Developmental Aspects of the Renal Response to Hypoxemia in the Lamb Fetus

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SUMMARY The effects of fetal hypoxemia on renal hemodynamics and renal function were studied in two groups of chronically catheterized young (<120 days of gestation) and near-term lamb fetuses (>130 days). Fetal hypoxemia produced, in both groups, a significant decrease in renal blood flow (RBF) and a significant increase in the filtration fraction. However, the glomerular filtration rate (GFR) did not change significantly, suggesting that the renal vasoconstriction associated with fetal hypoxemia was more important at the efferent than at the afferent arteriolar level. In the group of near-term fetuses, the decrease in RBF correlated closely with changes in plasma renin activity (PRA) (r = —0.77). No changes in PRA were observed during hypoxemia in the group of young fetuses. After hypoxemia, reactive hyperemia associated with a significant increase in urinary prostaglandin excretion (PGE and PGF₂α) was observed in near-term fetuses but not in young fetuses. It also was demonstrated that fetal hypoxemia produced a significant increase in fetal plasma concentrations of vasopressin associated with an antidiuresis in all but one near-term fetus and in 50% of the young fetuses, suggesting that the ability of the fetal kidney to reabsorb free water is more developed in near-term fetuses. Finally, fetal hypoxemia had no effect on mean arterial pressure and heart rate in young fetuses; however, in near-term fetuses, a significant increase in blood pressure and a decrease in heart rate were observed. In summary, it appears that the response of the fetal kidney to hypoxemia depends on the degree of fetal maturation.


PERINATAL hypoxemia is now recognized as a major cause of renal failure in newborn infants (Daniels and James, 1976; Dauber et al., 1976; Anand et al., 1978). It has been demonstrated that severe hypoxemia during the neonatal period is associated with a decrease in urine volume and glomerular filtration rate (GFR) (Guignard et al., 1976) and with an impairment in the renal mechanism of acid-base regulation (Torrado et al., 1974).

Similar derangements in fetal renal function also have been observed following fetal asphyxia (Daniel et al., 1975, 1978). Daniel et al. (1975, 1978) showed a decrease in urinary output and GFR, and an increase in urine osmolality and urinary solute excretion after either partial or complete occlusion of the umbilical cord in chronically catheterized fetal lambs. Cohn et al. (1974) also have demonstrated that fetal hypoxemia produces a decrease in fetal renal blood flow and an increase in fetal arterial pressure and peripheral resistance.

The present protocol was designed to study the developmental pattern of the fetal renal response to pure hypoxemia in absence of fetal acidoses and hypercapnia and to evaluate the possible factors modulating this response. More specifically, the present study was designed to determine simultaneously the effect of hypoxemia on fetal renal blood flow, glomerular filtration rate, and excretion of water and electrolytes. The interrelationship between the changes in renal hemodynamics and renal function and the changes in fetal plasma catecholamines, plasma arginine vasopressin and plasma renin activity also were investigated during fetal hypoxemia. Finally, the ability of the fetal kidney to recover from an hypoxemic stress was investigated.

Methods

Animal Preparation and Surgical Procedures

Pregnant mixed-breed Dorset Suffolk ewes were obtained from a local source and the gestational age based on the induced ovulation technique (Jennings and Crowley, 1972).

Prior to surgery the animals were fasted for 48 hours. Anesthetization of the ewe and surgery on the fetus were performed as described previously (Gresham et al., 1972; Robillard et al., 1979, 1980).
Physiological Studies

During the physiological studies, the ewe was transferred into a small cart restricting it to an upright position. Two groups of chronically catheterized fetal lambs were studied. In the first group of nine fetuses, studies were performed between 106 and 119 days of gestation and in the second group 10 fetuses were studied between 131 and 141 days of gestation (term being 145 days).

In all animals the fetal glomerular filtration rate (GFR) was determined by a constant infusion of [125I]sodium iothalamate (Glofil, Abbott Laboratories). A priming dose of iothalamate was administered followed by a constant infusion of 0.1 μCi/kg per min in 5% dextrose solution at a rate of 0.09 ml/min. An equilibration period of 1 hour then was observed before the start of the first renal clearance period. After the equilibration period, three 20-minute control urine collections were performed in all fetuses.

After the control urine collection periods, fetal hypoxemia was induced by placing a bag over the ewe's head and having her breathe a mixture of 11.1% oxygen with the balance nitrogen. The oxygen-nitrogen gas mixture was pre-calibrated at an accuracy level of ±0.11% by Air Products Co., Penn. After allowing a 10-minute equilibration period to permit the fetal Po2 to reach a new equilibrium, two 10-minute urine collections were performed during hypoxemia. At end of the hypoxemia period, the bag was removed and, after allowing the fetus to recover for 1 hour, two 20-minute urine collections were made.

