

Fibrinogen Has a Rapid Turnover in the Healthy Newborn Lamb

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ABSTRACT. The half-lives for coagulation factors in the healthy newborn infant are not known and may be different than for the adult. We measured the half-life for fetal sheep fibrinogen and compared it to the half-life of adult sheep fibrinogen. Fibrinogen was purified from adult and fetal sheep plasma and radiolabeled with either ^{125}I or ^{131}I . The half-lives for these fibrinogens were determined in the adult sheep and newborn lamb. In addition, the fetal and adult sheep fibrinogens were compared by reptilase time, thrombin clotting time, sialic acid content, and the behavior of the N-glycans derived from these fibrinogens on the immobilized lectin, Sepharose-concanavalin A. Finally, the *in vivo* response of coinjected radiolabeled fibrinogens to increasing doses of infused thrombin was determined. The fetal sheep fibrinogen differed from the adult as indicated by a prolonged reptilase time and an increased sialic acid content (fetal: 10–11 residues/340 Kd versus adult: 8–9 residues/340 Kd). The latter was also reflected in differing chromatographic profiles for the N-glycans on Sepharose-concanavalin A. The half-lives for both the adult and fetal fibrinogen were significantly more rapid in the newborn lamb (fetal: 47 ± 2.0 h; adult: 46 ± 2.4 h, mean \pm SEM) than in the adult (fetal: 116 ± 6.5 h; adult: 121 ± 6.9 h). Finally, the adult and fetal sheep fibrinogen responded to thrombin in an identical fashion in the intact animal. In summary, both adult and fetal fibrinogen have faster half-lives in the lamb compared to the adult, despite a higher sialic acid content for the fetal fibrinogen. We speculate that the more rapid turnover of coagulation proteins in the newborn may be of significance when planning replacement therapy for congenital or acquired coagulopathies. (*Pediatr Res* 23: 249–252, 1988)

the response of the “fetal” fibrinogen to thrombin *in vivo*. Many of these questions can only be asked ethically in an animal model of newborn coagulation. We have used the sheep model to investigate the clearance and response to thrombin of “fetal” and adult fibrinogen. Our results show that fibrinogen, whether adult or fetal, has a faster turnover in the newborn compared to the adult and that both adult and fetal fibrinogen have a similar response *in vivo* to thrombin.

MATERIALS AND METHODS

Animal model. Plasma for purification of fibrinogen was obtained from adult sheep and from fetal lambs at approximately 100 days gestation with term being 147 days gestation. To obtain the fetal plasma, the pregnant ewe was given an epidural anesthetic supplemented with sedative doses of sodium pentobarbital by continuous intravenous infusion. The fetal lamb was partially delivered in a breech position through a uterine incision. Silastic catheters were placed in the fetal lamb's femoral artery and the fetal blood obtained.

Fibrinogen purification. Blood for fibrinogen purification was collected into polypropylene tubes containing 3.8% sodium citrate and EACA. A fraction of the blood obtained was centrifuged at $3000 \times g$ for 20 min and multiple aliquots of the plasma sample frozen at -70°C for future coagulation assay. The remaining plasma was used for fibrinogen purification according to the method of Straughn and Wagner (7). In brief, the fibrinogen was prepared by absorbing plasma with aluminum gel followed by three precipitation steps with β -alanine. The final precipitate was resuspended in a citrate saline solution and frozen. Purified fetal fibrinogen was labeled with ^{125}I and adult fibrinogen with ^{131}I using the iodine monochloride technique (8). The final fibrinogen solution had greater than 95% clottable protein content, showed the expected three bands on reduced sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and after iodination more than 95% of the radioactivity was clottable with thrombin. In addition, human albumin (Connaught Laboratories, Toronto, Canada) was labeled with ^{125}I for kinetic studies, either in separate animals or in animals receiving ^{131}I -labeled fibrinogen.

