient plasmas (heparin treated).

![Fig. 4. Scatterplots of APTT/s, comparing results obtained with (top) reagent 91/558-ACL (x) vs Manchester reagent–Schnitger & Gross (y); (middle) Automated APTT-ACL (x) vs Manchester reagent–Schnitger & Gross (y); and (bottom) reagent 91/558-ACL (x) vs Automated APTT-ACL (y), where x and y represent the natural logarithms of APTT/s. In each panel, the straight (dashed) line represents the orthogonal regression line for the normal (n = 24) plus ex-vivo heparin (n = 58) samples. The equation for this line (n = 82) is: top, y = 1.34 + 0.68x (r = 0.95); middle, y = 1.48 + 0.69x (r = 0.94); and bottom, y = 0.20 + 0.98x (r = 0.98). The regression equation for the ex-vivo heparin samples alone (n = 58) is: top, y = 1.24 + 0.70x (r = 0.83); middle, y = 1.25 + 0.74x (r = 0.79); and bottom, y = 0.02 + 0.94x (r = 0.94). For comparison, the in-vitro heparin points (see Fig. 3) are represented as well (connected by the solid line).](diagram)
results determined with these ex vivo heparin samples differed from that determined with the in vitro samples (Fig. 4, top). A similar difference was observed for the relation between results by Automated APTT (Organon Teknika) and by Manchester reagent (Fig. 4, middle). In contrast, the relation between reagent 91/558 and Automated APTT results was practically the same for ex vivo and in vitro heparin samples (Fig. 4, bottom), and the scatter of ex vivo data points in the latter was less wide than in the first two comparisons.

**Pooled patients’ plasmas.** Pooled patients’ plasmas were prepared from specimens with low INR (1.2–1.3), to minimize the effect of oral anticoagulation on the APTT. The APTTs were determined with 5 different instruments (Table 3). The coagulation times determined with the mechanical instruments (Schnitger & Gross, KC10) were longer than those with the photooptical instruments. Coagulation times determined with reagent 91/558 were plotted against those obtained with Automated APTT (Fig. 5). For each instrument, the relationship was practically linear, with all $r > 0.9993$. However, the relation between reagent 91/558 or Automated APTT and Manchester reagent was not linear (not shown).

**Relation between APTT and ex vivo heparin activities.** Log APTT values of ex vivo heparin samples were plotted against the anti-factor Xa activities in these samples (Fig. 6, left) and showed very wide scatter. Some samples had relatively long APTT with low anti-Xa activities. In some samples the effect of concomitant oral anticoagulation was very high (INR > 5), which in part may account for the long APTT values. The relation between APTT and anti-Xa, based on results for pooled normal plasma and the pooled ex vivo heparin plasmas H1, H2, and H3, is also shown in Fig. 6. The line connecting the four pooled plasma points is at the lower limit of the scatter of individual patients’ points. Wide scatter was also observed when APTT values (log scale) of the same samples were plotted against the anti-IIa activities (Fig. 6, right).

The correlation between anti-Xa and anti-IIa activities of these samples was much better ($r = 0.87$, not shown), in agreement with previous studies [16].

**Heat degradation study.** The clotting times for assays performed with reagent 91/558 that had been stored at 4 °C did not change during 3 months (Fig. 7). When reagent 91/558 was stored at 37 °C and 44 °C, the clotting times were prolonged. The APTT ratio (abnormal APTT:normal APTT) tended to increase with increasing storage temperature.

**Long-term stability.** A program for regular monitoring of reagent 91/558 stored at low temperature (−70 °C) was not provided. After 4 years’ storage at −70 °C, reagent 91/558 was tested with the same lot of pooled normal plasma (Table 4). The coagulation times were practically the same as the initial values.

<table>
<thead>
<tr>
<th>Pooled normal plasma</th>
<th>—</th>
<th>—</th>
<th>1.0</th>
<th>39.4 ± 0.7</th>
<th>36.8 ± 2.2</th>
<th>35.1 ± 1.5</th>
<th>35.6 ± 0.6</th>
<th>37.6 ± 0.9</th>
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</thead>
<tbody>
<tr>
<td>Pooled patient H1</td>
<td>0.16</td>
<td>0.18</td>
<td>1.2</td>
<td>66.3 ± 1.7</td>
<td>63.3 ± 1.9</td>
<td>53.6 ± 0.7</td>
<td>55.8 ± 1.4</td>
<td>61.9 ± 1.9</td>
</tr>
<tr>
<td>Pooled patient H2</td>
<td>0.42</td>
<td>0.32</td>
<td>1.3</td>
<td>112.2 ± 2.2</td>
<td>109.4 ± 2.2</td>
<td>92.7 ± 4.6</td>
<td>99.3 ± 3.8</td>
<td>104.8 ± 5.3</td>
</tr>
<tr>
<td>Pooled patient H3</td>
<td>0.61</td>
<td>0.51</td>
<td>1.3</td>
<td>208 ± 7</td>
<td>205 ± 7</td>
<td>177 ± 8</td>
<td>171 ± 8</td>
<td>180 ± 13</td>
</tr>
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</table>

*Mean ± SD of 6 separate runs.

