Influence of Fetal Extracellular Volume Contraction on Renal Reabsorption of Bicarbonate in Fetal Lambs

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Summary

In order to determine the renal threshold for bicarbonate in the fetal lamb and factors that may influence renal reabsorption of bicarbonate in the fetus, 18 acute fetal lamb preparations (110-140 days of gestation) were studied. In the first series of experiments involving nine fetuses, the renal bicarbonate threshold of normal fetuses varied from 12.0 to 23.5 mM/liter with a mean value of 17.7 ± 1.37 mM/liter. This is significantly lower ($P < 0.005$) than the value measured in five adult sheep of 28.7 ± 1.68 mM/liter. There was a significant and positive correlation between the fetal plasma bicarbonate at threshold level and the fetal kidney weight, as well as fetal age. In a second series of experiments, the excretion of bicarbonate and sodium was studied in nine fetuses before and after dehydration by peritoneal dialysis. After peritoneal dialysis there was a significant decrease in urinary pH ($P < 0.025$), bicarbonate excretion ($P < 0.001$), sodium excretion ($P < 0.001$), fractional excretion of sodium ($P < 0.001$), and a significant increase in bicarbonate reabsorption ($P < 0.01$). When glucose was replaced by mannitol in the peritoneal dialysis fluid the effects on bicarbonate reabsorption paralleled those when glucose was present in dialysis fluid. It was also shown that when glucose was given intravenously to the fetus, up to a plasma concentration of 200 mg/100 ml, there was no effect on the fetal renal reabsorption of bicarbonate. These data indicate that the low threshold for bicarbonate reabsorption by the fetal kidney is not due to a limited capacity to increase bicarbonate or sodium reabsorption and suggest that the fetal kidney is able to respond to volume depletion by increasing its reabsorption in bicarbonate and electrolytes.

Speculation

A number of factors have been suggested to explain the low bicarbonate threshold observed during fetal life and during the early newborn period. These include the immaturity of the nephron, the oncotic pressure in the peritubular capillary circulation, the influence of distribution of intrarenal blood flow, the role played by the renin-angiotensin aldosterone system, and the effect of α and β adrenergic stimulation.

The present study suggests that the state of high extracellular fluid volume that exists during fetal life and the early newborn period significantly influences the maturation of the renal threshold for bicarbonate.

The regulation of acid-base homeostasis by the mature kidney involves the reabsorption of filtered bicarbonate on the one hand and the excretion of hydrogen ion as titrable acid and ammonium on the other.

The ability of the fetal kidney to excrete fixed acids and to establish an adult-type gradient of pH between blood and urine

has been previously investigated (30, 41). Vaughn and associates (41) have shown that the excretion of titrable acid and ammonium per unit of body weight was considerably less in the fetal lamb than in adult animals. Smith (30) demonstrated that even when renal compensation to metabolic acidosis in the fetus is limited as compared with that of the adult, the fetal lamb kidney is able to increase hydrogen ion excretion significantly in response to prolonged and profound metabolic acidosis in a manner qualitatively similar to the adult animal. Recently, Moore (18), demonstrating a clearcut pH gradient between blood and urine in fetal lambs after infusion of Na_2SO_4 , suggested that a possible lack of avidity for renal reabsorption of Na^+ in exchange of H^+ might explain the defect of urinary acidification in the fetus. However, these studies still do not exclude a functional tubular immaturity in the transport of bicarbonate to explain the low capacity of the fetus for urinary acidification.

It is well known that the physiologic acidosis seen in newborn animals is due to a low renal plasma bicarbonate threshold (7, 19). This decreased threshold for bicarbonate can be markedly increased in newborn puppies under the stimulus of extracellular volume contraction (19). However, the results found in newborn animals may not necessarily be extrapolated to the fetus where the integrity of acid-base balance depends much on the proper functioning of the placenta and where the fetal-maternal-amniotic fluid complex constitutes a three-compartment system in close relationship.

The present study was designed: (1) to investigate the capacity of the fetal lamb kidney to reabsorb bicarbonate and (2) to use this model for the exploration of factors that might influence the fetal threshold for bicarbonate excretion.

METHODS

Studies of renal bicarbonate transport were carried out on a total of 18 fetal lambs, divided in two groups. Control values were determined from five adult nonpregnant sheep. The fetuses were weighed at the end of the experiment and their age determined as previously described (12, 24). The adult ewes were weighed before the experiment.

