Impaired Renal Acidification in Infants with Fetal Alcohol Syndrome

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ABSTRACT. Urinary acidification was studied in six unrelated infants with fetal alcohol syndrome and eight healthy age-matched infants. Creatinine clearance, fractional sodium excretion, plasma renin activity, and plasma aldosterone were normal in all patients but fractional potassium excretion was lower in the patients than in the controls ($p = 0.0001$). After ammonium chloride loading, minimum urine pH was significantly higher in FAS patients than in control subjects (5.5 ± 0.1 and 4.7 ± 0.1 , respectively, $p = 0.00005$). Net acid excretion was also lower in the patients (24.5 ± 1.7 Eq/min) than in the controls (27.8 ± 2.1 Eq/min, $p = 0.008$). Following sodium bicarbonate loading, fractional bicarbonate excretion was significantly higher ($p = 0.00005$) and fractional potassium excretion significantly lower ($p = 0.002$) in the patients than in the controls with comparable blood pH and bicarbonate levels. Treatment with chlorothiazide lowered plasma potassium and raised plasma bicarbonate to normal levels ($p = 0.05$). Concomitantly, fractional sodium excretion, fractional potassium excretion, and urinary net acid excretion increased significantly ($p = 0.01$). We conclude that patients with fetal alcohol syndrome have a defect in distal acidification and potassium excretion which cannot be attributed to abnormal aldosterone secretion. (*Pediatr Res* 19: 850-853, 1985)

Abbreviations

FAS, fetal alcohol syndrome
FE_K, fractional excretion of potassium
PHCO₃, plasma bicarbonate concentration
NH₄, ammonium
TA, titratable acid
NAE, net acid excretion
RTA, renal tubular acidosis

While evaluating patients with the FAS it became apparent that episodes of hyperchloremic metabolic acidosis associated with alkaline urine were common during the period of dehydration. Review of the literature showed no report of impaired renal acidification associated with the syndrome (1-4). Therefore, a prospective study of renal acidification was performed in six infants with FAS to determine if there is any relationship between perinatal ethanol exposure and impaired urinary acidification in the development of metabolic acidosis.

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PATIENTS AND METHODS

The subjects for this study were six infants selected from a larger prospective study on fetal dysmorphogenesis among alcoholic women. The mothers of the affected infants had been consuming alcohol heavily prior to and throughout pregnancy. None was addicted to any other drugs. All satisfied the criteria for alcoholism as defined by the criteria committee of the National Council on Alcoholism (5). The six selected infants of these women were recognized as having the pattern of altered growth and morphogenesis characteristic of FAS (6, 7). The pertinent clinical features shared by these children are shown in Table 1. All presented in early infancy with symptoms including poor feeding, vomiting, polyuria, dehydration, and failure to thrive. A pattern of impaired renal acidification aggravated by volume contraction was repeatedly noted in each patient. Fluid therapy improved this clinical condition and the acid-base disorder; however, hyperchloremic metabolic acidosis developed and persisted with each subsequent episode of dehydration. Normal blood concentrations of glucose, calcium, phosphate, and alkaline phosphatase were obtained in all patients. Blood pressure measurements taken on several occasions were normal. None had malabsorption syndromes, glucosuria, aminoaciduria, proteinuria, hyperphosphaturia (defined as tubular reabsorption of phosphate 85%), or hypercalciuria (urine calcium/urine creatinine 0.25). Intravenous pyelography and renal ultrasonography disclosed no abnormalities. Most infants were fed a combination of breast milk, commercial formula, and cereals, providing an average daily caloric intake of 105 (range 100-110) kcal/kg at the time of study.

