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Blood Coagulation Changes after Hypoxemia: a Fetal Lamb Model

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Summary

The effects of fetal hypoxemia on blood coagulation (platelet count, prothrombin time, partial thromboplastin time, fibrinogen, factors II, V, VI, VIII, IX, X, XI, XII, XIII and von Willebrands activities, fibrin degradation products, and fibrin monomer) were evaluated in nine chronically catheterized fetal lambs early in the third trimester of pregnancy (107–110 days gestation). Seven chronically catheterized fetal lambs of similar gestational ages served as controls. The hypoxemic episode (pO_2 14 mm Hg) was maintained for 1 hr in the experimental group during which time there were only minimal changes in P_{CO2}, arterial pressure, heart rate, and pH. Epinephrine and norepinephrine levels increased significantly in the stressed animals—22 pg/ml pre- to 1025 pg/ml postepinephrine, and 475 pg/ml pre- to 2292 pg/ml postnorepinephrine. There were no significant changes in blood coagulation factor activities related to the hypoxic stress although, one fetus who experienced acidemia did develop a transient increase in fibrin monomer. Slight through significant increases in factor VIII coagulant activity (4.0%), von Willebrand activity (5.9%), and factor XII activity (4.3%) occurred in both the hypoxemic and control fetal lambs. These changes were associated with minimal increases in the white blood cell count (15%) and slight decreases in the mean arterial pressure (3.9 mm Hg), hemoglobin (1.2 g), and hematocrit (2.9%) and may have been related to the blood loss of 25% that occurred as a result of sampling in both groups. There were no differences between the hypoxic and control animals' levels of coagulation factor activities when measured during an 18-day follow-up period except for a slight increase in factor X activity (10%) in the control animals not apparent in the nine hypoxic animals. Thus an episode of severe fetal hypoxemia in the absence of hypotension, acidosis, and hypercarbia does not lead to acute or chronic alterations in blood coagulation factor activities in the fetal lamb.

Speculation

The absence of a coagulopathy in the fetal lamb during and after severe intrauterine hypoxemia, may be related to adaptation to lower intrauterine pO_2 levels, to an immature coagulation system unable to respond to stress, and/or to the ability of the placental circulation to maintain a normal pH in the presence of severe hypoxemia.

Postnatal hypoxemia in newborn infants has been associated with variable abnormalities of blood coagulation (1, 2, 7, 12, 18, 20, 26 30). Some authors report changes consistent with intravascular coagulation (7), while others suggest the changes are related to decreased synthesis of coagulation factors by the liver (2, 18). Stresses, including hypoxemia before delivery, have also been associated with variable alterations of blood coagulation; some authors suggest an acceleration of the maturation of blood coagulation factor activities (1, 28). The variability of reported changes in blood coagulation associated with hypoxemia before and after delivery may be the result of the influences of other often associated stresses such as hypotension, hypercarbia, acidosis, and hypothermia which are not easily separated from hypoxemia in the clinical setting. Since the blood coagulation system is maturing throughout gestation (3, 4, 13) the influence of any stress may also depend upon the degree of maturation at the time of the insult.

To isolate and examine the effects of hypoxemia on the developing blood coagulation system, in the absence of other stresses, a fetal lamb model has been developed (17). In this report blood coagulation in the fetus was examined before, during, and after a 1 hr exposure to severe hypoxemia. The hypoxemic episode was induced early in the third trimester of pregnancy during which time there are few developmental changes in blood coagulation (17). The abnormalities usually associated with hypoxemia including acidosis, hypotension, and hypercarbia were minimal thereby allowing examination of the effects of a relatively pure state of hypoxemia.

MATERIALS AND METHODS

Pregnant mixed breed Dorset-Suffolk ewes were obtained from existing sources and were housed throughout the study at the University of Iowa Animal Quarters.

The gestational ages of the sheep fetuses were known based on induced ovulation techniques (15). Before surgery, the animals were fasted for 48 hr. Anesthesia of the ewe and surgery on the fetus were performed as described previously (17, 25).