The threshold for bicarbonate excretion in the first group of nine fetuses (120-147 days of gestation) was compared to bicarbonate threshold in five adult sheep.

The fetuses were prepared using an acute intrauterine fetal preparation. In the ewes, spinal anesthesia was induced with 4 ml of a 2% lidocaine hydrochloride (43) solution, repeated when required during the experiment. The uterus was exposed and incised, and the hind leg of the fetus was delivered. Polyethylene catheters were placed into a pedal vein and artery, and advanced approximately 10-15 cm after local anesthesia was induced with lidocaine hydrochloride. Through the same uterine

incision, the umbilical cord was located and exposed. The urachus was identified and ligated distally and a 3.5 French infant feeding tube was advanced 4–6 cm into the fetal bladder. The sight of urine flowing in the catheter was utilized to ensure proper placement of this catheter. The hind limb was replaced in the uterine cavity, and the fetal membrane, uterine cavity, and abdominal wall were closed.

After completion of the surgical procedures, control collections were obtained to measure glomerular filtration rate, bicarbonate reabsorption, and electrolyte (Na, K, Cl) excretion. Thereafter, the fetal plasma bicarbonate concentration was lowered well below the renal threshold by intravenous infusion of 1.5 M hydrochloric acid (HCl) in 5% dextrose and water at a rate of 0.1 ml/min, corresponding to 0.02–0.05 ml/min/kg of fetal body weight. After additional collection periods the infusion of HCl was stopped and a solution of 10% sodium bicarbonate containing 40 mEq/liter of potassium was infused at the same rate (0.1 ml/min) and serial collections of urine at 15-min intervals were made until the renal threshold for bicarbonate was passed. Renal bicarbonate threshold was defined arbitrarily as the level of bicarbonate in plasma at which the bicarbonate excretion in urine is 20 μ Eq/100 ml of glomerular filtration rate (GFR) (7, 38).

Control adult values for bicarbonate threshold were also determined in five adult sheep. After determination of control values for blood gases and electrolytes (Na^+ , K^+ , Cl^-), ammonium chloride was added to their morning feeding for 3 days to lower the concentration of plasma bicarbonate below the renal threshold. On the day of the experiment, the animal was immobilized with curare after insertion of an endotracheal tube. Respiration was regulated with a Harvard respiratory pump (44) adjusted to maintain the arterial pCO_2 tension at approximately 34 mm Hg. The normal pCO_2 in adult sheep has previously been shown to range between 30 and 34 mm Hg (5, 13). Control collections were then completed to determine glomerular filtration rate, bicarbonate reabsorption, and electrolyte (Na, K, Cl) excretion. Thereafter, a solution of 10% hypertonic sodium bicarbonate containing 40 mEq/liter of potassium was infused at 1 ml/min (0.02–0.04 ml/min/kg of body weight) and serial collections of urine at 15-min intervals were made until the renal bicarbonate threshold was passed. Potassium was added to the bicarbonate solution to maintain the plasma potassium concentration as close as possible to normal control values.

In the second group of nine fetuses (114–142 days of gestation), using an acute exteriorized fetal preparation, reabsorption and excretion of bicarbonate were studied before and after peritoneal dialysis used to produce a state of extracellular volume contraction. The fetuses were completely exteriorized as previously described (30) and kept at 39° on a warm table adjacent to and level with the uterus. After three 15-min control collection periods during which glomerular filtration rate, reabsorption of bicarbonate, and excretion of electrolytes (Na, K, Cl) were monitored, 50 ml/kg fetal body weight of a solution of Dianeal 4.25% (45) containing 4 mEq/liter of potassium and previously warmed at 39° was placed in the fetal peritoneal cavity. After allowing 15 min for equilibration, three experimental periods of 15 min each were performed to evaluate the same parameters as those of the control periods. At the end of the last experimental period, the dialysis fluid was removed from the peritoneal cavity and compared to the predialysis solution of Dianeal for osmolality, sodium, potassium, and chloride concentration.

Additional studies were carried out to determine whether the glucose present in the solution of Dianeal 4.25% could modify the renal bicarbonate reabsorption. Using the same acute exteriorized fetal preparation, three experiments were completed in which the solution of Dianeal was replaced by a solution of mannitol 4.25% containing the same electrolyte mixture as Dianeal to which we added 4 mEq/liter of potassium. Also, in order to monitor possible changes in fetal renal bicarbonate

reabsorption when fetal plasma glucose increased, two chronic fetal lambs were studied using the preparation described by Gresham (12).

