Regulatory response of intramembranous absorption of amniotic fluid to infusion of exogenous fluid in sheep

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Faber, J. Job, and Debra F. Anderson. Regulatory response of intramembranous absorption of amniotic fluid to infusion of exogenous fluid in sheep. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R236-R242, 1999.—Six fetal sheep were operated on at 118 to 121 days of gestation. The pulmonary end of the trachea was connected to the gastric end of the esophagus with a section of tubing. This left urine as the only source of amniotic fluid and intramembranous absorption as sole exit. Multiple indwelling fetal vascular, intra-amniotic, allantoic, and a fetal bladder catheter were placed. Beginning 5 days after surgery, all urine was drained from the bladder and immediately reinfused into the amniotic sac to monitor urine production rate. After 4 days of urine infusion alone, the urine infusion was augmented for 6 days with an intra-amniotic infusion of Ringer solution. Amniotic and allantoic fluid volumes were measured at autopsy. During the period of Ringer infusion, intramembranous absorption of amniotic fluid increased by more than 1,191±186 (SE) ml/day (P<0.002) and the rates of Na⁺ and Cl⁻ absorption increased to more than five times (P<0.005) and eight times (P<0.005) their initial values. Only one of six fetuses had polyhydramnios. It is concluded that intramembranous absorption of amniotic fluid makes a strong regulatory adjustment in response to an abnormal increase in inflow of exogenous fluid.

fetus; conceptus; extrafetal water; homeostasis; polyhydramnios; oligohydramnios; urine; lung fluid

THE VOLUME OF AMNIOTIC fluid in sheep fetuses of 125 to 135 days of gestational age is of the order of 700 ml (27). There are only two primary sources and two primary routes of clearance of amniotic fluid during the latter half of gestation (5). The sources are fetal urine and lung fluid, and the routes of clearance are fetal swallowing and intramembranous absorption (4, 19), where the term intramembranous absorption denotes absorption into the fetal capillaries of the chorionamnion including capillaries covering the surface of the placenta and the cord.

Because amniotic fluid turns over at a rate of about once per day, the rates of fluid production and absorption must be equal to prevent the volume from becoming progressively larger or smaller. This implies a form of regulation with a time constant of less than 1 day. Although each of the two inflows and two outflows have been demonstrated to change under the influence of various stimuli (2, 4, 9, 12, 20), such changes could constitute disturbances of a normal volume or corrections of an abnormal volume. We report the new finding that intramembranous absorption increases when amniotic fluid inflow is increased by Ringer infusion and that under the conditions of these present experiments intramembranous absorption assists in the regulation of amniotic fluid volume.

The principle of the experimental protocol was to prevent the amniotic inflow of fluid from the lung and the absorption of amniotic fluid by swallowing. This left urine production as the source of amniotic fluid and intramembranous absorption as the path of exit. We measured urine inflow into the amnion by continuous drainage of urine from the fetal bladder and immediate reinfusion of the drained urine into the amnion. In what we will call the first period of the experiment, this procedure was continued for 4 days to approximate a new steady state. In the second period (of 6 days), the urinary source of amniotic fluid was continued but augmented by an intra-amniotic infusion of Ringer solution. Amniotic fluid collected at autopsy demonstrated the existence of near normal amniotic fluid volumes (except in 1 case), indicating that intramembranous absorption had almost completely adapted to a great increase in inflow of fluid into the amnion.

In the sheep, there is also an extrafetal allantoic sac outside the amnion that contains about 400 ml of fluid (27). Inflow into the allantoic sac is fetal urine, flowing through the urachus at a rate of one-third to one-half of the production rate of urine (26). Allantoic fluid is absorbed into the chorionallantoic membrane (11). In the present experiments, none of the urine was infused into the allantoic sac; elimination of urine inflow has been reported to cause the allantoic sac to empty in about 2 days (27).

METHODS

Animals and surgery. Six time-bred ewes were obtained from Oregon State University and operated on at 118 to 121 days of gestational age. All surgical and experimental procedures were approved by the Institutional Animal Care and Use Committee. Anesthesia was induced with 400 mg ketamine and 10 mg diazepam intravenously and continued after intubation with approximately 1% halothane as needed in a 50/50 mixture of oxygen and nitrous oxide. Strict sterility was maintained.