Pharmacokinetic studies. The half-lives of the labeled fetal and adult fibrinogens and albumin were determined in the healthy newborn lamb during the 1st wk of life and in the healthy adult sheep. The adult sheep received 100 μCi of ^{125}I -labeled fetal fibrinogen, 100 μCi of ^{131}I -labeled adult fibrinogen, and/or 100 μCi of ^{125}I -labeled albumin, whereas the newborn lambs received 20 μCi of ^{125}I -labeled fetal fibrinogen, 20 μCi ^{131}I -labeled adult fibrinogen, or 20 μCi of ^{125}I -albumin. Baseline and subsequent blood samples (2 ml) were drawn in heparinized syringes for measurement of fibrinogen (9), hematocrit, total protein (refractometer) and ^{125}I -fibrinogen, ^{131}I -fibrinogen, and/or ^{125}I -albumin. In each case the whole blood samples were spun at 3500 rpm for 15 min and 1-ml plasma samples frozen for measurement of the radioactivity in a well-type γ -scintillation counter that allowed

There is a growing appreciation that the neonatal coagulation system has unique features compared to the adult. For example, the levels of the coagulation factors vary with gestational and postnatal age, and the ability of the newborn coagulation system to generate thrombin and inhibit thrombin is impaired compared to the adult (1, 2). However, there is little information available on the comparison of the structure and pharmacokinetics of components of the coagulation system in the newborn with the adult. The most extensive information exists for fibrinogen which is thought to exist in a fetal form with an increased sialic acid content in the newborn infant (3–6). There is no information on the pharmacokinetics of fibrinogen in the healthy infant or on

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for correction if both ^{125}I and ^{131}I were used. Less than 1% of total counts were not precipitated with absolute ethanol, which indicates that more than 99% of the label was protein bound.

Thrombin infusion experiments. In these experiments both ^{125}I -fetal fibrinogen and ^{131}I -adult fibrinogen were injected into healthy adult sheep ($n = 3$) at least 24 h before study. On the day of study, two catheters were placed in the jugular veins. Exogenous bovine thrombin was infused into the right jugular vein at increasing doses (0.1–1.2 U/kg/min). Blood samples were obtained as described above from the left jugular vein over the subsequent 2-h time period.

Evidence for a "fetal" fibrinogen. To confirm the presence of a "fetal" fibrinogen in the fetal lamb, both a 2-U thrombin clotting time, performed with and without calcium, and a reptilase time were performed in both fetal lamb plasma samples and adult sheep plasma samples. Sialic acid analyses of the purified fetal and adult fibrinogens were performed in duplicate using mild acid hydrolysis followed by the periodate oxidation-thiobarbituric acid method of Aminoff (10) using N-acetyl neuraminic acid (Sigma Chemical Co., St. Louis, MO) as a standard. N-glycans were produced from fibrinogen by hydrazinolysis as described by Fukuda *et al.* (11) except that the glycans were desalted after cleavage and rotary evaporation on Biogel P-2 instead of Sephadex G-25. Chromatography of the isolated N-glycans on Sepharose-concanavalin A was carried out as previously described (12). Sialic acid analysis of the separated, concentrated fractions was performed. The final calculations of sialic acid content per molecule assumed a mol. wt. of 340 Kd and an $E^{1\%}$ value of 15.5 (13).

Statistical analysis. Results are reported as means \pm SEM. The half-life of the fibrinogen was calculated using linear regression in the β -portion of the curve. Comparisons between the adult and newborn plasmas were performed with Student's unpaired t test and a $p < 0.05$ was considered significant. For multiple comparisons the Bonferroni correction was used and $p < 0.01$ was considered significant.

