Maternal DDAVP-induced hyponatremia preserves fetal urine flow during acute fetal hemorrhage

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Desai, Mina, Zhice Xu, Catalina Guerra, Nathash Kallichanda, and Michael G. Ross. Maternal DDAVP-induced hyponatremia preserves fetal urine flow during acute fetal hemorrhage. Am J Physiol Regul Integr Comp Physiol 285: R373–R379, 2003. First published April 10, 2003; 10.1152/ajpregu.00765.2002.—Maternal administration of DDAVP induces maternal and fetal plasma hyponatremia, accentuates fetal urine flow, and increases amniotic fluid volume. Fetal hemorrhage represents an acute stress that results in fetal AVP secretion and reduced urine flow rate. In view of the potential therapeutic use of DDAVP for pregnancies with reduced amniotic fluid volume, we sought to examine the impact of maternal hypotonicity during acute fetal hemorrhage. Chronically catheterized pregnant ewes (130 ± 2 days) were allocated to control or to DDAVP-induced hyponatremia groups. In the latter group, tap water (2,000 ml) was administered intragastrically to the ewe followed by DDAVP (20 μg bolus, 4 μg/h) and a maintenance intravenous infusion of 5% dextrose water for 4 h to achieve maternal hyponatremia of 10–12 meq/l. Thereafter, ovine fetuses from both groups were continuously hemorrhaged to 30% of estimated blood volume over a 60-min period. DDAVP caused similar degree of reductions in plasma sodium and osmolality in pregnant ewes and their fetuses. In response to hemorrhage, DDAVP fetuses showed greater reduction in hematocrit than control fetuses (14 vs. 10%). Both groups of fetuses demonstrated similar increases in plasma AVP concentration. However, the AVP-hemorrhage threshold was greater in DDAVP fetuses (22.5%) than in control (17.5%). Hemorrhage had no significant impact on plasma osmolality, electrolyte levels, or cardiovascular responses in either group of fetuses. Despite similar increases in plasma AVP, DDAVP fetuses preserved fetal urine flow rates, with values threefold those of control fetuses. These results suggest that under conditions of acute fetal stress of hemorrhage, maternal DDAVP may preserve fetal urine flow and amniotic fluid volume.

rapid induction of hyponatremia; arginine vasopressin; amniotic fluid volume; pregnancy; sheep

AMNIOTIC FLUID VOLUME is dependent on a balance of fluid secretion (fetal urine flow and lung liquid) and fluid resorption (fetal swallowing and, in sheep and perhaps humans, intramembranous flow) (4, 8, 21). Fetal urine is the principal source of amniotic fluid, with production rates in the near-term ovine or human fetus of ~1,000 ml/day (5, 14). Fetal endocrine responses to intrauterine stress, including increased AVP secretion, may reduce fetal urine flow rates and thus amniotic fluid volume (oligohydramnios) (23, 24). Consequently, oligohydramnios may be a marker of fetal compromise and is associated with significant perinatal morbidity and mortality. In addition, oligohydramnios presents an ongoing risk of umbilical cord compression and fetal hypoxia.

To address the risks of oligohydramnios, clinicians have increased amniotic fluid volume with infusions administered via transabdominal or transcervical catheters. Alternatively, amniotic fluid volume may be increased by augmentation of fetal fluid production. Our laboratory has developed a model of maternal plasma hypotonicity that results in an increase in ovine amniotic fluid volume (13, 15, 22). A similar phenomenon has been noted in human studies (11). The model uses maternal oral water hydration and administration of a V2 receptor agonist, DDAVP, to prevent a maternal urinary diuresis. Fetal hypotonicity occurs in response to maternal hypotonicity and results in increased fetal urine production. It is thus postulated that DDAVP therapy may be useful for the prevention and/or treatment of reduced amniotic fluid volume. To date, DDAVP fetal effects have been examined only in euhydrated, nonstressed ovine fetuses (15, 22).

Fetal hemorrhage represents an acute stress that results in fetal AVP secretion and reduced urine flow rate (6, 20). In view of the potential therapeutic use of DDAVP for pregnancies with reduced amniotic fluid volume, we sought to examine the impact of maternal DDAVP in “stressed” fetuses.

