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Disseminated intravascular coagulation
endotoxin
Hageman factor (coagulation factor XII)
lysozyme
newborns

Hageman Factor and Disseminated Intravascular Coagulation (DIC) in Newborns and Rabbits

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Summary

There was no significant difference in the levels of factor XII between sick newborns and normal age-matched controls, although the levels of both groups were lower than normal older children. Detailed coagulation studies on 44 sick infants revealed 11 to have disseminated intravascular coagulation (DIC). In those with DIC, the mean Hageman factor was 20% and in those without DIC, 25% ($P > 0.05$). Rabbits given a constant infusion of lysozyme (which inhibits factor XII) showed laboratory evidence of endotoxin-induced DIC. The data suggest that neither reduced factor XII levels nor Hageman factor inhibition provided protection from DIC. The data further suggest that other coagulation pathways might be involved in order to elicit the DIC.

Speculation

Since Hageman factor activation is thought to be involved in initiating the coagulation mechanism and activation of fibrinolytic, kinin, and complement systems and therefore may be involved in basic pathophysiologic reactions, this study was undertaken to determine whether physiologic reductions in this factor might be protective in any way. Although it was found that newborns had lower factor XII levels than older children or adults and that the levels were lower in the younger infants and were therefore thought to be due to a developmental delay, no protection from the development of acquired coagulopathies could be detected. A significant number of sick neonates were found to have a variant form of DIC (reduced plasma factors II, V, VIII, and fibrinogen, but normal platelet counts instead of thrombocytopenia). It is speculated that these infants' platelets were not responsive to the DIC-provoking event due to a developmental platelet dysfunction.

The role of Hageman factor (coagulation factor XII) in activating the intrinsic or plasma coagulation mechanism is well known. More recently, factor XII has been reported to be involved directly or indirectly in activating the fibrinolytic, kinin, and complement systems (7, 19). Thus, it has been postulated that factor XII and its activation may play a pivotal role in the initiation of many pathophysiologic reactions ranging from inflammation to shock. Newborn infants are known to have reduced Hageman factor levels and normal adult levels are not achieved until 7-14 days of age (1, 8). The biologic significance of this reduction in Hageman factor is not known.

Experimental and human data suggest that activation of factor XII may be instrumental in producing the coagulopathy of DIC (9-11). Pretreatment of rabbits with lysozyme, which prevents the activation of Hageman factor (16), reportedly protects the animals for developing liquoid-induced DIC (14). The purpose of this study was to investigate the levels of factor XII and their role in sick newborns with associated coagulopathies and to study the effect of factor XII inhibition on endotoxin-induced DIC in rabbits. The data indicate that neither reduced factor XII levels nor Hageman factor inhibition provided protection from DIC. The data also suggest that in both the experimental model and sick neonates another coagulation pathway must be involved in order to elicit the DIC.

MATERIALS AND METHODS

HUMAN STUDIES

Neonates admitted to the Intensive Care Nurseries from 1969–1975 were evaluated after informed consent was obtained. No blood products had been given before the initial study. Citrated platelet-poor plasma was obtained by methods previously described for newborns (6, 21). Platelet counts were performed in the Clinical Pathology Laboratory at the University Hospital by standard hematologic technique. Citrated platelet poor plasma was used for assays of factors XII (24), VIII (24), V (22), II (17), and fibrinogen (20). In some instances, fibrinolytic assays were performed and consisted of plasma plasminogen and plasminogen activator levels by employing a fibrin plate technique (4).

ANIMAL STUDIES

Rabbits, California strain, of either sex, weighing 1.0 kg were used. *Escherichia coli* endotoxin $011B_4$ (Difco Laboratories, Detroit, Mich.) solutions were prepared fresh daily in normal saline solution. Lysozyme (Nutritional Biochemical Corp., Cleveland, Ohio) was dissolved in sterile normal saline. Whole blood was obtained from the rabbits by two syringe technique and anticoagulated in a ratio of 1 volume 3.8% sodium citrate to 9 volumes whole blood. The anticoagulated blood was centrifuged at 8,000 rpm for 20 min at 4° to obtain platelet-poor plasma. The plasma was used for partial prothrombin time (PTT) (24), prothrombin time (18), and assays of factors XII, VIII, V, II, and fibrinogen by methods described under "Human Studies." Platelet counts were performed on whole blood using phase microscopy (2). Because of the rapid plasma disappearance rate of lysozyme, adequate levels could be achieved only by using constant infusion by way of a Harvard pump. The model consisted of a young rabbit with a Teflon catheter in the external jugular vein which was used for the constant infusion of lysozyme and a similar catheter placed in the carotid artery which was used for blood sampling. This preparation stayed intact and thrombus free for up to 6 hr. It was found that an infusion of 20 mg/kg/min of lysozyme regularly prolonged the PTT. The effect was noted within 10 min and could be maintained indefinitely. Similarly treated animals using saline or phosphate-buffered saline showed no change in their coagulation studies.