RESULTS

Evidence for a fetal fibrinogen. Because the newborn lamb has a lower fibrinogen concentration than the adult sheep, the adult plasma was diluted in saline to a similar fibrinogen concentration as in the lamb. The fetal plasma showed a significantly prolonged reptilase time compared to the adult sheep plasma and a similar trend for the thrombin clotting time in the absence but not in the presence of Ca^{++} (Table 1). Analysis of the sialic acid content showed that fetal fibrinogen samples contained considerably more sialic acid (10.4, 10.5 residues/molecule) than adult fibrinogen (8.8, 8.8 residues/molecule) (Table 2). Furthermore, the isolated N-glycan fraction from fetal fibrinogen largely passed through Sepharose-concanavalin A (60%) whereas less than 10% of glycans from adult fibrinogen passed through the lectin column. This confirmed the increased sialic acid content of the fetal fibrinogen and indicated that the majority of glycans on adult fibrinogen was biantennary whereas most fetal glycans were more branched (12).

Turnover studies. The pharmacokinetic curves for both fetal and adult fibrinogen were markedly different in the newborn lamb compared to the adult sheep (Figs. 1 and 2). The half-lives for both fetal and adult fibrinogen were significantly shorter in the newborn lamb compared to the adult sheep (40% of the adult value) (Table 3). The half-life of ^{125}I -albumin in the lamb was 67% of the value in the adult sheep (Table 3).

Response to thrombin infusion. Figure 3 shows the response of the fetal and adult fibrinogen to an infusion of thrombin (the same thrombin used for the thrombin clotting times). At a dose of 1 U/kg/min of thrombin, both the adult and fetal fibrinogen showed an increased consumption that was comparable for both fibrinogens.

Table 1. Coagulation tests (mean \pm SEM)

Test	Fetus	Diluted adult sheep
<i>n</i>	5	5
Fibrinogen (g/liter)	1.3 \pm 0.15	1.3
TCT* with Ca^{++}	20.4 \pm 0.53†	23.3 \pm 0.21
TCT without Ca^{++}	54 \pm 7.5	44 \pm 0.8
Reptilase (s)	29 \pm 1.8†	19 \pm 0.7

* Thrombin clotting time.

† A significant difference between the fetus and adult sheep of <0.01 .

Table 2. Sialic acid content of fetal and adult sheep fibrinogen

	Fetal fibrinogen	Adult fibrinogen
Sialic acid content (residue/molecule)	10.4, 10.5	8.7, 8.8
% Distribution of N-glycans on Sepharose-concanavalin A*		
Unbound fraction	61%	8%
Bound fraction	39%	92%

* Determined from the sialic acid content of the N-glycans samples after chromatography on Sepharose-concanavalin A.

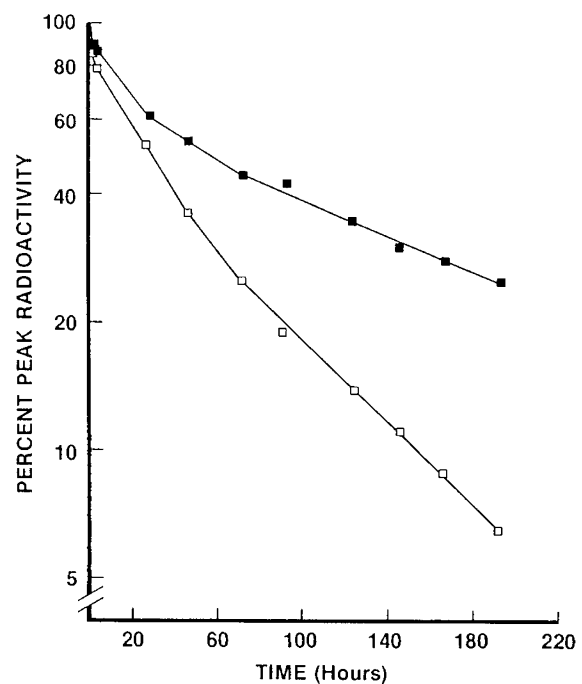


Fig. 1. The plasma survival curves of radiolabeled fetal lamb fibrinogen in the adult sheep (\blacksquare , $n = 5$) and newborn lamb (\square , $n = 5$). The fetal fibrinogen had a consistently faster turnover in the lamb compared to the sheep. The values are all expressed as the mean with all SEM less than 5%.

