Exchange transfusion activates coagulation and alters the coagulation profile in newborn infants

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Summary
Exchange transfusion (ET) with adult blood is a standard procedure for neonates with severe hyperbilirubinemia. How ET affects newborn coagulation system remains, however, largely unknown. Thus, we prospectively evaluated the effect of ET on thrombin formation and coagulation profile in 18 newborns (22 ETs). Prothrombin fragment F1+2 and thrombin-antithrombin complexes increased considerably during ET while platelets were significantly reduced. Protein C increased less (p<0.001) and factor VIIIc more (p<0.001) than expected based on their levels in the infused blood. Further, in vitro thrombin generation initiated by 5 pH1 tissue factor was analysed. Before the first ET, newborn endogenous thrombin potential (ETP) and thrombin peak remained at ≈60% of adult control plasma levels, but the lag time to thrombin burst in newborn plasma was ≈45% shorter than the lag time in adult plasma. At the end of the first ET, the thrombin burst still started ≈35% earlier in newborn than adult plasma, whereas ETP and thrombin peak were increased to >90% of adult levels. ETP and peak remained elevated at adult levels until the beginning of the second ET. APC-induced reductions in newborn ETP remained unaltered throughout the first ET. The reductions of ETP by APC were less pronounced in newborn than adult plasma (p<0.0001). We conclude that ET is associated with multiple procoagulant changes and increased in vivo thrombin formation. This ET-induced procoagulant challenge may be of clinical significance in sick newborns already prone to bleeding and thrombotic complications.

Keywords
Blood coagulation, exchange transfusion, newborn, protein C, thrombin

Introduction
The coagulation system is not fully developed at the time of birth, and most coagulation factors and anticoagulants circulate at lower levels in newborns than in older children or adults (1, 2). Hemorrhagic or thrombotic complications, however, rarely occur in healthy newborns reflecting the apparent balance in the system.

Newborn infants with severe hyperbilirubinemia are treated by exchange transfusion (ET). ET causes alterations in newborn physiology and may lead to life-threatening complications, the sickest newborns being at the greatest risk (3–5). Coagulation system-related complications of ET include both thromboses and hemorrhages, potentially even disseminated intravascular coagulation (4, 6, 7). On the other hand, ET may be successfully used to treat neonatal sepsis (8–10) in which the combined systemic inflammation and coagulopathy may lead to multi-organ failure and death (11). How ET affects the coagulation system in the newborn remains, however, largely unknown.

During ET infant blood is almost completely exchanged for adult blood containing adult procoagulants and anticoagulants but no platelets. Consequently, thrombocytopenia, altered concentrations of clotting factors, and other relevant perturbations of the coagulation system may occur. Neonatal plasma proteins differ from their adult counterparts (12, 13), and the neonatal endothelium and platelets face the challenge of interacting with an altered plasma coagulation system after ET. Earlier studies on newborn pro- and anticoagulants in relation to ET have reported
elevations in coagulation factor and anticoagulant concentrations after ET (14, 15). These concentrations have corresponded to the levels in the infused blood.

We studied the effects of ET on thrombin formation in vivo, measured individual pro- and anticoagulant factors, and analysed tissue factor-induced thrombin formation and the effects of activated protein C (APC) in vitro. We report ET-induced activation of the newborn coagulation system associated with altered in-vitro regulation of thrombin best summarized as an ET-induced procoagulant challenge.

Materials and methods

Study population

The study was conducted in the Hospital for Children and Adolescents in Helsinki, Finland and was approved by the ethics committee of the hospital. Informed consent was obtained from the parents prior to entry into the study. Eighteen patients requiring a total of 22 ETs were enrolled into the study between November 2003 and April 2005. All ETs were performed due to hyperbilirubinemia with or without anemia (Table 1). Samples were obtained across only the first ET in 16, across both ETs in four, and across only the second ET in two patients.