Indwelling catheters were inserted into a maternal sapheous artery and vein. After the abdomen was opened, an incision was made into the uterus. The fetal chorionicamnion was sutured to the uterus before extracting the hind legs of the fetus. Indwelling catheters were inserted into the fetal pedal arteries and veins and advanced approximately 22 cm toward the aorta and inferior caval vein. An amniotic fluid catheter was sutured to the skin closure. The fetal bladder was catheterized through a suprapubic incision, and a second
amniotic fluid catheter was tied to the skin at the site of the incision. The edges of the chorioamnion were carefully apposed before the uterus was closed with interrupted sutures. No more than one catheter emerged between any pair of sutures. The uterine closure was then oversewn with a continuous suture. A new uterine incision was made over the fetal head. One end of a vinyl U-shaped tube was inserted into the esophagus toward the stomach and the other end into the trachea toward the lungs. The distal esophagus and trachea were ligated. A carotid artery and jugular vein were cannulated with vinyl catheters. A third amniotic fluid catheter was sewn to the fetal skin closure. The membranes and uterus were closed as previously described. Two allantoic fluid catheters were inserted into the allantoic sac. All catheters were tunneled subcutaneously and emerged at the flank of the ewe where they were kept in a pouch sewn to the skin.

One million units of penicillin G were administered as prophylaxis against streptococci, one-half of it into the amniotic fluid and one-half into the allantoic fluid. No other antibiotics were used. Aerobic and anaerobic fetal blood cultures throughout the experimental protocols confirmed the adequacy of the sterile surgical and antibiotic regimens.

Experimental protocol. The animals were given 5 days for postoperative recovery. On the 5th day after surgery, they were placed in a stanchion where they remained for the duration of the protocol with free access to water at all times. They were fed twice daily. Five of the six animals were subjected to the standard protocol, shown in Fig. 1. The protocol used on the first animal differed in details as will be indicated.

On day 5, we opened the fetal catheters for measuring pressures and taking samples of fetal arterial blood, fetal urine, and the amniotic and allantoic fluids. After this, we began the continuous drainage of fetal urine from the bladder into a sterile jar by means of a method adapted from Harding et al. (14). A relay kept the volume in the jar at a level corresponding to about 50 ml by switching the power to a Gilson Minipuls3 roller pump. The pump returned all fetal urine into the amniotic sac through one of the indwelling amniotic fluid catheters. Integration of the activity of the roller pump over 24-h periods yielded a daily record of the volume of urine drained and infused into the amniotic sac with an accuracy of ±2%.

On day 9, after 4 days of urine drainage and infusion, we again measured pressures and took samples. The infusion of urine was then continued but augmented by an infusion of Ringer solution into another of the amniotic fluid catheters by means of a second roller pump. The mean daily volumes of Ringer were measured at the infusion containers and are given in Table 1 and Fig. 2. The composition of the Ringer solution was (in mM) 130 Na, 110 Cl, 28 lactate, 4 K, and 3 Ca2+. The combined urine and Ringer infusion was continued during the second period of the protocol for 6 days.

On day 15 we again measured pressures and took samples. In five fetuses, we injected 125I-albumin (human, Mallinkrodt) into the amniotic fluid for the unrelated purpose of refining a tracer dilution method for measuring amniotic fluid volume. Filtration of an aliquot of stock solution of the labeled albumin through a nominal 30-kDa filter showed 99% retention. Samples were taken for the next 7 h and counted in a Wallac 1470 gamma scintillation counter.

The ewe and the fetus were then killed by means of an intravenously administered commercial euthanasia solution, as approved by the Institutional Animal Care and Use Committee. An immediate autopsy was done. The amniotic fluid and if present allantoic fluids were collected and measured. The fetuses were inspected, and the positions of the catheters were verified.