RESULTS

CLINICAL STUDIES

Normal and sick neonates were grouped according to gestational ages; a full term infant was defined as greater than and a premature as less than 37 weeks gestational age. Of the sick infants, 75% of problems were due to respiratory distress syndrome (RDS), 6% to sepsis, and 10% to miscellaneous causes such as abruptio placentae, pneumonia, hypoxia secondary to fetal distress, and so forth Figure 1 demonstrates the distribution of the factor XII levels in the normal full term and premature infants. As can be seen, 73% of the premature infants had 30% or less factor XII levels whereas 82% of the full term infants demonstrated levels of factor XII above 30%. The data suggested that the smaller the infants, the lower the factor XII levels. Thus, in three gestational age groups (37–42, 33–37, and 27–33 weeks) the factor XII levels were 47% ± 4 in 21 infants, 33% ± 4 in 13, and 27% ± 6 in 10. A comparison of the factor XII levels in both normal and sick newborns is depicted in Table 1. There was no significant difference between the normal and sick infants in either group. Although full term infants had higher factor XII levels than prematures and the normal infants had higher levels than sick infants, the data was not significantly different between these groups. Detailed coagulation analysis was performed on 44 of the 77 sick neonates. The largest number of patients were found in the respiratory distress syndrome and the results are shown on Table 2. There were 32 such infants and the results of all the infants are shown in the first column of the table. The RDS infants were arbitrarily divided into those with thrombocytopenia and a group with reduced factor VIII levels. No significant difference in the factor XII levels was noted in these groups. When the infants were subdivided on the basis of the severity of RDS, it was found that the factor XII level was lower (17% activity) in the severe group (20 infants) than in the patients with mild to moderate (23% factor XII activity) disease (12 infants).

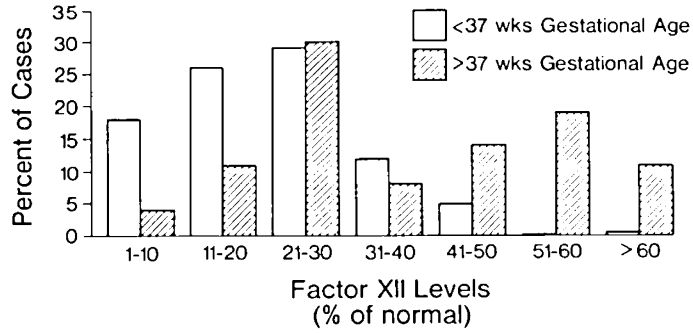

Fig. 1. Factor XII levels in normal full term and premature newborns.

Table 1. *Hageman factor in normal and sick newborns*[1]

	Normal	Sick	*P*
All newborns (mean ± SEM)	39 ± 4	32 ± 4	>0.05
Gestational age			
>37 weeks (mean ± SEM)	47 ± 4 (21)	41 ± 5 (25)	>0.05
<37 weeks (mean ± SEM)	30 ± 5 (23)	23 ± 3 (52)	>0.05

[1] The Hageman factor is expressed as a percentage of normal. Numbers in parentheses are number of newborns.

When all of the sick neonates with hypofibrinogenemia were studied, it was found that they clustered into two groups. *Group I*, which consisted of 14 infants, had reduced factor VIII levels and *group II* was comprised of 14 infants who demonstrated normal to increased factor VIII activity. The results are shown in Table 3. As can be seen, the *group I* infants demonstrated a more severe coagulopathy with regard to factors II, V, VIII, and XII when compared to the *group II* infants. There was no difference in the types of disease entities seen within the two groups. (In *group I* there were eight cases of RDS and asphyxia, three babies born of abruptio placentae, one pneumonia, one sepsis, and one congenital heart disease case. In *group II*, there were eight cases of RDS with asphyxia, two with abruption, and four with pneumonia.)

Of all the sick infants, 11 were found to have classic disseminated intravascular coagulation (*e.g.*, thrombocytopenia with reduced factors II, V, VIII, and fibrinogen). In those with DIC, the factor XII levels were 20% activity and those without were 25% activity.

The time of obtaining the blood samples for factor XII assay in the sick infants who did not receive plasma or whole blood transfusion before study was not critical. In 69 determinations, the factor XII levels were 30% if the samples were obtained between 0 and 12 hr after birth, 25% between 12 and 24 hr, and 24% between 24 and 48 hr.

