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Hydrops in Fetal Sheep from Rapid Induction of Anemia

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ABSTRACT. We operated on 14 singleton fetal sheep at 126 ± 3 d gestation and produced nonimmune anemia in 12 of them to study the mechanisms responsible for hydrops. Two fetuses served as controls. Partial exchange transfusions were performed daily to lower the hematocrit while we measured arterial blood gas tensions; IIb concentration; oxygen saturation; arterial oxygen content; aortic, central venous, and umbilical venous pressures; heart rate; plasma protein concentration; and colloid osmotic pressure. Hydrops developed in six of the fetuses and did not develop in six others, although both groups were anemic to the same degree, had similar total amounts of blood withdrawn based on kilograms of dry weight, and had similar dry weights. The fetuses who had hydrops became anemic more rapidly than the nonhydropic fetus (5.2 ± 1.9 *versus* 8.3 ± 2.7 d; $p < 0.05$) and had more blood exchanged each day (197 ± 56 *versus* 113 ± 28 mL/kg dry body wt/d; $p = 0.008$). Umbilical venous pressures increased in both hydropic and nonhydropic fetuses, but the central venous pressure became elevated only in the hydropic fetuses. Changes in heart rate, arterial pH and blood gas tensions, arterial oxygen content, plasma protein concentration, colloid osmotic pressure, and aortic pressure were similar in both groups. At autopsy the hydropic fetuses had 78 ± 47 mL of ascites and 20 ± 26 mL of pleural fluid. The water content of the hydropic fetuses and of the hydropic fetuses' placentas was greater than that of the nonhydropic fetuses. We conclude that a more rapid development of anemia is associated with hydrops in fetal sheep. The fetal sheep that became hydropic also had an elevated central venous pressure. (*Pediatr Res* 35: 560–564, 1994)

Abbreviations

IVC, inferior vena cava

Both immune and nonimmune fetal anemia have been associated with the development of hydrops fetalis. The mechanism underlying hydrops formation with anemia of either immune or nonimmune origin is unexplained. Most investigators have attributed the fetal edema to an increase in microvascular permeability to protein or to a reduction in plasma colloid osmotic pressure resulting from fetal hypoproteinemia (1–4).

We and others have performed rapid atrial pacing in the chronic fetal sheep preparation to model hydrops fetalis resulting from supraventricular tachycardia (5–7). Consistently, we have observed that edema forms early during the pacing protocol and has always been coincident with the appearance of an increased central venous pressure but in the absence of hypoxemia, acidosis, or hypoproteinemia. From these findings we concluded that an increased central venous pressure is the primary mechanism for hydrops formation with rapid atrial pacing in fetal sheep (5). We hypothesized that an elevated venous pressure would also be associated with the development of hydrops in cases of anemia.

We performed this experiment to produce simple, progressive, nonimmune anemia in fetal sheep to study the development of hydrops as the anemia advanced and to find whether a relationship existed between the development of hydrops and an increase in central venous pressure. We chose to produce nonimmune anemia to have more control over the rate of production of anemia and to eliminate other variables that might be introduced resulting from hemolysis.

MATERIALS AND METHODS

Surgical methods. We successfully operated on 14 single gestation ewes that ranged in gestation from 120 to 131 d. The nonhydropic fetuses' gestational age was 125 ± 3 d, and the hydropic fetuses' gestational age was 126 ± 4 d (mean ± SD). Using epidural anesthesia with 3 mL of 1% tetracaine HCl (Pontocaine Sanofi Winthrop Pharmaceuticals, New York, NY) accompanied by a continuous i.v. infusion of ketamine (1 g/L normal saline) for intraoperative sedation, we made a single, midline abdominal incision in the ewe; then, by previously described procedures, we inserted catheters into an artery and a vein in the fetal hock and advanced them such that their tips were in the fetal descending aorta and the abdominal IVC (5, 8). Next, we extended the initial uterine incision, extracted both fetal hind limbs, and delivered the fetus to the level of the xyphoid process. We then made a 2-cm midline incision in the fetal abdomen above the umbilicus, bluntly dissected free the intraabdominal umbilical vein, and inserted a polyvinyl catheter (inside diameter, 0.030 inches, outside diameter, 0.050 inches) directly into the vein, advancing it only 1 cm such that the tip rested in the vein proximal to the ductus venosus. After securing the catheter with a purse string suture, we closed the fetal abdominal incision. Before returning the fetus to the uterine cavity, we attached a large bore basket catheter to the fetal abdominal wall to measure amniotic fluid pressure. Then we returned the fetus to the uterine cavity, directed all plastic catheters through the ewe's abdomen to a site on her flank, and stored the catheters in a plastic bag attached to her flank. Postoperatively, we administered Combiotic (penicillin and dihydrostreptomycin; Pfizer Inc. Agriculture Division, New York, NY), 3 mL intramuscularly, daily for 5 d, and injected gentamicin (5 mg) and penicillin G (600 000 U) into the amniotic fluid daily. We gave each ewe buprenorphine (Norwich Eaton, Norwich, NY) (20 mg) intramuscularly daily for 3 d for analgesia. All animals recovered for at least 5 d before we began any experiments.