Table 1. Urine and Ringer inflows and autopsy fluid volumes in 6 successive experiments on fetal sheep

<table>
<thead>
<tr>
<th>Animal</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autopsy weight, kg</td>
<td>4.35</td>
<td>3.31</td>
<td>3.09</td>
<td>3.33</td>
<td>4.69</td>
<td>3.09</td>
</tr>
<tr>
<td>Gestational age at autopsy, days</td>
<td>139</td>
<td>135</td>
<td>134</td>
<td>135</td>
<td>133</td>
<td>136</td>
</tr>
<tr>
<td>Urine flows during second period (Ringer infusion), ml/day</td>
<td>875</td>
<td>995</td>
<td>1,158</td>
<td>1,161</td>
<td>1,044</td>
<td>1,411</td>
</tr>
<tr>
<td>First period, ml/day</td>
<td>1,089</td>
<td>826</td>
<td>617</td>
<td>853</td>
<td>503</td>
<td>1,026</td>
</tr>
<tr>
<td>Difference, ml/day</td>
<td>-214</td>
<td>169</td>
<td>541</td>
<td>308</td>
<td>542</td>
<td>365</td>
</tr>
<tr>
<td>Ringer flow infused, ml/day</td>
<td>759</td>
<td>1,278</td>
<td>1,252</td>
<td>1,113</td>
<td>1,257</td>
<td>1,342</td>
</tr>
<tr>
<td>Excess inflow (difference of urine flows plus Ringer flow), ml/day</td>
<td>545</td>
<td>1,447</td>
<td>1,793</td>
<td>1,421</td>
<td>1,799</td>
<td>1,727</td>
</tr>
<tr>
<td>Duration of infusion, days</td>
<td>8.57</td>
<td>5.79</td>
<td>5.79</td>
<td>5.81</td>
<td>5.73</td>
<td>5.69</td>
</tr>
<tr>
<td>Excess volume (excess inflow times duration), ml</td>
<td>4,671</td>
<td>8,378</td>
<td>10,381</td>
<td>8,256</td>
<td>10,307</td>
<td>9,826</td>
</tr>
<tr>
<td>Autopsy volume, amniotic plus allantoic fluids, ml</td>
<td>300</td>
<td>505</td>
<td>1,190</td>
<td>4,060</td>
<td>1,650</td>
<td>1,530</td>
</tr>
<tr>
<td>Excess volume reabsorbed (excess volume minus autopsy volume), ml</td>
<td>4,371</td>
<td>7,873</td>
<td>9,191</td>
<td>4,196</td>
<td>8,657</td>
<td>8,296</td>
</tr>
<tr>
<td>Excess rate of reabsorption (excess volume reabsorbed divided by duration), ml/day</td>
<td>510</td>
<td>1,360</td>
<td>1,587</td>
<td>722</td>
<td>1,511</td>
<td>1,458</td>
</tr>
</tbody>
</table>

Animal 4 had polyhydramnios. Excess volume is after subtraction of autopsy volumes. Excess rate of reabsorption is mean 1,191 ± 186 (SE) ml/day (P < 0.002).
Methods and analytical procedures. For pressure measurements, the fetal arterial, venous, and amniotic fluid catheters were connected to calibrated sterile Abbott Transpac-IV transducers and a Gould TA2000 polygraph recorder. Fetal arterial and venous pressures are reported with respect to amniotic fluid pressure. Intrauterine (amniotic fluid) pressure is reported in the standing ewes with respect to atmospheric pressure at gauge level, which was at an arbitrary height of 43 cm above the floor of the stanchion.

Fetal arterial blood samples, urine samples, and amniotic (and if possible allantoic) samples were taken for the measurement of blood gases, pH, and hemoglobin concentrations (Instrumentation Laboratories IL306 and IL482) and urine, amniotic fluid, and plasma Na⁺ and Cl⁻ concentrations (Beckman Lablyte 810 electrode system). Freezing point depression osmolalities were determined with an Advanced Micro Osmometer, model 3MO.

Additional samples were taken into EDTA and immediately placed on ice for radioimmunoassays of circulating angiotensin I concentrations and plasma renin activity, as described (3), and for circulating angiotensin II concentrations. Samples for angiotensin II were extracted on Varian Bond Elut LRC C18 columns, eluted with 1% trifluoroacetic acid in 60% acetonitrile and 40% water, and dried. The dried material was redissolved in the assay buffer supplied in the Peninsula Laboratories Angiotensin II RIA kit RIK7002 and processed by radioimmunoassay.