Five patients were born prematurely, between gestational weeks 23 and 32, and they needed mechanical ventilation at the time of the study. The primary comorbidities in these five patients in addition to hyperbilirubinemia were as follows: 1) Respiratory distress syndrome (RDS), asphyxia; 2) RDS, pulmonary hypertension, bacterial sepsis (five days after ET); 3) RDS, asphyxia, hypoxic-ischemic encephalopathy, intraventricular haemorrhage (IVH) grade II (detected prior to the first ET), systemic coagulopathy; 4) RDS, IVH grade I (detected prior to ET); 5) Asphyxia, systemic coagulopathy. All the ventilated patients received antibiotic therapy (ampicillin and gentamycin). C-reactive protein (CRP) of patient 1 was 21 mg/l on the day of the second ET and decreased thereafter, the other four patients had CRP levels <10 mg/l around ETs (normal value for CRP <10 mg/l). The blood cultures remained negative, and no clinical signs of infection were observed in these five patients until the septic infection in patient 2 five days after ET. In the remaining 13 patients, born at term, hyperbilirubinemia was the only medical problem. One of these 13 patients received ampicillin and gentamycin but, in retrospect, proved to be free of infection based on further clinical observation, CRP values (<10 mg/l), and negative blood cultures.

Data of the first ETs (n=16) and of the second ETs (n=6) were analyzed separately. Furthermore, data of the first ETs were analyzed separately in the group of all patients (n=16) and in a group of patients not requiring mechanical ventilation (n=12). Results of the whole group of 16 patients are presented and, unless otherwise stated, the results were similar in the group of all 16 patients and in the group of 12 patients not requiring mechanical ventilation. Of the six patients undergoing the second ET, three required mechanical ventilation at the time of the study (patients 1, 3, and 5; see above). Because of the small number of second transfusions all six patients were analyzed together. The basic data of the patients and the indications for ETs are summarized in Table 1.

Exchange transfusion and blood sampling

ET was performed with composite blood (200 ml/kg). Blood was drawn with 5 ml or 10 ml syringes from an arterial line while simultaneously infusing the composite blood into a venous line. Composite blood, prepared by mixing stored red cells and fresh frozen plasma and anticoagulated by citrate-phosphate-dextrose, was provided by the Finnish Red Cross Blood Transfusion Service. During ET, a dose of 206 mg calcium glutionate (13.5 mg Ca²⁺) was infused after every 100 ml of exchanged blood. The investigational sampling was performed from the arterial line immediately at the beginning of the ET, at the end of the ET, and 2 hours after completing the ET. Prior to the investigational sampling, 3–5 ml of blood was first drawn from the arterial line and discarded (during ET) or infused back into the patient (at 2-h sample) after the investigational sampling. Samples from the infused composite blood were collected at the beginning of ET from the actual intravenous line used for infusion into the patient. The point of collection was after the water-heating coil, and thus this sampling site was theoretically the best possible site to exclude the activation of coagulation during blood storage as well as the activation during handling and warming prior to the actual procedure. Adult control blood samples (n=9) were collected from the antecubital veins and cord control samples (n=8) from the umbilical vein at uncomplicated deliveries of healthy term newborns. Nine parts of blood were collected on one part of 0.109 M trisodium citrate. Patient samples and composite blood samples were separated by centrifugation (2,000 g for 10 min) without delay and stored at −70°C until assayed. Control plasma (adult and cord) was centrifuged twice at 2,000 g for 10 min. Pooled adult control plasma from healthy adult volunteers was used as an internal control to the measurements performed with the Thrombogram (Thrombinoscope BV, Maastricht, Netherlands) (see below).