Studies of renal function were performed when the patients were clinically stable in the absence of dehydration. Eight healthy age-matched infants were similarly studied and served as controls. Although the normal infants were larger than the FAS infants, group mean values for weight were not significantly different. The average weight was 7.18 kg in the infants with FAS and 7.21 kg in the control group. All the urines were collected under mineral oil for 5 hr by means of bladder catheterization. Ten-milliliter aliquots of urine were then aspirated under oil in an air-tight syringe, and the pH, NH₄, and TA were measured within 5 min of collection. Arterialized capillary blood samples were obtained at the midpoint of each urine collection. In both blood and urine specimens, pH determinations were made using direct-reading electrodes on a Mark 2 Digital Acid-Base Analyzer (Radiometer, Copenhagen, Denmark). Urinary acidification was studied using the short ammonium chloride test of Edelmann *et al.* (8, 9). Urinary NH₄, TA, NAE, and bicarbonate were analyzed by methods previously described (10, 11). Alkalinization of the urine was accomplished in all infants using the standard protocols of the bicarbonate titration test (12). Creatinine content of plasma and urine was measured with a Beckman Creatinine Analyzer 2. Sodium and potassium were measured by flame photometry. Glomerular filtration rate was

Table 1. Clinical features observed in infants with the FAS

Feature	Patient					
	1	2	3	4	5	6
Age (mo)/sex	12/F	10/F	10/M	8/F	7/F	6/M
Growth deficiency (10th percentile)						
Prenatal	+	+	+	+	+	+
Postnatal	+	+	+	+	+	+
Facial characteristics						
Short palpebral fissures	+	+	+	+	+	+
Hypoplastic philtrum	+	+	+	+	+	+
Hypoplastic maxilla	+	+	+	+	+	+
Microcephaly (3rd percentile)	+	+	+	+	+	+
Central nervous system						
Developmental delay	+	+	+	+	+	+
Hypotonia	+	+	+	+	+	+
Skeletal						
Joint anomalies	+	+	+	+	+	+
Altered palmar crease pattern	+	+	+	+	+	+
Cardiac anomaly	+	+	+	+	+	+

* Syndactyly of the second and third toes bilaterally and limitation of motion at elbows.

† Atrial septal defect.

estimated by clearance of endogenous creatinine (urinary concentration of creatinine times urinary flow rate)/plasma concentration of creatinine. The fractional excretion of a substance was calculated according to the standard formula (clearance of the substance/creatinine clearance $\times$ 100). Fasting and supine plasma renin activity was measured by radioimmunoassay (E. R. Squibb and Sons, Inc., Princeton, NJ). Plasma aldosterone was determined using a standard radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, CA).

The possibility of a primary defect in potassium excretion was explored by examining the urinary acidification following oral diuretic therapy (13). Chlorothiazide, 10 mg/kg/day, was administered orally for a period of 5 days. Before and after this diuretic agent, timed urine collections and blood samples were obtained. The specimens were analyzed for bicarbonate, pH, sodium, potassium, NH_4 , and TA. The protocol for this study was approved by the Institutional Review Board, the University of Medical Science of Iran, and informed written maternal consent was obtained for all patients prior to study. Statistical analysis was done using the Student's *t* test for paired or unpaired variables. All values represent the mean $\pm$ SD.

RESULTS

Initial biochemical data in FAS patients are summarized in Table 2. Baseline FE_K in FAS group was significantly lower than the control group ($p = 0.0001$). Clearance of creatinine, plasma concentrations of aldosterone, plasma renin activity, and fractional excretion of sodium were normal in all subjects.

After ammonium chloride loading the urine pH fell significantly in both patient and control subjects (Table 3). Urine pH fell below 5.5 in only one of the six FAS infants with a mean decrease of the group to 5.5 ± 0.1 . This was significantly higher than the minimum urinary pH in eight control subjects in whom urinary pH after acid loading was 4.7 ± 0.1 , ($p = 0.00005$). In all of the control subjects urine pH fell to 4.8 or less. The fall in PHCO_3 was more pronounced in patients than in controls. Maximum NH_4 excretion ($p = 0.0002$) and net acid excretion ($p = 0.008$) were lower in FAS patients than in controls. TA accounted for a higher percentage of NAE in FAS subjects compared to control subjects (52 ± 0.3 and 47 ± 0.7 , respectively) ($p < 0.05$). FE_K was significantly lower ($p = .00005$) in FAS patients compared to the controls.