MATERIALS AND METHODS

Animals and surgery. Twelve mixed-breed pregnant ewes with singleton fetuses were studied. The care and use of animals were approved by the Animal Research Committee of Harbor-University of California Los Angeles Medical Center and were in accordance with the American Association for the Prevention of Cruelty to Animals.

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Accreditation of Laboratory Animal Care and National Institutes of Health Guidelines. The sheep were housed indoors in individual study cages and acclimated to a 12:12-h light-dark lighting regimen with food (alfalfa pellets) and water provided ad libitum. They were deprived of food 24 h before surgery.

The gestational age at the time of surgery was 125 ± 2 days. Surgical anesthesia was induced by an intramuscular injection of ketamine hydrochloride (20 mg/kg) plus atropine sulfate (50 μg/kg) and subsequently maintained by maternal endotracheal ventilation with 1 l/min oxygen and 1–2% isoflurane. The uterus was exposed by midline abdominal incision, and a small hysterotomy was performed to expose a fetal hindlimb. The fetal femoral vein and artery were catheterized (Tygon, ID = 1.0 mm; OD = 1.8 mm), and catheters were threaded to the inferior vena cava and abdominal aorta, respectively. The maternal vein and artery were similarly catheterized with polyethylene catheters (8-Fr). The fetal bladder was catheterized (Tygon, ID = 1.3 mm; OD = 2.3 mm) via cistotomy, and fetal urachus was ligated to eliminate urine flow to allantoic cavity. An intraperitoneal plastic catheter (Catheter Medicut, CA) was inserted for measurement of amniotic fluid pressure. The uterus and maternal abdomen were closed in layers. Catheters were exteriorized to the maternal flank and placed in a cloth pouch sewn to the ewe’s side. At the end of surgery, an equivalent volume of 0.15 M NaCl replaced any fluid lost from the amniotic cavity.

A minimum of 5 days of postoperative recovery was allowed before experimental studies. During the first 3 days of the recovery period, antibiotics were administered intravenously twice daily to the ewe (1 g chloramphenicol, 967 mg oxacillin sodium, 72 mg gentamicin sulfate) and fetus (35 mg oxacillin sulfate, 8 mg gentamicin sulfate). Maternal and fetal catheters were flushed daily with heparinized saline (10 IU/ml), subsequently filled with sodium heparin solution (10 and 1,000 IU/ml, respectively), and sealed with sterile plastic caps.

Experimental protocol. All experiments were performed on conscious animals standing in their holding cages. In all cases, fetal arterial pH was >7.3, and the fetal urine osmolality was <200 mosmol/kgH2O before study.

Chronically catheterized pregnant ewes (130 ± 2 days) were allocated either to a control-hemorrhage group (n = 6) or to a DDAVP-infused hemorrhage group (n = 6). The fetal bladder was drained by gravity. During a 2-h baseline period, maternal and fetal arterial blood pressure, including amniotic fluid pressure, was monitored.

Tap water was administered along with DDAVP to induce maternal hyponatremia. In prior studies, the administration of DDAVP alone does not induce hyponatremia in the ovine model. Conversely, the administration of tap water alone does not induce hyponatremia in the absence of DDAVP. However, administration of both induces hyponatremia (13, 16). Thus, in the DDAVP-hemorrhage group, 2,000 ml of tap water (warmed to 37°C) was administered via nasoruminal tube to the pregnant ewe over a period of 30 min. This was followed immediately with an intravenous bolus of 20 μg DDAVP and 4 μg/h DDAVP infusion together with a maintenance intravenous infusion of 5% dextrose water. Maternal plasma sodium concentration was rapidly decreased from baseline levels by 1.8 ± 0.3 meq/l over a period of 3 h and was maintained at the level for a further 1 h by titration of the rate of dextrose water infusion. Thus the total duration of DDAVP infusion was 4 h.