In seven fetuses of less than 120 days and in seven fetuses over 130 days of gestation, renal blood flow (RBF) was measured as previously described (Heymann et al., 1977; Robillard and Weitzman, 1980) using radioactive microspheres (15 ± 3 μm), which incorporated either 85Sr, 46Sc, or 141Ce into their structure (3M Co.). Immediately after the three control urine collection periods, approximately 4.0 x 10⁶ microspheres were suspended in 3 ml of 0.9% saline solution and were injected throughout a 30-second period into the fetal femoral vein catheter and then immediately flushed with 3 ml of 0.9% saline solution (Robillard and Weitzman, 1980). Femoral arterial blood was collected during a period of 3 minutes beginning 20 seconds before the injection of microspheres and at a withdrawal rate of 2.91 ml/min using a Harvard infusion withdrawal pump to obtain a lower body independent reference sample. A second bolus of radioactive microspheres, labeled differently from the first bolus, was injected at the end of the second urine collection during hypoxemia. Finally, a third bolus of radioactive microspheres was injected at the end of the second urine collection period during recovery. Previous studies by our group (Robillard and Weitzman, 1980) demonstrated that, under control conditions, microsphere injection does not modify the fetal renal parameters. Moreover, in a way to assess if there were no recirculation of microspheres during hypoxemia, 9- and 15-μm microspheres marked with different radioactive labels were injected in four fetuses before and during hypoxemia into the inferior vena cava and radioactivity was determined in the fetal liver as previously described (Rudolph and Heymann, 1967). Before hypoxemia, the per cent of total injected counts distributed to the liver was 0.21 ± 0.03% using 15-μm microspheres and 18.1 ± 6.5% using 9-μm microspheres. During hypoxemia the per cent of total injected count remained stable at 0.28 ± 0.06% using 15-μm spheres but increased to 24.2 ± 9.2% after administration of 9-μm spheres. Therefore, no significant recirculation of microspheres was observed during hypoxemia when 15-μm spheres were used.

At the midpoint of each urine collection period, arterial blood was obtained from the fetus for determination of plasma concentrations of [125I]sodium iothalamate, plasma electrolyte concentrations (Na⁺, K⁺, Cl⁻), plasma osmolality, hematocrit, total plasma proteins, plasma lactate concentration, arterial blood pH, and blood gases (P0₂ and PCO₂).

Plasma renin activity (PRA), arginine vasopressin (AVP), and catecholamines (epinephrine and norepinephrine) were determined before hypoxemia, during the last period of hypoxemia, and at the end of the recovery period.

At the end of each urine collection period, arterial blood was collected for determination of electrolyte (Na⁺, K⁺, Cl⁻) concentration, urine osmolality, urine concentration of [125I]sodium iothalamate, and urinary prostaglandin (PGE and PGF₂α) concentrations.

Fetal blood samples were replaced with an equal amount of maternal blood to avoid any hemodynamic effects of sampling.

Fetal arterial, venous, and amniotic pressures were recorded continuously in every experiment, using Statham P23Db pressure transducers (Statham Instruments Division, Gould, Inc.) and a Beckman R-611 recorder. Fetal mean arterial blood pressures (MABP) were corrected relative to concomitant amniotic pressures. Fetal heart rate was monitored with a cardiograph triggered from the fetal arterial pressure pulse wave. At the end of the experiment, the fetus and ewe were killed separately with lethal doses of KCl solution.

Analytical Methods

Blood for pH, PCO₂, and PO₂ was collected anaerobically in heparinized glass syringes, and measurements were made immediately with the appropriate pH, PCO₂, and PO₂ electrodes at 39°C using a Radiometer PHM 72 MK2 acid-base analyzer (Radiometer Co.). Plasma and urine concentrations of sodium and potassium were determined by flame photometry using lithium as an internal standard (IL443-flame photometer) and chloride by potentiometric titration (Radiometer CMT10 chloride ti-
trator). Plasma and urine osmolality were measured by freezing point depression using an Advanced osmometer. Concentrations of $^{[38]}$Sodium iothalaminate in plasma and urine were determined by $\gamma$ emission of $^{131}$I, using a Beckman-300 spectrometer. Protein content of fetal serum was determined using a refractometer (National Instruments, Co.).

All blood samples for AVP and PRA measurements were collected in chilled tubes containing potassium EDTA, kept on ice and centrifuged within a few minutes at 4°C. PRA was determined by radioimmunoassay of generated angiotensin I (Haber et al., 1989). AVP extraction and assay were done within 3 weeks by shipping frozen samples air freight in dry ice from Iowa City to Torrance, California. Plasma samples were extracted using the bentonite extraction procedures of Skowsky et al. (1974), and AVP was measured by radioimmunoassay using antibody R-71 (Weitzman and Fisher, 1974), and AVP was measured by radioimmunoassay of generated angiotensin I (Haber et al., 1989). AVP extraction and assay were done within 3 weeks by shipping frozen samples air freight in dry ice from Iowa City to Torrance, California. Plasma samples were extracted using the bentonite extraction procedures of Skowsky et al. (1974), and AVP was measured by radioimmunoassay using antibody R-71 (Weitzman and Fisher, 1978). This antisera is specific for AVP and has no significant cross-reactivity with arginine vasotocin or oxytocin. Norepinephrine and epinephrine determination were collected under ice, then immediately frozen at —70°C. Urine samples were extracted with ethylacetate and separated into classes by silicic acid chromatography. The PGE's were determined by radioimmunoassay using antisera that has equal reactivity for PGE$_1$, and PGE$_2$, and PGF's using an antisera that has 100% reactivity with PGF$_2\alpha$, and 70% cross-reactivity for PGF$_1\alpha$. The technique, reliability, and full characterization of each assay have been reported previously (VanOrden and Farley, 1973; VanOrden et al., 1977).

Gamma emission generated from the microspheres was measured from both fetal kidneys and reference femoral arterial blood samples. Immediately after the kidneys had been removed from the fetus, they were weighed, cut into sagittal sections of less than 1 g, and placed in counting vials containing a predetermined amount of 10% formalin in such a way as to prevent air tissue interfaces that could alter the counts. Energy window ranges were set between 210 and 275 keV for $^{85}$Sr, 367-578 keV for $^{48}$Sc, and 74 and 102 keV for $^{141}$Ce counts in tissue. However, to obtain true $^{85}$Sr and $^{141}$Ce counts, isotope separation was performed using standard calculations (Heymann et al., 1977).