During initial studies the ewes with their chronically catheterized fetuses were transferred into small carts restricting them to upright positions. Group one consisting of nine chronically catheterized lamb fetuses, 107–110 days gestation were exposed to hypoxemia. Group two consisting of seven chronically catheterized fetal lambs of the same gestational age (107–110 days) were studied concurrently but not exposed to hypoxemic stress.

Blood samples were obtained from the fetuses via the chronic catheters in both groups of animals before beginning the study. After removing an initial 1 ml of blood to clear the line of saline, a sample of blood totaling 9 ml was obtained for the various measurements to be described. After the initial 9-ml collection of blood, hypoxemia was induced in the nine fetal lambs in group 1 by placing a bag over the ewe's head and having her breathe a mixture of 11.1% oxygen balanced with nitrogen. The oxygen-nitrogen gas mixture was precalibrated with an accuracy level ± 0.11% by Airproducts Company of Pennsylvania. At 30 min into the experiment, a second 9-ml blood sample was obtained from hypoxemic and control fetuses. Hypoxemia was concluded after a total of 60 min by removing the bag and having the ewe breathe room air. Prolonging the hypoxemic episode beyond 60 min results in acidosis, and often hypotension and death to the fetus. These events would preclude evaluation of the acute and chronic effects of pure hypoxemia and is the reason for using the 60-min exposure period. Third and fourth samples of blood totaling 9 ml each were obtained from each of the fetal lambs at 90 and 210 min after the start of hypoxemia (30 min and 2½ hr from the completion of the

hypoxemic stress). The animals were then returned to their permanent quarters.

During the initial studies, 0.5 ml of the blood was collected anaerobically in heparinized plastic syringes and measurements of pH, P_{CO_2}, and pO_2 were immediately determined with the appropriate pH, P_{CO_2}, pO_2 electrodes at 39°C using a radiometer pH amp 72 NK2 acid base analyzer (Radiometer Company, Denmark). Protein content of fetal serum was determined on 0.5 ml of clotted blood using a refractometer (Medical Instruments Company, Baltimore, MD). Hemoglobin, hematocrit, and white blood cell counts were measured using a Coulter Model S on a 0.5 ml aliquot of the blood that was anticoagulated with EDTA. Platelet counts were also obtained on this aliquot using phase microscopy. Epinephrine and norepinephrine were determined on a 2-ml aliquot collected in 40 μl of anticoagulant containing 90 mg/ml ETA, 60 mg/ml glutathione. A radioenzymatic assay (CAT-A-KIT, Upjohn Company, Kalamazoo, MI) as described previously by Passon and Peuler (22) was used for the epinephrine and norepinephrine determinations.

Coagulation factor activities were measured on plasma from 2.7-ml aliquots of blood anticoagulated with 0.3 ml of 0.05 M sodium citrate, pH 5.0. Standard techniques for measuring the prothrombin times (29), and partial thromboplastin times (24) were used. Specific factor activities II, V, VII, VIII, IX, X, XI, and XII were measured using one-stage assays measuring the ability of the test plasmas to correct plasma known to be deficient in the factor to be tested (21). Human deficient plasmas were used and an adult sheep standard pool consisting of plasma from 10 nonpregnant ewes was used for calibration. Fibrinogen concentration was measured as clottable protein (8). Von Willebrand factor activity was measured using washed formalin fixed frozen platelets according to the method of Brinkhous and Read (5). All activities measured are quoted as percents of a reference standard pool of sheep plasma obtained from 10 nonpregnant ewes. Plasma digestion products of fibrinogen/fibrin were measured in serum from a 0.5 ml aliquot of blood allowed to clot in 5 μl of a solution of 0.05 M EACA, 0.125 M $CaCl_2$, 10 U/ml thrombin. The method of Merskey *et al.* (19) using rabbit anti-sheep fibrinogen antibody and sheep fibrinogen coated red blood cells was used. Fibrin monomer was measured according to the method of Kisker *et al.* (16) on plasma from 2.7-ml aliquots of blood collected in 0.3 ml of anticoagulant containing 2% EDTA, 10 mg/ml soybean trypsin, 100 U/ml heparin pH 7.4. Sheep fibrinogen and anti-sheep fibrinogen antibody were used throughout.

