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Dansylcadaverine
fetus

fibrin-stabilizing factor (*factor XIII*)
neonate

Fibrin-stabilizing Factor (*Factor XIII*) in the Fetus and the Newborn Infant

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Extract

Using a fluorescent method based on the ability of the thrombin and calcium-activated fibrin-stabilizing factor (factor XIII) to incorporate dansyl cadaverine into casein, measurements were made in plasma samples from 52 healthy neonates and 25 fetuses between the 17th and 24th gestational weeks. Forty healthy adults and 63 samples from pregnant women were used as controls. The measured values ranged from 3 to 21 units/ml of plasma for neonates and 1 to 14 for fetuses, compared with 7 to 42 for the adult normal and 3 to 15 for the pregnant women populations.

Speculation

It is conceivable that the relatively low fibrin-stabilizing factor activity during fetal life and immediately after birth, corresponding, respectively, to mean values of about 0.25 and 0.5 of that found in normal adults, may afford some safeguard against lasting coagulation damage.

In recent years much interest has been focused on fibrin-stabilizing factor (FSF or *factor XIII*), the plasma zymogen of the transamidating enzyme responsible for the covalent cross-linking of fibrin molecules during clotting. Its chemical properties (for review see Reference 8) and variations of the plasma levels of the factor in different pathologic conditions have been the subject of several studies (*e.g.*, 11, 13, 18, 19, 22). For newborn infants, some authors reported low values (1, 3, 7, 23), whereas others have found normal adult levels (6, 14, 17, 21, 24). However, all of these investigations were carried out with bioassays, such as differential clot solubilities, which from the quantitative point of view are of questionable reliability.

A method based on the ability of the thrombin and calcium ion-activated *factor XIII* to incorporate a fluorescent amine (dansyl cadaverine) into casein has already been employed successfully (9, 10) for the quantitative evaluation of the mode of inheritance of the *factor XIII* genetic deficiency disease. In the present study, determinations were made with the use of this method on 52 healthy and 36 sick neonates and also in 25 fetuses obtained by legal abortion. Forty healthy adults and 63 samples from pregnant women were used as controls.

MATERIALS AND METHODS

CLINICAL MATERIAL

Fifty-two (45 from Malmö, 7 from Evanston) apparently healthy full term neonates were studied from normal pregnancies and vaginal delivery after 38–42 weeks of gestation. The Malmö material also included 25 fetuses obtained by induced abortion; mothers were healthy and their pregnancies were terminated on sociomedical grounds. The fetuses were removed by abdominal hysterotomy between the 17th and 24th gestational weeks. Crown-heel lengths measured between 17 and 30 cm. Informed consent was obtained in accord with the Helsinki Declaration. The 36 sick newborns from Malmö comprised 10 with hyaline membrane disease (respiratory distress syndrome) and 26 with other neonatal disorders (mixed group).

COLLECTION OF BLOOD

Blood was drawn into plastic syringe by puncture of a cubital or a scalp vein. The technique described by Ekelund *et al.* (4) was used in the fetuses. Plasma was prepared as described previously (12) and was stored in plastic tubes at -70°. In the neonates from Evanston cord blood was obtained from the freely flowing placental end of the cord before delivery of placenta. In the sick newborn infants blood specimens were obtained 2–24 hr after delivery from an indwelling plastic catheter inserted in one of the umbilical arteries for therapeutic and diagnostic reasons.

DETERMINATION OF FIBRIN-STABILIZING FACTOR

A slight modification of the method described by Lorand *et al.* (10) was used; otherwise, unless indicated, volumes and conditions were those given in Table II of Reference 10. After "desensitizing" the fibrinogen in plasma by heating at 56° for 4 min in the presence of glycerol (50%) followed by rapid cooling, thrombin (topostasin, 25 NIH units in the system (25)) and calcium chloride (3 mM) were added in order to allow activation of FSF. Dithiothreitol (0.2 M) was used as a reducing agent. The incorporation reaction was started by admixing of monodansyl cadaverine (26) (2 mM in Tris-chloride, pH 7.5) and 3% casein. The reaction was terminated by addition of 10% trichloroacetic acid. The protein precipitate was then washed repeatedly in ethanol-ether (1/1). After drying at room temperature, the precipitate was solubilized by digestion with 0.5 ml trypsin (Trypure (27), 25 Anson units/g or 0.05 mg, in 0.1% ammonium bicarbonate buffer, pH 7.9) for 12 hr. Finally a mixture containing Tris-HCl buffer (0.05 M, pH 8.0), urea (8 M), and sodium dodecyl sulfate (0.5%) was added. The fluorescence intensity of the solution was read against a 4 mM monodansyl cadaverine standard and data are given in terms of this latter by expressing rate of amine incorporation as FSF units per milliliter of citrated plasma according to the formula given by Lorand *et al.* (10). A minor volume correction had to be made to account for dilution by the addition of trypsin.

RESULTS

NORMAL ADULTS

In 22 apparently healthy nonpregnant women aged 18–40 years, the level of fibrin-stabilizing factor was found to vary between 11 and 35 units/ml (mean 21 FSF units/ml plasma, SD ± 6.6) and in 18 healthy men aged 25–45 years, between 16 and 32 units (mean 22 FSF units/ml plasma, SD ± 4.3). No significant sex difference was found ($P > 0.05$). The mean of the whole adult material was 21 FSF units/ml plasma (SD ± 5.6), in agreement with earlier reports with this method (9). Values for pregnant women appear to be lower (Fig. 1).