## Computations and Data Analysis

RBF was determined according to the following formula: RBF (ml/min) = total kidney counts × reference flow from the femoral artery (ml/min) / total femoral blood counts. Renal vascular resistance (RVR) was determined according to the following formula: RVR (mm Hg/ml per min) = RPP / RBF where RPP is the renal perfusion pressure estimated to be equal to the aortic pressure minus the inferior vena caval pressure and RBF is the renal blood flow expressed in ml/min. The filtration fraction (FF) was determined according to the following formula: FF(%) = GFR/RFF when GFR is the glomerular filtration rate (ml/min) and RPF is the renal plasma flow (ml/min).

Osmolar clearance was determined as follows: $C_{\text{osm}}$ (ml/min) = (U$_{\text{osm}}$ - V)/$P_{\text{osm}}$, in which U$_{\text{osm}}$ and P$_{\text{osm}}$ represent urine and plasma osmolality in mOsm/kg H$_2$O and V the urinary flow rate in ml/min. The solute free water clearance ($C_{\text{sfw}}$) was calculated as the difference between the urine flow (V) and the osmolar clearance ($C_{\text{osm}}$): $C_{\text{sfw}}$ = V - $C_{\text{osm}}$. $T^{18}H_2O$ represents negative free water clearance or free water reabsorption.

Since the control and experimental values were determined for each subject, a paired t-test was employed to determine statistical significance. The term “significant” is used throughout the paper to describe changes with a total $P$ value of less than 0.05 in a two-sided significance limit. Since two tests actually were completed for each variable, the critical value of $t$ actually used corresponds to $\alpha = 0.05/2$ rather than $\alpha = 0.05$ to maintain the desired level ($P$ value) under the use of multiple tests (Miller, 1966).

When values obtained from fetuses below 120 days were compared to values obtained from fetuses over 130 days, an unpaired $t$-test was used. Regression lines and associated correlation coefficients were computed by least-squared formulas. The results are presented as mean ± standard error.

## Results

### Effect of Hypoxemia on Fetal Arterial Blood Values

The effects of hypoxemia on fetal arterial blood values in fetuses of less than 120 days and fetuses over 130 days of gestation are presented in Table 1. During hypoxemia, the fetal P$O_2$ decreased significantly in both groups of fetuses, whereas the blood pH remained stable. The P$CO_2$ values showed small but significant decreases (−3.6 ± 0.66 mm Hg) from control values. Except for plasma potassium concentration, which increased significantly in both groups and the plasma osmolality values during hypoxemia in fetuses of less than 120 days of gestation. In fetuses over 130 days of gestation, there were small but significant increases in the plasma sodium, chloride, and osmolality during hypoxemia. The hematocrit, total protein concentrations, and plasma lactate concentrations also increased significantly during hypoxemia in both groups of fetuses.

One hour after hypoxemia, fetal blood pH decreased below control values in both groups and the plasma lactate remained high. Moreover, in fetuses
Table 1  Effect of Hypoxemia on Fetal Arterial Blood Values

<table>
<thead>
<tr>
<th></th>
<th>&lt;120 days (n = 9)</th>
<th>&gt;130 days (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>H</td>
</tr>
<tr>
<td>pH</td>
<td>7.37 ±0.01</td>
<td>7.36 ±0.01</td>
</tr>
<tr>
<td>Pco2 (mm Hg)</td>
<td>42 ±1</td>
<td>38* ±1</td>
</tr>
<tr>
<td>Po2 (mm Hg)</td>
<td>22 ±1</td>
<td>12* ±1</td>
</tr>
<tr>
<td>Na+ (mEq/l)</td>
<td>146 ±1</td>
<td>145 ±1</td>
</tr>
<tr>
<td>K+ (mEq/l)</td>
<td>3.8 ±0.1</td>
<td>4.3* ±0.1</td>
</tr>
<tr>
<td>Cl- (mEq/l)</td>
<td>106 ±1</td>
<td>107 ±1</td>
</tr>
<tr>
<td>Osmolality (mOsm/kg H2O)</td>
<td>292 ±2</td>
<td>294 ±2</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>32 ±1</td>
<td>35* ±1</td>
</tr>
<tr>
<td>Total proteins (g/100 ml)</td>
<td>3.3 ±0.1</td>
<td>3.7* ±0.1</td>
</tr>
<tr>
<td>Lactate (mg/100 ml)</td>
<td>13 ±2</td>
<td>35* ±2</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. n, number of animals; C, control period; H, hypoxemia period; R, recovery period.

* P < 0.05 when C compared to H; f P < 0.05 when C compared to R.

Effect of Hypoxemia on Circulating Fetal Vasoactive Substances (Table 2)

No significant differences were observed between young fetuses (<120 days) and near-term fetuses (>130 days) when control plasma values for plasma renin activity (PRA), plasma arginine vasopressin (pAVP), and plasma catecholamines (norepinephrine (NE) and epinephrine (E)) were compared.

During hypoxemia, pAVP and plasma catecholamine (E, NE) concentrations increased significantly in both groups of fetuses, whereas a significant rise in PRA was observed only in near-term fetuses. It was found also that during hypoxemia PRA, pAVP, plasma epinephrine, and plasma norepinephrine concentrations were significantly (P < 0.05) lower in young than in near-term fetuses.

At the end of the recovery period, all values but pAVP were back to control levels in fetuses of less than 120 days whereas only PRA and NE returned to pre-hypoxemia levels in near-term fetuses.