In both fetuses and nonpregnant adult sheep, GFR was determined as described previously (24) using a constant infusion of sodium [^{125}I]iothalamate (46) (approximately 0.1 $\mu\text{Ci}/\text{min}/\text{kg}$). Arterial blood samples were collected anaerobically in heparinized glass syringes via an arterial catheter at the midpoint of each urine collection and analyzed immediately for pH and pCO_2 . Urine was also collected anaerobically in a glass syringe, kept on ice, and analyzed immediately after collection for total CO_2 content and pH.

Blood and urine pH and blood pCO_2 were measured at normal body temperature of 39° with a Radiometer PHM72 MK2 acid base analyzer (47) with appropriate pH and pCO_2 electrodes. Urine total CO_2 was determined by the method of Van Slyke and Neill (40) using a Natelson microgasometer. Urine bicarbonate concentration was calculated from the Henderson-Hasselbalch equation. The values for pK' were calculated for each urine sample according to the formula $\text{pK}' = 6.33 - 0.5\sqrt{B}$, where B represents the total cation concentration, estimated as the sum of sodium plus potassium expressed in equivalents per liter (14). The solubility coefficient used for CO_2 in urine was 0.0309. Blood bicarbonate was determined from pH and pCO_2 values using the Siggaard Andersen nomogram (29).

The concentration of sodium and potassium in blood and urine were measured with a flame photometer (48). Chloride in blood and urine was measured with a chloridometer (49). Blood and urine osmolality were determined with an osmometer (50). Concentration of total protein was determined by the biuret reaction and concentration of albumin was calculated from the percentage of total protein accounted for by albumin as determined by electrophoresis on cellulose acetate (8). Glucose in blood was measured by the glucose-oxidase method (27). Sodium [^{125}I]iothalamate was counted in blood and urine on a Beckman Gamma 300 radiation counter (51).

Data were evaluated using the *t*-test for paired data to compare control to experimental values in the same group of animals, and for unpaired data for comparisons between groups. The regression curves were derived by the method of least squares.

RESULTS

DETERMINATION OF RENAL THRESHOLD FOR BICARBONATE EXCRETION IN FETAL SHEEP

Control arterial blood values and urine pH from nine fetal lambs prior to the infusion of hydrochloric acid and bicarbonate titration are presented in Table 1.

In the adult nonpregnant ewes, mean arterial blood values for pH, pCO_2 , bicarbonate, and electrolytes, determined before and after induction of metabolic acidosis, are presented in Table 2. The postacidification values were determined when the animals were on a respirator so as to maintain their pCO_2 values as close as possible to normal control values.

Values for plasma bicarbonate observed at threshold in fetal lambs and adult sheep are summarized in Table 3. Mean values for plasma bicarbonate at a threshold of 17.7 mmol/liter in fetal lambs is significantly lower than the mean value of 28.7 mmol/liter found in adult sheep ($P < 0.001$), despite the fact that mean fetal pCO_2 of 43.9 at threshold is significantly higher than mean adult pCO_2 of 32.8 mm Hg ($P < 0.01$). Arterial blood pH at threshold in the fetuses is also significantly lower than adult arterial pH ($P < 0.001$).

Plasma bicarbonate at threshold shows a significant correlation ($r = 0.76$) and a statistically significant positive regression ($P < 0.01$) when related to fetal kidney weight (Fig. 1). There is also a significant correlation ($r = 0.60$) and a significant positive regression ($P < 0.05$) when plasma bicarbonate at threshold is presented as a function of fetal age. However, a multiple regres-

Table 1. Control acid-base status in nine fetal lambs

Fetus	Age, days	Weight, kg	Blood						Urine	
			pH	pCO ₂ , mm Hg	HCO ₃ ⁻ , mmol/liter	mEq/liter			pH	% FE _{HCO₃} ¹
						Na ⁺	K ⁺	Cl ⁻		
71	136	3.15	7.29	50.0	23.6	150	4.6		6.83	2.1
73	130	2.60	7.41	47.0	29.3	136	3.3		7.10	30.0
74	134	3.00	7.21	52.0	20.0	146	4.5		7.26	36.3
76	136	3.15	7.36	51.0	28.4	134	3.4		7.59	17.5
80	120	1.80	7.38	39.5	23.0	132	4.9	104	6.50	1.0
82	144	4.30	7.35	39.3	21.3	132	5.6	101	5.70	0.08
83	147	4.70	7.37	43.1	24.5	133	4.3	104	6.88	1.5
84	139	3.60	7.37	40.0	22.7	133	4.6	106	6.88	3.0
85	145	4.41	7.42	38.1	24.8	137	4.4	98	7.20	26.9
Mean			7.35	44.4	24.17	137	4.4	102.6		
SE			0.02	1.86	1.01	2.17	0.23	1.39		

¹Percentage of filtered bicarbonate excreted.