When urinary bicarbonate excretion was studied at normal PHCO_3 concentration, FAS patients excreted a higher percentage of the filtered load than in controls ($p = 0.00005$, Table 4)

Table 2. Initial laboratory data in six infants with FAS (mean $\pm$ SD)*

	Control	FAS
Urine pH	5.9 ± 0.1	6.0 ± 0.3
Plasma pH	7.36 ± 0.1	7.35 ± 0.1
PHCO_3 (mEq/liter)	21 ± 0.5	20.9 ± 0.4
PK (mEq/liter)	5.3 ± 0.1	5.4 ± 0.1
C_{Cr} (ml/min)	22.5 ± 2.8	22 ± 2.5
FE_K (%)	28 ± 1.8	$23 \pm 1.3^\dagger$
FE_{Na} (%)	0.4 ± 0.1	0.5 ± 0.1
PRA (ng/ml/h)	7.8 ± 0.7	7.9 ± 0.8
Plasma aldo (g/dl)	60 ± 5	62 ± 6

* Abbreviations: PK, plasma potassium; C_{Cr} , creatinine clearance; FE_{Na} , fractional sodium excretion; aldo, aldosterone.

† $p = 0.0001$.

Table 3. Results of acidification in response to ammonium chloride administration (mean $\pm$ SD)*

	Controls	FAS	<i>p</i> †
Urine pH	4.7 ± 0.1	5.5 ± 0.1	0.00005
Plasma pH	7.33 ± 0.1	7.32 ± 0.1	NS‡
PHCO_3 (mEq/liter)	16.7 ± 0.4	15.8 ± 0.2	0.0003
PK (mEq/liter)	5.3 ± 0.2	5.5 ± 0.2	NS
FE_K (%)	29 ± 1.6	23.5 ± 1.3	0.00005
$\text{U}_{\text{NH}_4}\text{V}$ (Eq/min)	14.3 ± 1.0	11.7 ± 0.8	0.0002
$\text{U}_{\text{TA}}\text{V}$ (Eq/min)	13.5 ± 1.1	12.8 ± 0.9	NS
$\text{U}_{\text{NAE}}\text{V}$ (Eq/min)	27.8 ± 2.1	24.5 ± 1.7	0.008

* Abbreviations: PK, plasma potassium; $\text{U}_{\text{NH}_4}\text{V}$, urinary ammonium excretion; $\text{U}_{\text{TA}}\text{V}$, urinary titratable acid excretion; $\text{U}_{\text{NAE}}\text{V}$, urinary net acid excretion.

† Unpaired *t* test.

‡ Not significant ($p > 0.05$).

Table 4. Summary of data following bicarbonate titration tests (mean $\pm$ SD)*

	Controls	FAS	<i>p</i> †
Urine pH	7.39 ± 0.1	7.40 ± 0.2	NS‡
Plasma pH	7.38 ± 0.1	7.37 ± 0.1	NS
PHCO_3 (mEq/liter)	23.1 ± 0.1	22.8 ± 0.3	NS
PK (mEq/liter)	5.1 ± 0.2	5.2 ± 0.1	NS
FE_K (%)	32.5 ± 3.5	26 ± 1.0	0.002
FE_{HCO_3} (%)	0.8 ± 0.1	3.5 ± 0.2	0.00005
FE_{Na} (%)	1.3 ± 0.2	1.5 ± 0.1	NS

* Abbreviations: PK, plasma potassium; FE_{Na} , fractional sodium excretion.

† Unpaired *t* test.

‡ Not significant ($p > 0.05$).

indicating a mild to moderate bicarbonate leak in FAS subjects. During bicarbonate loading, FE_K was again significantly lower in patients than in controls.

Treatment with chlorothiazide lowered plasma potassium and raised PHCO_3 (Table 5). Concomitantly, fractional excretion of sodium, FE_K , and NAE increased and urinary pH decreased significantly. The percentage of NH_4 excreted during diuretic therapy was higher than urinary TA excretion (50 ± 0.6 and $49 \pm 0.4\%$, respectively).

