Polyuria and impaired renal blood flow after asphyxia in preterm fetal sheep

J. S. Quaedackers, V. Roelfsema, C. J. Hunter, E. Heineman, A. J. Gunn, and L. Bennet

The Liggins Institute, The University of Auckland, Auckland, New Zealand

Submitted 13 October 2003; accepted in final form 3 November 2003

Quaedackers, J. S., V. Roelfsema, C. J. Hunter, E. Heineman, A. J. Gunn, and L. Bennet. Polyuria and impaired renal blood flow after asphyxia in preterm fetal sheep. Am J Physiol Regul Integr Comp Physiol 286: R576–R583, 2004. First published November 6, 2003; 10.1152/ajpregu.00592.2003.—Renal impairment is common in preterm infants, often after exposure to hypoxia/asphyxia or other circulatory disturbances. We examined the hypothesis that this association is mediated by reduced renal blood flow (RBF), using a model of asphyxia induced by complete umbilical cord occlusion for 25 min (n = 13) or sham occlusion (n = 6) in chronically instrumented preterm fetal sheep (104 days, term is 147 days). During asphyxia there was a significant fall in RBF and urine output (UO). After asphyxia, RBF transiently recovered, followed within 30 min by a secondary period of hypoperfusion (P < 0.05). This was mediated by increased renal vascular resistance (RVR, P < 0.05); arterial blood pressure was mildly increased in the first 24 h (P < 0.05). RBF relatively normalized between 3 and 24 h, but hypoperfusion developed again from 24 to 60 h (P < 0.05, analysis of covariance). UO significantly increased to a peak of 249% of baseline between 3 and 12 h (P < 0.05), with increased fractional excretion of sodium, peak 10.5 ± 1.4 vs. 2.6 ± 0.6% (P < 0.001). Creatinine clearance returned to normal after 2 h; there was a transient reduction at 48 h to 0.32 ± 0.02 ml·min⁻¹·g⁻¹ (vs. 0.45 ± 0.04, P < 0.05) corresponding with the time of maximal depression of RBF. No renal injury was seen on histological examination at 72 h. In conclusion, severe asphyxia in the preterm fetus was associated with evolving renal tubular dysfunction, as shown by transient polyuria and natriuresis. Despite a prolonged increase in RVR, there was only a modest effect on glomerular function.

acute renal failure; perfusion; prematurity

PRETERM INFANTS are well known to be at increased risk of acute renal failure as well as transient fluid and electrolyte disturbances particularly in the first few days of life (9, 19, 52). The etiology of neonatal acute renal failure is most often associated with hemodynamic disturbances such as hypotension, hypovolemia, and exposure to acute asphyxia (44, 48). It has been proposed that since preterm infants have a low basal glomerular filtration rate, which is very close to basal renal blood flow (RBF), the pathogenesis of this renal impairment is predominantly prerenal, i.e., secondary to reduced intrarenal blood flow (45). However, there is little direct experimental evidence.

Moderate hypoxia in the near-term fetus is associated with an acute decrease in fetal urine production (41), whereas during prolonged hypoxia urine output (UO) and glomerular filtration rate and urine osmolality rapidly normalize (14). Similarly, in near-term fetal sheep prolonged moderate hypoxia was associated with an increase in RBF, which returned to control values during recovery (8, 22). However, in contrast with the effects of moderate hypoxia, exposure to acute asphyxia, with acute hypotension and profound hypoxia (5), commonly results in significant postinsult hemodynamic perturbations. For example, clinical studies in near-term infants have demonstrated that asphyxia is associated with reduced RBF velocity on the first, but not the third, day of life using Doppler ultrasound (2, 34).

There are only limited data on the effect of asphyxia on RBF in the preterm fetus, which suggest that RBF is acutely reduced during asphyxia but normal 2 and 4 h after asphyxia despite impaired glomerular filtration and a small increase in renal vascular resistance (RVR; 38). This is somewhat surprising since, in contrast, recent evidence using continuous blood flow monitoring suggests that exposure to asphyxia in the premature fetal sheep leads to prolonged secondary hypoperfusion in other peripheral vascular beds, including the femoral and gastrointestinal beds (4, 5). These data raise the possibility that similar changes in renal perfusion would lead to significant impairment of renal function.