Fetal arterial, venous, and amniotic pressures were recorded continuously during the experiments using Stratham P23Db pressure transducers (Stratham Instruments Division, Inc.) and a Beckman R-611 recorder. Fetal heart rate was monitored with a cardiotachometer triggered from the fetal arterial pressure pulse.

Baseline values before the introduction of hypoxemia were compared in the two groups using one way analysis of variance. The acute changes that occurred during (30 min) and after the institution of hypoxemia (90 and 120 min) were compared in the two groups using a repeated measures analysis of variance. A significant interaction with hypoxemia would be indicated by a difference in the response across time in group one hypoxemic as compared to group two control. If the interaction test is not significant one can assume any changes occurring with time are parallel in the two groups. If the interaction test was not significant, analysis for parallel changes with time in the two groups was carried out.

After the completion of the initial experiments serial blood samples were obtained from both control and hypoxemic animals to determine if there were any long-term influences of the hypoxic stress on the subsequent development of fetal blood coagulation. Samples of blood were obtained from the fetal lambs approximately three times a week during an 18-day follow-up period.

Multiple linear regression was used to complare the patterns of changes in the groups during the 18 days after the episode of acute hypoxemia. Preliminary analysis indicated that all changes during this 18-day period were adequately described by straight line regressions. General linear models techniques were therefore used to test for differences in rates of change (slopes) for the two groups. Duncan's multiple comparison procedure was used.

RESULTS

In Tables 1 and 2 are presented the baseline values and values at 30, 90, and 120 min during the initial experiments. There were no significant differences between the groups at time zero. Values obtained on samples during hypoxemia (30-min samples) and following hypoxemia (90- and 120-min samples) did reveal certain changes. As was expected because of the phlebotomy, there was a significant decrease in the values for hemoglobin and hematocrit in both groups. There was a slight but significant increase in the white blood cell count in both groups. Mean arterial pressure decreased in both groups but remained within physiologic range. These changes in the hematocrit, hemoglobin, white blood cell count, and arterial pressure were parallel in both groups and therefore would not be attributed to hypoxemia (Table 1).

Changes specific to the hypoxemic group included a decrease in the partial pressure of oxygen from a mean of 25.8 ± 1.4 mm

Table 1. *Physiologic measures during hypoxemia*[1]

	Time 0 (before hypoxemia)		Time 30 min (during hypoxemia)		Time 90 min (30 min after hypoxemia)		Time 210 min (2½ hr after hypoxemia)	
	Hypoxic group 1 (N = 9)	Control group 2 (N = 7)	Hypoxic group 1 (N = 9)	Control group 2 (N = 7)	Hypoxic group 1 (N = 9)	Control group 2 (N = 7)	Hypoxic group 1 (N = 9)	Control group 2 (N = 7)
Hemoglobin (g/dl)	9.4 ± 0.6	8.6 ± 0.2	9.6 ± 0.6	8.6 ± 1.8	9.0 ± 0.6	8.1 ± 0.2	7.8 ± 0.5[2]	7.4 ± 0.2[2]
Hematocrit (%)	30.4 ± 1.8	27 ± 1.1	31.8 ± 2.0	27.8 ± 0.8	29.8 ± 2.1	26.2 ± 0.7	27.1 ± 1.6[2]	24.4 ± 0.9[2]
White blood count ($mm^3/10^3$)	5.1 ± 1.4	3.2 ± 0.6	5.7 ± 1.5[2]	3.7 ± 0.6[2]	5.5 ± 1.5[2]	3.9 ± 0.7[2]	5.5 ± 1.2[2]	4.3 ± 0.8[2]
Mean arterial pressure (mm Hg)	41.0 ± 1.8	36.1 ± 2.3	43.3 ± 1.8	35.6 ± 1.6	41.5 ± 1.7	35.4 ± 1.9	33.5 ± 5.1[2]	34.6 ± 2.0[2]
Heart rate (beats/min)	191 ± 5	190 ± 14	180 ± 8	187 ± 17	212 ± 12	187 ± 7	214 ± 11	189 ± 5.8
PO_2 (mm Hg)	25.8 ± 1.4	24.9 ± 1.0	14.2 ± 1.0[3]	23.6 ± 1.3	24.4 ± 1.9	24.5 ± 0.7	23.8 ± 2.2	25.6 ± 0.9
P_{CO_2} (mm Hg)	41.6 ± 1.4	38.9 ± 0.8	35.5 ± 1.4[3]	39.8 ± 1.6	39.4 ± 1.8	38.9 ± 1.8	42 ± 1.8	40.5 ± 2.0
pH	7.38 ± 0.01	7.4 ± 0.01	7.39 ± 0.02	7.4 ± 0.01	7.36 ± 0.2	7.4 ± 0.01	7.37 ± 0.02	7.4 ± 0.01
Total protein (g/dl)	3.1 ± 0.13	3.2 ± 0.16	3.3 ± 0.10	3.2 ± 0.19	3.2 ± 0.1	3.2 ± 0.2	3.1 ± 0.1	3.2 ± 0.2
Epinephrine (pg/ml)	22 ± 12	12.5 ± 2.5	1025 ± 560[3]	17.4 ± 4.8	43.8 ± 19	20 ± 5.5	81 ± 46	20 ± 7.1
Norepinephrine (pg/ml)	475 ± 195	338 ± 61	2292 ± 820[3]	427 ± 116	732 ± 256[3]	444 ± 75	697 ± 296	546 ± 117