DISCUSSION

The results of our study demonstrate the presence of tubular dysfunction in patients with FAS despite preservation of normal glomerular function. Five of six patients were unable to lower their urine pH to less than 5.5, and all six had a blunted increase in urinary excretion of TA and NH_4 in response to ammonium

Table 5. Effect of chlorothiazide on renal acidification in six infants with FAS (mean $\pm$ SD)*

	Chlorothiazide therapy		<i>p</i> †
	Before	After	
Wt (kg)	7.18 $\pm$ 1.6	7.10 $\pm$ 1.5	NS
Urine pH	6.1 $\pm$ 0.1	5.0 $\pm$ 0.1	0.05
Plasma pH	7.34 $\pm$ 0.1	7.36 $\pm$ 0.1	0.05
PHCO ₃ (mEq/liter)	21 $\pm$ 0.1	22 $\pm$ 0.5	0.05
PK (mEq/liter)	5.5 $\pm$ 0.2	5.1 $\pm$ 0.1	0.01
FE _K (%)	21 $\pm$ 1.5	27.2 $\pm$ 1.1	0.001
FE _{Na} (%)	0.4 $\pm$ 0.1	4.9 $\pm$ 0.2	0.00001
U _{NH4} V (Eq/min)	11.5 $\pm$ 0.2	12.9 $\pm$ 0.5	0.01
U _{TA} V (Eq/min)	12.1 $\pm$ 0.1	13.2 $\pm$ 0.4	0.05
U _{NAE} V (Eq/min)	23.6 $\pm$ 0.3	26.1 $\pm$ 0.9	0.01

* Abbreviations: PK, plasma potassium; FE_{Na}, fractional sodium excretion; U_{NH4}V, urinary ammonium excretion; U_{TA}V, urinary titratable acid excretion; U_{NAE}V, urinary net acid excretion.

† Paired *t* test.

chloride administration. During bicarbonate infusion, the amount of bicarbonate excreted in the urine at normal PHCO₃ remained below 5% in all six FAS patients. These findings are characteristics of distal rather than proximal RTA (9, 14, 15). Because PHCO₃ concentrations and blood pH were normal prior to the acid loading, the inability to lower urine pH in our patients is consistent with incomplete distal RTA (16). In patients with incomplete distal RTA, NH₄ excretion characteristically constitutes a greater than normal fraction of urinary acid both before and during induced acidosis (16). In our patients with FAS NH₄ excretion presented a smaller fraction of total acid produced, and the absolute excretion was also lower than that observed in control subjects. Since NH₄ excretion and final acidification occur primarily in the collecting duct (17), the impairment of this aspect of tubular function might be expected in FAS patients.

All of our patients with FAS also had an abnormality in tubular secretion of potassium. Despite the impairment in renal potassium excretion, hyperkalemia did not develop during acid loading, suggesting the presence of an extrarenal mechanism of potassium adaptation (18). Since urinary potassium excretion primarily reflects secretion in the distal nephron, probably at the level of the cortical collecting duct (19, 20), the decreased FE_K is further evidence for a distal tubular dysfunction in FAS patients.

Defective potassium excretion can result from decreased delivery of sodium to the distal nephron (21–23), a defect in aldosterone secretion (24–26), or a primary defect in tubular secretion of potassium (27–29). Decreased sodium delivery to the distal nephron cannot account for the impairment in urinary potassium excretion observed in FAS patients. As shown in Table 4, our patients were able to increase urinary sodium excretion in response to sodium bicarbonate administration, but their ability to augment potassium excretion was markedly decreased compared to control subjects suggesting a defect in distal sodium reabsorption.

Since hyporeninemic hypoaldosteronism can result in renal acidosis and impaired potassium excretion (24–26), the integrity of the renin-aldosterone axis was examined in FAS infants. The results demonstrated normal values for plasma renin activity and plasma aldosterone concentration (Table 2), thus excluding hypoaldosteronism as the cause of the impaired potassium excretion.