In the present study we examined the hypothesis that exposure to severe asphyxia in the preterm sheep fetus would lead to secondary renal hypoperfusion and that this would be associated with impaired glomerular and tubular function. Renal histological examination and immunohistochemical staining for disruption of cytoskeletal anchorage of Na-K-ATPase after 3 days recovery were undertaken to assess the presence of renal damage.

METHODS

Animal preparation. All procedures were approved by the Animal Ethics Committee of the University of Auckland. Singleton Romney/Suffolk fetal sheep were instrumented using sterile techniques at 97–98 days of gestation (term = 147 days) under general anesthesia induced by Alfaxalone (2.5 mg/kg; Schering Plough Animal Health, Wellington, NZ) and maintained with 2–3% halothane in oxygen (4). Ewes were given 5 ml of Streptycin (Stockguard Laboratories, Hamilton, New Zealand) intramuscularly for prophylaxis before the start of surgery. The uterus was exposed via a midline incision, and the fetal hindlimbs and abdomen were exteriorized. Polyvinyl catheters were placed in the left femoral artery and vein to measure fetal blood pressure. The fetal bladder was exposed via a paramedian incision, and a blunted polystyrene catheter was inserted to allow continuous gravity drainage of fetal urine. The left fetal kidney was exposed via a paravertebral incision, and a size 2SB ultrasonic flow probe (Transonic Systems, Ithaca, NY) was placed around the left renal artery to continuously record RBF. The uterus was then closed in layers, and the upper body of the fetus was exteriorized via a second uterine incision. A polystyrene catheter was placed in the right brachial artery for blood sampling. Two multiperforated catheters were placed in the amniotic sac for reading of intra-amniotic pressure and the return of collected urine. Electrocardiogram (ECG) electrodes (Cooner Wire, Chatsworth, CA) were sewn across the chest to record the fetal ECG.

An inflatable silicone occluder was placed around the umbilical cord.
of all fetuses for the induction of asphyxia (In Vivo Metric, Healdsburg, CA). All fetal leads were exteriorized through the maternal flank, and a maternal long saphenous vein was catheterized. Antibiotics (80 mg gentamicin, Pharmacia and Upjohn, Perth, Australia) were administered into the amniotic sac before closure of the uterus.

After surgery sheep were housed together in the vivarium in separate metabolic cages with ad libitum access to water and food. They were kept in a temperature-controlled room (16 ± 1°C, humidity 50 ± 10%), in a 12:12-h light-dark cycle. A period of 6–7 days postoperative recovery was allowed, during which time antibiotics were administered intravenously daily to the ewe (600 mg Crystapen, Biochemie, Vienna, Austria, for 4 days and 80 mg gentamicin, daily for the first 3 days). Fetal arterial blood was taken daily from the brachial artery for blood gas analysis for the assessment of fetal health. Catheter patency was maintained by continuous infusion of heparinized saline (20 U/ml at 0.2 ml/h).

**Experimental design.** Fetal mean arterial pressure (MAP) and venous pressure (MVP) corrected for maternal movement by subtraction of intra-amniotic fluid pressure, fetal heart rate (FHR) derived from the ECG, and RBF were recorded continuously from 24 h before umbilical cord occlusion until 72 h afterward. Data were stored to disk by custom software for off-line analysis (Labview for Windows, National Instruments, Austin, TX). Urine was collected continuously using a collection bag on an occlusion collector, hourly volume was measured, and urine was returned to the amniotic sac after sterilization using a 0.2-µm syringe filter (Acrodisc, Gelman Laboratory, Ann Arbor, MI).