Experimental methods. We produced anemia in 12 of the fetuses by repeated partial exchange transfusions of 30 or 40 mL/pass depending on whether we estimated the fetal weight at the

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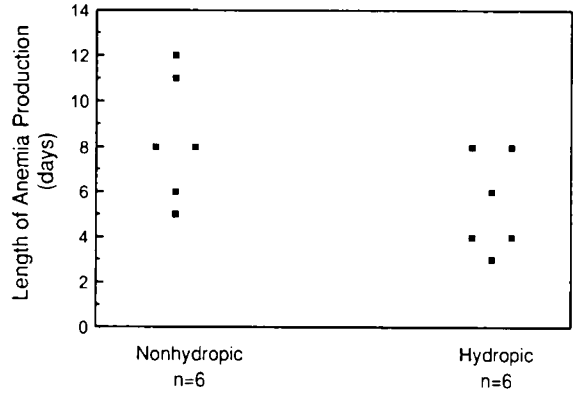

Fig. 1. Number of days over which fetal sheep were made anemic, nonhydropic *vs* hydropic. The nonhydropic fetal sheep were made anemic over 8.3 ± 2.7 d. The hydropic fetal sheep were made anemic over 5.2 ± 1.9 d ($p < 0.05$; unpaired t test). Some overlap of the groups exists.

time of surgery to be less or more than 3 kg. Each day we conducted two to four exchanges at 1.5- to 2-h intervals. We centrifuged the blood withdrawn at 2200 rpm at 21°C for 10 min; then we discarded the red cells and mixed the plasma with an aliquot of 0.9% sodium chloride to equal the initial volume of blood withdrawn. We then reinfused the resulting mixture of plasma and normal saline into the fetus for 1 h.

During each experiment we measured the following values: vascular pressures (aortic, IVC, and umbilical venous), heart rate, arterial pH and blood gas tensions, hematocrit, Hb, oxygen saturation, oxygen content, total plasma protein concentration, and plasma colloid osmotic pressure during a baseline period and then daily after the withdrawal of blood and reinfusion of the plasma/saline mixture. We measured vascular pressures with Statham p23dB transducers, recorded the pressures on a Gould 2800S recorder (Gould Inc., Cleveland, OH), and obtained the heart rate from the phasic aortic pressure tracing. We measured pH and arterial blood gas tensions with a Corning 178 pH/blood gas analyzer (Corning Medical and Scientific, Medfield, MA). We determined the Hb, oxygen saturation, and oxygen content values with an IL 482 Co-oximeter (Instrumentation Laboratory, Lexington, MA) and measured a spun hematocrit. We measured total protein concentration with the Bradford reaction and colloid osmotic pressure with a Wescor colloid osmometer (Wescor Inc., Logan, UT).

We continued to perform the partial exchange transfusions for as many days as possible, desiring to achieve an increase in central venous pressure. However, at the first indication of labor, determined by amniotic fluid pressure waves and an increase in the plasma protein concentration, we killed the ewe and fetus with Beuthanasia solution (Schering-Plough Animal Health, Phoenix, AZ). Two of the fetuses served as operated controls and were killed 5 d after surgery for determination of wet to dry ratios.