Table 2  Effect of Hypoxemia on Circulating Fetal Vasoactive Substances

<table>
<thead>
<tr>
<th></th>
<th>&lt;120 days</th>
<th>&gt;130 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=9</td>
<td>n=8</td>
</tr>
<tr>
<td>PRA (ng/ml per hr)</td>
<td>4.1±1.7</td>
<td>3.8±1.2</td>
</tr>
<tr>
<td>AVP (mU/ml)</td>
<td>0.53±0.2</td>
<td>10.1*±2</td>
</tr>
<tr>
<td>E (ng/ml)</td>
<td>0.02±0.004</td>
<td>1.38*±0.06</td>
</tr>
<tr>
<td>NE (ng/ml)</td>
<td>0.54±0.15</td>
<td>3.40*±0.69</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. n, number of animals; PRA, plasma renin activity; AVP, arginine vasopressin; E, epinephrine; NE, norepinephrine; C, control periods; H, hypoxemia periods; R, recovery periods.

* P < 0.05 when C compared to H; † P < 0.05 when C compared to R.
### Table 3 Effect of Hypoxemia on Fetal Renal Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>&lt;120 days</th>
<th></th>
<th>&gt;130 days</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C (n = 7)</td>
<td></td>
<td>H (n = 7)</td>
<td></td>
</tr>
<tr>
<td>T-RBF (ml/min)</td>
<td>40 ±4</td>
<td>28* ±3</td>
<td>44</td>
<td>58 ±5</td>
</tr>
<tr>
<td>RBF (ml/min per gKW)</td>
<td>±0.21</td>
<td>1.70*</td>
<td>±0.18</td>
<td>2.68</td>
</tr>
<tr>
<td>RVR (mm Hg/ml per min)</td>
<td>±0.01</td>
<td>1.48*</td>
<td>±0.18</td>
<td>1.06</td>
</tr>
<tr>
<td>FF (%)</td>
<td>±0.7</td>
<td>9.9*</td>
<td>±1.6</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>MABP (mm Hg)</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Heart rate (beats/min)</td>
<td>±10</td>
<td>180</td>
<td>±12</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. n, number of animals; T-RBF, total renal blood flow; RBF, renal blood flow; RVR, renal vascular resistance; FF, filtration fraction; MABP, mean arterial blood pressure; C, control periods; H, hypoxemia periods; R, recovery periods. Total kidney weight was 16.1 ± 0.7 g for fetuses <120 days and 29.6 ± 1.5 g for fetuses >130 days.

* P < 0.05 when C compared to H; †P < 0.05 when C compared to R.

The role of PRA and pAVP in modulating changes in total renal blood flow (T-RBF) and MABP during hypoxemia also were studied. In fetuses of less than 120 days gestation, multiple regression analysis demonstrated that neither PRA nor pAVP significantly influenced the change in T-RBF. The partial coefficients of correlation were -0.32 and 0.18, respectively. In fetuses over 130 days of gestation, multiple regression analysis demonstrated a high partial coefficient of correlation between changes in T-RBF and PRA values (r = -0.77) and a low partial correlation between changes in T-RBF and pAVP (r = 0.50). There was no significant correlation between changes in MABP and PRA or pAVP in fetuses over 130 days, the coefficient of correlation being, respectively, -0.17 and -0.03.

#### Effect of Hypoxemia on Fetal Renal Function

The effects of hypoxemia on glomerular filtration rate (GFR), renal concentrating ability, and electrolyte excretion are shown in Table 4.

During hypoxemia, the mean values for urinary flow rate (V) and GFR (C_{GFR}) remained stable. However, there was a significant increase in U_{OSM} and a significant decrease in C_{H,O} in both groups of fetuses. No significant changes in C_{GFR} were found.

When data from individual fetuses were examined (Fig. 1), simultaneous decreases in C_{H,O} and urinary flow rate were observed during hypoxemia in five of nine fetuses of less than 120 days and nine out of 10 fetuses over 130 days, suggesting that, in these fetuses, hypoxemia was accompanied by a
TABLE 4 Effect of Hypoxemia on Fetal Renal Function

<table>
<thead>
<tr>
<th></th>
<th>&lt;120 days (n = 9)</th>
<th></th>
<th>&gt;130 days (n = 10)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>H</td>
<td>R</td>
<td>C</td>
</tr>
<tr>
<td>V (ml/min)</td>
<td>0.41 ± 0.04</td>
<td>0.34 ± 0.06</td>
<td>0.40 ± 0.08</td>
<td>0.81 ± 0.14</td>
</tr>
<tr>
<td>Cioth (ml/min)</td>
<td>1.73 ± 0.10</td>
<td>1.57 ± 0.30</td>
<td>2.04 ± 0.20</td>
<td>3.94 ± 0.40</td>
</tr>
<tr>
<td>UNa-V (µEq/min)</td>
<td>18.3 ± 2.9</td>
<td>29.9 ± 5.8</td>
<td>40.8† ± 9.8</td>
<td>37.0 ± 7.8</td>
</tr>
<tr>
<td>FE Na+ (%)</td>
<td>7.1 ± 1.1</td>
<td>15.3 *†</td>
<td>12.9 f†</td>
<td>6.2 ± 1.1</td>
</tr>
<tr>
<td>FECl- (%)</td>
<td>4.2 ± 0.8</td>
<td>11.9 *†</td>
<td>14.6 f†</td>
<td>3.6 ± 0.9</td>
</tr>
<tr>
<td>UK-V (µEq/min)</td>
<td>2.6 ± 0.6</td>
<td>3.5 ± 2.5</td>
<td>3.9 f†</td>
<td>11.3 ± 3.3</td>
</tr>
<tr>
<td>FEK+ (%)</td>
<td>38.1 ± 11.4</td>
<td>51.0 ± 13.8</td>
<td>47.2 f</td>
<td>67.9 ± 13.7</td>
</tr>
<tr>
<td>Uosm (mosm/kg H2O)</td>
<td>124 ± 1.6</td>
<td>218 *†</td>
<td>258 f†</td>
<td>171 ± 2.9</td>
</tr>
<tr>
<td>Curo (ml/min)</td>
<td>0.24 ± 0.02</td>
<td>0.09 *†</td>
<td>0.07 f†</td>
<td>0.38 ± 0.10</td>
</tr>
<tr>
<td>Curi (ml/min)</td>
<td>0.17 ± 0.02</td>
<td>0.25 ± 0.06</td>
<td>0.32 f†</td>
<td>0.43 ± 0.01</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. C, control periods; H, hypoxemia periods; R, recovery periods; n, number of animals; V, urinary flow rate; Cioth, clearance of iothalamate; UV, urinary excretion of electrolytes; FE, fractional excretion of electrolytes.