Experiments were conducted at 103–104 days gestation. Fetuses received either sham occlusion (n = 6) or asphyxia induced by rapid inflation of the umbilical cord occluder for 25 min (n = 13) with sterile saline of a defined volume known to completely inflate the occluder. Successful occlusion was confirmed by observation of an immediate sharp rise in MAP and a rapid fall in FHR (4, 5). Blood samples were taken at 15 min before occlusion, 5 and 17 min during occlusion, and 30 min, 2, 4, 8, 24, 48, and 72 h postocclusion for pH and blood gas determination (Ciba-Corning Diagnostics 845 blood gas analyzer and co-oximeter), glucose, and lactate measurements (YSI model 2300, Yellow Springs, OH). Blood samples were taken for measurement of creatinine, sodium, potassium, osmolality, atrial natriuretic peptide (ANP), and plasma renin activity (PRA) at 15 min before occlusion, and 30 min, 2, 4, 8, 24, 48, and 72 h postocclusion (Auckland Healthcare Laboratory Services, Auckland, NZ). ANP and PRA were also measured at 17 min during asphyxia. Plasma ANP levels were measured by ovine specific radioimmunoassay as previously reported (10). PRA was calculated as the amount of angiotensin I produced per liter of plasma per hour (18). Paired urine samples were taken for measurement of urine composition in 8 of the 13 asphyxia group fetuses; insufficient urine samples were available for analysis in the sham control group. On collection, blood was transferred into chilled test tubes and spun at 4°C (3,000 rpm) for 15 min. Plasma and urine were stored at −80°C for subsequent analysis.

On completion of the experiment at 72 h the ewe and fetus were killed with an intravenous overdose of pentobarbital sodium. Fetuses were removed, weighed, and examined for the presence of hydrops fetalis, and samples of the fetal kidney were taken for histological examination. Tissue specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned (4 µm), and stained with hematoxylin and eosin (H & E). Immunohistochemical staining of Na,K-ATPase was performed after blocking nonspecific binding sites. The primary antibody (MA3–929, mouse anti-sheep α1-subunit of Na,K-ATPase, Affinity Bioreagents, Golden, CO) was incubated for 48 h at 4°C at a concentration of 1:100. After repeated washes the slides were exposed to secondary horse anti-mouse antibody for 24 h at 4°C and ABC reagent for 1 h at room temperature (Vector Laboratories, Burlingame, CA). Slides were developed with diaminobenzidine (DAB).

**Data analysis and statistics.** Renal arterial vascular resistance (RVR) was calculated using the formula (MAP – MVP)/absolute RBF (mmHg•min/ml). Creatinine clearance (ClCr) and fractional excretion of sodium and potassium (FeNa, FeK) were calculated as follows

\[ ClCr = \frac{\text{urine}_{\text{Creat}} \times \text{volume}}{\text{plasma}_{\text{Creat}} \times \text{kidney wt}} \]

\[ FE_{Na} = \frac{\text{urine}_{\text{Na}}}{\text{plasma}_{\text{Na}}} = \frac{\text{urine}_{\text{Creat}}}{\text{plasma}_{\text{Creat}}} \]

Statistical analysis was performed using SPSS (SPSS, Chicago, IL). The effect of asphyxia on continuous variables was tested by ANOVA, with time treated as a repeated measure to allow for repeated sampling. For comparisons over time, baseline levels were used as a covariate. For the analysis of the occlusion and 1-h postocclusion periods, data were compared with the mean of the hour before occlusion (baseline). For the analysis of the long-term recovery data (1–72 h postocclusion), the baseline period was taken as the mean of the 12 h before occlusion. These data were analyzed in 12-h blocks by repeated measurement ANOVA. If a significant effect of group was found for the first 12 h, since rapid changes occurred for many parameters within this interval, individual time points were compared by ANOVA, adjusted using the baseline levels as the covariate. The within-subjects relationship of RBF and ClCr was determined by regression analysis using the method of Bland and Altman (7). Proportions were compared using the Fisher exact test. Statistical significance was accepted when P < 0.05. Data are presented as means ± SE.