Table 2. Acid-base status before and after induction of metabolic acidosis in five nonpregnant ewes

Nonpregnant ewes (<i>n</i> = 5)	Blood						Urine pH
	pH	pCO ₂ , mm Hg	HCO ₃ ⁻ , mmol/liter	mEq/liter			
				Na ⁺	K ⁺	Cl ⁻	
Before acidification							
Mean	7.49	32.6	25.0	144.8	4.12	108	
SE	0.007	0.82	0.80	1.01	0.42	1.15	
After acidification							
Mean	7.29	32.5	15.4	139.0	4.70	115	5.87
SE	0.03	1.60	1.49	1.86	0.14	2.61	0.03

Table 3. Values of plasma at threshold for bicarbonate

	No.	pH	pCO ₂ , mm Hg	HCO ₃ ⁻ , mmol/liter	K ⁺ , mEq/liter	Cl ⁻ , mEq/liter
Fetus	71	7.27	50.3	22.7	4.6	
	73	7.10	40.1	12.0	4.7	
	74	7.13	49.2	15.5	4.5	
	76	7.00	58.3	13.7	4.6	
	80	7.28	31.7	14.4	4.9	106
	82	7.31	47.8	23.5	5.1	101
	83	7.32	40.0	20.0	5.6	106
	84	7.33	39.9	20.5	4.9	103
	85	7.26	38.6	17.1	5.4	104
$\bar{x}$		7.22	43.98	17.71	4.92	104.0
SE		0.03	2.67	1.37	0.12	0.94
Adult ewe	4	7.50	32.0	25.1	4.3	113
	5	7.54	36.0	31.4	3.6	117
	6	7.60	34.0	33.5	4.7	110
	7	7.50	32.0	25.0	4.3	113
	8	7.58	30.0	28.5	4.3	109
$\bar{x}$		7.54	32.8	28.70	4.24	112.4
SE		0.02	1.07	1.68	0.17	1.39
t		6.04	2.99	4.90	3.16	4.96
P		0.001	0.01	0.001	0.005	0.001

sion analysis shows a high correlation of fetal kidney weight with fetal age ($r = 0.87$) and a low partial correlation of age with plasma bicarbonate at threshold. Therefore, the plasma bicarbonate at threshold correlates primarily with fetal kidney weight and fetal age does not account for an independent source of variation.

EFFECTS OF EXTRACELLULAR VOLUME CONTRACTION ON RENAL REABSORPTION OF BICARBONATE IN FETAL SHEEP

The effect of extracellular volume contraction on bicarbonate reabsorption was evaluated in nine acute fetal preparations between 114 and 142 days of gestation.

Blood and urine values before and after contraction of the extracellular fluid volume in the fetus are summarized in Table 4. There is no significant difference in arterial blood values for pH, pCO₂, PO₂, or in arterial plasma concentrations for bicarbonate, sodium, chloride, and osmolarity before and after extracellular volume contraction. There is a small but significant increase ($P < 0.001$) of 0.3 mEq/liter in plasma concentration of potassium after volume contraction. There is no significant change in total protein and albumin concentration. However, there is an increase in hematocrit values from 45.8 ± 1.8 to 47.1 ± 2.3 .

Urine values presented in Table 4 show that urine flow, GFR, and the amount of bicarbonate filtered are stable before and after peritoneal dialysis. However, there is a significant increase in reabsorbed bicarbonate ($P < 0.01$) and a significant decrease in urinary pH ($P < 0.025$) and urinary excretion of bicarbonate ($P < 0.001$), sodium ($P < 0.001$), and chloride ($P < 0.001$) after contraction of the extracellular fluid volume in the fetus.

In five animals the dialysis fluid from the peritoneal cavity was collected at the end of the experiment and compared to the pre-experimental solution of Dianeal for sodium, potassium, chloride, and osmolarity, as shown in Table 5. There was a significant decrease ($P < 0.001$) in the concentrations of Na⁺, K⁺ and Cl⁻ in the peritoneal dialysis fluid at the end of the dialysis period, indicating a transfer of water from the fetal extracellular space to the peritoneal cavity.

