Intramembranous absorption rate is unaffected by changes in amniotic fluid composition

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Anderson, D., Q. Yang, A. Hohimer, J. Faber, G. Giraud, and L. Davis. Intramembranous absorption rate is unaffected by changes in amniotic fluid composition. Am J Physiol Renal Physiol 288: F964–F968, 2005.—Experiments were performed to determine the effect of amniotic fluid dilution on the rate of intramembranous absorption. Seven fetal sheep at 118 days gestation were instrumented with a shunt between the trachea and esophagus and arterial and venous vascular catheters. In addition, the urachus of the fetal bladder was ligated, and a catheter was placed in the bladder. Ligation of the urachus does not interfere with urine flow into the amnion. After 5 days of recovery, fetuses were randomly assigned to one of two protocols; all fetuses completed both protocols. In the fetuses in the control period, continuous urine flow measurement was begun. In the fetuses assigned to the isovolumic dilution protocol, continuous urine flow measurement was also begun and, in addition, amniotic fluid was continually exchanged with lactated Ringer solution on an isovolumic basis. After 3–4 days, fetal blood pressures and amniotic fluid volumes were determined. Amniotic fluid volumes were determined by drainage. Each fetus was then assigned to the remaining protocol. The presence of the tracheal-esophageal shunt and ligation of the urachus allowed the rate of intramembranous absorption to be calculated. Isovolumic exchange showed no effect on fetal vascular pressures, blood-gas values, or urine production. We could demonstrate no effect of isovolumic dilution of amniotic fluid on its volume. However, we were able to demonstrate an inverse relationship between amniotic fluid volume and intramembranous absorption (P < 0.02).

The rate of change in volume of amniotic fluid is determined by the difference between the inflow of fluid and the outflow of fluid into the amniotic space. The most significant sources of fluid are fetal urine and lung fluid secretions, whereas the loss of fluid occurs primarily through swallowing and intramembranous absorption (1–3, 8). Intramembranous absorption refers to the movement of fluid from the amniotic fluid into the fetal blood perfusing the amnion and chorion (11) and ranges from ~250 to 500 ml/day (8). While intramembranous absorption rate will change in response to a variety of fetal perturbations including hypoxia (13, 16), esophageal ligation (9), and changes in the osmotic gradient between the fetal plasma and the amniotic fluid (7), the basis for any regulatory control over the intramembranous absorption rate is unknown.

The present series of experiments was designed to test the hypothesis that the rate of intramembranous absorption is independent of the composition of the amniotic fluid. The contribution of intramembranous absorption was isolated by continually measuring urine flow and eliminating the contributions of lung fluid secretion and fetal swallowing through creation of a fistula between the esophagus and the trachea. Under these conditions, the composition of the amniotic fluid is dependent on the contribution of the urine flow and the contribution of diffusional exchange between the amniotic fluid and the fetal plasma. In an attempt to determine whether the composition of the amniotic fluid affected the rate of intramembranous absorption, both were measured under control conditions and after amniotic fluid was isovolumically diluted with lactated Ringer solution.

MATERIALS AND METHODS

All surgical and experimental procedures have been approved by the Institutional Animal Care and Use Committee (IACUC) of the Oregon Health and Sciences University.

Surgical protocol. Seven pregnant sheep carrying single fetuses were obtained from a commercial source and underwent surgery at 118 ± 1 (SE) days of gestation. Ewes were premedicated with 7.5 mg of atropine, 400 mg of ketamine, and 10 mg of diazepam. After intubation, anesthesia was maintained using 0.5–1.5% halothane or isoflurane in a mixture of oxygen and nitrous oxide. Gas concentrations were adjusted, and additional doses of diazepam were given when necessary to guarantee an adequate surgical plane of anesthesia in both the ewe and the fetus.