At the end of DDAVP infusion, fetuses from both groups (i.e., controls and DDAVP hemorrhage) had blood samples taken to represent 0% hemorrhage, after which they were continuously hemorrhaged to 30% of estimated blood volume over a 60-min period at a rate of 0.5% of estimated fetal-placental blood volume per minute. Blood volume was estimated to be 110 ml/kg body wt (3); fetal weight was determined from an established formula (17); fetal body wt (kg) = 0.096 × gestational age (days) – 9.2228.

After hemorrhage, animals were monitored for an additional 20 min. Throughout the basal and experimental periods, maternal and fetal arterial blood was withdrawn at timed intervals for measurement of pH, blood gases, hematocrit, plasma electrolyte composition, osmolality, and AVP concentration. In addition, fetal urine samples were collected for determination of urine flow rate, osmolality, and sodium, potassium, and chloride concentrations. The total volume of fetal blood withdrawn during maternal DDAVP infusion and basal period was replaced with an equal volume of heparinized maternal blood withdrawn before each experiment and filtered through a 20-μm antimicrobial filter.

Analytic methods. Maternal and fetal arterial blood pressure and amniotic fluid pressure were monitored continuously by means of a Beckman R-612 recorder (Beckman Instruments, Fullerton, CA) and Statham P23 pressure transducers (Garret, Oxnard, CA). All signals were digitized at 50 Hz and acquired on an IBM-compatible computer. Heart rate and systolic, diastolic, and mean arterial pressures were calculated from pressure tracings by means of Advanced CODAS software (DataQ Instruments, Akron, OH).

Blood pH, arterial PCO2, and arterial PO2 values were measured at 39°C with a Radiometer BM 33 MK2-PHM 72 MKS acid-base analyzer system (Radiometer, Copenhagen, Denmark). Plasma and urinary electrolyte levels were determined with a Nova 5 electrolyte analyzer (Nova Biomedical, Waltham, MA). Osmolality was measured by freezing-point depression on an Advanced Digimatic Osmometer (model MO, Advanced Instruments, Needham Heights, MA). Plasma AVP levels were assessed by radioimmunoassay as previously described (25). The technique employed in our laboratory is sensitive to 0.8 pg AVP/ml plasma (0.16 pg/tube). The intra- and interassay coefficients of variation were 6 and 9%, respectively. Circulating DDAVP levels were measured with the AVP radioimmunoassay; DDAVP shows 34.5% cross-reactivity with our AVP antibody. AVP and DDAVP concentrations are reported as uncorrected immunoreactive AVP (irAVP).

Statistical analysis. Changes in response to DDAVP were analyzed by repeated-measures ANOVA with Dunnett’s post hoc tests (compared timed values with control/basal values). Comparison of responses of DDAVP and control fetuses to hemorrhage was determined with two-way repeated-measures ANOVA. Best-fit regression models (linear, second-order polynomial) were used to determine thresholds for hemorrhage-induced irAVP secretion, defined as percent blood volume withdrawal at which plasma irAVP values significantly increased above basal values. Regression analysis used all individual data points. All values are expressed as means ± SE.

RESULTS

The maternal and fetal cardiovascular and arterial parameters during the basal period were within previously published ranges and representative of nonstressed animals (2) (Table 1). Furthermore, basal maternal plasma sodium (146.2 ± 0.9 meq/l), chloride (113.7 ± 0.4 meq/l), and potassium (4.2 ± 0.1 meq/l)
concentrations and osmolality (303.6 ± 2.3 mosmol/kg H2O) were similar to those observed in ad libitum-fed, hydrated pregnant sheep. Likewise, these indexes in fetal plasma were within normal range for this gestational age (10) (sodium, 140 ± 0.8 meq/l; chloride, 106.8 ± 0.9 meq/l; potassium, 4.1 ± 0.1 meq/l; osmolality, 298.7 ± 0.8 mosmol/kg H2O).