DISCUSSION

The hemostatic system in the newborn infant has unique features when compared to the hemostatic system in the adult. One aspect of interest is the evidence for "fetal" forms of coagulation factors (3–5, 14–16) and their influence on the turnover of the coagulation protein in physiologic and pathophysiologic states. Because these studies would be generally considered unethical in the healthy human infant, they necessitate the use of an animal model. The fetal sheep was chosen for our study because the fetal sheep has been described as one model for the newborn coagulation system (17) and the lamb fibrinogen may

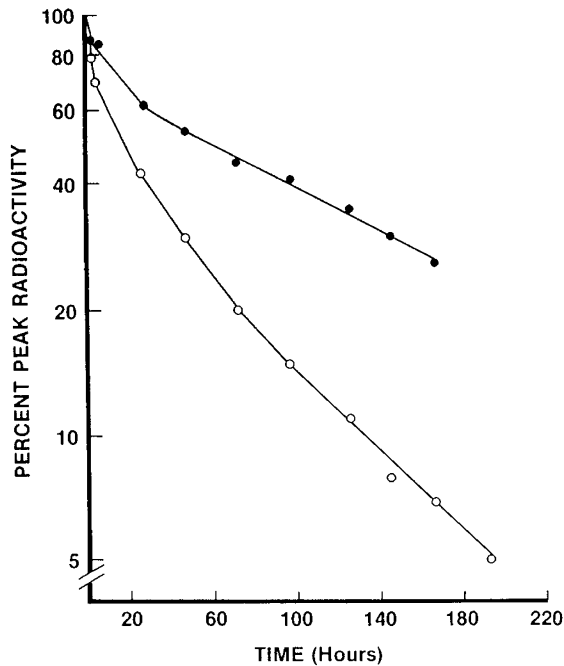


Fig. 2. The plasma survival curves of radiolabeled adult sheep fibrinogen in the adult sheep (●, *n* = 7) and newborn lamb (○, *n* = 10). The adult fibrinogen had a consistently faster turnover in the lamb compared to the sheep. The values are expressed as the mean with all SEM less than 5%.

Table 3. Apparent plasma half-lives of albumin and fibrinogen measured in newborn lamb and adult sheep*

	Newborn (h)	Adult (h)
¹²⁵ I-albumin	144 ± 23.4 (4)†	214 ± 17.8 (5)
¹²⁵ I-fetal sheep fibrinogen	47 ± 2.0 (5)†	116 ± 6.5 (5)
¹³¹ I-adult sheep fibrinogen	46 ± 2.4 (10)†	121 ± 6.9 (7)

* Mean ± SEM (*n*) where *n* is the sample number.

† Statistical difference between the newborn and adult of *p* < 0.01.

exist in a “fetal” form (18). Herein we show that sheep fibrinogen, whether fetal or adult, has a more rapid clearance in the healthy newborn lamb compared to the adult sheep. In addition, both the fetal and adult sheep fibrinogen respond in a similar fashion *in vivo* to a challenge with thrombin even though carbohydrate analyses revealed significant differences between the fetal and adult fibrinogen in sialic acid content and in the degree of branching of the N-glycans. Thus, the increased sialic acid content likely contributed to the *in vitro* demonstration of a dysfibrinogenemia in the lamb but did not contribute to the more rapid clearance of fibrinogen in the lamb.