At autopsy, we weighed the fetuses and measured the quantity of fetal ascites and pleural fluid accumulations and their protein concentrations and the volume of amniotic fluid, if possible. Then we determined the total water content of the fetus and of the uterus and placenta together by the wet-to-dry method (9). The water content of the uterus and placenta were taken together because of the difficulty in precise separation.

Statistical methods. We separated the fetuses into either hy-

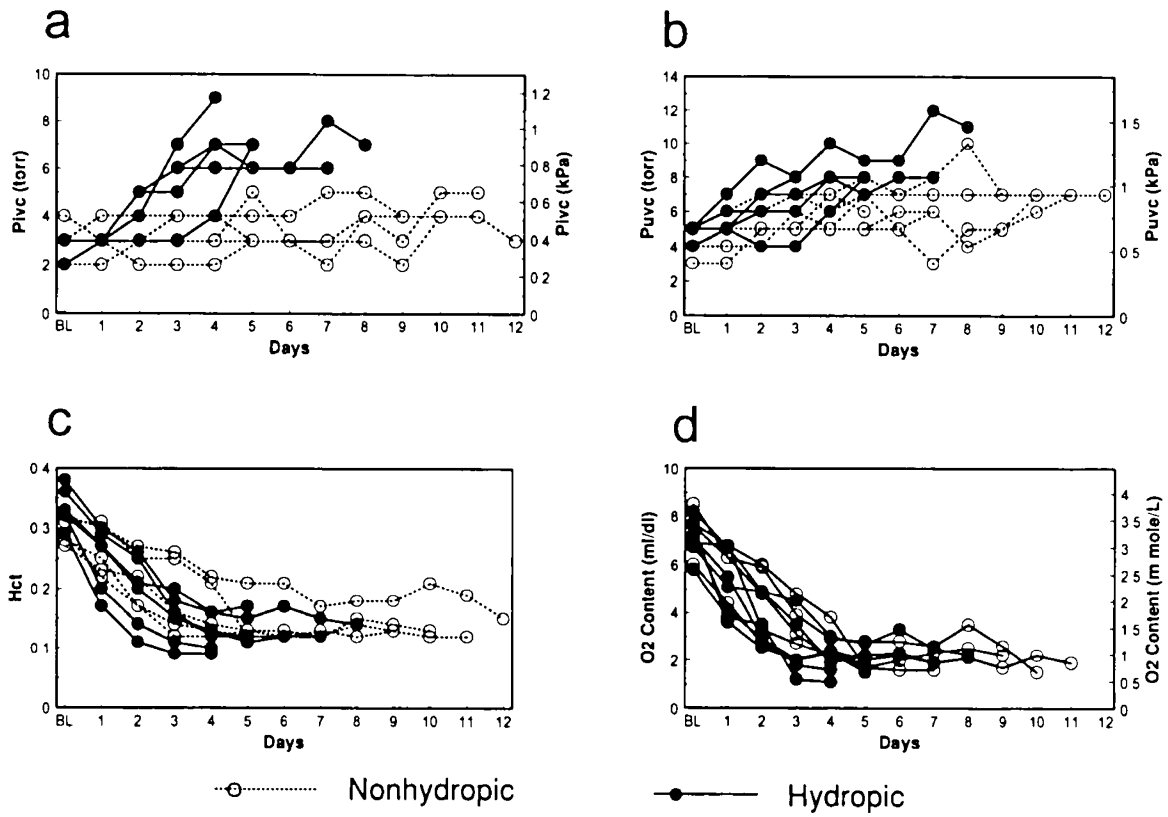

Fig. 2. *a*, Time course for change in IVC pressure. Some of the points overlap. IVC pressure does not change in the nonhydropic fetuses, whereas in the hydropic fetuses it increased from 0.31 to 0.91 kPa ($p < 0.001$). *b*, Time course for change in umbilical venous pressure. Umbilical venous pressure increased from 0.51 to 0.91 kPa in the nonhydropic group ($p < 0.025$) and from 0.67 to 1.04 kPa in the hydropic group ($p < 0.025$). *c*, Time course for change in hematocrit (*Hct*). The hematocrit value decreased to 0.31 in the nonhydropic fetuses and to 0.32 in the hydropic fetuses, but the decrease occurred over 5.2 ± 1.9 d in the hydropic fetuses and over 8.3 ± 2.7 d in the nonhydropic fetuses ($p < 0.05$). *d*, Time course for change in arterial oxygen content. Oxygen content decreased from 3.27 to 0.91 mmol/L in the nonhydropic fetuses ($p < 0.05$) and from 3.00 to 0.77 mmol/L in the hydropic fetuses ($p < 0.05$).