**RESULTS**

**Blood composition measurements during asphyxia.** Before each experiment, all fetuses had normal blood gas, acid-base, and glucose and lactate status according to the standards of our laboratory and were not statistically different between groups. Umbilical cord occlusion was associated with the development of profound hypoxia and mixed respiratory and metabolic acidosis. Fetal arterial pH fell to 6.888 ± 0.013 at 17 min of cord occlusion (vs. 7.367 ± 0.004 in sham controls, P < 0.05) and normalized by 8 h postocclusion; at 72 h, fetal pH was 7.381 ± 0.008. Fetal arterial PO2 (PaO2) fell to 8.8 ± 0.6 at 17 min of asphyxia (vs. 23.2 ± 1.0 mmHg in sham controls, P < 0.05), rapidly normalized after occlusion, and subsequently increased moderately (27.2 ± 1.0 vs. 22.9 ± 0.7 mmHg in sham controls at 72 h, P < 0.05). Fetal arterial PCO2 (PaCO2) increased during occlusion to 134.9 ± 3.3 mmHg at 17 min (vs. 51.2 ± 2.1 mmHg in sham controls, P < 0.05) and returned to control values by 30 min postocclusion. Fetal arterial glucose decreased to 0.51 ± 0.1 at 17 min of occlusion (vs. 0.93 ± 0.0 mmol/l in sham controls, P < 0.05). Postocclusion fetal glucose increased rapidly and remained mildly elevated to the end of the study (1.12 ± 0.1 vs. 0.92 ± 0.1 mmol/l in sham controls, P < 0.05). Fetal arterial lactate increased during occlusion to 6.22 ± 0.2 at 17 min (vs. 0.58 ± 0.1 mmol/l in sham controls, P < 0.05) and postocclusion gradually normalized by 48 h; at 72 h lactate was 0.79 ± 0.0 vs. 0.66 ± 0.1 mmol/l in sham controls (not significant).

**Cardiovascular measurements during asphyxia.** Asphyxia led to progressive bradycardia with initial hypertension followed by the development of profound hypotension at the end of occlusion (Fig. 1). The nadir of MAP was 9.4 ± 0.5 mmHg (vs. 35.8 ± 0.6 mmHg in sham controls, P < 0.05), and FHR fell to 59 ± 4 beats/min at the end of occlusion (vs. 172 ± 3 beats/min in sham controls, P < 0.05). RBF was markedly reduced by asphyxia, in a triphasic pattern. At the onset of occlusion RBF fell rapidly to 0.8 ± 0.3 ml/min at 4 min of occlusion (vs. 13.8 ± 1.1 ml/min in sham controls, P < 0.05).
R578 ASPHYXIA AND THE PRETERM KIDNEY

Cardiovascular measurements during recovery. Postocclusion RBF transiently returned to sham control values (Fig. 1). This was followed by a secondary fall in RBF from ~25 min, with a marked elevation of RVR, such that RBF was significantly reduced compared with sham controls for the first 2 h (Fig. 2). Although the initial marked postocclusion increase in RVR became attenuated from 3 h onward, RVR was significantly elevated for most of the remaining recovery period, with an associated reduction in RBF (Fig. 2). RVR was transiently normalized between 8 and 18 h and appeared to be resolving toward the end of recovery, such that values of RVR and RBF were not significantly different from sham controls. There was significant rebound hypertension for the first 2 h postocclusion, and MAP remained significantly elevated during much of the first 24 h postocclusion (P < 0.05), progressively returning to baseline thereafter (Fig. 2).

Fetal heart rate (Fig. 1) showed a brief rebound tachycardia at the end of occlusion, peaking at 6 min at 243 ± 5 (vs. 182 ± 2 beats/min in sham controls, P < 0.05); however, subsequently FHR was not significantly different from sham controls (data not shown).

Renal function. UO was markedly decreased during occlusion to a mean of 1.7 ± 0.3 ml-kg^{-1}-h^{-1} [vs. 9.1 ± 0.5 ml-kg^{-1}-h^{-1} at baseline (Fig. 3, P < 0.05)]. Postocclusion, UO remained significantly decreased for 1 h followed by a marked increase in UO between 3 and 12 h, maximal at 3 h (22.2 ± 5 ml-kg^{-1}-h^{-1}, P < 0.05). UO then returned to control values for the remainder of the study (Fig. 3).

ClCr (Fig. 3) was reduced during the first 2 h postocclusion (0.22 ± 0.03 vs. 0.45 ± 0.04 ml-min^{-1}g^{-1} at baseline, P < 0.05) and again at 48 h to 0.32 ± 0.02 ml-min^{-1}g^{-1} (P < 0.05). The reduction at 48 h corresponded in time with the lowest level of RBF in the final 48 h. There was a positive within-subjects correlation between RBF and ClCr, r^2 = 0.16 (Fig. 4, P = 0.002).