We chose to study the clearance of fibrinogen because of its importance in blood clotting and because of the evidence for a “fetal” form of fibrinogen in the human infant. An increased sialic acid content is one of the main differences between human newborn and adult fibrinogen and is responsible for the delayed polymerization observed for the human fetal fibrinogen *ex vivo* (3–5). We were able to demonstrate similar abnormalities in fetal sheep fibrinogen compared to the human infant. Conceivably the presence of an increased sialic acid content and an altered N-glycans branching profile in the fetal sheep fibrinogen molecule could result in a different response to thrombin *in vivo* as well as *in vitro*. We tested this hypothesis by directly infusing thrombin into the adult sheep containing both ¹²⁵I-fetal and ¹³¹I-adult fibrinogens and demonstrated their similar response to thrombin. This observation indicates that the different carbohydrates carried by these fibrinogens are unlikely to influence the role of fibrinogen during intravascular coagulation. This conclu-

sion agrees with the reports of Nishibe and Takahashi (19) and Gilman *et al.* (20) who found that neither deglycosylation nor the inhibition of glycosylation during biosynthesis changed the coagulant properties of fibrinogen. The physiologic reason for the increased sialic acid content of fetal sheep fibrinogen is not obvious. Other fetal proteins such as human fetal fibronectin (21) and human placental fibronectins (22) also contain a higher carbohydrate content than in the adult plasma. For fibronectin, as the carbohydrate content increases, the binding between fibronectin and collagen decreases. Whether the high sialic acid of fetal sheep fibrinogen alters functional properties *in vivo* is unknown.

Information on pharmacokinetics of coagulation factors in the healthy human full-term or premature infant is sparse and largely confined to the sick infant. Premature human infants with varying degrees of the respiratory distress syndrome show shortened ¹²⁵I-fibrinogen survivals (half-life of 2.8 to 2.1 days) compared to healthy adults (4.5 days) (23, 24). One cannot be sure if these shortened fibrinogen survivals reflect a physiologic phenomena or a disease process. Indeed, antithrombin III survivals are reported to be shorter in reasonably healthy infants requiring an exchange transfusion for hyperbilirubinemia compared to the adult (25). In addition, small animals show a faster turnover of proteins in general compared to large animals, likely due to an increased basal metabolic rate. The shorter half-life for albumin in the newborn lamb compared to the adult sheep is compatible with this observation. The shorter half-life for at least one coagulation factor, fibrinogen, in a healthy newborn animal compared to the adult is likely in large part to be due to an increased metabolic rate. The similar rates of clearance observed for either fibrinogen in the lamb or the adult sheep (Figs. 2 and 3) indicate that, probably, differences in sialic acid content and the branched nature of the N-glycans do not contribute toward the catabolism of fibrinogen. A similar conclusion was made by Barsigian *et al.* (26) who calculated similar half-lives for rabbit native fibrinogen and aglycofibrinogen in rabbits. Also, the early work of Wong *et al.* (27) demonstrated that plasma clearance of transferrin in rabbits was independent of any preceding desialylation *in vivo*. If other coagulation factors have a faster clearance in the newborn compared to the adult this information may have clinical relevance in the treatment of inherited disorders in the infant.

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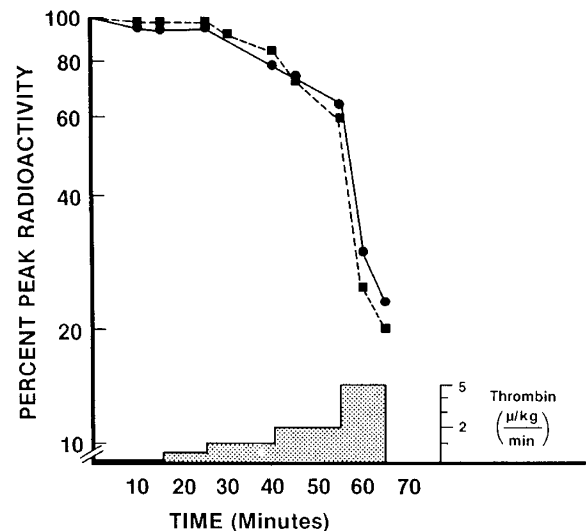


Fig. 3. The response of ¹³¹I-labeled adult fibrinogen (■) and ¹²⁵I-labeled fetal fibrinogen (●) to an infusion of increasing doses of thrombin in the adult sheep. Both fibrinogens responded to the infused thrombin in a similar fashion.

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