A Case of Bicarbonate-losing Renal Tubular Acidosis with Defective Carboanhydrase Activity

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Donckerwolcke, R. A., van Stekelenburg, G. J., and Tiddens, H. A. (1970). *Archives of Disease in Childhood*, 45, 769. **A case of bicarbonate-losing renal tubular acidosis with defective carboanhydrase activity.** A girl of 20 months had primary proximal renal tubular acidosis with a lowered renal threshold for bicarbonate. There was no carboanhydrase inhibition after acetazolamide administration, suggesting that defective carboanhydrase activity may have been causative.

Renal tubular acidosis (RTA) is classically defined as tubular insufficiency in renal excretion of H^+ ions, out of proportion to, or occurring in the absence of glomerular insufficiency (Elkinton *et al.*, 1960). Rodriguez Soriano and co-workers have considerably expanded this concept; they differentiate between *proximal* RTA associated with a disturbance in reabsorption of HCO_3^- , and a *distal* type associated with a defect in excretion of H^+ . These defects can occur in combination, but will considerably exceed in importance any possibly associated glomerular disturbance (Rodriguez Soriano *et al.*, 1967; Rodriguez Soriano and Edelmann, 1969). Morris (1969) prefers a classification on a physiopathological basis and suggests the terms 'rate type RTA' (bicarbonate wasting) and 'gradient type RTA' (impaired excretion of H^+).

Several reports have described conditions in which either a disturbance in reabsorption of HCO_3^- , or in excretion of acid, constitutes one aspect of a complex clinical picture. These conditions are usually classified as secondary (Royer and Broyer, 1967; Rodriguez Soriano and Edelmann, 1969).

On the other hand there are the isolated disturbances of as yet obscure aetiology, which are referred to as primary (Rodriguez Soriano *et al.*, 1967). Theoretical considerations and experimental findings (Rector *et al.*, 1960; Schwartz, Falbriard, and Relman, 1958) suggest that such a disturbance may be based on renal carboanhydrase deficiency. The present study supports this hypothesis.

Case Report

The patient was born at term after an uneventful pregnancy and parturition; her birthweight was 3500 g.

She is the sixth child of non-consanguineous parents; the first child has cerebral palsy, and the third child a cleft palate. At 11 weeks she was admitted to hospital elsewhere for vomiting, diarrhoea, and a tendency to hypothermia; she was then noted to have a persistently low serum total CO_2 (14 mEq/l.).

At 20 months she was admitted for investigation of her retarded growth (Fig. 1). She was small and thin, both height and weight being well below the 3rd centile.

The musculature was hypotonic; reflexes were normal. Blood pressure, ECG, and chest *x*-ray were normal. Eyes: besides convergent squint, there was a bilateral band-keratopathy which progressed during the period of observation.

Griffith's IQ test at a chronological age of 127 weeks gave a mental age of 84 weeks and a general IQ of 66.

Bone age 3 months (at age 20 months). Intravenous pyelography: renal size and excretion normal; no nephrocalcinosis.

Chromosomes (J. O. van Hemel) were normal.

Methods

The acid-base status in blood (pH , P_{CO_2} , HCO_3^- , and total CO_2) was determined by the Astrup method; Na and K by flame photometer; Cl colorimetrically, phosphate, creatinine, and insulin (anthrone method) colorimetrically; glucose by the glucose-oxidase method; Ca by visual titration with EGTA using ortho-OH-naphthol-blue as indicator; urea by urease reaction in combination with indophenol reaction. Gastric acid secretion after histamine stimulation was measured during 3 consecutive 30-minute periods.

Urine pH was measured with a pH electrode; titrable acid by immediate titration to pH 7.4 with 0.1 N NaOH, ammonium content colorimetrically, using the indophenol reaction.

The total CO_2 of the urine was determined by the method of van Slyke and Neill (1924) adapted by Mook (1930) using a microgasometer. The P_{CO_2} and bicar-

FIG. 1.—Growth rate.

bonate concentration were calculated from the Henderson-Hasselbach equation.