Coagulation assays on samples from the study patients

The plasma samples were analyzed for antithrombin, protein C, factor V, factor VII, and factor VIIIc activities. Free protein S antigen, prothrombin fragment F1+2, thrombin-antithrombin

### Table 1: Clinical data of the newborns (n=18) treated by exchange transfusion (ET).

<table>
<thead>
<tr>
<th>Clinical information</th>
<th>Gestational age (weeks)</th>
<th>37.6 (23–39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)</td>
<td>3120 (660–3980)</td>
<td></td>
</tr>
<tr>
<td>Male/Female</td>
<td>9/9</td>
<td></td>
</tr>
<tr>
<td>Cesarean section</td>
<td>8/10</td>
<td></td>
</tr>
<tr>
<td>1 min Apgar score</td>
<td>9 (1–9)</td>
<td></td>
</tr>
<tr>
<td>Age at first ET (days)</td>
<td>2 (0–13)</td>
<td></td>
</tr>
<tr>
<td>Duration-first ET (minutes)</td>
<td>97 (60–153)</td>
<td></td>
</tr>
</tbody>
</table>

### Indications for ET

| Rh-immunization              | 7                       |
| Other immunization           | 6                       |
| Nonimmune hemolysis          | 1                       |
| Hyperbilirubinemia of prematurity | 4                      |
complexes (TAT), and von Willebrand factor (vWF) antigen and activity were also measured. Prothrombin fragment F1+2 and TAT were analyzed using Enzygnost F1+2 micro kit and Enzygnost TAT micro kit, respectively, by Behringwerke AG, Marburg, Germany. Antithrombin and protein C activities were measured with chromogenic assays (Berichrom Antithrombin III and Berichrom Protein C, Dade Behring, Liederbach, Germany) using an automated coagulometer (BCS, Dade Behring, Liederbach, Germany). Coagulation factor V and VII clotting activities were measured with prothrombin time (Thromborel S, Dade Behring, Liederbach, Germany) based clotting assays utilizing commercial factor deficient plasmas (Dade Behring, Liederbach, Germany). FVIIIc activities were assayed by one-stage APTT-based assay and FVIII-deficient plasma (Pathromtin SL, Clotting Factor VIII-Deficient Plasma, Dade Behring). For the measurement of free protein S antigen, a latex-based method (IL Test Free Protein S, Instrumentation Laboratory) was performed. Von Willebrand factor antigen was determined using STA-Liatest vWF–kit (Diagnostica Stago, Asnières, France) with STA Compact analyser. Von Willebrand factor ristocetin cofactor activity was determined using Packs-4 aggregometer (Helena BioSciences Europe, Sunderland, UK) with lyophilised platelets (Helena BioSciences Europe) and Ristocetin A SO4 (America Biochemical and Pharmaceutical Ltd., Marlton, NJ, USA). An in-house normal plasma pool calibrated with the international standard for FVIII and von Willebrand Factor in Plasma (WHO International Institute for Biological Standards and Control, NIBSC; Hertfordshire, UK) was used as a standard.

**Thrombin generation**

Thrombogram (Thrombinoscope BV, Maastricht, Netherlands) was used to measure the formation of thrombin. The experiment was performed according to manufacturers’ instructions and previously published information (16). Briefly, thrombin generation was measured by monitoring the splitting of fluorogenic substrate by thrombin in a tissue factor-triggered plasma sample and by comparing this thrombin activity with the constant known thrombin-like activity of thrombin calibrator (alpha-2-macroglobulin-thrombin complex, Thrombinoscope BV) in a parallel sample. The coagulation was triggered by 5 pM tissue factor and 4 µM phospholipids (phosphatidylserine : phosphatidylcholine : phosphatidylethanolamine 1 : 3 : 1).

Plasma sample (80 µl) with the trigger (20 µl) or the calibrator (20 µl) was pipetted into 96-well plates. Twenty µl of FluCa (containing 2.5 mM fluorogenic substrate, Z-Gly-Gly-Arg-AMC, and 100 mM CaCl2) were then added into the wells, and color formation at 390 and 460 nm was monitored for one hour. The measurements were taken every 20 seconds. During the experiment, Thrombinoscope software program compared the absorbance readings from the triggered wells with the corresponding calibrator wells. In triggered wells, increasing fluorescence not only reflects free thrombin activity but also thrombin-alpha-2-macroglobulin activity (17). The thrombin-alpha-2-macroglobulin activity was mathematically subtracted from the total amidolytic activity by the computerized algorithm yielding a free thrombin generation curve over time. In control experiments, adding up to 2.0 µM alpha-2-macroglobulin to adult plasma did not have any influence on the thrombin generation curves confirming the validity of the algorithm (data not shown).