<table>
<thead>
<tr>
<th>Anti-Xa</th>
<th>Anti-IIa</th>
<th>INR</th>
<th>APTT/s (reagent 91/558)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IU/mL</td>
<td>IU/mL</td>
<td></td>
<td>Schnitger &amp; Gross</td>
</tr>
<tr>
<td>Pooled normal plasma</td>
<td>—</td>
<td>—</td>
<td>1.0</td>
</tr>
<tr>
<td>Pooled patient H1</td>
<td>0.16</td>
<td>0.18</td>
<td>1.2</td>
</tr>
<tr>
<td>Pooled patient H2</td>
<td>0.42</td>
<td>0.32</td>
<td>1.3</td>
</tr>
<tr>
<td>Pooled patient H3</td>
<td>0.61</td>
<td>0.51</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Fig. 5. Relation between two APTT reagents based on analyses of deep-frozen pooled plasmas with five instruments: coagulometer according to Schnitger & Gross (○), KC10 (△), Electra-900 (●), ACL-300 (■), and Sysmex CA 5000 (□).

Four pooled plasmas were used: one normal and three patient (H1, H2, and H3). Each point is the mean of six measurements (linear scale). A regression line was calculated for each instrument.
Discussion

Synthetic phospholipids can be used to prepare a PTT reagent with adequate response to heparin. In the present study we investigated the feasibility of preparing a lyophilized synthetic phospholipid reagent with acceptable characteristics for monitoring heparin therapy. This may be the first step towards development of a reference reagent for standardization of heparin therapy. Precautions were taken to avoid oxidation of the phospholipids during preparation of the reagent. In previous studies we used kaolin as activator but here we replaced this with colloidal silica, which does not sediment as rapidly as kaolin and is compatible with nephelometric instruments such as the ACL-300. The activity of the silica depends on the particle diameter: Activity per unit mass peaks at ~40 nm and then decreases as the surface area decreases.

Lyophilization of the mixture of liposomes and silica induced a slight prolongation of the coagulation times (Fig. 1). The mechanism of the prolongation is not known, but limited coalescence of the colloidal silica particles or liposomes cannot be excluded. The reproducibility of the reagent preparation should be investigated.

Coagulation times were also influenced by the duration of incubation of plasma with reagent (Fig. 2). Interestingly, the coagulation time of normal plasma was shortened by increasing incubation time, but in the presence of heparin.

Table 4. APTT/s of pooled normal plasma determined with reagent 91/558 in 1992 and 1996 after plasma and reagent were stored at −70 °C.

<table>
<thead>
<tr>
<th>Year</th>
<th>Schnitger &amp; Gross Mean</th>
<th>Range</th>
<th>ACL-300 Mean</th>
<th>Range</th>
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<tr>
<td>1992</td>
<td>39.9</td>
<td>39.1–42.2</td>
<td>35.9</td>
<td>35.7–36.1</td>
</tr>
<tr>
<td>1996</td>
<td>39.9</td>
<td>39.0–40.6</td>
<td>36.1</td>
<td>33.7–37.1</td>
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</table>

*In each year, 20 determinations were performed in one run.
ence of heparin a minimum coagulation time was observed after 2–4 min. Consequently, the response to heparin (i.e., APTT ratio) increased with the incubation time. A fixed incubation time of 5 min was chosen because use of longer incubation times would be less economical.

Storage of reagent 91/558 at 37 °C and 44 °C resulted in prolonged APTT values and APTT ratios (Fig. 7), suggesting that the heat stability of the reagent may need improvement. No deterioration was observed when the reagent was stored at 4 °C for 3 months. Shipment of reagent 91/558 during hot weather may thus require cooling bags to avoid deterioration. After 4 years of storage at −70 °C, reagent 91/558 showed no evidence of significant deterioration (Table 4).