Twenty infants were given plasma transfusions after the initial studies were performed. Sixteen of these could be evaluated in follow-up. However, the data did not give a clue to the possible half-life of the factor XII. In all infants, the pretransfusion factor XII level was 27% and the post-transfusion level was 49%. The post-transfusion levels were determined at variable times,but usually within 48 hr after the infusion. The data suggested that the baseline factor XII levels were reached 72 hr after the previous transfusion.

Table 2. *Platelet and coagulation factor levels in respiratory distress syndrome (RDS)*

	All RDS neonates (32)[1]	RDS neonates with thrombocytopenia (5)[1]	RDS neonates with reduced factor VIII (10)[1]	Normal range (<37 weeks gestational age)
Platelets ($\times 10^3$/mm^3)	259[2]	114	255	275
	93-420	93-143	103-398	150-400
Factor II (%)	23	23	16	40
	1.0-60	12-30	2-23	20-60
Factor V (%)	50	49	32	100
	15-100	33-80	15-44	50-150
Factor VIII (%)	70	78	30	120
	13-145	19-115	13-40	60-180
Fibrinogen (mg/dl)	180	134	150	240
	84-365	84-211	84-281	175-350
Factor XII (%)	20	23	19	30
	6-45	9-36	6-32	15-45
Gestational age (weeks)	32	34	30	
	24-40	31-36	24-31	

[1] Number of neonates.
[2] Mean value and range.

Table 3. *Factor XII levels in hypofibrinogenemic (<150 mg/dl) sick newborns with and without reduced factor VIII levels*

	Group I, reduced VIII (14)[1]	*Group II*, normal or ↑ VIII (14)[1]	
Platelets ($\times 10^3$/mm^3)	240	247	
	(21-398)[2]	(80-420)	
Factor II (%)[3]	15	25	
	(2-30)	(9-41)	
Factor V (%)	28	44	
	(2-40)	(8-82)	
Factor VIII (%)	32	95	
	(13-45)	(51-210)	
Fibrinogen (mg/dl)	109	103	
	(0-145)	(37-136)	
Factor XII (%)	13	29	P <0.1
	(1-27)	(13-50)	P >0.05
Gestational age (weeks)	20	35	
	(23-40)	(27-40)	

[1] Number of neonates.
[2] Range.
[3] Percentage of normal.

Fibrinolytic studies in 20 premature and 20 full term infants revealed no significant differences in plasminogen levels (1.0 unit ± 0.1 versus 0.7 unit ± 0.1) or plasminogen activator content (110 mm^2 ± 22 versus 50 mm^2 ± 15).

Factor XII levels in over 100 older children and adults have consistently been in the range of 75-100% (90% ± 10).

ANIMAL MODEL FOR HAGEMAN FACTOR INHIBITION

Pooled, citrated platelet poor plasma was obtained from normal rabbits. Saline or various concentrations of lysozyme were added to plasma aliquots. After appropriate incubation periods, the partial thromboplastin times and prothrombin times were calculated. Compared to saline controls, significant prolongations of only the PTT was seen with lysozyme. The PTT for a mixture of saline and rabbit plasma was 24 sec, whereas plasma plus lysozyme was greater than 250 sec. Adding normal plasma to the lysozyme-treated rabbit plasma partially corrected the abnormality; however, adding human plasma which was congenitally deficient in factor XII did not provide correction. By using the infusion rate of 20 mg/kg/min or greater of lysozyme, the PTT was significantly prolonged in normal rabbits, and, there was no change in either the plasma prothrombin time at the same time the PTT was prolonged or in the platelet count, factors II, V, or VIII levels. However, Hageman factor activity fell 43-61% from baseline. After discontinuing the infusion, Hageman factor activity returned to normal within 20 min. In the endotoxin experiments, rabbits were prepared with 0.1 mg/kg endotoxin given intravenously. Twenty-four hours later, the animals were infused with either sterile normal saline or lysozyme. The infusions were continued for 2 hr. A second endotoxin injection of the same quantity was given 15 min after the start of the infusion, that is, at a time when the PTT was grossly prolonged in the lysozyme-treated animals. Blood was then obtained at 4 hr for measurement of the white blood cells, platelets, factor XII, and fibrinogen concentration. The results are shown in Table 4. As can be seen, lysozyme infusion prevented the fall in factor XII levels, but did not influence endotoxin-induced leukopenia, thrombocytopenia, or fibrinogen reduction.