There was a decrease of 31.8% in the osmolarity and a 44.6% decrease in the glucose concentration in the peritoneal dialysis

fluid at the end of the experimental period. The fall in osmolarity (31.8%) was greater than the decrease in concentration of electrolytes (16.1%), suggesting that the glucose in dialysis fluid was passing from the peritoneal cavity to the fetal circulation. There was, however, no significant rise in arterial blood glucose in the fetus at the end of the dialysis period.

EFFECTS OF GLUCOSE ON BICARBONATE REABSORPTION IN FETUS

In order to verify whether the transfer of glucose from the dialysis fluid to the fetus could modify the response of the kidney to extracellular volume depletion, the glucose in the dialysis fluid was replaced by mannitol in three acute preparations. As shown in Table 6, there was no significant difference in GFR before and after peritoneal dialysis with mannitol. However, there was a significant decrease in excreted bicarbonate ($P < 0.025$) and sodium ($P < 0.025$) and a significant increment ($P < 0.1$) in reabsorbed bicarbonate. There was also a decrease (not significant) in urinary pH. These results paralleled those when Dianeal containing glucose was used as peritoneal dialysis fluid (Table 4).

In a second series of experiments using two chronic fetal

Fig. 1. Relationship between fetal kidney weight and plasma bicarbonate at threshold.

models, the arterial plasma glucose concentration was increased over 200 mg/100 ml without contracting the fetal extracellular volume. During this experiment the amount of bicarbonate reabsorbed decreased by a mean of $1.2 \pm 0.08 \mu\text{Eq/ml GFR}$ when the GFR and arterial blood values remained stable.

DISCUSSION

Since the renal secretory capacity of hydrogen ions involves the reabsorption of bicarbonate and since presently available data on the fetus (30, 41) deal only with the excretion of ammonium and titrable acid to explain the metabolic acidosis in the fetus, the present study was designed to investigate the contribution of bicarbonate reabsorption to acid secretion during fetal life.

In the first part of this study it is demonstrated that the values of plasma bicarbonate at threshold for renal bicarbonate excretion are lower in the fetal lamb than in nonpregnant adult ewes. It is also shown that the level of plasma bicarbonate at threshold increases significantly with fetal kidney weight ($P < 0.01$).

In the present study, the bicarbonate threshold was determined in the adult nonpregnant ewes after they had received 3

Table 5. Analysis of dialysis fluid before and after peritoneal dialysis in five fetuses

	mEq/liter			Osmolarity, mOsm/kg H ₂ O	Glucose, g/100 ml
	Na	K	Cl		
Before (n = 5)					
$\bar{x}$	138.8	4.32	103.4	497.2	3.96
SE	0.37	0.13	0.24	1.06	0.01
After (n = 5)					
$\bar{x}$	117.0	3.32	86.4	339.2	2.19
SE	1.54	0.13	1.56	18.7	0.09
P	<0.001	<0.001	<0.001	<0.001	<0.001

Table 4. Summary of blood and urine values before and after peritoneal dialysis in nine fetuses¹

	Before		After		
	$\bar{x}$	SE	$\bar{x}$	SE	P
Blood					
pH	7.358	0.007	7.352	0.01	N.S.
pCO ₂ , mm Hg	41.1	0.84	41.4	0.87	N.S.
pO ₂ , mm Hg	22.3	1.82	22.5	1.49	N.S.
HCO ₃ ⁻ , mmol/liter	22.7	0.37	22.7	0.58	N.S.
Na ⁺ , mEq/liter	136.5	1.15	136.2	1.05	N.S.
K ⁺ , mEq/liter	4.8	0.14	4.5	0.12	<0.001
Cl ⁻ , mEq/liter	99.7	0.84	99.4	0.97	N.S.
Total protein, g/100 ml	3.67	0.21	3.61	0.15	N.S.
Albumin, g/100 ml	1.99	0.11	1.92	0.09	N.S.
Osmolarity, mOsm/kg	283	2.8	285	3.3	N.S.
Hematocrit	45.8	1.8	47.1	2.3	N.S.
Urine					
Flow, ml/min	0.47	0.10	0.37	0.12	N.S.
GFR, ml/min	0.88	0.10	0.82	0.09	N.S.
pH	7.18	0.12	6.91	0.19	<0.025
F _{HCO₃} ⁻ , $\mu\text{Eq/ml GFR}$	22.70	0.37	22.70	0.58	N.S.
R _{HCO₃} ⁻ , $\mu\text{Eq/ml GFR}$	19.10	1.14	20.60	0.90	<0.01
E _{HCO₃} ⁻ , $\mu\text{Eq/ml GFR}$	3.61	0.85	2.04	0.80	<0.001
E _{Na} ⁺ , $\mu\text{Eq/ml GFR}$	14.70	3.70	8.72	2.95	<0.001
C _{Na} ⁺ /C _{loth} × 100	10.70	2.68	6.30	2.10	<0.001
E _{Cl} ⁻ , $\mu\text{Eq/ml GFR}$	10.53	2.84	5.97	2.19	<0.001
C _{Cl} ⁻ /C _{loth} × 100	10.40	2.70	6.00	2.20	<0.001