When applicable, 2.5 nM activated protein C (APC) was mixed with the coagulation trigger just before adding the trigger to the plate. APC (Hyphen BioMed, Neuville-sur-Oise, France) was produced by activation of plasma-derived human protein C by agarose bound thrombin. An aliquot of pooled internal control plasma was used in each Thrombogram assay.

**Statistical analysis**

Mann-Whitney U test and Wilcoxon signed rank test were used for non-paired and paired comparisons, respectively. Kruskall-
Wallis and Dunn's test were used for non-paired multiple comparisons. Spearman R correlation coefficients were calculated. Data are expressed as means and standard errors of mean (SEM) or as box and whiskers plots (median, 25th and 75th percentile, minimum, and maximum). Data were analyzed using GraphPad Prism 3.0 (San Diego, CA, USA).

**Results**

**Activation of coagulation at the end of the first exchange transfusion**

The levels of both F1+2 and TAT increased significantly during ET (Fig. 1). Two hours after completing ET the levels of F1+2 and TAT had decreased, but they remained significantly higher (p<0.05) than at the beginning of ET. When the four patients requiring ventilatory support were excluded from the analysis, the 2-hour levels of TAT did not differ from pre-ET levels. The levels of F1+2 and TAT in the infused plasma were invariably low (F1+2 0.38 ± 0.03 nM; TAT 1.8 ± 0.2 µg/l) and within normal range for adults (F1+2 <1.1 nM; TAT <4.0 µg/l).

**Coagulation factors, anticoagulants and platelets during the first ET**

The coagulation factor and anticoagulant levels at the beginning and end of the first ET and in the infused blood are presented in Table 2. At the beginning of ET the levels corresponded with reference values for the newborns (1) with low levels of all the measured anticoagulants and factor VII, adult-like levels of factors V and VIIIc and increased levels of vWF.

AT, PC, and PS increased significantly (p<0.001) during ET. Of the procoagulants, FVII and FVIIIc increased significantly (p<0.05), whereas FV and vWF decreased (p<0.05). In the group of 12 patients not requiring mechanical ventilation, the increase in FVII was not statistically significant. At the end of ET the levels of PS, AT, FV, FVII, and vWF in newborn plasma were similar to those in the infused blood. In contrast, protein C levels remained lower (p<0.001) and FVIIIc higher (p<0.001) than the levels in the infused blood (Table 2).

All the anticoagulants decreased (p<0.05) during the two-hour period following ET. PC decreased significantly more (p<0.01) than AT or PS (PC −38 ± 3%, AT −7 ± 2%, PS −6 ± 2%; decrease from end-ET level). FV and FVII maintained their post-ET levels, but FVIIIc decreased from the unexpectedly high levels reached at the end of ET (p<0.01).

ET was associated with a marked decrease in the platelet count (Pre-ET 272 ± 23 10^9/l vs. post-ET 45 ± 4 10^9/l; p<0.001). Six out of 16 patients (38%) received platelet transfusion after the first ET.

**Increased thrombin formation potential and short lag time to thrombin burst at the end of the first ET**

At the beginning of ET the endogenous thrombin potential (ETP, area under the thrombin generation curve) of newborn plasma was similar to that of cord plasma but was only ~60% of the ETP in adult plasma (p<0.001) (Fig. 2). The newborn thrombin peak was also ~60% of adult levels (p<0.001) and ~90% of cord plasma levels. Lag time to the initiation of thrombin formation (defined as reaching 10 nM thrombin) and time to thrombin peak were ~40–45% shorter in newborn plasma than in adult plasma at the beginning of ET (p<0.001 for both comparisons).