**DDAVP infusion.** DDAVP and oral water induced significant maternal and fetal hyponatremia. Maternal plasma sodium concentrations (Fig. 1A) decreased rapidly during the first hour (146.2 ± 0.9 to 138.9 ± 0.9 meq/l), followed by a gradual decrease during the next 2 h (135.7 ± 0.9 and 134.8 ± 0.8 meq/l), meeting the study objective of 10- to 12-meq/l decrements below the basal period. Thereafter, the sodium concentration remained stable at this level over 1 h (134.6 ± 0.9 meq/l). A similar pattern in reduction of maternal plasma chloride concentrations (Fig. 1B) and plasma osmolality (Fig. 1C) was observed. Maternal plasma potassium concentrations (Fig. 1D) decreased with the initiation of hyponatremia but did not demonstrate a further reduction. Maternal hematocrit (Fig. 1E) decreased significantly from basal value at all time periods. The 20-μg bolus and 4 μg/h infusion of DDAVP resulted in maintained irAVP levels at ~160 pg/ml (Fig. 1F). Maternal and fetal arterial blood pressure, heart rate, blood gases, pH, and hemoglobin remained largely unchanged during the period of DDAVP infusion (Table 1).

In response to maternal hyponatremia, fetal plasma sodium concentrations (Fig. 2A) decreased consistently but more slowly over the same time period. For instance, fetal sodium concentrations sequentially decreased from 140.1 ± 0.8 to 136.9 ± 0.5, 133.8 ± 0.5, and 131.7 ± 0.5 meq/l over a 3-h period, after which it was maintained at 131.0 ± 0.5 meq/l. Fetal plasma chloride (Fig. 2B) and plasma osmolality (Fig. 2C) showed a similar decline. Fetal plasma potassium concentrations, unlike maternal plasma potassium concentrations, did not change during the study. Similarly, fetal hematocrit and plasma irAVP levels were unaltered throughout the study, with no evidence of suppression of irAVP secretion below the basal levels after maternal water loading (Table 1). In association with hyponatremia, fetal urinary flow significantly increased from 0.17 ± 0.05 to 0.28 ± 0.04 ml kg⁻¹ min⁻¹ and remained elevated throughout the study (Fig. 2D). This was accompanied by a nonsignificant tendency toward decreased urine osmolality (Fig. 2E) and sodium concentration (Fig. 2F).

**Hemorrhage.** During hemorrhage, fetal hematocrit decreased significantly in both the control-hemorrhage and DDAVP-hemorrhage fetuses (Fig. 3A). Control fetuses demonstrated a 9% reduction in hematocrit over the 60-min hemorrhage, compared with a 13% decrease among DDAVP fetuses (Table 2, P < 0.01). However, hemorrhage had no significant impact on plasma osmolality, electrolyte levels, or cardiovascular responses, including arterial pH, Pco2, or Po2 in either group of fetuses (Table 2).

Plasma irAVP levels increased significantly in both control-hemorrhage (0.9 ± 0.2 to 9.7 ± 2.1 pg/ml) and DDAVP-hemorrhage fetuses (1.2 ± 0.2 to 7.0 ± 0.8 pg/ml) (Fig. 3B). The regression analysis of plasma irAVP vs. percent hemorrhage revealed the best-fit equation. For control hemorrhage: plasma irAVP = 0.94 – 0.101 (n) + 0.0124 (n)² (r = 0.84; P < 0.001). For DDAVP hemorrhage: plasma irAVP = 1.29 – 0.013 (n) + 0.004 (n)² (r = 0.72; P < 0.001). n Represents the percent blood volume withdrawal. The hemorrhage threshold for irAVP secretion in control-hemorrhage fetuses occurred at 17.5% blood volume withdrawal, whereas in DDAVP-hemorrhage fetuses, it occurred at...
22.5% ($P < 0.01$). The slope of the regression lines (which represent the sensitivity of the hormonal response to hemorrhage) was also significantly different ($P < 0.05$) between the two groups of fetuses.

Fetal urine volume decreased significantly with the progression of hemorrhage. However, the decrement was significantly greater in the control-hemorrhage fetuses, such that these fetuses exhibited a 75% decrease in urine flow rate (0.5 ± 0.02 to 0.12 ± 0.01 ml/min, $P < 0.001$) compared with 43% seen in the DDAVP-hemorrhage fetuses (0.61 ± 0.02 to 0.35 ± 0.01 ml/min, $P < 0.01$). This marked difference in urine flow rate with the advent of hemorrhage becomes more evident when the urine volume flow rate is expressed as percentage of basal urine volume (Fig. 3C). At the conclusion of hemorrhage, DDAVP fetuses maintained urine flow at rates threefold the control fetuses.