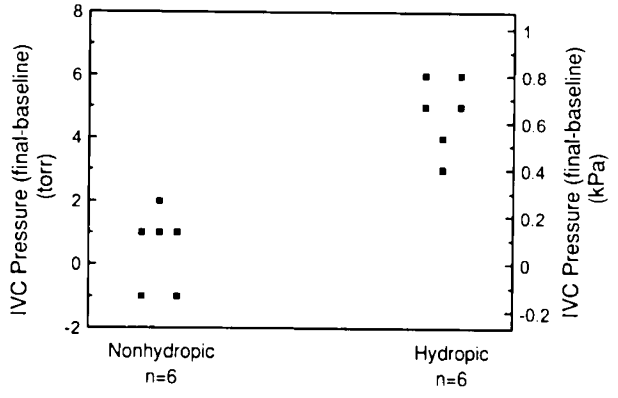

Fig. 3. Final baseline IVC pressures, nonhydropic *vs* hydropic. The differences between the final and the baseline IVC pressures in the nonhydropic fetuses were significantly less than those of the hydropic fetuses ($p < 0.001$; unpaired t test). This difference separated the fetuses into two distinct groups.

dropic or nonhydropic groups based on whether they had ascites and pleural effusions. We compared all data between the nonhydropic and hydropic groups by the unpaired t test, and we made comparisons within each group by two-way analysis of variance. We considered a p value less then 0.05 as statistically significant.

This protocol was reviewed and approved by the Baylor College of Medicine Animal Protocol Review Committee.

RESULTS

All the fetuses that were bled became anemic; hydrops developed in six, and six remained nonhydropic. The fetuses that did not have hydrops became anemic over a period of 8.3 ± 2.7 d, whereas those becoming hydropic were made anemic over a period of 5.2 ± 1.9 d (mean ± SD; $p < 0.05$; unpaired t test). The fetuses that did not become hydropic had an average of 113 ± 28 mL of blood/kg dry body weight/d withdrawn, 896 ± 187 mL of blood/kg dry body weight total, whereas the fetuses with hydrops had 197 ± 56 mL of blood/kg dry body weight/d withdrawn or 914 ± 128 mL of blood/kg dry body weight total (mean ± SD; $p = 0.008$ for milliliters of blood per kilogram dry body weight per day; $p = 0.84$ for total milliliters per kilogram dry body weight; unpaired t test). We used the dry body weight in calculating the amount of blood withdrawn per kilogram per day and the total amount of blood withdrawn per kilogram to alleviate the problem of the hydropic fetuses having a greater weight because of water accumulation. By using this method, we did not underestimate the amounts of blood withdrawn in the hydropic fetuses. Figure 1 shows that some overlap exists between the two groups with respect to the time during which the anemia was produced.

Figure 2*a* shows that the IVC pressure increased from 0.31 ± 0.07 kPa to 0.91 ± 0.16 kPa (2.3 ± 0.5 torr to 6.3 ± 0.8 torr) only in the hydropic group of anemic fetuses without any significant change in the IVC pressure in the nonhydropic fetuses (mean ± SD; $p < 0.001$; unpaired t test). The umbilical venous pressure increased in both nonhydropic and hydropic fetuses but to a greater extent in the hydropic fetuses (Fig. 2*b*). Figure 2*c* and 2*d* shows that the hematocrit value and the arterial oxygen content decreased in both groups. Figure 3 shows that when the baseline central venous pressure was subtracted from the final value for central venous pressure, the fetuses fell into two distinct, hydropic and nonhydropic groups.

The production of anemia by partial exchange transfusion did not affect aortic pressure. The heart rate increased in both groups but achieved significance only in the hydropic group (Table 1). Production of anemia resulted in small decreases in pH and arterial oxygen tension and increases in arterial carbon dioxide tension in both hydropic and nonhydropic fetuses. Repeated partial exchange transfusions resulted in a 64% reduction in the Hb level in the nonhydropic group and a 69% reduction of the Hb level in the hydropic group. The plasma protein concentration and plasma colloid osmotic pressure increased with development of anemia in both groups of fetuses (Table 2).