Surgery was performed using sterile procedures. An abdominal incision was made on the ewe. The uterus was exposed, and an incision was made in the uterus over the fetal head. To ensure good apposition of the fetal membranes during closure of the uterus, the membranes were sutured to the uterus using interrupted stitches. The fetal head was exposed. Polyvinyl catheters were placed in a jugular vein and a carotid artery. One end of a short length of large-bore tubing was placed in the esophagus, directed toward the stomach, and the other end was placed in the trachea, directed toward the lungs. This created a fistula between the lungs and the stomach, preventing lung fluids from making any contribution to the amniotic fluid and preventing any removal of amniotic fluid through fetal swallowing, yet allowing movement of fluid between the lungs and stomach. The cranial ends of the trachea and esophagus were separately ligated. The fetal incisions were repaired. A catheter for measuring amniotic fluid pressure was attached to the fetal neck. A second catheter, consisting of a length of large-bore tubing attached to a screw-top vial with multiple side holes, was attached to the side of the neck. This catheter would be used for later drainage of amniotic fluid (7). The fetal head was returned to the uterus, and the uterine incision was repaired.

A second uterine incision was made over the hindquarters of the fetus. As before, the membranes were securely attached to the myometrium. The fetal hindquarters were removed from the uterus.
amniotic fluid catheter not located near the infusion catheter. Lactated Ringer solution was chosen because its composition mimics that of amniotic fluid with respect to its ionic composition. Therefore, the nonelectrolytes in the amniotic fluid would undergo dilution without major changes in the ionic composition and the osmolality of the amniotic fluid. Thus the amniotic fluid was diluted without changing its volume. Urine flow was also measured continuously during this period and returned as in the control period. At the end of the dilution period, arterial and venous blood pressures were measured, plasma, urine, and amniotic fluid samples were collected, and the amniotic fluid volume was measured. Therefore, the only net inflow in both the control and the dilution experiments was the urine produced by the fetus.

On completion of one protocol, the fetus was assigned to the remaining protocol. Six fetuses completed both protocols, with the seventh fetus completing two control and two wash protocols. Its results were averaged before being included in the statistical analyses. In one fetus during the control period, and in another during the dilution period, the urinary bladder catheter failed to drain. Although urine still entered the amniotic fluid by natural urination, its volume could not be determined. These two periods yielded relevant data only with regard to the change in amniotic fluid volume due to exchange.

Analytic techniques. Arterial, venous, and amniotic fluid pressures were measured using sterile Abbot Transpac-IV transducers. Pressures were recorded using Macintosh-based software. All transducers were calibrated to better than 1% each day and zeroed before each measurement. Vascular pressures are referred to amniotic fluid pressure as zero.

Heparinized arterial blood samples were collected for determination of blood gases, pH, oxygen content (Instrumentation Laboratories IL306 and IL482), and hematocrit. Plasma and amniotic fluid samples were frozen for later determination of Na⁺, K⁺, and Cl⁻ concentrations (Beckman Labyte 810 electrode system) and freezing-point depression osmolalities (Advanced Micro Osmometer, model 3MO).

The Ringer solution was commercial lactated Ringer USP with a stated composition of (in meq/l) 130 Na⁺, 4 K⁺, 110 Cl⁻, 28 lactate, and 3 Ca²⁺ and an osmolality of 275 mosmol/kgH₂O. Necropsy. At the completion of the experiment, the ewes were euthanized using a commercial euthanasia solution approved by the IACUC. Necropsies were performed to verify uterine healing, catheter position, and no leakage from the incision site. All fetuses appeared normal and of appropriate size for gestational age.

### RESULTS

Fetal age was comparable during the control period (128 ± 2 days) and during the amniotic fluid dilution period (131 ± 1 days). There were no statistically significant differences in arterial blood-gas values between the two groups (Table 1). The two groups of fetuses were also indistinguishable hemodynamically. During the control period, fetal arterial blood pressure was 44 ± 1 mmHg, venous blood pressure was 3.1 ± 1.0 mmHg (n = 6), and heart rate was 177 ± 7 beats/min (bpm). During the amniotic dilution period, fetal arterial blood pressure was 45 ± 1 mmHg, venous blood pressure was 3.8 ± 1.1 mmHg, and heart rate was 181 ± 10 bpm. During the control period, the following determinations were made in fetal arterial blood: pH = 7.35 ± 0.01, Pco2 = 51.3 ± 1.2 Torr, Po2 = 21 ± 1 Torr, oxygen content = 8.1 ± 0.6 ml O₂/dl blood, and hematocrit = 33 ± 2%. Amniotic fluid dilution had no statistically significant effect on these values. During this period, pH = 7.37 ± 0.01, Pco2 = 49.1 ± 0.8 Torr, Po2 = 21 ± 1 Torr, oxygen content = 8.1 ± 0.6 ml O₂/dl blood, and hematocrit = 33 ± 2%. Amniotic fluid dilution had no statistically significant effect on these values. During this period, pH = 7.37 ± 0.01, Pco2 = 49.1 ± 0.8 Torr, Po2 = 21 ± 1 Torr, oxygen content = 8.1 ± 0.6 ml O₂/dl blood, and hematocrit = 33 ± 2%.
22 ± 1 mmHg, oxygen content = 8.6 ± 0.5 ml O2/dl blood, and hematocrit = 34 ± 2% (n = 6 due to equipment failure). Thus amniotic fluid dilution had no discernable effect on the fetus.