Statistics. Data are presented as means ± SE. Statistical significance (P < 0.05) of any changes between day 5, day 9 (end of period 1), and day 15 (end of period 2) were determined by analysis of variance for repeated measures (24). Other tests are identified in the results.

RESULTS

Control data. Autopsy weights and gestational ages are shown in Table 1. The data recorded at the start of the protocols (5 days after surgery) were normal for fetal sheep of this gestational age (1, 8). Fetal arterial and venous pressures were 43 ± 2 and 1.9 ± 0.6 mmHg. Fetal arterial blood samples yielded a pH of 7.354 ± 0.007, a PCO₂ of 51 ± 1, and a PO₂ of 19 ± 1 torr, a hemoglobin concentration of 11 ± 1 g/dl, and a hematocrit of 35 ± 1%. None of these variables was statistically significantly changed on days 9 and 15.

Mean intrauterine pressures on days 5, 9, and 15 were 12.1 ± 0.8, 12.1 ± 1.5, and 13.2 ± 1.9 mmHg, and none of these values differed statistically significantly from each other.

Daily fluid volumes. Data are presented in the same order (the order in which the experiments were done) in the text, Table 1, and Fig. 2. Figure 2 shows the daily infusions of urine (which, unless some urine escaped via the urachus, would equal urine production) into the amnion during the 4 days of the first period as well as the urine infusions during the second period of the protocol (the second period lasted 9 days in fetus 1). Figure 2 also shows the daily volumes of infused Ringer solution during the second period of the protocols.

The first animal yielded autopsy volumes of only 300 ml of amniotic fluid and no allantoic fluid, indicating that the rates of Ringer infusion could be substantially increased, and this was done in the five subsequent experiments. Because the data for animal 1 were incomplete and not taken at the standardized times (Fig. 1) that applied to the remaining five fetuses, data on animal 1 were not used except as noted. They did not contradict any of the reported data.

Figure 2 shows a decrease in mean urine drainage during the Ringer infusion in the first fetus. The subsequent experiments all showed an increase in urine drainage during the Ringer infusion periods, with a mean increase of 389 ± 71 ml/day (P < 0.01).

Only one animal (animal 4) was found to have polyhydramnios at autopsy; we failed to separate the amniotic and allantoic volumes in this animal but the
combined volume amounted to 4,060 ml (Table 1). The mean amniotic fluid volume of the remaining five fetuses was near normal at 812 ± 241 ml. Two of those five fetuses (animals 3 and 6) had 500 and 540 ml of allantoic fluid at autopsy. The mean allantoic fluid volume in the remaining three fetuses was only 25 ml. Thus the mean allantoic fluid volume of the five fetuses in which it could be measured was 223 ± 122 ml.

It was assumed that amniotic fluid volume was close to a steady state at the end of the first period (day 9) at the end of the second period of the protocol (day 15) because any depletion of existing fluid cannot have been but small in comparison to the infused volumes. Thus the total volume of intramembranous amniotic fluid absorption was approximately equal to the volume of urine infused in the first period and equal to the sum of the volumes of urine and Ringer infused during the second period.

However, to ensure that any calculated increase in intramembranous absorption during the second period would not be overestimated, we made the extremely conservative assumption that all of the combined (amniotic plus allantoic) fluids collected at autopsy had accumulated during the Ringer infusion period (were "left over") and that only the differences between the infused volumes (urine plus Ringer) and the autopsy volumes had been removed by intramembranous absorption during the second period of the protocol.

Thus, for animal 6 in Table 1, the total urine infused in the first period of 4.0 days was 4,105 ml or 1,026 ml/day. During the second period (of 5.69 days), total urine infused was 8,029 ml or 1,411 ml/day and the total Ringer infused was 7,640 ml or 1,342 ml/day. Combined urine and Ringer infused in the 2nd period was 8,029 + 7,640 = 15,669 ml. This combined infused volume in the second period was corrected for the "left over" autopsy fluids of 1,530 ml, which left a net total reabsorbed volume of 14,139 ml, for a net intramembranous absorption rate of 2,485 ml/day during the second period. This net rate of 2,485 ml/day was 1,458 ml/day in excess of the absorption rate of 1,026 ml/day during the period of urine infusion alone.