Fetal hemorrhage did not alter the maternal blood composition and cardiovascular responses in either the maternal DDAVP-infused or the untreated, control pregnant ewes. However, as stated earlier, the former group of ewes continued to maintain the lower plasma osmolality and electrolyte levels compared with the control pregnant ewes.

**DISCUSSION**

In the present study, maternal DDAVP was administered intravenously simultaneously with water. This methodology enabled a rapid and titrated reduction of maternal plasma sodium and plasma osmolality. Plasma sodium decreased 8%, stabilizing at 2 h after DDAVP. Plasma osmolality decreased by 7% and stabilized at 3 h after DDAVP. Consistent with previous studies (13, 22), maternal hematocrit decreased immediately by 15%, although it demonstrated only a 9% decrease at the conclusion of the stabilization. This may reflect red blood cell swelling secondary to plasma hyposmolality (7) or, alternatively, result from splenic red blood cell release. If extrapolated to a percent volume expansion, these results suggest a 9% plasma volume expansion at 4 h after DDAVP administration. The extrapolated degree of volume expansion based on hematocrit is likely an underrepresentation of the actual degree of plasma volume expansion. Despite the reduced maternal hematocrit, there was no change in fetal hematocrit (and likely fetal plasma volume) in response to DDAVP. These results are consistent with previous studies that demonstrated that mater-
nal blood volume significantly increased in response to DDAVP-induced hyponatremia (from 80 ± 15 to 93 ± 14 ml/kg), whereas fetal blood volume did not change (16).

Maternal plasma volume expansion may be of potential fetal benefit, as relative contraction of maternal plasma volume has been demonstrated to be associated with the development of fetal growth retardation, maternal preeclampsia, preterm labor, and oligohydramnios (4, 8). The lack of evidence for an increase in fetal plasma volume provides reassurance that fetal fluid retention will not occur in response to induced hyponatremia. Thus the fetus is able to effectively excrete plasma water while maternal DDAVP inhibits maternal urinary diuresis, facilitates maternal blood volume expansion, and potentially increases maternal uterine/placental blood flow (3, 17).

Fetal plasma composition demonstrated similar reductions in the degree of plasma sodium and osmolality compared with maternal plasma, although with slower rate of change. Fetal plasma sodium stabilized at 3 h and plasma osmolality at 4 h (as evidenced by a stable osmolality during the hemorrhage period after the 4-h basal period). This likely represents the delay due to maternal-to-fetal transplacental water transfer. In response to DDAVP, fetal urine flow rates increased significantly by 80%. If extrapolated over a 24-h period, this increased urine flow would contribute 700 ml to the amniotic cavity. Notably, there was no effect of plasma hypotonicity on fetal blood pressure or heart rate.

During hemorrhage, removal of ~107 ml of blood in both the control and study groups was calculated on the basis of estimated blood volume. As there was no evidence of plasma or blood volume expansion in the DDAVP fetuses, as measured indirectly via hematocrit and hemoglobin determination, it is likely that this calculation resulted in identical or similar degrees of blood volume withdrawal in both groups. In mature mammals, hormonal changes after hemorrhage, in-
cluding increased plasma concentrations of AVP (1), serve to maintain mean arterial pressure and restore blood volume. Consistent with prior studies (19, 20), hemorrhage stimulated increases in fetal plasma irAVP in both groups. Inasmuch as hemorrhage did not change either plasma sodium or osmolality, the increase AVP was likely a direct response to reduced blood volume.

There were marked differences in the response of DDAVP fetuses compared with controls after hemorrhage. The greater reduction in hematocrit among DDAVP fetuses likely reflects increased fetal plasma water in response to loss of intravascular volume. It is likely that maternal plasma hyponatremia facilitates transplacental water transfer into the fetal intravascular compartment, suggesting that the hyponatremia facilitated restoration of plasma volume in response to hemorrhage.