Osmalolities and electrolyte concentrations. Osmalolities and electrolyte concentrations for fetal plasma, fetal urine, and amniotic fluid are summarized in Table 1. The values collected after amniotic fluid dilution (expressed as a percentage of the control value and compared with 100%) showed no statistically significant changes for any of these variables. The osmotic gradient and concentration gradients for Na⁺, K⁺, and Cl⁻ between fetal plasma and amniotic fluid were also examined. When the control period was compared with the dilution period, there were no statistically significant changes.

Urine flow and amniotic fluid volume. The relationship between urine flow and amniotic fluid volume was examined. Using least squares linear regression, no statistically significant relationships could be demonstrated during the control period, during the period of amniotic fluid dilution, or after the data from both of these periods were combined.

Intramembranous absorption. After amniotic fluid drainage, each control period and each dilution period began with a known volume of fluid. Because the contributions of lung fluid secretion and swallowing to amniotic fluid volume have been eliminated, and urine production is known, total intramembranous absorption is equal to the difference between the initial volume and the final volume plus the total urine volume. The results are reported in Table 2. Urine production and intramembranous absorption are available for only six fetuses due to failure of the bladder catheter in two fetuses. While each of the two fetuses successfully completed both a control period and an amniotic fluid dilution period, urine production could only be determined in one period for each fetus. There were no statistically significant differences for the two groups of fetuses with respect to the amniotic fluid volume drained at the end (P = 0.62; n = 7), urine production (P = 0.38; n = 6), or intramembranous absorption rate (P = 0.44; n = 6).

The relationship between the amniotic fluid volume after the control period and the amniotic fluid volume after the dilution period was examined. A strong and highly significant relationship existed between the two (P = 0.0005; r² = 0.93). Therefore, amniotic fluid dilution had no demonstrable effect on the tendency of a fetus to have a relatively high or relatively low amniotic fluid volume (Fig. 1).

### Table 1. Osmalologies and electrolytes in various fluids in 7 fetal sheep after completion of control period and dilution period

<table>
<thead>
<tr>
<th>Fluid Type</th>
<th>Osmolality (mosmol/kg H2O)</th>
<th>Na⁺ (meq/l)</th>
<th>K⁺ (meq/l)</th>
<th>Cl⁻ (meq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal plasma</td>
<td>284 ± 3</td>
<td>136.4 ± 1.8</td>
<td>3.6 ± 0.2</td>
<td>110.3 ± 3.2</td>
</tr>
<tr>
<td>Fetal urine</td>
<td>111 ± 9</td>
<td>27.7 ± 2.7</td>
<td>5.4 ± 1.9</td>
<td>14.4 ± 1.6</td>
</tr>
<tr>
<td>Amniotic fluid</td>
<td>263 ± 5</td>
<td>137 ± 3.3</td>
<td>6.1 ± 2.3</td>
<td>98 ± 2.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7, except n = 6 for fetal urine and amniotic fluid osmolality during dilution studies. Brackets denote concentration.

### Table 2. Comparison between control period and dilution period

<table>
<thead>
<tr>
<th>Period</th>
<th>Drained amniotic fluid volume (n = 7), ml</th>
<th>Intramembranous absorption (n = 6), ml</th>
<th>Intramembranous absorption rate, ml/h</th>
<th>Duration of experiment, h</th>
<th>Total volume of fluid exchanged, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control period</td>
<td>892 ± 240</td>
<td>3,111 ± 366</td>
<td>3,081 ± 558</td>
<td>79 ± 5</td>
<td>11,039 ± 1,581</td>
</tr>
<tr>
<td>Dilution period</td>
<td>1,104 ± 371</td>
<td>2,844 ± 597</td>
<td>2,526 ± 588</td>
<td>85 ± 5</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE.