* P < 0.05 when C compared to H; † P < 0.05 when C compared to R.

Pure increase in free water reabsorption. The Uosm levels found during fetal hypoxemia (Fig. 2) correlated very closely with gestational age in fetuses over 130 days of gestation (r = 0.86, P < 0.005); however, there was no correlation between plasma arginine vasopressin levels and urine osmolality or fetal age during hypoxemia in either group of fetuses.

A significant increase in fractional excretion of sodium (FENa+) and chloride (FECl-) was observed in both groups of fetuses during hypoxemia (Table 4). An increase in mean urinary Na+ and Cl− excretion was also observed; however, only fetuses of less than 120 days of gestation showed a significant increase in Cl− excretion during hypoxemia. Fractional excretion of K+ and mean urinary K+ excretion...
tion did not change significantly during hypoxemia in either group of fetuses.

One hour after hypoxemia, the mean fetal GFR was significantly higher than control values in fetuses over 130 days. In seven of nine fetuses of less than 120 days, GFR increased. The percent increase in GFR during recovery, when compared to control in fetuses of less than 120 days (16 ± 7%), was not significantly different from the percent change found in older fetuses (35 ± 11%). Mean values for urinary excretion of Na⁺, K⁺, and Cl⁻ were significantly higher than control values during the recovery phase of hypoxemia in both groups of fetuses. The increase in electrolyte excretion paralleled increases in \( C_{\text{osm}} \) and \( C_{\text{osm}} \) during recovery when compared to control values (Table 4).

### Effect of Hypoxemia on Fetal Urinary Prostaglandin Excretion

The changes in urinary prostaglandin (\( \text{UpGE} \) and \( \text{UpGF}_{2\alpha} \)) excretion during and after hypoxemia are presented in Table 5. Using non-parametric statistics, it was found that, during control periods, the urinary excretion rates of PGE and PGF₂α, expressed in ng/min, were significantly higher in fetuses of more than 130 days than in fetuses of less than 120 days gestation. When these values were corrected for kidney weight, urinary excretion rates of PGE and PGF₂α still were higher in fetuses over 130 days than in fetuses of less than 120 days, but the values were not statistically different.

During hypoxemia \( \text{UpGE} \) and \( \text{UpGF}_{2\alpha} \) did not change significantly in either group of fetuses (Table 5). After hypoxemia, \( \text{UpGE} \) and \( \text{UpGF}_{2\alpha} \) increased, respectively, in four of five, and in five of five fetuses over 130 days gestation. Moreover, during the recovery period, the \( \text{UpGE} \) and \( \text{UpGF}_{2\alpha} \) levels expressed either in ng/min or ng/min per g of kidney weight were significantly higher in fetuses over 130 days than in the group of fetuses of less than 120 days gestation.

### Discussion

#### Influence of Hypoxemia on Fetal Blood Values

Analysis of the fetal renal response to hypoxemia has been complicated by the various techniques and experimental conditions employed (Daniel et al., 1975, 1978; Walker et al., 1977). In the present study, using chronically catheterized fetuses, fetal hypoxemia was produced without any significant changes in arterial pH, and fetal \( \text{Pco}_2 \) was maintained within normal range (Comline and Silver, 1970) despite a slight decrease secondary to maternal hyperventilation. However, there were small but significant increases in plasma potassium and lactate concentrations during hypoxemia and a significant decrease in fetal pH 1 hour after hypoxemia (Table 1), corresponding to a return from hyperventilation to normal breathing by the ewe. Fetal hypoxemia was accompanied by a significant rise in hematocrit and total protein concentration in both groups of fetuses and a significant increase in plasma osmolality in the group of near-term fetuses, suggesting that during fetal hypoxemia there is a shift of water from the vascular to the extravascular space. Battaglia et al. (1958) have suggested previously that a rise in intracellular osmotic pressure would be at the origin of the water movement out of the vascular space during hypoxemia.