At autopsy, the two controls that underwent operations weighed 3.80 ± 0.55 kg, the nonhydropic fetuses weighed 3.22 ± 0.69 kg, and the hydropic fetuses weighed 3.47 ± 0.55 kg (mean ± SD; $p = 0.47$). The two controls that underwent operations and the nonhydropic group of fetuses did not have measurable ascites or pleural fluid. The hydropic group had 78 ± 47 mL of ascites with a protein concentration that was 0.76 that of plasma and 20 ± 26 mL of pleural fluid that had a protein concentration that was 0.67 that of plasma (mean ± SD). The dry weight of the nonhydropic fetuses was 616 ± 62 g; the dry weight of the hydropic fetuses was 608 ± 58 g (mean ± SD). The body water content of the nonhydropic fetuses was 80.9 ± 0.7% of wet weight and the body water content of the hydropic fetuses was 83.2 ± 1.5% of wet weight (mean ± SD; $p < 0.025$; unpaired t test). The water content of the two controls that underwent operations was 80.2 ± 0.9% of their wet weight. The nonhydropic placenta-uterus unit weighed 1.117 ± 0.214 kg; the hydropic unit weighed 2.184 ± 0.510 kg ($p < 0.01$). The water content of the uteri and placentas of the nonhydropic fetuses was 86.8 ± 1.0% compared with 91.6 ± 1.5% of the wet weight of the uteri and placentas of the hydropic fetuses (mean ± SD; $p < 0.005$; unpaired t test). The volume of amniotic fluid in two hydropic fetuses was 1.600 L and 0.918 L; the volume in three nonhydropic fetuses was 0.450 L, 0.235 L, and 0.638 L; and the volume in the two control fetuses was 0.375 L and 0.417 L.

DISCUSSION

Our studies show that the rapid production of severe anemia (5 ±2 d) led to generalized edema associated with an elevated central venous pressure and placental edema in the fetal sheep. When a similar degree of anemia was produced for a longer time period (8 ± 3 d), generalized and placental edema did not occur, and the central venous pressure was not elevated above control values.

Heart failure developing in the fetuses that were bled more rapidly with a resultant increase in IVC pressure is an attractive explanation for the appearance of hydrops. Hydrops only devel-

Table 1. *Effect of partial exchange transfusions on heart rate and mean vascular pressures in fetal sheep**

Condition	Time (d)	HR (beats/min)	PAo (kPa)	P_{UV} (kPa)	P_{IVC} (kPa)
Nonhydropic/baseline	0	175 ± 15	5.12 ± 0.63	0.51 ± 0.16	0.43 ± 0.13
Nonhydropic/final	8.3 ± 2.7†	189 ± 19	5.47 ± 0.27	0.83 ± 0.11†	0.49 ± 0.14
Hydropic/baseline	0	180 ± 8	5.56 ± 0.13	0.67 ± 0.14	0.31 ± 0.07
Hydropic/final	5.2 ± 1.9†‡	198 ± 13†	5.47 ± 1.07	1.04 ± 0.18†	0.91 ± 0.16†§

* Conversion: 1 kPa = 7.5006 torr. Nonhydropic, $n = 6$; hydropic, $n = 6$. Mean ± SD. HR, heart rate; Ao, aortic; UV, umbilical vein.

† Value different from baseline, two-way analysis of variance, $p < 0.025$.

‡ Nonhydropic and hydropic final values different, unpaired t test, $p < 0.05$.

§ Nonhydropic and hydropic final values different, unpaired t test, $p < 0.001$.