A significant relationship (P < 0.02) between amniotic fluid volume and intramembranous absorption rate was demonstrated (Fig. 2). From this result, it became apparent that there is an interaction between amniotic fluid volume and intramembranous absorption; those fetuses with a low amniotic fluid volume also have a high intramembranous absorption, whereas those fetuses with a relatively large amniotic fluid volume have a relatively low rate of intramembranous fluid absorption after both the control and the dilution periods.

### DISCUSSION

The present series of experiments describes the relationship between intramembranous absorption and amniotic fluid volume in fetal sheep in which swallowing and lung secretions have been eliminated. Esophageal ligation eliminates one of the pathways for fluid to leave the amniotic compartment. Without the contribution of intramembranous absorption, one would predict that polyhydramnios would result with the elimination of this pathway. While it has been reported that inflation of an occluder around the esophagus will cause amniotic fluid volume to increase from ~580 to 1,550 ml (8), the long-term responses are less clear. Wintour et al. (17) have reported no change in amniotic fluid volume after surgical ligation of the esophagus. This is in contrast to Matsumoto et
Intramembranous absorption is known to be a significant pathway for fluid movement from the amniotic fluid to the fetus. Water injected into the amniotic fluid will enter the fetus, even in the presence of esophageal ligation (9), as will arginine vasopressin (10). Intramembranous absorption will increase in response to severe hypoxia (14, 16) or in response to excess volumes of Ringer solution (6). Increases in intramembranous absorption would serve to limit increases in amniotic fluid volume. While intramembranous absorption is dependent on the crystalloid osmotic pressure difference between amniotic fluid and fetal plasma, this effect is relatively weak and significant volumes of fluid appear to also move through an additional pathway that is permeable to protein (7). In the present series of experiments, there were no significant changes in amniotic fluid osmolality.

In fetuses exposed to prolonged hypoxia, Matsumoto et al. (14) measured an increase in urine flow with no increase in amniotic fluid volume. From these results, they concluded that intramembranous absorption also increased. Because our fetuses appeared to be well oxygenated throughout the study, we do not believe that intramembranous absorption underwent any alteration as a result of hypoxia.

Matsumoto et al. (12) also measured an increase in vascular endothelial growth factor after hypoxia, concluding that vascular endothelial growth factor leads to greater vessel growth in the membranes and enhanced fluid uptake via intramembranous absorption. While vascular endothelial growth factor was not measured in the fetuses in the experiments reported here, it could potentially provide an explanation for the normal variability of amniotic fluid volume seen in near-term pregnant sheep.

In a separate series of experiments (18), we examined the effects of amniotic fluid dilution in fetuses with intact tracheas and esophagi. Intramembranous absorption rates could not be measured in these fetuses because unknown amounts of fluid were entering the amniotic fluid from the lungs and unknown amounts of fluid were leaving the amniotic fluid through swallowing. However, it was possible to examine the effect of isovolumic dilution of amniotic fluid with lactated Ringer solution on amniotic fluid volume. The experimental protocol of these experiments was identical to those just presented except that the trachea and esophagus of each fetus were intact. Surprisingly, amniotic dilution under these conditions resulted in a near doubling of the amniotic fluid volume compared with control (Fig. 3). It is known that the volume of lung secretions does not appear to regulate amniotic fluid volume (4). In
addition, the role of swallowing in the regulation of amniotic fluid volume is not clear (15). However, a comparison of the results from the experiments in which the trachea and esophagus are intact to these experiments with a shunt between the trachea and the esophagus can only lead to the conclusion that either swallowing or lung secretions are making an, as yet, undetermined contribution to establishing amniotic fluid volume. This would be consistent with the speculation of Matsumoto et al. (13) that fetal lung secretions contain a factor that increases intramembranous permeability. Further investigation will be necessary to identify any possible regulatory factor.

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GRANTS

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