Table 1 shows the calculations for all animals. It can be seen that mean intramembranous reabsorption increased highly significantly from the first to the second period by a mean of 1,191 ± 186 (SE) ml/day (P < 0.002). On the average, intramembranous absorption during the Ringer infusion period was more than 2.5 times that during the period of urine infusion alone.

Electrolytes. Table 2 shows the concentrations of Na⁺ and Cl⁻ in fetal plasma, fetal urine, and in the amniotic and allantoic fluids. The concentrations of Na⁺ and Cl⁻ in fetal urine had increased on day 15 (P < 0.01 for both) but remained well below those of the infused Ringer solution (130 and 110 mM).

The concentrations of Na⁺ and Cl⁻ in the amniotic fluid remained below those of fetal plasma. However, the differences between the concentrations in fetal plasma and those in the amniotic fluid had decreased after the Ringer infusion, statistically insignificantly so for Na⁺ (P < 0.1) but significantly for Cl⁻ (P < 0.05).

During the second period (combined urine and Ringer infusion) there was a large increase in the inflowing electrolyte loads, due to the increases in infused daily volumes and the increases in the urinary electrolyte concentrations. Again, the conservative assumption was used, i.e., that all electrolyte recovered in the amniotic and allantoic fluids at autopsy had accumulated in the extrafetal fluids during the Ringer infusion period. Thus the autopsy amounts were subtracted before calculating the intramembranous absorption rates during the second period. With that assumption, the logarithmic mean of the ratios of intramembranous absorption (meq/day) in the second and first periods of the protocol. Thus the autopsy amounts were subtracted before calculating the intramembranous absorption rates during the second period. With that assumption, the logarithmic mean of the ratios of intramembranous absorption (meq/day) in the second and first periods of the protocol.

<table>
<thead>
<tr>
<th>Day 5</th>
<th>Day 9</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>Na⁺</td>
<td>136±1</td>
</tr>
<tr>
<td></td>
<td>Cl⁻</td>
<td>106±1</td>
</tr>
<tr>
<td>Urine</td>
<td>Na⁺</td>
<td>33±4</td>
</tr>
<tr>
<td></td>
<td>Cl⁻</td>
<td>16±3</td>
</tr>
<tr>
<td>Amniotic fluid</td>
<td>Na⁺</td>
<td>115±5</td>
</tr>
<tr>
<td></td>
<td>Cl⁻</td>
<td>82±5</td>
</tr>
<tr>
<td>Allantoic fluid</td>
<td>Na⁺</td>
<td>79±12</td>
</tr>
<tr>
<td></td>
<td>Cl⁻</td>
<td>45±9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 animals. Significantly lower than values on days 5 and 9: *P < 0.05 and †P < 0.01.

Table 3 lists the fetal plasma renin activities and the circulating angiotensin I and angiotensin II concentrations. We expected these con-
The enormous loss of $^{125}$I-albumin in the short period of time between injection and autopsy was entirely unexpected. In 8 h more than one-third of the injected radioalbumin had disappeared from the extrafetal fluids in fetuses that could not swallow. Tomoda et al. (22) reported that radioalbumin disappeared from the amniotic fluid at a rate of 4.9% per hour and assumed that this rate of disappearance was mostly due to fetal swallowing. For comparison, a loss rate of 4.9% per hour would have left 67% of the injected radioactivity after 8 h.

Radioalbumin is known to be cleared from the allantoic fluid, be it at a slow rate, corresponding to about 10% per day (27). It appears therefore that uptake of radioalbumin through an intramembranous pathway is possible and at times physiological function. We conclude that radioalbumin disappearance by way of the intramembranous pathway may have caused past estimates of fetal swallowing of amniotic fluid to be too high. This possibility is in accord with the observations that the swallowing rates obtained by direct measurement of swallowing by Harding et al. (14) and Ogundipe et al. (17) are smaller than those obtained with $^{125}$I-albumin. This is not to say that in unoperated or noninfused fetuses intramembranous absorption of albumin is as fast as was observed in the present experiments.