Table 2. *Effect of partial exchange transfusions on arterial blood gas tensions, Hb, Hct, COP, and total protein concentration in fetal sheep**

Condition	pH	$Paco_2$ (kPa)	Pao_2 (kPa)	O_2 content (mmol/L)	Hb (g/L)	Hct	COP (kPa)	Protein (g/L)
Nonhydropic/baseline	7.40 ± 0.04	6.36 ± 0.40	2.67 ± 0.37	3.27 ± 0.42	92.0 ± 11.0	0.31 ± 0.04	2.15 ± .023	36.0 ± 3.0
Nonhydropic/final	7.35 ± 0.05†	7.12 ± 0.43†	2.04 ± 0.27†	0.91 ± 0.19†	33.0 ± 2.0†	0.13 ± 0.02†	2.65 ± 0.37†	41.0 ± 4.0†
Hydropic/baseline	7.41 ± 0.03	6.24 ± 1.01	2.80 ± 0.33	3.00 ± 0.45	93.0 ± 6.0	0.32 ± 0.02	2.21 ± 0.21	36.0 ± 2.0
Hydropic/final	7.32 ± 0.08†	6.86 ± 0.99†	2.04 ± 0.35†	0.77 ± 0.19†	29.0 ± 5.0†	0.12 ± 0.02†	2.56 ± 0.40†	39.0 ± 4.0†

* Conversion: 1 kPa = 7.5006 torr. Mean ± SD. Nonhydropic, $n = 6$; hydropic, $n = 6$. $Paco_2$, arterial carbon dioxide tension; Pao_2, arterial oxygen tension; Hct, hematocrit; COP, colloid osmotic pressure; protein, total protein concentration.

† Final value different from baseline, paired t test, $p < 0.05$.

oped in fetuses whose inferior cava pressure was elevated. Other than the differences in time during which the fetuses were made anemic, why an increased central venous pressure in response to anemia developed in some fetuses but not in others remains elusive and cannot be answered by our data. A high state of cardiac output may have been produced in both groups of fetuses because the umbilical venous pressure increased in both groups and the arterial oxygen content decreased in both groups, but the longer time taken to produce anemia in the nonhydropic fetuses may have allowed time for compensation of the failure to occur, preventing the increase in IVC pressure and hydrops.

We previously demonstrated that an increase in central venous pressure results in both increased transvascular fluid filtration and in impairment of lymphatic return of fluid back to the venous circulation, resulting in edema (10, 11). *In vivo,* central venous pressure is the outflow pressure for the thoracic duct, and relatively small increases in central venous pressure in the fetal sheep are capable of impairing return of lymph to the circulation, resulting in edema. Our finding that an elevated umbilical venous pressure does not necessarily correspond with an elevated central venous pressure is in accordance with perinatologists not necessarily finding a relationship between the umbilical venous pressure and hydrops at cordocentesis (12).

None of the other experimental models of fetal anemia have achieved an Hb concentration as low as 40 g/L, which may explain why these experiments were unsuccessful in producing hydrops (13, 14). This lack of correlation between degree of anemia and hydrops is supported by other experiments in fetal lambs showing that experimental anemia alone does not result in hydrops (13, 14).

The degree of placental edema in the hydropic group of fetuses is striking. Hydrops is an exaggeration of the normal accumulation of water by the fetal compartment (fetus, placenta, and amniotic fluid) that is occurring throughout pregnancy, and the ultimate source of this water is the mother (15). The factors that are responsible for fetal edema are likely responsible for edema of this entire fetal compartment. It is possible that the edematous placenta impairs normal fluid exchange between the mother and the fetus, promoting hydrops formation. Alternatively, the edema of the placenta as well as the generalized edema of the fetus could result from an increase in vascular protein permeability, although we think this less likely because hypoproteinemia was not present in our study.

Obviously, large volumes of fetal blood were manipulated in this study. Although particular attention was given to sterile technique, it is possible that our fetuses were infected; we do not have cultures to prove that they were not. From our previous experience, infected fetuses have had severe acidosis and hypoxemia and have had a foul odor at autopsy, occasionally with a small amount of blood-tinged ascites. Although the ascites and pleural fluid protein concentrations of 0.76 and 0.67 of plasma values appear elevated, these results are similar to values that we have obtained in other studies (5) and are most compatible with pressure-induced transvascular filtration of fluid.

In summary, rapid production of severe anemia by partial exchange transfusions in fetal sheep causes hydrops associated with an increased central venous pressure and placental edema. Production of the same degree of anemia for a longer period of time is not associated with hydrops, placental edema, or an increased central venous pressure. An increased umbilical venous pressure found with anemia in fetal sheep does not assure an increased central venous pressure.

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