[1] Mean ± S.E.

[2] Mean values in groups 1 and 2 are significantly different ($P = <0.05$) from time 0 but the changes are parallel in both groups.

[3] Mean values in group 1 are significantly different ($P = <0.05$) from mean values in group 2 therefore, differences are likely related to hypoxemia.

Table 2. *Coagulation factor activities during hypoxemia*[1]

	Time 0 (before hypoxemia)		Time 30 min (during hypoxemia)		Time 90 min (30 min after hypoxemia)		Time 210 min (2½ hr after hypoxemia)	
	Hypoxic	Control	Hypoxic	Control	Hypoxic	Control	Hypoxic	Control
	group 1 (N = 9)	group 2 (N = 7)	group 1 (N = 9)	group 2 (N = 7)	group 1 (N = 9)	group 2 (N = 7)	group 1 (N = 9)	group 2 (N = 7)
Platelets ($mm^3/10^3$)	366 ± 35	392 ± 45	384 ± 37	394 ± 43	389 ± 35	403 ± 43	394 ± 29	383 ± 33
Prothrombin time (sec)	16.3 ± 0.8	16.1 ± 0.4	15.8 ± 0.4	15.8 ± 0.2	15.9 ± 0.5	16.2 ± 0.3	16.3 ± 0.7	15.9 ± 0.3
Partial thromboplastin time (sec)	55.7 ± 4.2	54.2 ± 3.0	55.2 ± 3.8	53.6 ± 2.8	53.1 ± 4.1[2]	52.5 ± 2.9[2]	53.6 ± 3.9	52.9 ± 3.2
Thrombin time (sec)	21.5 ± 0.9	22.7 ± 2.6	20.4 ± 0.7	22.7 ± 2.1	19.3 ± 2.2	22.3 ± 2.0	21.9 ± 6.7	22.5 ± 2.0
Fibrinogen (% of adults)	45.4 ± 3.9	51.7 ± 1.2	47.1 ± 3.4	55.2 ± 1.2	44.8 ± 2.8	50.5 ± 1.1	43.8 ± 2.9	50.0 ± 1.1
II (% of adults)	34.5 ± 2.2	45.0 ± 3.0	34.0 ± 2.3	44.8 ± 3.1	35.6 ± 2.4	45.6 ± 2.0	33.7 ± 2.4	45.1 ± 3.6
V (% of adults)	54.8 ± 5.9	61.5 ± 6.0	58.6 ± 6.6	69.5 ± 4.6	56.4 ± 5.8	66.0 ± 5.8	55.7 ± 6.1	61.2 ± 4.1
VII (% of adults)	46.2 ± 3.0	52.7 ± 4.4	49.2 ± 2.9	54.3 ± 3.8	51.3 ± 6.1	57.1 ± 3.8	47.8 ± 3.4	55.3 ± 5.1
VIII (% of adults)	24.8 ± 3.3	20.5 ± 3.4	25.4 ± 2.5	24.5 ± 2.9	29.1 ± 3.4[2]	25.0 ± 3.9[2]	29.4 ± 3.4[2]	23.7 ± 3.4[2]
IX (% of adults)	30.5 ± 2.9	27.3 ± 3.0	31.6 ± 2.8	30.8 ± 3.4	29.0 ± 3.2	27.1 ± 2.9	27.7 ± 3.0	28.6 ± 3.5
X (% of adults)	31.4 ± 3.2	39.3 ± 3.0	34.6 ± 3.1	44.0 ± 3.1	33.5 ± 3.1	40.7 ± 3.0	32.4 ± 3.6	39.8 ± 3.2
XI (% of adults)	24.2 ± 3.6	26.7 ± 6.6	23.7 ± 3.7	31.7 ± 6.8	23.0 ± 3.3	30.0 ± 5.7	22.4 ± 3.0	29.1 ± 6.3
XII (% of adults)	29.1 ± 5.9	23.8 ± 4.3	33.2 ± 5.1[2]	25.8 ± 4.0[2]	35.3 ± 6.0[2]	25.3 ± 3.9[2]	32.1 ± 5.4[2]	24.2 ± 3.1[2]