Fetal plasma irAVP increased significantly in both DDAVP and control fetuses, although peak levels were significantly greater in controls compared with DDAVP groups. Because the fetal blood volume responses to hemorrhage may involve transplacental fluid exchange, placental transfusion, and interstitial fluid shifts, the pattern of blood withdrawal may condition the AVP response. For instance, in term ovine fetuses with the use of a serial acute withdrawal approach, blood losses of 17–19% (9) and 20–30% (18) were necessary to significantly increase plasma AVP levels. Consistent with these results, control fetuses

Table 2. Arterial blood values in response to hemorrhage in control and DDAVP fetuses

<table>
<thead>
<tr>
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<th>Control Fetuses (n = 6)</th>
<th>DDAVP Fetuses (n = 6)</th>
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<tbody>
<tr>
<td></td>
<td>0% Hemorrhage</td>
<td>30% Hemorrhage</td>
</tr>
<tr>
<td>Plasma osmolality, mosmol/kgH2O</td>
<td>301.8 ± 0.7</td>
<td>300 ± 1.5</td>
</tr>
<tr>
<td>Plasma sodium, meq/l</td>
<td>139.3 ± 1.3</td>
<td>139.2 ± 0.9</td>
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<tr>
<td>Plasma chloride, meq/l</td>
<td>105.6 ± 2.5</td>
<td>106.2 ± 2.4</td>
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<tr>
<td>Hematocrit, %</td>
<td>31.2 ± 0.7</td>
<td>28.2 ± 0.4*</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>8.9 ± 0.4</td>
<td>8.1 ± 0.5</td>
</tr>
<tr>
<td>Diastolic MAP, mmHg</td>
<td>59 ± 1</td>
<td>48 ± 1</td>
</tr>
<tr>
<td>Systolic MAP, mmHg</td>
<td>62 ± 3</td>
<td>60 ± 2</td>
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<tr>
<td>MAP, mmHg</td>
<td>53 ± 1</td>
<td>51 ± 2</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>166 ± 4</td>
<td>161 ± 5</td>
</tr>
<tr>
<td>PaO2, mmHg</td>
<td>23 ± 2</td>
<td>22 ± 2</td>
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<tr>
<td>PaCO2, mmHg</td>
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<td>49 ± 2</td>
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<tr>
<td>pH</td>
<td>7.39 ± 0.01</td>
<td>7.38 ± 0.01</td>
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Values are means ± SE. Impact of hemorrhage: *P < 0.001. Control vs. DDAVP fetuses: †P < 0.01.
demonstrated a hemorrhage threshold for increased irAVP at 17.5%. The reduced peak irAVP responses and the elevated threshold (22.5%) in DDAVP fetuses are likely due to suppressive effects of plasma volume restoration and/or the induced plasma hypotonicity.

In response to hemorrhage, fetal urine flow rates significantly decreased in both groups. Similar to the cardiovascular and AVP responses, fetal urine flow responses were significantly abated in DDAVP compared with control fetuses. As DDAVP does not cross the ovine placenta (12, 13), the reductions in urine flow rate are likely a result of combined fetal plasma AVP and/or cardiovascular responses. Our studies previously demonstrated that fetal urine flow increases in response to hyponatremia are dependent on the degree of hyponatremia. In the present study, the abated reduction in urine flow rate in DDAVP fetuses indicates that hypotonicity maintains fetal urine flow during hemorrhage.

Thus the results of the present study indicate that induced plasma hyponatremia results in protective effects in fetuses during acute hemorrhage. Although it is unknown if the beneficial/protective effects of DDAVP are a direct result of the reduced fetal plasma osmolality or a consequence of enhanced maternal transplacental flow, DDAVP-induced hyponatremia reduces the urinary responses to hemorrhage. We speculate that DDAVP and oral water may be of value in the prevention and/or treatment of oligohydramnios in pregnancies with evidence of fetal stress due to hemorrhage.

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DISCLOSURES

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