¹ GFR: glomerular filtration rate; F: filtered; R: reabsorbed; E: excreted; C/C_{loth} × 100: percentage of filtered electrolytes excreted.

Table 6. Effect of peritoneal dialysis with mannitol in three fetuses¹

	Plasma						Urine				
	pH	pCO ₂ , mm Hg	pO ₂ , mm Hg	HCO ₃ ⁻ , mmol/ liter	Na ⁺ , mEq/liter	Osmolar- ity, mOsm/ kg	GFR, ml/min	μEq/min/ml GFR			
								pH	R _{HCO₃} ⁻	E _{HCO₃} ⁻	E _{Na⁺}
Before (n = 3)											
$\bar{\chi}$	7.31	39.4	30.01	19.81	133.6	283.4	2.44	7.21	15.48	4.16	16.15
SE	0.02	3.68	3.68	1.43	1.15	2.81	0.67	0.22	3.01	1.71	4.57
After (n = 3)											
$\bar{\chi}$	7.30	40.3	30.06	19.44	132.7	285.5	2.69	7.10	16.93	2.54	10.56
SE	0.04	3.12	3.12	1.23	1.07	0.85	0.66	0.31	2.25	1.22	2.95
P	N.S.	N.S.	N.S.	N.S.	N.S.	<0.1	N.S.	N.S.	<0.1	<0.025	<0.025

¹ GFR: glomerular filtration rate; R: reabsorbed; E: excreted.

days of ammonium chloride. Then, it could have been possible that they were volume contracted. However, during the period of ammonium chloride injection the adult sheep were not restricted in water and their weight difference before and after ingestion of ammonium chloride was not statistically different. Therefore, it seems very unlikely that the difference in the bicarbonate threshold values between the fetuses and the adult nonpregnant ewes was secondary to phenomena other than developmental.

Anatomic immaturity of the nephron may be a factor that explains the low threshold in the fetus. Previous studies in newborn animals indicated that the increase in kidney weight with age reflects the predominant growth of the proximal tubule (9, 21) and suggested that early morphologic glomerular preponderance is paralleled by functional glomerular preponderance. Recently, however, Horster and Valtin (15), Spitzer and Brandis (33), and Solomon (32), using micropuncture studies, showed in different newborn animal models that proportionality between filtration and reabsorption of sodium and water in superficial proximal tubules is maintained through development, and that functional glomerulotubular balance is present throughout the entire period of renal maturation. Micropuncture studies, however, are not available in the fetus for comparison. Thus it is not possible, at this time, to state whether or not the low threshold for bicarbonate in the lamb fetuses represents functional glomerulotubular imbalance.

Other factors such as plasma pCO₂ tension, plasma chloride concentration, body potassium stores, carbonic anhydrase activity, and stimuli affecting sodium reabsorption may also play a role in explaining the low renal threshold for bicarbonate found in the fetuses. In the present study the plasma bicarbonate at threshold is lower in the fetuses than in adult ewes despite significantly higher pCO₂ values in the fetus ($P < 0.01$). It is well known that an increase in arterial pCO₂ *in vivo* will increase bicarbonate reabsorption (23). Therefore, the higher pCO₂ values in the fetuses would tend to increase bicarbonate reabsorption.

The presence of a lower chloride concentration in the fetuses versus the adult ewes ($P < 0.001$) has probably no direct effect on the low fetal renal threshold for bicarbonate. The mechanism by which chloride influences renal bicarbonate reabsorption seems to be indirect and related to changes in extracellular volume (4, 28). It is shown that when chloride is selectively depleted, it is not the low plasma chloride concentration but rather the contracted extracellular fluid volume that is most important in maintaining the relatively high rate of hydrogen secretion (4). Kurtzman (17) has also shown that expansion of effective extracellular volume results in a decreased chloride and bicarbonate reabsorption.