FULL TERM NEWBORNS AND FETUSES

Values for fibrin-stabilizing factor in neonates from Malmö ranged from 6 to 21 units/ml plasma (mean 11 FSF units/ml plasma, SD ± 3.4). In the 25 fetuses, the results fell between 1 and 14 units/ml plasma (mean 5 FSF units/ml plasma, SD ± 3.5). The difference between the fetal and the newborn levels was statistically significant ($P < 0.001$), as was also the difference between the neonatal and the adult material ($P < 0.001$). In the group of seven neonates from Evanston the FSF levels ranged only from 3 to 6 units/ml plasma (mean 5 FSF units/ml plasma).

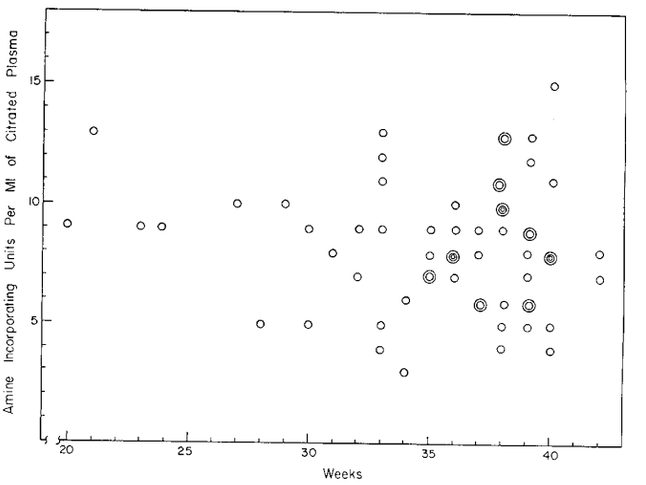

Fig. 1. Fibrin-stabilizing factor (*factor XIII*) values in plasma (*ordinate*) during various stages (*abscissa*) of pregnancy.

Table 1. *Comparative data for fibrin stabilizing factor (factor XIII) using method described by Lorand et al. (10)*

Subjects	Source	No. tested	Units/ml plasma Range	Mean	±SD
Full term neonates	Malmö	45	6–21	11	3.4
Sick neonates	Evanston	7	3–6	5	
RDS[1]		10	7–12	8.5	1.9
Mixed group		26	3–24	10.6	4.7
Fetus[2]	Malmö	25	1–14	5	3.5
Adult controls	Malmö	40	11–35	21	5.6
	Literature[3]	72	7–42	19	5.5
Heterozygotes of hereditary deficiency	Literature[3]	13	5–18	12	3.3
Homozygote deficients	Literature[3]	7		0.2	0.1

[1] Respiratory distress syndrome.

[2] No correlation with gestational age (17–24 weeks) could be detected.

[3] Literature values from Lorand *et al.* (9).

SICK NEWBORNS

Values for fibrin-stabilizing factor were 7–12 units/ml plasma (mean 8.5; SD ± 1.9) for the 10 neonates with respiratory distress syndrome (gestational age of 29–40 weeks), and 3–24 units/ml plasma (mean 10.6; SD ± 4.7) for the 26 infants (gestational age of 26–40 weeks) with other neonatal disorders. There was no significant difference ($P > 0.05$) between these two groups.

Our results to date are summarized in Table 1. For purposes of comparison included in the table are literature values obtained by the same method for 72 control individuals, for 7 homozygote hemorrhagic patients afflicted with the hereditary deficiency of total absence of functional fibrin stabilizing factor; and for 13 individuals, parents of these patients, classified as heterozygotes for this deficiency trait (9). No one in the latter group showed a noticeable hemorrhagic tendency. It is interesting to note that mean values for fibrin-stabilizing factor in the plasma of full term neonates are similar to those found in the adult heterozygote group. A number of newborn and fetal samples yielded results approximating the homozygotic deficiency state.

DISCUSSION

Zuch *et al.* (24) sought to explain apparent low values of fibrin-stabilizing factor in terms of a lower concentration of sulfhydryl groups in plasma, due perhaps to a not fully developed liver function in neonates. The assays used in our studies did include a sufficiently high concentration of dithiothreitol so as to measure the zymogen which could maximally be activated by thrombin and calcium ions. Inasmuch as the values in neonates were still significantly lower than those in adults, some other explanation must be found. We are inclined to think that there may be a mechanism which somehow regulates the concentration of fibrin-stabilizing factor in plasma so as to maintain the possible physiologic advantage seemingly tilted towards fibrinolysis during infancy as suggested by Ekelund *et al.* (4, 5). According to this concept, neonates would require an added protection against fibrin deposits and this would be achieved by a lowering of the concentration of factors which promote clotting (*e.g.*, prothrombin) and by a higher than adult concentration of the components participating in lysis (*e.g.*, plasminogen). Fibrin-stabilizing factor should fall in the former category because its functioning leads to a highly elastic clot structure (8, 16) which is very resistant to lysis. It is possible that the relatively low FSF activity during fetal life and immediately after birth, coupled with low values of other clotting factors and high values of fibrinolytic agents, can afford some extra safeguard against permanent damage caused by excessive coagulation. It might be mentioned that Ambrus *et al.* (1, 2) suggested that there might be a relation between high levels of FSF and incidence of hyaline membrane in preterm babies. As seen in Table 1, our results to date do not seem to bear out this conclusion.

Also, it may be mentioned that postnatal studies on 20 normal babies indicate that adult levels of FSF (range of 13–30 units/ml plasma) have been achieved by about 3 weeks of age.

CONCLUSION

Functional *factor XIII* levels in plasma are significantly reduced during fetal life and in neonates (measured immediately after birth), in comparison with values found in the normal adult population. Hitherto, no significant difference could be found between healthy and sick neonates, although the latter group included 10 infants with hyaline membrane disease.

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