XIII (% of adults)	51.9 ± 2.5	44.8 ± 3.5	54.1 ± 2.5	46.3 ± 3.6	52.6 ± 2.9	46.1 ± 4.5	51.1 ± 3.1	45.7 ± 3.8
Fibrin degradation products (μg/ml)	0.73 ± 0.08	0.94 ± 0.12	0.93 ± 0.20	0.86 ± 0.12	0.86 ± 0.20	1.03 ± 0.11	0.73 ± 0.09	0.86 ± 0.12
von Willebrand activity (% of adult)	25.6 ± 1.8	27.9 ± 4.0	31.8 ± 3.3[2]	28.7 ± 3.0[2]	31.3 ± 2.7[2]	29.9 ± 3.5[2]	33.0 ± 3.8[2]	32.4 ± 4.0[2]
Fibrin monomer (μg/ml)	5.2 ± 1.5	6.1 ± 1.7	9.0 ± 1.6	6.0 ± 1.4	7.5 ± 1.6	6.0 ± 1.7	6.6 ± 1.4	6.7 ± 1.7

[1] Mean ± S.E.

[2] Mean values in groups 1 and 2 are significantly different ($P = <0.05$) from time 0 but the changes are parallel in both groups.

Hg at time zero to 14.2 ± 1.0 mm Hg at 30 min (Table 1). The P_{CO_2} was also slightly lower during hypoxemia in these animals but remained within a physiologic range. The initial P_{CO_2} of 41.6 ± 1.4 mm Hg before the institution of hypoxemia decreased to 35.5 ± 1.4 mm Hg after 30 min of hypoxemia. Of interest there was no significant change in pH although one of the hypoxic animals did develop some acidosis at 90 min (pH 7.26). Both epinephrine and norepinephrine increased substantially in the hypoxic animals. The initial mean level of epinephrine 22.2 pg/ml increased to a mean of 1025 pg/ml at 30 min. Norepinephrine was 475 pg/ml in the hypoxic animals before the institution of hypoxia and increased to 2292 pg/ml at 30 min.

There were no changes in blood coagulation factors attributable to hypoxemia (see Table 2). All changes in coagulation factor activities during the initial experiments were parallel in both groups. These changes included an approximate 2-sec shortening of the partial thromboplastin time and a 4 to 6% increase in the activities of factors VIII, XII, and von Willebrand's factor activity. Although the mean value for fibrin monomer increased from 5.2 μg at time zero to 9 μg at 30 min in the hypoxic group the increase was not significant as it was due to one animal who developed an increased level of fibrin monomer of 16 μg/ml at 30 min. This animal was also the animal who experienced acidemia. The levels of the other blood coagulation factors II, V, VII, X, XI, fibrinogen, and the platelet counts were stable throughout the experiment.