It is thus possible that this low fetal plasma chloride concentration may be secondary to the high extracellular fluid volume

found normally in the fetus (10, 42) and may parallel the low fetal renal threshold for bicarbonate.

Plasma potassium concentration was found to be higher in the fetuses than in adult ewes ($P < 0.005$). It has long been known that an increase in plasma potassium concentration will reduce bicarbonate reabsorption by limiting intracellular hydrogen ions available to the exchange mechanism (22). However, the fetuses with high plasma potassium concentrations also have the highest values for plasma bicarbonate at threshold. Therefore, it seems unlikely that the difference in renal bicarbonate threshold values between fetal and adult animals is related to the high fetal plasma potassium concentration.

The presence of an active carbonic anhydrase enzyme has been demonstrated in the fetal lamb during the last half of gestation (26). It has been shown in acute (31) and chronic (25) fetal preparations that the renal response to administration of a carbonic anhydrase inhibitor, acetazolamide, is similar to that observed in the adult sheep. Thus, the low threshold for renal bicarbonate reabsorption found in the fetus is probably not secondary to a lack of fetal renal carbonic anhydrase activity. However, enzyme kinetic studies are needed to clearly demonstrate that point.

The stimuli affecting tubular sodium reabsorption will also modify reabsorption of filtered bicarbonate (36). Kurtzman (17) has shown previously in adult dogs that expansion of extracellular volume, known to depress sodium reabsorption (35), decreased bicarbonate reabsorption. Moore (19) demonstrated that the decreased threshold for bicarbonate found in puppies 1–4 weeks of age can be markedly increased under the stimulus of extracellular volume concentration.

In the second part of this study a mild contraction of extracellular fluid volume, using peritoneal dialysis, was induced in the fetuses to determine whether factors that stimulate sodium reabsorption in adult and newborn animals (17, 19) have an effect upon fetal bicarbonate reabsorption. These experiments show that, parallel to the contraction of fetal extracellular volume, there is a significant increase in bicarbonate reabsorption ($P < 0.01$) and a significant decrease in urine pH ($P < 0.025$), excreted bicarbonate ($P < 0.001$), sodium ($P < 0.001$), and chloride ($P < 0.001$).

It is then possible that the state of high extracellular fluid volume found normally in the fetus is a major determinant of bicarbonate reabsorption by the fetal kidney. The present data shows that during fetal peritoneal dialysis water is removed from the fetus and added to the peritoneal cavity. It is also demonstrated that during this process there is an increase in bicarbonate and electrolyte reabsorption in the fetus. However, even if the present data suggest a parallelism between a mild contraction of extracellular volume and bicarbonate reabsorption during fetal life, this study does not permit evaluation of the exact amount of effective extracellular fluid volume removed and the

effect of this procedure on placental exchange of solute and water. Furthermore, it is possible that physical factors other than the contraction of extracellular volume alone may have influenced the present findings.

Oncotic pressure within the postglomerular microcirculation has been shown to influence proximal reabsorption of electrolytes in adult experimental animals (34) and is regulated by peripheral plasma protein concentration and filtration fraction (6). In the present study, there is no modification in peripheral plasma protein concentration; however, filtration fraction was not determined. It is possible that the extracellular volume depletion may have influenced the filtration fraction in the direction of increasing bicarbonate and electrolyte reabsorption. The small rise in hematocrit found at the end of the dialysis period, even if not significant, may also have influenced the tubular function by changing the viscosity of the blood and the flow and pressure in peritubular capillaries (3, 16). It is then conceivable that factors other than the contraction of extracellular volume might have influenced the increase in bicarbonate reabsorption during the fetal peritoneal dialysis.

It is also possible that the increase in fractional bicarbonate and electrolyte reabsorption by the fetal kidney may be related to an increase in aldosterone secretion during the period of fetal peritoneal dialysis. However, the present study was not designed to investigate this aspect.

The use of an acute experimental preparation may also have modified the response to volume depletion. Spinal anesthesia, as used in the present study, is not known to have any effect on the metabolic and cardiovascular functions of the fetus (2); on the other hand, it is known that operative and anesthetic stresses might affect kidney function, especially the tubular reabsorption of water (12). However, since in the present study every fetus is used as its own control, it is clear that the results obtained after peritoneal dialysis are not artifacts.

