Intramembranous absorption rate is unaffected by changes in amniotic fluid composition

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Intramembranous absorption rate is unaffected by changes in amniotic fluid composition. Am J Physiol Renal Physiol 288: F964–F968, 2005; doi:10.1152/ajprenal.00407.2004.—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 amnio. 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 the 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).

fetal fluid balance

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.

Surgeries were 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.

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Catheters were placed in a fetal pedal artery and vein. An incision was made on the fetal abdomen, over the bladder. The neck of the bladder was ligated, preventing urine entry into the allantoic sac. Therefore, the total urine output entered the amniotic fluid. A catheter was placed in the bladder. The fetal abdominal incision was repaired. Additional amniotic fluid catheters were placed on the fetal hindlimb and at the abdominal incision. Two large-bore catheters attached to screw-top vials were also positioned on the fetus, one at the hock and one near the abdominal incision. About a half-liter of saline was added to the uterus. Addition of the fluid aided in the return of the fetus to the uterus and replaced any fluid lost during surgery on the fetus. The uterine incision was repaired. The vascular catheters were filled with a 50% heparin solution. One million units of penicillin were added to the amniotic fluid; no other antibiotics were routinely used. The fetal catheters were then exteriorized, and the maternal incision was repaired. The catheters were stored in nylon pouches attached to the flank of the ewe. The ewes received 0.6 mg of buprenorphine twice a day for 2 days for routine postoperative pain prevention. The sheep were given 5 ± 1 days (range 3–9 days) for recovery. Experimental protocol. On the day of the experiment, the ewe was brought to the laboratory and placed in a stanchion with free access to food and water and the ability to stand or lie at will. Continuous urine flow measurement was started. The system for continuous measurement of urine flow has been previously described (7). Briefly, the bladder catheter was allowed to continuously drain into a sterile jar instrumented with electrodes. The outflow of the jar was connected to a parallel system. The system used the same-diameter pump tubing as the urine-return system and was placed in the same roller pump, fluid was pumped only when the urine-return system was triggered; daily measurement of this fluid volume allowed determination of urine volume.

Amniotic fluid volumes were determined using a modification of the technique developed by Dickson et al. (5). This was accomplished by connecting each of the three large-bore amniotic fluid catheters to evacuated bottles and draining the amniotic fluid for a minimum of 20 min while the urine pump was stopped. In every case, drainage continued for ~5 min after the time point when significant amounts of fluid no longer entered the evacuated bottles. Once the drainage was completed, the volume of fluid collected was measured. In the first two experiments, the volume of the drained amniotic fluid was measured and returned to the uterus. However, in subsequent experiments, 1 liter of warm lactated Ringer solution was returned. The use of lactated Ringer solution had the advantage of minimizing the risk of infection and establishing a baseline amniotic fluid volume that was identical for all fetuses. This also allowed us to more easily compare the results from these experiments with our previously published experiments (18). The duration of the experiments was long enough to allow the establishment of a new steady state, making the initial composition of the replacement fluid of no great consequence.

Fetuses were then randomly assigned to either the control protocol or the amniotic fluid dilution protocol. However, all fetuses participated in both protocols. The control protocol consisted of measuring urine flow continuously for 3 days; the returned urine constituted the only inflow into the amnion. At the end of this period, arterial and venous blood pressures were measured, plasma, urine, and amniotic fluid samples were collected, and the amniotic fluid volume was measured.

The 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 rezeroed 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 IL 306 and IL 482), and hematocrit. Plasma and amniotic fluid samples were frozen for later determination of Na+, K+, and Cl− concentrations (Beckman Lablyte 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 Ca++ 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.

The Ringer solution was used for the initial determination of blood gases, pH, oxygen content (Instrumentation Laboratories IL 306 and IL 482), and hematocrit. Plasma and amniotic fluid samples were frozen for later determination of Na+, K+, and Cl− concentrations (Beckman Lablyte 810 electrode system) and freezing-point depression osmolalities (Advanced Micro Osmometer, model 3MO).

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 ± 0.0 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 ± 1.4 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, P O 2 = 51.3 ± 1.8 Torr, P O 2 = 21 ± 1 Torr, oxygen content = 8 ± 0.06 ml O 2/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, P O 2 = 49.1 ± 0.8 Torr, P O 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.

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

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Osmolality, mosmol/kgH₂O</th>
<th>[Na⁺], meq/l</th>
<th>[K⁺], meq/l</th>
<th>[Cl⁻], meq/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control experiments</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<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</td>
<td>6.1±2.3</td>
<td>98±2</td>
</tr>
<tr>
<td>Isovolumetric dilution experiments</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetal plasma</td>
<td>281±2</td>
<td>139.5±1.0</td>
<td>3.7±0.2</td>
<td>111.6±1.4</td>
</tr>
<tr>
<td>Fetal urine</td>
<td>94±7</td>
<td>22.7±2.8</td>
<td>3.9±2.4</td>
<td>13.4±1.9</td>
</tr>
<tr>
<td>Amniotic fluid</td>
<td>261±3</td>
<td>141±4</td>
<td>4.1±0.2</td>
<td>104±3</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.

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 1,530 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

![Graph](http://ajprenal.physiology.org/)

Fig. 2. Relationship between amniotic fluid volume and rate of intramembranous absorption by least squares linear regression. Amniotic fluid volume and intramembranous absorption rate are inversely related. P < 0.02; 95% confidence limits shown.

![Graph](http://ajprenal.physiology.org/)

Fig. 3. Relationship between amniotic fluid volume during the control period and after a period of isovolumic dilution using lactated Ringer solution. Solid lines describe the intact group and the trachea-esophageal (TE) fistula group. Dashed lines represent fetuses with intact tracheas and esophagi (data from Ref. 18). The slope of the line describing the intact group of fetuses is significantly greater than the slope of the line describing the fetuses with the trachea-esophageal shunt (P < 0.0006).
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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