Relatively few changes in coagulation factor activities occurred during the 18-day follow-up. In Table 3 are presented the average changes in activity per day for the hypoxic and control groups and the *P* values for Duncan's test of the equality of the groups. As can be seen the only significant difference between the two groups occurred with respect to a slight increase in factor X activity in the control animals not seen in the hypoxic group ($P = 0.013$). The overall increase in factor X activity in the control group during the 18 day period was 10.3% while there was no significant change in factor X activity in the hypoxemic animals.

Table 3. *Changes in blood coagulation factors during 18 days after hypoxemia*

	Hypoxia (N = 9)	Control (N = 7)	
Factor	Average change/day	Average change/day	P
Prothrombin time	+0.35 sec/day	+0.88 sec/day	0.323
Partial thromboplastin time	+0.19 sec/day	−0.05 sec/day	0.301
Thrombin time	−0.05 sec/day	+0.05 sec/day	0.379
Fibrinogen	0.00%/day	0.00%/day	0.801
II	−0.24%/day	+0.09%/day	0.285
V	−0.23%/day	−0.01%/day	0.703
VII	−0.01%/day	+0.02%/day	0.944
VIII	−0.15%/day	−0.15%/day	0.997
IX	−0.07%/day	−0.26%/day	0.172
X	−0.08%/day	+0.57%/day	0.013[1]
XI	+0.16%/day	+0.60%/day	0.203
XII	+0.23%/day	+0.41%/day	0.701
XIII	+0.90%/day	−0.12%/day	0.106
Fibrin degradation products	−0.13μgm/day	−0.11μgm/day	0.934

[1] $P < 0.05$.

DISCUSSION

In study of newborn premature infants with hypoxemia secondary to respiratory distress syndrome or birth asphyxia, Markarian *et al.* (18) demonstrated decreased levels of factor VIII coagulant activity in cord blood samples in the asphyxiated infants (44 ± 8.1%) as compared to normal premature infants (106 ± 9.4%). Markarian's studies also demonstrated a progressive decrease in the levels of prothrombin, factor VIII, factor X, and factor V in infants who developed severe hypoxemia and died in the neonatal period (18). These latter findings were interpreted as evidence of liver injury and a decrease in the synthesis of liver dependent coagulation factor activities. Hathaway and Henderson (11) found similar results in newborn puppies when they were subjected to 24 hr of severe hypoxemia. Appleyard and Cottom (2) found that hypoxemic infants failed to respond appropriately to the administration of vitamin K, a result that also suggested liver injury. Chadd *et al.* (6) in examining infants with hypoxemia found

evidence of lower platelet counts, increased fibrin degradation products, increased thrombin clotting times, and lowered kaolin cephalin clotting times. The findings of Chadd *et al.* (6) were interpreted as suggestive of disseminated intravascular coagulation rather than liver failure. Chessels and Wiggelsworth (7) also reported that the combination of hypoxemia and acidosis particularly when associated with hypothermia produced abnormalities of coagulation indicative of disseminated intravascular coagulation. In contrast to the findings of a number of authors (2, 6, 7, 18) Perlman and Dvilansky (23) did not find any abnormalities in 14 asphyxiated, small-for-dates and premature infants other than slightly lower platelet counts. Thomas (28) suggested that prenatal stress actually accelerated the maturation of blood coagulation and increased levels of blood coagulation factors V, and VIII were seen in infants with fetal distress studied by Hathaway *et al.* (12). This variability in the results reported by different authors probably reflects variations in the timing and duration of the hypoxemic stress, variations in the maturity of the infants studied, and variations in the severity of acidosis, hypercarbia, hypotension, and hypothermia that may accompany hypoxemic episodes.

The chronically catheterized fetal lamb model has provided us with a tool whereby the study of hypoxemia not complicated by severe acidosis, or hypotension and in a constant temperature environment can be evaluated at a specific time of maturation. The present studies were carried out in fetal lambs of gestational ages 107–112 days. Gestation in newborn lambs is approximately 145 days, thus these animals were studied during the early part of the third trimester of pregnancy, a period where we have previously shown that there are relatively few developmental changes occurring in coagulation factor activities (17).