Having dismissed hypoaldosteronism as the cause of the impaired potassium excretion, we had to consider the possibility of primary defect in potassium secretion in FAS patients. To examine this possibility, each subject was treated with chlorothiazide. Thiazides are known to enhance potassium excretion by increasing tubular flow rate, providing more sodium to the distal sodium-potassium exchange sites and causing volume depletion with concomitant stimulation of aldosterone secretion (13). Ad-

ministration of chlorothiazide produced marked diuresis of both sodium and potassium. Plasma potassium decreased and PHCO₃ rose to normal levels. Concomitantly NAE increased significantly. It is unlikely that this effect was due to extracellular volume contraction, since the change in body weight at this point was negligible. Therefore, it seems logical to speculate that the kaliuretic and acidification response to chlorothiazide in our FAS patients was the consequence of enhanced sodium delivery to the cation-exchange sites in the distal nephron where aldosterone exerts its effect (19, 20).

In the presence of sufficient mineralocorticoid activity, an increase in distal delivery of sodium increases the normally observed lumen-negative potential difference (27, 28). This increase in potential difference then promotes hydrogen ion secretion. Since distal potassium secretion is also dependent on the lumen-negative potential difference created by sodium reabsorption (19, 20), the urinary potassium excretion would similarly be increased. A defect in sodium reabsorption at the distal nephron would result in a decreased potential difference, and hence in a decrease in urinary acidification (27–30).

The findings of the combined tubular defects in distal acidification and potassium excretion in the presence of adequate levels of plasma aldosterone and inability to lower the urine pH during acidemia are consistent with a voltage-dependent type of distal RTA (29, 30). Such patients are also recognized by inability to lower urine pH and to increase potassium excretion in response to maneuvers which enhance lumen-oriented electro-negativity in the distal nephron (31, 32). While we did not infuse Na₂SO₄ to our patients, the fall in urine pH and the increase in potassium excretion observed after chlorothiazide administration rather suggests that our patients had a reversible type of voltage-dependent distal RTA (30). The resulting hyperchloremic metabolic acidosis in this variant of distal RTA is distinguished from that of type 4 RTA by the accompanying normal plasma aldosterone concentration (33, 34).

The similarity in the present study in the pattern of impaired hydrogen ion and potassium excretion among the patients with FAS, suggest a singular mode of etiology. Intrinsic damage to the cells of the distal nephron caused by ethanol appears to be the most likely possibility. However, further studies will be needed to determine the pathophysiologic mechanisms responsible for the impaired renal acidification in FAS patients. Regardless of the mechanism, FAS should be added to the list of conditions associated with secondary RTA.

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Epidermal Growth Factor in Mouse Milk during Early Lactation: Lack of Dependency on Submandibular Glands

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ABSTRACT. Using a specific and sensitive radioimmunoassay, we found epidermal growth factor (EGF) in mouse milk during early lactation in normal and sialoadenectomized mice. Levels of EGF peaked around the 6th day postpartum and decreased thereafter up to day 12. Sephadex G-50 column chromatography of milk from normal and sialoadenectomized mice showed a single immunoreactive component comigrating with purified 6045 dalton EGF from the mouse submandibular gland. The concentrations of EGF were similar in milk collected from the breast and the stomach of the offspring immediately after feeding. The molecular profiles, concentrations, and ontogeny of EGF in milk of control and sialoadenectomized mice were also similar, suggesting that the submandibular gland is

not the major source of EGF in mouse breast milk. (*Pediatr Res* 19: 853-856, 1985)

Abbreviations

EGF, epidermal growth factor
SMG, submandibular gland
RIA, radioimmunoassay
PBS, phosphate-buffered saline

EGF is present in high concentrations in mouse SMG and in lesser concentrations in many mouse and human tissues and body fluids (1-5). EGF stimulates accelerated proliferation and differentiation of skin, corneal epithelium, and lung tissue *in vitro* and induces a cascade of events that results in increased mitogenic activity. *In vivo* EGF accelerates eyelid opening and tooth eruption in newborn mice (6), and promotes functional maturation of the intestine (7) and lungs (8). Functional EGF receptors have been found in many embryonic tissues (9).

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