### Table 5  Effect of Hypoxemia on Fetal Urinary Prostaglandin Excretion

<table>
<thead>
<tr>
<th></th>
<th>&lt;120 days ((n = 4))</th>
<th>&gt;130 days ((n = 5))</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{UpGE} ) ((\text{ng/min}))</td>
<td>0.41 ± 0.08</td>
<td>1.66 ± 1.02</td>
<td>0.05</td>
</tr>
<tr>
<td>( \text{UpGE} ) ((\text{ng/min per gKW}))</td>
<td>0.026 ± 0.007</td>
<td>0.058 ± 0.038</td>
<td>NS</td>
</tr>
<tr>
<td>( \text{UpGF}_{2\alpha} ) ((\text{ng/min}))</td>
<td>0.82 ± 0.14</td>
<td>1.14 ± 0.30</td>
<td>NS</td>
</tr>
<tr>
<td>( \text{UpGF}_{2\alpha} ) ((\text{ng/min per gKW}))</td>
<td>0.085 ± 0.031</td>
<td>0.038 ± 0.014</td>
<td>NS</td>
</tr>
<tr>
<td>( \text{UpGF}_{2\alpha} ) ((\text{ng/min}))</td>
<td>0.74 ± 0.52</td>
<td>3.94 ± 0.81</td>
<td>0.05</td>
</tr>
<tr>
<td>( \text{UpGF}_{2\alpha} ) ((\text{ng/min per gKW}))</td>
<td>0.048 ± 0.009</td>
<td>0.124 ± 0.041</td>
<td>0.05</td>
</tr>
<tr>
<td>( \text{UpGF}_{2\alpha} ) ((\text{ng/min}))</td>
<td>0.56 ± 0.18</td>
<td>5.01 ± 1.95</td>
<td>0.05</td>
</tr>
<tr>
<td>( \text{UpGF}_{2\alpha} ) ((\text{ng/min per gKW}))</td>
<td>0.034 ± 0.019</td>
<td>0.169 ± 0.149</td>
<td>NS</td>
</tr>
<tr>
<td>( \text{UpGF}_{2\alpha} ) ((\text{ng/min}))</td>
<td>0.76 ± 0.30</td>
<td>3.72 ± 1.42</td>
<td>NS</td>
</tr>
<tr>
<td>( \text{UpGF}_{2\alpha} ) ((\text{ng/min per gKW}))</td>
<td>0.046 ± 0.016</td>
<td>0.125 ± 0.048</td>
<td>NS</td>
</tr>
<tr>
<td>( \text{UpGF}_{2\alpha} ) ((\text{ng/min}))</td>
<td>0.74 ± 0.20</td>
<td>7.31 ± 2.99</td>
<td>0.05</td>
</tr>
<tr>
<td>( \text{UpGF}_{2\alpha} ) ((\text{ng/min per gKW}))</td>
<td>0.046 ± 0.010</td>
<td>0.248 ± 0.101</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*Values are mean ± SEM, \( n \), number of animals; NS, not significant.*
EFFECTS OF HYPOXEMIA DURING FETAL LIFE/Robbilard et al. 135

Influence of Hypoxemia on Fetal Renal Hemodynamics and Circulating Vasoactive Substances

The renal hemodynamic response to hypoxemia was similar in young and near-term fetuses. Renal blood flow decreased and renal vascular resistance increased proportionately in both groups of fetuses during hypoxemia. Similar results were observed by Cohn et al. (1974) and Millard et al. (1979) in unanesthetized fetal lambs over 120 days of gestation subjected to hypoxemia. In the present study the significant increase in filtration fraction associated with absence of changes in GFR suggest that during fetal hypoxemia the renal vasoconstriction is more important at the efferent than at the afferent arteriolar level and that fetal GFR is not necessarily plasma flow dependent. Similar findings also have been made by Alward et al. (1978) in newborn piglets following the simultaneous production of hypoxemia, hypercarbia, and acidosis.

In addition, we also demonstrated that, in near-term fetuses, the decrease in renal blood flow during hypoxemia was associated with an increase in PRA, pAVP, and plasma catecholamine concentrations. However, the decrease in renal blood flow correlated more closely with changes in PRA ($r = -0.77$) than with changes in pAVP concentrations. Because of the small number of near-term fetuses in which plasma catecholamine concentrations were determined, we were unable to evaluate the effect of the rise in plasma catecholamine on the decrease in renal blood during hypoxemia. However, both the absence of changes in PRA and the absence of a significant correlation between the decrease in RBF and the increase in pAVP concentrations ($r = 0.18$) during hypoxemia in the group of young fetuses, suggest that the stimulation of the sympathetic-adrenal system may participate in the regulation of RBF during hypoxemia in very immature fetuses. Furthermore, the increase in total plasma catecholamine concentrations during hypoxemia was significantly ($P < 0.005$) lower in young (5.04 ± 0.47 ng/ml) than in near-term fetuses (18.73 ± 3.28 ng/ml), whereas the percentage change in RBF was not significantly different between both groups of fetuses (−30 ± 6% in <120 days and −33 ± 10% in >120 days). These observations suggest that the renal vasculature in young fetuses (<120 days) is more sensitive to circulating catecholamines than in near-term fetuses. In support of this hypothesis, Jose et al. (1974) demonstrated that, during infusion of varying concentrations of epinephrine into the renal artery of newborn (45–56 days) and adult dogs, there was an increased vascular sensitivity to epinephrine in puppies, suggesting that maturation played a role in the renal vascular response to catecholamines. However, it remains possible that the near-term fetuses reached maximum renal vasoconstriction at lower levels of plasma catecholamines or that there may be other factors that inhibit or limit renal vasoconstriction during hypoxemia in the more mature fetuses. In favor of this last hypothesis, Millard et al. (1979), using fetal lambs between 125 and 140 days of gestation, demonstrated that prostaglandins protected the fetal renal blood flow against massive vasoconstriction during hypoxemia.

During the recovery period, the fetal renal blood flow returned to control levels in young fetuses and significantly exceeded control levels (reactive hyperemia) in near-term fetuses. Factors that modulate the return of the fetal renal blood flow to control levels or permit reactive hyperemia in near-term fetuses presently are unknown. However, previous studies done in adult animals suggest that reactive hyperemia following renal artery occlusion is mediated by prostaglandins (Herbaczynaska and Vane, 1974; Spielman and Osswald, 1978). In the present study we demonstrated that significant increases in urinary excretion of PGE and PGF$_{2a}$ accompanied the post-hypoxic renal hyperemia in near-term fetuses; however, in young fetuses there was no evidence of reactive hyperemia nor was there a rise in urinary prostaglandins excretion. These findings suggest that prostaglandin synthesis by the fetal kidney may modulate the renal hyperemic response after hypoxemia in near-term fetuses.