FE_{Na} (Fig. 3) was increased during the first 24 h postocclusion, reaching a maximum of 10.5 ± 1.4% at 2 h (vs. 2.6 ± 0.6% at baseline, P < 0.05). FE_{K} gradually increased, later than the increased FE_{Na}, to a maximum of 23.1 ± 3.8% at 24 h (vs. 4.3 ± 0.5% at baseline, P < 0.05) (Fig. 3). Urine osmolality was increased immediately postocclusion, maximal at 24 h (317 ± 26 vs. 149 ± 12 mosmol/kgH_2O at baseline, P < 0.05), returning to control values by 72 h (Fig. 3).

Fig. 1. Time sequence of changes in fetal mean arterial blood pressure (MAP), heart rate (FHR), renal blood flow (RBF), and renal vascular resistance (RVR) in sham controls (open circles, n = 6) and in fetuses exposed to umbilical cord occlusion for 25 min (filled circles, n = 13) from 60 min before occlusion to 60 min after release of occlusion or sham occlusion. The shaded area shows the period of occlusion or sham occlusion. Data are 1-min averages, shown as means ± SE. *P < 0.05, asphyxia group vs. sham controls, analysis of covariance (ANCOVA).
Plasma ANP and PRA. ANP was markedly increased during occlusion to 327 ± 65 pmol/l (vs. 35 ± 2 pmol/l in sham controls, \( P < 0.05 \), Fig. 5). After asphyxia ANP remained mildly increased during the first 8 h, with a maximum of 123 ± 45 pmol/l at 4 h, after which plasma ANP returned to control levels. PRA (Fig. 5) was decreased postocclusion and returned to baseline values by 24 h.

Postmortem examination, renal histology, and immunohistochemistry. Mild subcutaneous edema was observed in 5 of 13 asphyxia group animals vs. no edema in 6 sham control group animals (not significant, Fisher exact test). Frank ascites and pleural effusions were not seen in any fetus in either group. Average fetal body weight was 1,770 ± 66 g in the asphyxia group vs. 1,645 ± 61 g in the sham group (not significant). Immunohistochemical staining of Na,K-ATPase demonstrated no disruption of the predominantly basolateral cytoskeletal anchorage of Na,K-ATPase. Histological examination of H & E-stained sections by light microscopy confirmed that there...
was no tubular or glomerular injury after 3 days recovery in either the asphyxia or sham control groups.

**DISCUSSION**

Despite exposure to a near-terminal episode of asphyxia, associated with a severe reduction in RBF, preterm fetal sheep showed transient renal dysfunction during recovery, with no histological injury observed 3 days later. Recovery of renal function was characterized by a marked increase in UO and FENa during the first 24 h, which was associated with a moderate secondary rise in plasma ANP levels and a fall in PRA. We report for the first time that there was a prolonged secondary increase in RVR during recovery, which was just beginning to resolve after 3 days. Renal perfusion was maintained in part in the first 24 h by a mild increase in fetal blood pressure and in part by the transient period of relative normalization of RVR around 12 h postinsult. When blood pressure returned to control levels the increased RVR led to a prolonged reduction in RBF. Despite this continuing renal hypoperfusion in the 2nd and 3rd days of recovery, during this interval ClCr was only significantly but mildly impaired when RBF was most markedly reduced.

However, consistent with studies in the near-term fetus, this initial adaptation was rapidly lost, with a change in the mechanism of renal hypoperfusion from active vasoconstriction, with arterial hypertension, to a near pressure-passive fall secondary to worsening hypotension (29, 30). From ~4 min after the start of occlusion renal resistance fell progressively, with a reciprocal increase in RBF, although flow never reached control levels. Once overt hypotension developed, from 9 min of occlusion, RBF fell in parallel with the fall in blood pressure. The mechanism of this loss of renal vascular tone is unknown, but it is not unique to the renal vascular bed. In the near-term fetus the initial vasoconstriction during asphyxia is mediated by sympathetetic neural activity (30). Potentially, loss of vasoconstriction could reflect loss of sympathetic activity; however, this seems relatively improbable given that circulating catecholamines increase markedly during prolonged asphyxia (24). Levels of circulating ANP were markedly increased toward the end of cord occlusion in the present study by nearly 10-fold, consistent with the effect of inhalational hypoxia (11). ANP has direct renal vasodilatory effects in the fetal and postnatal sheep (49), and its effects are enhanced after ischemia in the adult dog (1). Interestingly, administration of ANP is reported to reduce renal damage in experimental models of...
ischemic acute renal failure (15); thus the present results raise the possibility that the increase in endogenous ANP may help limit renal injury during fetal asphyxia by reducing the duration of intense vasoconstriction.