ETP and thrombin peak increased significantly (p<0.01) in newborn plasma during ET reaching nearly the corresponding adult levels (Fig. 2). Lag time and time to thrombin peak were both prolonged during ET (p<0.001), but remained shorter at the end of ET in newborn plasma than in adult plasma (p<0.001).

**Resistance of newborn plasma to APC and its persis-tence across the first ET**

APC decreased ETP at the beginning of ET by 31 ± 5% and the thrombin peak by 47 ± 5% (Fig. 3). These decreases were similar to APC-induced decreases in cord plasma but significantly less (p<0.001) than APC-induced decreases in adult plasma (decrease in adult ETP −79 ± 4%; decrease in adult peak thrombin −82 ± 3%). The apparent resistance of newborn plasma to the effects of APC remained virtually unaltered across ET; at the end of ET, APC decreased the newborn ETP by 35 ± 4% and the thrombin peak by 47 ± 3% (Fig. 3B).

**Clinical correlates of thrombin generation**

F1+2, TAT, ETP, peak thrombin, and lag time to thrombin formation before and after the first ET and the proportional increase in F1+2, TAT, and ETP (the respective level measured after Table 2: Coagulation factor and anticoagulant levels (U/ml). The results are presented for the whole group (n=16) and for the 12 patients not requiring mechanical ventilation at the beginning and at the end of the first exchange transfusion (ET) and in the transfused blood. The post-ET levels differed significantly (p<0.05) from the levels at the beginning of ET except for factor VII in the group of 12 patients. The post-ET levels were similar with the corresponding levels in transfused blood expect for protein C and factor VIIIc, which both differed significantly (p<0.001) from the corresponding levels measured from the transfused blood.

<table>
<thead>
<tr>
<th>Antithrombin</th>
<th>Protein C</th>
<th>Protein S</th>
<th>Factor V</th>
<th>Factor VII</th>
<th>Factor VIIIc</th>
<th>von Willebrand factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning, n=16</td>
<td>0.58 ± 0.03</td>
<td>0.32 ± 0.02</td>
<td>0.53 ± 0.03</td>
<td>1.10 ± 0.05</td>
<td>0.76 ± 0.05</td>
<td>0.91 ± 0.05</td>
</tr>
<tr>
<td>Beginning, n=12</td>
<td>0.59 ± 0.03</td>
<td>0.32 ± 0.02</td>
<td>0.51 ± 0.01</td>
<td>1.18 ± 0.05</td>
<td>0.81 ± 0.05</td>
<td>0.94 ± 0.05</td>
</tr>
<tr>
<td>End, n=16</td>
<td>0.70 ± 0.02</td>
<td>0.64 ± 0.02</td>
<td>0.68 ± 0.03</td>
<td>0.97 ± 0.06</td>
<td>0.83 ± 0.05</td>
<td>1.13 ± 0.05</td>
</tr>
<tr>
<td>End, n=12</td>
<td>0.70 ± 0.02</td>
<td>0.65 ± 0.01*</td>
<td>0.69 ± 0.03</td>
<td>0.91 ± 0.07</td>
<td>0.88 ± 0.04*</td>
<td>1.16 ± 0.04*</td>
</tr>
<tr>
<td>Transfused, n=16</td>
<td>0.75 ± 0.02</td>
<td>0.79 ± 0.03</td>
<td>0.72 ± 0.03</td>
<td>0.93 ± 0.05</td>
<td>0.91 ± 0.05</td>
<td>0.93 ± 0.05</td>
</tr>
<tr>
<td>Transfused, n=12</td>
<td>0.72 ± 0.02</td>
<td>0.80 ± 0.03</td>
<td>0.71 ± 0.03</td>
<td>0.96 ± 0.05</td>
<td>0.96 ± 0.06</td>
<td>0.85 ± 0.05</td>
</tr>
</tbody>
</table>

All patients, n=16

Patients not requiring mechanical ventilation, n=12
ET divided by the respective level measured before ET) were not correlated with gestational age, birth weight, the amount of transfused blood (ml/kg), duration of ET (in minutes), pre- and post-ET bilirubin level, or with pre- and post-ET hemoglobin.