DISCUSSION

Reduced factor XII levels in humans can either be due to a deficiency of this factor (8) or intravascular activation with subsequent reduction, as suggested by Mason and his associates (9, 10). The finding that normal neonates have reduced factor XII levels could be interpreted, therefore, as either due to physiologic developmental delay or as evidence of activation of the intrinsic coagulation mechanism. The data in this study indicate that both normal and sick neonates had similar factor XII levels. In addition, even in those infants who had manifested severe activation of the coagulation mechanism, *i.e.*, disseminated intravascular coagulation, showed no significant difference between the factor XII levels in that syndrome versus either normal infants or infants who had other coagulopathies. These results would suggest that the reduced levels in newborns are predominantly due to a developmental dalay and do not manifest activation of the intrinsic coagulation mechanism. Like the rabbits, the reduction in factor XII in the newborns did not seem to provide protection against acquired induced coagulopathies, particularly DIC. The fact that a consumption coagulopathy could indeed develop in the neonate with Hageman factor deficiency and that the level of factor XII did not necessarily decrease during the activation phase strongly suggests that the neonate, like the lysozyme-treated rabbit, can activate his coagulation pathway.

In this study, an *in vivo* model for inactivating Hageman factor was developed in rabbits. Contrary to the experience with liquoid-induced DIC (14), endotoxin-induced DIC could not be prevented in the lysozyme-treated animals of this study. Previous investigators have suggested that endotoxin activates Hageman factor and this was the trigger for the mechanism of activation of the intrinsic coagulation mechanism (11, 12). In addition, intravascular coagulation was reported not to occur in

Table 4. *Lysozyme infusion in endotoxin-treated rabbits*[1]

	0 hr	4 hr		% Change	
	(11)[2]	NSS (5)[1]	Lysozyme (6)[1]	NSS	Lysozyme
WBC (no./mm³)	7,137 ± 997[3]	1,073 ± 385	2,175 ± 766	85% ↓	70% ↓
Platelets (×10³/mm³)	257 ± 34	51 ± 10	143 ± 62	80% ↓	44% ↓
Fibrinogen (mg/dl)	317 ± 23	234 ± 20	200 ± 33	26% ↓	37% ↓
Factor XII (% of normal rabbit control)	100 ± 12	72 ± 4	121 ± 35	28% ↓	21% ↑

[1] All animals were prepared with 0.1 mg/kg endotoxin 24 hr before the study. At 24 hr (0 hr) all animals were given a second endotoxin injection plus either normal saline solution (NSS) or lysozyme and blood was obtained 4 hr later.

[2] Number of animals.

[3] Mean ± SEM.

the fowl with either liquoid or endotoxin (25). It is known that the fowl is congenitally deficient in factor XII. However, Shen *et al.* (23), by using an antibody against factor VIII and therefore inhibiting the early phases of intrinsic coagulation, found that intravascular coagulation could be induced by endotoxin in rabbits and suggested that the endotoxin probably triggers clotting by both the intrinsic and extrinsic systems. Müller-Berghaus and Schneberger (15) were not able to prevent the generalized Shwartzman reaction in rabbits by using lysozyme inhibition of factor XII in the thoratrast-endotoxin model. It is known that intravascular coagulation plays a major role in the production of the generalized Shwartzman reaction (5). Müller-Berghaus and Schneberger (15) concluded that endotoxin triggers DIC by a mechanism different from that of the intrinsic coagulation mechanism.

A number of investigators have stressed the important role that the blood platelets may have in triggering the mechanism for intravascular clotting. It is certainly conceivable that endotoxin activates the extrinsic coagulation mechanism by way of platelet injury (5) or, as proposed by Walsh (26), that platelets may act in the intrinsic mechanism through activated factor XI, thus bypassing factor XII.

Although activation of the coagulation mechanism through the platelets is an attractive explanation, the data on the human neonates in this study would suggest that perhaps another mechanism, undescribed at the present time, may be involved. Of all of the sick infants in this study, only 11 were found to have the classic hematologic manifestations of DIC (thrombocytopenia with reduced factors II, V, VIII, and fibrinogen). A significant number of sick neonates, however, were found to have normal platelets in the face of reduced factors II, V, VIII, and fibrinogen. Since these infants had neither hemophilia nor evidence of a hyperfibrinolytic state, the data could be interpreted as indicating a form of disseminated intravascular coagulation without the classic platelet changes. Since no attempt was made to study the half-life of the platelets in these infants, it is possible that production of platelets was keeping up with the destruction. It is known that human neonates, particularly prematures, have a transient but definite platelet dysfunction so that an alternative view could be that thrombocytopenia did not develop in these patients because of their lack of responsiveness to the event which was allowing coagulation factor activation and consumption (3, 13). Thus the hypothesis of Walsh regarding activation of the coagulation mechanism by way of platelet interaction with other activated clotting factors other than Hageman factor may not be tenable at this age.