Since glucose increases bicarbonate reabsorption in man (11) and in dogs (37), it was important to determine whether the passage of glucose from the dialysis fluid to the fetus during the process of extracellular volume contraction could have influenced the renal bicarbonate reabsorption in the fetus. It is improbable since it was not possible to demonstrate a significant increase in fetal plasma glucose at the end of the dialysis period. It is possible, however, that glucose passing from the peritoneal fluid to the fetus was cleared rapidly by the placenta (1) or used by the fetus (39). Furthermore, when the fetal plasma glucose was increased above 200 mg/100 ml in the chronic preparation there was no effect on renal bicarbonate reabsorption. Finally, using three acute fetal preparations, mannitol was substituted for glucose in the dialysis solution in order to produce extracellular volume contraction. The results of these studies (Table 6) indicate that the effects of extracellular volume contraction on bicarbonate reabsorption paralleled the results obtained when glucose was used as osmotic agent in the dialysis fluid (Table 4). Thus it is clear that the increase in bicarbonate reabsorption and the decrease in fractional excretion of sodium are not secondary to an effect of glucose on fetal bicarbonate reabsorption.

In summary, it has been demonstrated that the fetal threshold for renal bicarbonate excretion is lower than in the adult. This finding, added to the limited ability of the fetal kidney to excrete titrable acid and ammonia (30, 41), explains more completely why the fetal kidney is limited in its ability to establish adult-type gradient of pH between blood and urine. It has also been suggested that the fetal kidney is able to increase bicarbonate and sodium reabsorption under the stimulus of the contraction of the fetal extracellular fluid volume. It has been shown previously in adult animals (17, 20) and newborn puppies (19) that extracellular volume changes modify bicarbonate reabsorption. However, factors such as the oncotic pressure in the fetal peritubular capillary circulation, the influence of distribution in intrarenal blood flow during fetal life, the activity of the renin-angiotensin-aldosterone system, and the effect of α and β adrenergic stimulation in the fetus may have influenced bicarbonate reabsorption

during the period of peritoneal dialysis and need to be evaluated. Finally, it is also shown that glucose has no effect on bicarbonate and sodium reabsorption during fetal life.

CONCLUSION

The ability of the fetal kidney to excrete fixed acids and to establish an adult gradient of pH between blood and urine has been investigated previously (30, 41). In order to examine the capacity of fetal kidney to reabsorb bicarbonate, in the first part of the study it was shown that the values of plasma bicarbonate at threshold for renal bicarbonate excretion are lower in the fetal lambs than in adult nonpregnant ewes. It was also shown that the level of plasma bicarbonate at threshold increases significantly with fetal kidney weight ($P < 0.01$) and with fetal age ($P < 0.05$). In the second part of the study it was possible to show that contraction of fetal extracellular volume using peritoneal dialysis significantly increases bicarbonate reabsorption ($P < 0.01$) and significantly decreases urine pH ($P < 0.025$), excreted bicarbonate ($P < 0.001$), sodium ($P < 0.001$), and chloride ($P < 0.001$). Finally, it was demonstrated that the increment in bicarbonate reabsorption and the reduction in fractional excretion of sodium after peritoneal dialysis were not secondary to an effect of glucose on fetal bicarbonate reabsorption. The findings of this study suggest that the fetal kidney, under the stimulus of volume depletion, is not limited in its capacity to reabsorb sodium and bicarbonate.

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Cerebral cortex
fetus
phosphodiesterase

Cyclic Nucleotide Phosphodiesterase Activities of the Fetal and Mature Human Cerebral Cortex

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Summary

Phosphodiesterase activities were examined in the supernatant and pellet fractions of a 30,000 × g preparation of brain tissues from human fetuses and young adults. Differences in total activity and distribution of the high and low K_m activity enzymes for adenosine and guanosine 3',5'-monophosphate (cyclic AMP and cyclic GMP, respectively) were found. The mature cortex had 10 times more activity than the fetal brain for cyclic AMP hydrolysis and 15-20 times more activity for cyclic GMP hydrolysis. In the fetus, more activity for both nucleotides

at both high and low concentrations is associated with the supernatant fraction. With maturity, a shift in localization of high K_m activity for cyclic GMP and low K_m activities for both nucleotides to the particulate fraction is observed.

Michaelis constants for both mature and immature brains are similar. The K_m values for cyclic AMP are 10⁻⁴ and 10⁻⁵ M and 10⁻⁴ and 10⁻⁶ M for cyclic GMP. The V_{max} values differed by a factor of 10 between the high K_m and the low K_m forms for each substrate.