Influence of Hypoxemia on the Fetal Renin-Angiotensin System

The fact that PRA increased significantly during hypoxemia in near-term fetuses when no changes were seen in the group of young fetuses is difficult to explain. In previous experiments, Pipkin et al. (1974) were unable to demonstrate a rise in fetal PRA during hypoxemia in fetuses between 126 and 142 days of gestation and suggested that fetal hypertension occurring in near-term fetuses during hypoxemia was not due to increased renin production. In the present study, however, fetal PRA increased significantly in near-term fetuses despite a significant rise in mean arterial blood pressure, arginine vasopressin, and plasma potassium concentration, factors known to decrease PRA in fetal (Lumbers and Lewes, 1979; Robillard and Weitzman, 1980) and adult animals (Davis and Freeman, 1976). Since the fetal blood volume was maintained constant during the experiment, the rise in fetal PRA, seen in near-term fetuses during hypoxemia, could not be explained by changes in fetal blood volume. On the other hand, the state of activation of the renin-angiotensin system during fetal life and the decrease in fetal renal blood flow and increase in fetal sympathetic activity during hypoxemia may contribute, as in adult animals (Davis and Freeman, 1976; Zakheim et al., 1976), to increase PRA in near-term fetuses. The fact that the group of young fetuses was unable to increase PRA during hypoxemia cannot be explained by the present study. However, it is possible to speculate that the anatomical and/or physiological immaturity at the jux-
taglomerular cell level may be responsible for the lack of response to hypoxemia in fetuses of less than 120 days of gestation.

**Influence of Hypoxemia on Fetal Blood Pressure**

The failure to demonstrate an increase in blood pressure and a decrease in heart rate in fetuses of less than 120 days of gestation is in agreement with a previous study by Walker et al. (1979) in which it was suggested that the immaturity of the autonomic function early in gestation explained the difference in the response to hypoxemia between young and near-term fetuses. Moreover, in the present study, in accordance with previous studies (Comline and Silver, 1966; Jones and Robinson, 1975), we demonstrated that epinephrine and norepinephrine increased significantly in both groups of fetuses. We also found, as Comline and Silver (1966) did in exteriorized fetuses, that the rise in plasma catecholamine concentration was significantly higher in the older group of fetuses, suggesting that the degree of maturity of the sympathetic nervous system and/or the adrenal medulla may modulate catecholamine release during fetal hypoxemia as previously demonstrated by Hyman et al. (1978).

Finally, in our studies, the increase in blood pressure in the near-term fetuses did not correlate with either the rise in fetal PRA or the increase in pAVP concentrations.

**Influence of Hypoxemia on Water and Electrolyte Excretion by the Fetal Kidney**

We demonstrated in the present study that fetal hypoxemia influences free water reabsorption and electrolyte excretion by the fetal kidney. In previous studies it was suggested that the increase in fetal urine osmolality associated with hypoxemia (Daniel et al., 1975, 1978) was secondary to an increase in fetal pAVP (Alexander et al., 1972; Daniel et al., 1975 and 1978; Rurak, 1978). However, since fetal acidosis and hypercapnia accompanied fetal hypoxemia, interpretation of the previous results (Daniel et al., 1975, 1978) is very difficult.

In the present study, simultaneous decreases in $C_{\text{H}_2O}$ and $V$ not associated with significant changes in $C_{\text{urea}}$ were observed in the majority of near-term fetuses (nine of 10 fetuses) and in half of the young fetuses (five of nine fetuses) (Fig. 1), suggesting that fetal hypoxemia induces fetal diuresis. This antidiuretic effect of fetal hypoxemia, seen mainly in near-term fetuses (Fig. 2), probably was mediated through hypoxemia-mediated vasopressin release, since pAVP concentration rose in both groups of fetuses during hypoxemia. However, one can argue that, since there was no significant correlation between the fetal pAVP levels and fetal $U_{\text{urea}}$ during hypoxemia, there is no relationship between fetal antidiuresis and endogenous AVP secretion. However, the fact that maximal antidiuresis is achieved in fetal lambs at pAVP concentrations between 5 and 6 $\mu$U/ml (Robillard and Weitzman, 1980) is against this hypothesis, since the pAVP levels reached in the present studies during hypoxemia were above those levels. Therefore, the absence of correlation between fetal $U_{\text{urea}}$ and fetal pAVP only reflects the fact that the fetal pAVP levels were in excess of those needed to produce maximal antidiuresis during fetal life. Moreover, the finding of a higher proportion of near-term than young fetuses showing free water reabsorption during hypoxemia does not seem to be related to the presence of lower pAVP concentrations in young than near-term fetuses, but more on the increased ability of the fetal nephron to respond to AVP with increased gestational age (Fig. 2) as previously suggested (Robillard and Weitzman, 1980). Finally, the rise in pAVP concentration during fetal hypoxemia in the absence of fetal acidosis demonstrates, contrary to previous studies (Alexander et al., 1972; Daniel et al., 1975 and 1978; Rurak, 1978), that hypoxemia itself is a potent stimulus for pAVP secretion during fetal life. Similar findings also were reported for adult dogs by Anderson et al. (1978) and more recently by Stark et al. (1979) in fetal lambs.