With the introduction of hypoxemia, we found a significant decrease in the pO_2 from an initial level of 25.8 ± 1.4 mm Hg to a level of 14.2 ± 1.0 mm Hg (see Table 1). P_{CO_2}, although decreasing secondary to maternal hyperventilation in the hypoxemic animals, remained within a normal range (35.5 ± 1.4 mm Hg). The pH and mean arterial pressures also remained within a normal range during and immediately after the hypoxemic episodes though one animal did develop acidemia (pH 7.26) at 30 min into hypoxemia.

Of interest this animal also developed an increased level of fibrin monomer at 30 min (16 μg/ml). The fibrin monomer concentration returned to normal (6 μg/ml) 2½ hr after the hypoxemic episode. The increased level of fibrin monomer suggests that this animal may have experienced a transient episode of disseminated intravascular coagulation. Hardaway *et al.* (10) noted shortening of the clotting time in blood with lowered pH and Crowell and Houston (9) were able to demonstrate intravascular coagulation in dogs subjected to acidosis. The findings in our acidemic fetus supports the concept that acidosis may be a critical factor in initiating intravascular coagulation.

Significant increases in both epinephrine and norepinephrine occurred in the hypoxemic animals. Despite these catecholamine elevations, there were no changes in heart rate, and only minimal changes in coagulation factor activities and other hematologic measures. It has previously been demonstrated that epinephrine when given to adults, in addition to alterating heart rate and increasing blood pressure, results in significant increases in the white blood cell count, factor VIII, and von Willebrand ristocetin cofactor activity (14). Our fetal lambs 107–112 days gestation failed to show changes in heart rate, blood pressure, factor VIII activity, or von Willebrand factor activity in relation to the increases in catecholamines. The failure to demonstrate physiologic changes in heart rate and blood pressure in fetal lambs less than 120 days are in agreement with previous studies by Walker *et al.* (31). Failure to demonstrate a significant leukocyte or factor VIII response to catecholamines in the fetal lambs is in keeping with the lack of factor VIII and leukocyte response shown in preterm infants (27).

Minimal increases in the white blood cell count (15%), factor VIII (4%), factor XII (4.3%), von Willebrand ristocetin cofactor activity (5.9%), and slight decreases in mean arterial pressure (3.9 mm Hg), hemoglobin (1.2 g), and hematocrit (2.9%) were observed in both hypoxemic and control groups. Approximately 40 ml of blood was drawn from the fetal lambs during the initial experiments in both groups of animals. This volume represents approximately 25% of the estimated feto-placental blood volume at this gestational age and could explain the drop in hemoglobin, hematocrit, and the slight decrease in mean arterial pressure. How the other changes such as white blood cell count, factors VIII, XI, and von Willebrand ristocetin cofactor activities might also be related to this blood loss is unclear.

After the episode of hypoxemia, the animals were followed for 18 days. Samples were obtained from the fetuses three times a week to detect alterations of blood coagulation factor activities that might suggest a decreased synthesis by the liver or perhaps stimulation of maturation. No such changes were found. Changes in coagulation factor activities during the 18-day follow-up period as seen in Table 2 were minimal as expected from previous studies (17). Factor X activity did show a statistically significant increase during the 18-day period in control animals only. This increase in activity averaged 0.5% per day and was not seen in hypoxemic animals nor was a similar increase observed during this period of gestation in animals previously studied.

In summary, subjecting the lamb fetus to severe hypoxemia, pO_2 (14 mm Hg) lasting for 1 hr during the early part of the last trimester of pregnancy without acidosis is not associated with significant alterations of blood coagulation factor activities. There is no evidence that a hypoxic episode leads either to diminished or enhanced production of coagulation factors. Hypoxemia does not lead to activation of coagulation and disseminated intravascular coagulation although one of the fetuses who experienced acidemia did develop a transient increase in fibrin monomer concentration. Further study of exposure of fetuses to hypoxemia later in gestation such as just prior to delivery and the effects of acidemia in the fetus are needed before the potential complications of hypoxemic stress on blood coagulation are fully evaluated.

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