Purpose of study. We attempted to elicit a homeostatic response by challenging one of the four mechanisms that could conceivably help control amniotic fluid volume. It is generally accepted that the major inflows are fetal urine and lung fluid production and that the major routes of egress are fetal swallowing and intramembranous reabsorption (4, 19). Several studies have focused on the regulation of amniotic fluid volume by reducing the inflow of fluid by urinary drainage (15, 16, 27) or by augmenting the inflow by infusion of fluids of various compositions (12, 23). The present study differs from past experiments in the extent and durations of the infusions and by isolating a single absorptive mechanism, that of the intramembranous path.

The concept of intramembranous absorption was considered as long ago as 1961 (18) and more recently has been buttressed by convincing in vivo evidence by Brace and co-workers (4, 13). These workers routinely calculated intramembranous absorption on the basis that any inflow not removed by fetal swallowing constituted intramembranous absorption, where swallowing was calculated from the disappearance rates of radioalbumin, or the very similar disappearance rate of labeled red cells (12, 22). Thus intramembranous absorption of amniotic fluid may have been somewhat underestimated.

Interpretation of results. The present experiments ruled out lung fluid production and swallowing as routes of amniotic fluid production or removal. During the 4-day period of urine infusion alone, the rate of amniotic inflow was reduced by the absence of lung fluid flowing into the amnion but augmented by the one-third or more of the urine that normally enters the allantoic sac (26). Thus the total amniotic inflow of fluid in the first period must have been near normal or slightly high. Intramembranous absorption may already have been higher than normal because no fluid was removed by swallowing. Still, when challenged with a volume overload of Ringer solution in the second period, the intramembranous pathway responded with a further increase in the absorption of fluid to more than 2.5 times the rate in the first period. The increases in the relative rates of the electrolyte absorptions were
much greater. The present experiments do not prove that excess inflow of fluids other than Ringer would trigger a similar response; this would depend on how intramembranous absorption is effected and controlled. Control may depend on intrauterine volume or pressure, but the present experiments do not illuminate this question except for showing that most pressure and volume changes were small.

If urine production served a homeostatic role in volume control of amniotic fluid, it should have decreased during the volume loading of the amniotic fluid in the second period. However, it did the opposite, in accord with a similar finding by Gilbert and Brace (12). It appears that urine production serves a fetal need other than the control of amniotic fluid volume. The present results are consistent with the view that intramembranous absorption ensures the normalcy of volume by appropriately adapting to variations in amniotic fluid production, provided those variations are not too extreme.

Dickson and Harding (9) demonstrated that an induced decrease in extrafetal fluid volumes caused a reduction, rather than a compensatory increase, in the production rate of lung fluid. It appears then that lung fluid production, like urine production, may disturb but does not regulate amniotic fluid volume.

The regulatory role of fetal swallowing is controversial (19) and remains unresolved by the present experiments. Experimental esophageal ligation in fetal sheep does not reliably lead to polyhydramnios (25). However, in view of the present finding that intramembranous absorption vigorously responds to a potential volume abnormality, the observed normalcy of amniotic fluid volume after esophageal ligation does not rule out an additional regulatory role for swallowing.

Fate of excess water volume. The excess water and electrolyte absorbed into the fetal circulation from the amniotic fluid must have passed from the fetus into the mother. It is likely that removal of this excess of fetal somatic fluid was facilitated by the suppression of the fetal renin-angiotensin system (2).

Perspectives

The present study was not designed to elucidate the physiological mechanism that is responsible for intramembranous absorption. It often is assumed, although not made explicit, that intramembranous reabsorption is driven by a crystallloid osmotic pressure difference (7, 11, 15, 16, 20) because amniotic fluid is generally hypotonic with respect to fetal plasma. However, for a crystallloid osmotic pressure to exist, there must be a non-zero crystallloid reflection coefficient and, if so, much of the crystallloid content of the reabsorbed amniotic fluid would be sieved out and left behind in the amniotic sac. It is difficult to understand how such a mechanism could accommodate the fivefold increase in Na+ reabsorption and eightfold increase in Cl− reabsorption observed in the present experiments, and it could not account at all for the fast reabsorption of albumin.

For these reasons it is likely that the intramembranous path possesses at least an additional absorptive mechanism such as a hydrostatic-osmotic Starling mechanism, similar to the one that operates across the walls of somatic capillaries, or else that the choioamnion contains some form of lymphatic drainage.

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