Two patients in the study group had systemic coagulopathy after severe birth asphyxia (patients 3 and 5; see Methods). Patient 3 showed an extremely high D-dimer (547 mg/l, reference range <0.5 mg/l) on the day of birth. The first ET in this patient was performed at the age of three days when D-dimer had decreased to 7.8 mg/l. Pre-ET F1+2 (1.5 nM) and TAT (8.0 µg/l) and their behavior during the first ET was similar with other patients. In patient 5, D-dimer was 30 mg/l on the day of birth and 2.3 mg/l on the day of ET (at two days of age, second ET). F1+2 in this patient increased 10-fold (from 2.1 to 20.8 nM) during ET, representing the most pronounced F1+2 increase observed in the current study.

Coagulation system alterations during the second ET
Samples were obtained from six repeated ETs. The time interval from the first ET was from 16 to 29 h (mean 22 h). At the beginning of the second ET the levels of F1+2 and TAT did not differ from the corresponding levels measured at the beginning of the first ET or at two hours after completing the first ET. In remarkable similarity with the first ET, a marked increase in F1+2 and TAT levels was observed during the second ET (Fig. 1).

Figure 2: Thrombin generation in newborn infants before and after the first exchange transfusion (ET). Thrombin generation was measured at the beginning and at the end of the first ET (n=15) and compared with adult (n=9) and cord (n=8) controls. Error bars were omitted in panel A for clarity. Endogenous thrombin potential (ETP area under the thrombin generation curve) increased significantly during ET to adult levels whereas lag time to the thrombin burst (defined as reaching 10 nM thrombin) was still significantly shorter in post-ET newborn plasma than in adult plasma.

Figure 3: Thrombin generation in newborn infants in the presence or absence of 2.5 nM activated protein C (APC). The effect of APC on thrombin formation was measured in newborn plasma (n=15) at the beginning (A) and at the end (B) of the first exchange transfusion (ET) and compared with cord (A, C; cord n=8) or with adult controls (B, C; adults n=9). Error bars were omitted in panels A and B for clarity. APC diminished endogenous thrombin potential (ETP) significantly more in adult plasma than in newborn plasma at the beginning and at the end of ET (*p<0.001) (C).
At the beginning of the second ET the levels of procoagulants and anticoagulants did not differ significantly from the corresponding levels at the beginning of the first ET (Fig. 4A). Additionally, all the factors showed comparable levels at the end of the first and at the end of the second ET. PC increased less (p<0.05) and FVIIIc more (p<0.01) than expected on the basis of transfused blood also during the second ET. Despite similar individual factor levels at the beginning of the first and the second ET, ETP remained significantly elevated at adult levels from the end of the first ET until the beginning of the second ET (Fig. 4B). ETP further increased during the second ET (p<0.05).

The APC-induced decrease in ETP was significantly diminished at the beginning (-41 ±20%) and at the end (-47 ±12%) of the second ET when compared with the APC-induced decrease in ETP in adults (-79 ±4%, p<0.01 for both comparisons).

Discussion

ET induced considerable in-vivo activation of coagulation in the newborn, which was also observed after repeated ETs. The phenomenon was consistent since F1+2 and TAT increased across all of the 22 ETs. Though infusion of adult blood evidently increases the levels of most pro- and anticoagulants, there was no expectation of enhanced in-vivo thrombin formation. The infused blood did not show increased levels of coagulation activation markers. Low platelet counts induced by ET would also favor inhibition rather than elevation of thrombin formation. Finally, infusion of fresh frozen plasma to sick newborn infants previously helped to reduce in-vivo activation of coagulation (18). Thus, the ET-induced in-vivo thrombin escalation was a novel and surprising finding motivating the detailed analysis of the ET-induced changes in coagulation profile and in in-vitro thrombin regulation.