Within-run precision (Table 1) was in agreement with the goal of <3% CV proposed by the ICSH Panel on the PTT [19]. The Panel’s proposed goal for between-run precision (<4% CV) was achieved with deep-frozen normal plasma (Table 2). Between-run CVs tended to increase with increasing clotting times, as shown with some pooled patients’ plasmas (Table 2).

Despite proposals that a linear response of the APTT to heparin should be the aim [20], it is hard to achieve this in practice [2, 21]. With the three reagents used in this study, the dose–response curve for heparin between 0 and 0.1 IU/mL is less steep than the curve at higher heparin concentrations (Fig. 3). The response of the APTT to heparin is related to the phospholipid composition and concentration [11]. We used a phospholipid composition and concentration that could be expected to result in a nearly linear response [11]. The nonlinear dose–response curve of reagent 91/558 may be induced partly by the replacement of kaolin by colloidal silica and partly by the lyophilization.

The similar response of reagent 91/558 and Automated APTT to in vitro and ex vivo heparin samples (Fig. 4, bottom) may be related to the use of the same activator, i.e., silica. This may also explain the linearity of the relation between these reagents for analyses of pooled patients’ plasmas (Fig. 5). There was an obvious difference between ex vivo and in vitro heparin when Manchester reagent was correlated with either reagent 91/558 or Automated APTT (Fig. 4, top and middle). In vitro and ex vivo heparin may be adsorbed differently to silica and kaolin, which might account for the different responses of these samples in the APTT systems.

Several studies have shown that the incidence of deep vein thrombosis is reduced substantially in patients treated with heparin, the dose being adjusted by APTT monitoring [22–25]. Each of these studies used a single APTT reagent, so the clinical efficacy of the different APTT reagents cannot be compared. Nevertheless, good correlation between APTT reagents on the basis of determinations of ex vivo heparin samples suggests that these reagents have similar clinical efficacy. The use of correlation coefficients may be misleading when samples from apparently healthy individuals are included with the ex vivo heparin samples. For example, the correlation coefficient for the ex vivo heparin samples shown in Fig. 4 (bottom) was 0.94 but could have been increased to 0.98 by including the data from the 24 samples from healthy volunteers. Apparently the correlation coefficient depends on the relative numbers of samples from each group (normal subjects and patients), but there are no generally accepted guidelines for the numbers required.

The poor correlation between APTT values and anti-Xa or anti-IIa values in individual patient specimens (Fig. 6) is caused by the lack of specificity of the APTT for heparin. In this hospital full-dose heparin treatment of venous thrombosis is combined with oral anticoagulation from the beginning. Oral anticoagulation by vitamin K antagonists induces prolongation of the APTT but does not influence anti-Xa and anti-IIa results.

Interindividual variation of other factors, e.g., factor VIII, may also contribute to the dissociation between the APTT and heparin concentrations measured by anti-Xa and anti-IIa assays. Correlations were improved when individual specimens were replaced by pooled patients’ plasmas having different heparin contents but with minimal INR (Fig. 6). This suggests that the amounts of other factors influencing the APTT were similar in the pooled plasmas, and very likely the effect of interindividual variation of these factors is reduced by pooling.

Only a few clinical studies compare APTT and heparin assay in monitoring heparin treatment of deep venous thrombosis. Levine et al. [26] found no significant difference of recurrent venous thromboembolism and bleeding between patients monitored by APTT and those monitored by anti-Xa determinations. Holm et al. [27] suggested that both heparin assay and APTT may serve to identify patients whose heparin concentration is too low. Holm et al. [28] maintained that an anti-Xa assay seemed best suited for identifying patients at risk of bleeding, but APTT and thrombin time with recalcified plasma were also useful.

Some authors recommended the adoption of a reference APTT reagent for calibrating working APTT reagents [9]. At present, there is no consensus in the International Society on Thrombosis and Haemostasis Scientific and Standardization Committee and its subcommittee on control of anticoagulation concerning adoption of a reference APTT reagent for standardization of heparin monitoring. An advantage of using a reference APTT reagent for calibration of other APTT systems is the relatively high correlation coefficients that can be obtained (Figs. 4 and 5). Certification of clotting times of pooled patients’ plasmas by using certain instruments in relation to heparin concentrations (see Table 3) may be another step towards standardization of monitoring heparin therapy [29]. Such certification may be performed by multicenter studies.

In conclusion, a lyophilized synthetic phospholipid preparation can replace a natural phospholipid APTT reagent.
for monitoring heparin therapy, a finding that has possible applications for standardization.

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