After release of occlusion there was an initial rapid but transient recovery of RBF, followed by a secondary fall, such that mean RBF was significantly depressed for the first 2 h. This hypoperfusion occurred despite significant rebound hypertension as shown in Fig. 2 and was actively mediated, as demonstrated by the increase in RVR. This was accompanied by initial oliguria, reduced ClCr, and developing natriuresis (Fig. 3). Following this, polyuria developed with continuing natriuresis resolving between 13 and 24 h after reperfusion. The development of polyuria was broadly paralleled by relative normalization of RBF. The significance of this relationship is unknown but speculatively might reflect changes in renal metabolism during reversible injury.

Urinary osmolality remained increased until 48 h postocclusion, reflecting the overlapping time courses of the secondary increases in \( FE_{Na} \) and \( FE_{K} \) (Fig. 3). The later increase in potassium excretion, at the time that the natriuresis was resolving, is consistent with improving renal function allowing increased tubular sodium/potassium exchange. Glomerular function, as indicated by ClCr, was only briecllycreased tubular sodium/potassium exchange. Glomerular function after severe asphyxia in the preterm fetus. This overall pattern suggests primary, reversible tubular dysfunction. Clinically, this pattern is well described and may be more common than the classic pattern of oliguric renal failure in newborns (23, 31), although currently there are few data in preterm infants. The finding of normal renal architecture in the present study after 3 days recovery is consistent with the generally good clinical prognosis of polyuric renal failure (31). This and the finding of a normal distribution of Na,K-ATPase in the present study are consistent with the reported greater renal tolerance to ischemic injury in the immature rat, as shown by reduced Na,K-ATPase dislocation, compared with adult rats (50).

There was a mild reduction in ClCr at 48 h of recovery, corresponding in time with the most pronounced decrease in RBF in the chronic recovery phase. Within-subjects regression analysis demonstrated a positive relationship between RBF and ClCr in all but one subject. However, since this relationship accounted for <20% of variance in ClCr, and the effect of impaired RBF on ClCr appears to be predominant only with substantial reductions in blood flow, additional factors are likely to be important particularly within the normal range. There are limited data on the determinants of changes in RBF after premature birth; however, Doppler flow measurements suggest that RVR is initially elevated shortly after birth and progressively falls with time (13, 47). These studies found only a weak relationship between RBF velocity and UO (32) and that oliguria is typically seen only with marked reductions in renal velocity. These data are consistent with the present study and do not support the hypothesis that the glomerular filtration rate in the preterm kidney is more dramatically affected by small reductions in renal perfusion than is the case at later ages (45).

Plasma ANP has an important physiological role in regulating fluid balance by inhibiting tubular sodium reabsorption and increasing UO (37, 51). In the present study, plasma ANP levels were modestly but significantly elevated for the first 24 h after asphyxia. Although this broadly corresponds with the period of polyuria, ANP levels were still mildly elevated at 24 h, when polyuria had resolved. This suggests that ANP may have contributed to the development of polyuria and natriuresis but is unlikely to be the primary mechanism. Limited data from studies of hypoxia at this gestation suggest that circulating cortisol levels may also be elevated after asphyxia and thus also contribute to increased MAP, polyuria, and natriuresis (46). Although as noted above ANP is overall a vasodilator, its effects on RBF can be complex, with afferent arteriolar vasodilation and efferent vasoconstriction (43). Thus the combination of elevated RVR with elevated ANP in the first 24 h may reflect alterations in this balance or simply that the levels of circulating ANP were insufficient to overcome the background vasoconstrictor tone. This late rise in ANP was not due to fetal hypoxia, which resolved rapidly after reperfusion, or to hypotension, because blood pressure was modestly elevated at this time, or to fetal tachycardia. In the adult, release of hypoxia-inducible factor during hypoxia induces atrial ANP gene expression (12), and thus a brief insult can lead to prolonged effects on ANP.