Behavior of the most measured coagulation factors during ET was passive with apparently complete recovery of infused factors upon completion of ET: FV, FVII, AT, PS, and vWF showed similar levels in infused blood and in post-ET newborn plasma. In contrast, changes in PC and FVIIIc were more complicated. The approximately 80% recovery of transfused PC in post-ET plasma may suggest enhanced consumption of PC in the activated coagulation system. A similar behavior of PC was previously observed in association with small-volume plasma transfusion in sick newborns (18). The increase in FVIIIc over the levels of both pre-ET plasma and transfused blood was a consistent finding as it occurred in 18 of the 22 ETs. vWF release from endothelium as an explanation was excluded. Thus, feedback activation of FVIIIc by augmented thrombin or reactivity of FVIII during an acute phase response (19, 20) might play a role.

In post-ET plasma, the parameters of thrombin generation, i.e. ETP, thrombin peak, lag time, and the effects of APC, showed characteristics that were distinct from thrombin regulation in both intact newborn and adult plasma. ETP and the peak thrombin formation in newborn plasma increased from the pre-ET levels comparable with cord plasma to the post-ET levels comparable with adults. This shift was an expected one as total thrombin generation capacity in the newborn and adult plasma is mainly dependent on the concentration of prothrombin, which obligatorily rises when adult plasma is mixed with neonatal plasma (21, 22). In contrast to the shift in newborn ETP to adult levels during ET, lag time to thrombin burst in newborns remained shorter when compared with adults not only in pre-ET but also in post-ET plasma. The short lag time to thrombin burst in newborns has been attributed to low combined anticoagulant activities of tissue factor pathway inhibitor (TFPI), AT, and APC in newborn plasma (23, 24). Persisting short lag time would be consistent with proportionally lower synergistic anticoagulant capacity at the end of ET. Thus, the observed pattern of thrombin generation in post-ET plasma suggests a significant procoagulant shift upon ET; an enhanced potential for thrombin generation in association with preserved neonatal low anticoagulant defense. Why ETP was still increased to adult levels at the beginning of the second ET despite newborn-like levels of the various coagulation factors and anticoagulants cannot be directly explained by the current results. The half-life of prothrombin is longer than that of other main procoagulants (25) and thus it may persist longer in newborn circulation thereby contributing to the increased ETP.

To further explore the anticoagulant properties of post-ET plasma, the effects of exogenous APC were measured. In new-
born plasma, APC diminished thrombin formation potential significantly less than in adult plasma, which is consistent with previous reports (24, 26). Interestingly, however, the resistance to the effects of APC persisted after ET, and even after the second ET. Previously, resistance to APC in newborn plasma disappeared when TFPI and AT were raised to adult levels to promote synergistic anticoagulant activity of TFPI, AT and APC (24). TFPI was not measured in the current study. Since the levels of AT did rise with no concomitant increase in the effects of APC, the phenomenon of the inherent resistance of newborn plasma to APC may possibly be more complicated than a direct function of TFPI and AT. Overall, however, the post-ET resistance of newborn plasma to APC may reflect reduced synergistic anticoagulant activity, which would be in agreement with the other current findings of prevailing procoagulant state and reduced anticoagulant capacity in post-ET newborn plasma.

In conclusion, exchange transfusion with adult blood causes significant in-vivo activation of coagulation and alters the coagulation profile in newborn infants. In the pediatric population, sick newborns are at the greatest risk of hemorrhagic or thrombotic complications (27). Based on the numerous procoagulant changes induced by ET, thrombotic complications would seem a more likely risk. In the current study of mainly generally healthy, though severely hyperbilirubinemic, newborns complications were not observed. However, in sick newborns receiving intensive care, the potential risks of vascular complications increase and the marked ET-induced changes in coagulation homeostasis may become clinically significant.

Acknowledgement

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References