The data from this study suggests that the coagulation mechanism of the newborn is not activated by way of the intrinsic coagulation pathway and/or through platelet mechanisms. However, since evidence is presented that the coagulation mechanism is activated, activation would have to be through the extrinsic or tissue coagulation mechanism as suggested by the animal studies (15, 23).

CONCLUSION

There was no significant difference in the levels of factor XII between 77 sick newborns and 44 normal age-matched control infants although the levels in both groups were lower than normal older children. Detailed coagulation studies on 44 sick infants revealed 11 to have disseminated intravascular coagulation. In those with DIC, the mean Hageman factor was 20% and those without DIC, 25%. Rabbits given a constant infusion of lysozyme showed laboratory evidence of endotoxin-induced DIC. The data suggest that neither reduced factor XII levels in humans nor Hageman factor inhibition in rabbits provided protection from DIC. A significant number of sick infants were found to have a variant form of DIC. It was speculated that these infants' platelets were not responsive to the DIC-provoking event because of a developmental platelet dysfunction. Activation of the coagulation mechanism of the neonate, since it does not occur by way of the intrinsic plasma coagulation mechanism or through platelet interaction, most likely occurs by way of the extrinsic or tissue coagulation mechanism.

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Hypoplasia osteolysis, epiphyseal
muscle atrophy skeleton

Selective Muscle Fiber Hypoplasia and Epiphyseal Osteolysis

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Summary

This paper reports the investigation of a case associating grotesque skeletal deformities, selective hypoplasia of muscle fiber types I and IIA, and a decrease in skeletal mineral plus increased skeletal resorption. The investigation also included administration of synthetic salmon calcitonin, a hormone that reduces bone resorption. The patient, a 10-year-old female, presented with foreshortened, clawlike hands and feet, joint stiffness with contractures, marked muscle atrophy, and severe joint pain. Radiologic survey revealed muscle atrophy, osteoporosis and epiphyseal osteolysis. ^{47}Ca turnover was accelerated. Muscle biopsy revealed a population of hypoplastic muscle fibers, 10-22 μm in diameter, scattered singly or in small groups among a majority of larger morphologically normal fibers. Myofibrillar ATPase stains showed that all types I and IIA fibers present were hypoplastic, and all large fibers present were type IIB. Electron microscopic study of the skeletal muscle showed normal myofibrils but vacuolated mitochondria with disorganized cristae in the hypoplastic fibers. Synthetic salmon calcitonin given at 2 MRC units/kg 3 days a week produced a marked improvement in bone pain and reduced urinary excretion of hydroxyproline from 110.3 and 155.9 mg/24 hr during the two control periods to 136.2 and 84.2 mg/24 hr. Calcium metabolic balance changed from an average control value of +124.4 to +248 mg/day during calcitonin therapy. Iliac crest biopsy before calcitonin revealed increased uncalcified bone matrix and increased osteoclastic activity. After 3 months of calcitonin there was a decrease in the amount of uncalcified matrix and an apparent decrease in osteoclastic activity. Skin biopsy was normal.

Speculation

One may speculate as to the relationship between the muscle and bone abnormalities in the present case. Joint contractures are not uncommon in children with muscle disease, and it is likely that the joint deformities are at least in part related to the muscle hypoplasia and weakness. The osteomalacia and epiphyseal osteolysis, on the other hand, have not been reported in association with other types of congenital muscle disease, and appear much too severe to be secondary phenomena. The selective muscle fiber hypoplasia and severe osteomalacia are apparently two associated aspects of one clinical syndrome. Whether the mitochondrial abnormalities noted in the muscle reflect the underlying metabolic defect of this syndrome remains to be investigated.

We report a girl of Oriental-American Negro descent who was found to have the clinical features of foreshortened, clawlike hands and feet, joint stiffness, and contractures with severe joint pain and muscle atrophy. This characteristic clinical picture is explained by a selective muscle fiber hypoplasia affecting types I and IIA fibers, associated with a marked osteolytic process. To our knowledge, this is the first time that carpal and tarsal osteolysis has been associated with a selective muscle fiber hypoplasia.

MATERIALS AND METHODS

CASE REPORT

A 10-year-old girl was born by normal vaginal delivery after an uncomplicated full term pregnancy. The mother is Oriental and the father is an American Negro. The family history revealed a mother, two sisters, and a brother in good health. The father had muscle weakness during childhood and adolescence, and he was refused admission to the army because of it. A recent physical examination revealed normal muscle strength and mass. He refused a muscle biopsy. There was no history of musculo-