The renin-angiotensin system is also a significant potential contributor to changes in ClCr and renal perfusion. In the near-term fetus reduced perfusion pressure is a potent stimulator of renin release (42), which is mediated through activation of the sinoaortic receptors (33). However, there is conflicting evidence on the effect of hypoxia on PRA. Whereas postnatally, hypoxia is associated with increased PRA (3), no changes in PRA were observed during hypoxemia in the immature fetus (40, 41), and inhibition of the renin-angiotensin system during hypoxia has little effect on renal perfusion or function (36). These data are consistent with the present study, in which there was no significant change in PRA during cord occlusion.

There are few data on PRA during recovery from hypoxia (35). In the present study there was a consistent transient fall in PRA after asphyxia. The mechanism of this fall is unknown. The postasphyxial increase in ANP that parallels this fall may contribute, since infusions of ANP are reported to inhibit renin release in the isolated kidney (37) and in the adult dog (26), at least partly due to the increase in the filtered sodium load (51). An additional factor may be the increased renal perfusion pressure at this time, which is known to inhibit renin release (42). Finally, renal maturation is likely to be important. For example, in contrast with the present data, Lumbers et al. (35) found a sustained increase in PRA after severe asphyxia undertaken in fetal sheep at an even earlier stage of maturation, near-midgestation. It is striking that Lumbers et al. (35) found severe nonimmune hydrops fetalis after asphyxia (35), whereas in the slightly less immature (0.7 vs. 0.6 gestation) fetuses in the current study, despite a very similar insult, with a comparably severe degree of acidosis and hypotension, frank fetal hydrops was never seen, and mild skin subcutaneous edema only occurred in a minority of the fetuses. A subsequent study in the same model has shown that the pattern of recovery of renal function at 0.6 gestation was also very different from the present study (38). There was a more prolonged suppression of glomerular function at least to 4 h of recovery and no increase in UO or sodium loss, and without prolonged impairment of RBF, measured at 2 and 4 h of recovery (38).
These contrasting findings are consistent with the hypothesis that sustained activation of the renin-angiotensin system is an important mechanism in the development of fetal hydrops, through an increase in transplacental fluid uptake, as previously reviewed (17). Further, polyuria as seen in the current study may facilitate the excretion of such a fluid load and thus help alleviate the development of hydrops. The effect of maturation on the appearance of hydrops is intriguing and may relate to the greater anaerobic capacity of the younger fetuses, which permitted a greater tolerance to prolonged asphyxia (typically 30–40 min at 0.6 gestation vs. 25 min at 0.7 gestation) and thus exposed these younger fetuses to a more profound and prolonged period of hypotension and hypoperfusion (38). Alternatively, it may reflect a relatively greater maturity of intrarenal control mechanisms at the older age. Further research is clearly required to determine the specific factors controlling the appearance of hydrops in the immature fetus.

**Perspectives**

Premature infants frequently develop an early phase of intense polyuria with salt wasting after birth, which resolves over the first week (6, 19). The etiology is very poorly understood. The present study highlights two relevant aspects of preterm renal injury: first, that at least experimentally the immature kidney is relatively resistant to injury even after severe ischemia or asphyxia (38, 50); and second, that post-asphyxial renal dysfunction may be commonly manifested by polyuria and natriuresis (31). There is now increasing evidence from early electrophysiological and hemodynamic recordings that many preterm infants are exposed to significant events either in the immediate perinatal period or shortly after birth (27, 39); as illustrated by the present study adaptation to such events can lead to significant changes in systemic or regional perfusion (2, 16, 34). These intriguing observations raise the possibility that the common early, transient phase of polyuria in preterm infants may also be a consequence of early hypoxic-ischemic events and thus reflect reversible renal compromise rather than a purely maturational phenomenon.

**ACKNOWLEDGMENTS**

We are grateful to Dr. Karen Gibson and Prof. Eugenie Lumbers at the University of New South Wales, Australia for advice and Prof. Jelte de Haan at the University of Maastricht for critically reviewing the manuscript.

**GRANTS**

This study was supported by the Health Research Council of New Zealand, National Institutes of Health Grant RO-1 HD-32752, Lottery Health Board of New Zealand, and the Auckland Medical Research Foundation. J. S. Quedackers was supported by the Ter Meulen Fund, Royal Netherlands Academy of Arts and Sciences.

**REFERENCES**