Factors other than an increase in arginine vasopressin secretion also may have influenced the ability of the fetal kidney to reabsorb free water during hypoxemia. Levinsky et al. (1959) demonstrated in adult dogs that a decrease in GFR produced an increase in urine osmolality; however, significant alterations in fetal GFR were not observed in the present study during the period of hypoxemia. A significant decrease in RBF (Berliner and Davidson, 1957), increased concentration of circulating catecholamines, and stimulation of the renal nerve (Korner, 1963) are factors also known to affect renal water reabsorption. In the present study, the influence of those factors on the antidiuretic response to hypoxemia seen in some fetuses cannot be ruled out.

The effect of hypoxemia on fetal electrolyte excretion was characterized by significant increases in fractional excretion of sodium and chloride during hypoxemia and by significant increases in both fractional excretion and absolute excretion of sodium and chloride following hypoxemia in both groups of fetuses. In previous experiments, Alward et al. (1978) demonstrated in newborn piglets that production of hypoxemia associated with hypercarbia and acidosis increased absolute sodium excretion. In the present study, factors that could have contributed to the increase in electrolytes excretion during hypoxemia, and especially after hypoxemia, were not investigated. However, this effect of hypoxemia is probably multifactorial, where prostaglandins, catecholamines, and vasopressin—as well as alterations of enzymatic process involved in Na⁺ transport—have a role to play. The identification of factors responsible for increased sodium excretion during and after hypoxemia in immature animals may have important clinical implications since
hyponatremia has been described previously in asphyxia neonatorum (Feldman et al., 1970). In summary, it is demonstrated in the present study that: (1) the decrease in RBF during hypoxemia correlated closely with changes in plasma renin activity ($r = -0.77$) in the group of near-term fetuses. However, it is also suggested that the renal vasculature in young fetuses (<120 days) is perhaps more sensitive to circulating catecholamine than in near-term fetuses. (2) The absence of significant changes in GFR during hypoxemia while a significant decrease in RBF is observed suggests that during fetal hypoxemia the renal vasoconstriction is more important at the efferent than at the afferent arteriolar level and that this mechanism is present early in gestation. (3) Prostaglandin synthesis by the fetal kidney may modulate the renal hyperemic response following hypoxemia in near-term fetuses. In fetuses of less than 120 days, there was no evidence of reactive hyperemia nor was there a rise in urinary prostaglandins excretion. (4) Fetal hypoxemia produces a significant increase in fetal pAVP associated with an antidiuresis in all but one near-term fetus and in 50% of the young fetuses, suggesting that the ability of the fetal kidney to reabsorb free water is more developed in near-term fetuses. (5) The effect of hypoxemia on fetal arterial pressure and heart rate was significantly different when young fetuses were compared to near-term fetuses. It is suggested that the immaturity of the autonomic function early in gestation may explain this difference. (6) Finally, the fetal renin-angiotensin system is less sensitive to hypoxemia stress early in gestation, but this response increases as the fetus matures.

Acknowledgments

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Vasoactive Intestinal Polypeptide and the Canine Cerebral Circulation

DAVID A. WILSON, JOHN T. O’NEILL, SAMI I. SAID, AND RICHARD J. TRAYSTMAN

SUMMARY A potential role for cerebrovascular nerves containing vasoactive intestinal polypeptide (VIP) was examined in 24 anesthetized, ventilated dogs. Cerebral blood flow (CBF) was measured by either the cerebral venous outflow or microsphere method. Plasma VIP concentration was measured by radioimmunoassay. Hypercapnia (5% and 10% CO2) and hypoxia (7% O2) produced significant increases in cerebral venous outflow, but had no affect on arterial or cerebral venous VIP concentrations. Measurements of VIP in cerebrospinal fluid (CSF) made during 5% and 8% CO2 breathing also were not different from control values. VIP produced large dose-dependent increases in common carotid artery and temporalis muscle blood flow when injected or infused intraarterially; however, VIP had no effect on total or regional cerebral blood flow (rCBF) within the brain when administered in a similar manner. Unilateral perfusion of the cerebral ventricles with VIP produced significant increases (range: 11-80%) in CBF. These data are consistent with the possibility that local release of VIP from perivascular nerve endings could affect CBF. The unresponsiveness of canine cerebral vessels to blood-borne VIP may be due to the blood-brain barrier, since VIP dilates cerebral vessels when the barrier is bypassed by intraventricular infusion. These studies do not support the hypothesis that CBF changes induced during hypercapnia or hypoxia are mediated by VIP. Circ Res 48: 138-148, 1981

CEREBRAL blood vessels receive a rich complement of both adrenergic and cholinergic nerve fibers (Edvinsson and MacKenzie, 1977); however, despite intense investigation, the functional significance of these fibers remains controversial (Heistad and Marcus, 1978). Second, morphological studies have shown that another neurotransmitter may be involved. Third, transmural electric stimulation of isolated cerebral vessels evokes relaxation of vascular smooth muscle that is not inhibited by \( \alpha \)-adrenergic, \( \beta \)-adrenergic, cholinergic, histaminergic, or serotoninergic receptor site antagonists (Lee et al., 1978). However, Lee et al. (1976) also showed that vasocostriction was not inhibited by a antagonists, suggesting that either the \( \alpha \) receptor is unusual or that another neurotransmitter may be involved. Second, morphological studies have shown that many cerebral vessels contain fibers similar to type-P peptidergic nerve fibers found in the gastrointestinal tract and that some of these fibers contain vasoactive intestinal polypeptide (VIP) (Larsson et al., 1976a). VIP, originally isolated from porcine duodenum (Said and Mutt, 1970a), is a 28 amino acid residue peptide, structurally related to secretin and glucagon (Said and Mutt, 1972). In the central nervous system, VIP occurs in high concentrations in corti-
Developmental aspects of the renal response to hypoxemia in the lamb fetus.
J E Robillard, R E Weitzman, L Burmeister and F G Smith, Jr

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