Amino acids were determined by column chromatography; osmolality by cryoscopy.

Maximal concentrating capacity was examined after thirsting (Edelmann *et al.*, 1967a) and after vasopressin administration. Glucose reabsorption was measured by the method of Royer, Mathieu, and Habib (1963); GFR by endogenous creatinine clearance; and the renal contribution to acid-base regulation by the methods described by Edelmann *et al.* (1967b), with modifications to be discussed.

Urine collections employed an indwelling urethral catheter (urine was not collected under oil, since gases readily diffuse between urine and oil) (Oetliker and Rossi, 1969).

Acid loading test (Edelmann *et al.*, 1967c). NH_4Cl , 75 mEq/m.² was administered orally during a period of 60 minutes. Hourly urine collections were obtained before and during the 5 hours after NH_4Cl administration, for determination of pH, titrable acid (TA), and ammonium (NH_4^+). Arterialized blood samples were obtained before and 3 hours after acid load. From these data the hydrogen ion clearance index was calculated (Elkinton *et al.*, 1960; Peonides, Levin, and Young, 1965). Hydrogen ion clearance index = H^+ excretion (mEq/min./1.73 m²) $\times$ plasma CO_2 content (mEq/l.), where H^+ excretion = $\text{TA} + \text{NH}_4^+ - \text{HCO}_3^-$.

Bicarbonate titration. Bicarbonate reabsorption was investigated by the continuous infusion of a solution containing sodium bicarbonate, inulin, and 5% glucose. In the first study, bicarbonate threshold, tubular reabsorption, and the response to acetazolamide were examined (standardized or low flow method, Edelmann *et al.*, 1967b). After control collections, bicarbonate was infused at a rate calculated to produce an increase in serum bicarbonate of 1.5 mEq/l. per hr. When the bicarbonate threshold was reached, as judged by increase in urine pH to between 6.5 and 7.0, the rate of bicarbonate infusion was increased by a factor of 3 (to 0.45 mEq/min. NaHCO_3). Urine was collected at 30-minute intervals, at the midpoint of which blood samples were obtained from an indwelling needle that was flushed with slightly heparinized isotonic saline solution after each sampling. Over the entire range of serum HCO_3^- values for HCO_3^- reabsorption and excretion were determined. After 6 hours of Na bicarbonate infusion, when serum HCO_3^- had reached about 20 mEq/l., acetazolamide in a single dose of 12.5 mg./kg. was administered intravenously, and collections were continued for a further two 30-minute periods.

In a second study (high flow method), bicarbonate was infused at a rate of 0.45 mEq/min. during the entire investigation. After 4 hours at serum bicarbonate levels of about 25 mEq/l., T_m was measured over three 30-minute periods, after which acetazolamide in a single dose of 25 mg./kg. was administered intravenously, and collections were continued for a further three 30-minute periods. Curves of bicarbonate reabsorption and excretion were constructed and plotted (Pitts, Ayer, and Schiess, 1949).

Results

Blood/serum. Hb 10.1 g./100 ml., haematocrit 36%. Na 139 mEq/l., K 4.3 mEq/l., Cl 121 mEq/l., Ca 9.4 mg./100 ml., P 5.1 mg./100 ml., pH 7.11, Pco_2 28.5 mm. Hg, st. HCO_3^- 10.4 mEq/l., total CO_2 8.6 mEq/l.; urea 38 mg./100 ml., creatinine 0.5 mg./100 ml. PBI 4.8 μg ./100 ml., cholesterol 162 mg./100 ml., alkaline phosphatase 296 I.U.

Immunoglobulins: IgG 871 mg./100 ml., IgA 59 mg./100 ml.; IgM 70 mg./100 ml.

Gastric acid secretion. Free acidity 51–71 μEq /ml. (normal 15–95); total acidity 78–97 μEq /ml. (normal 25–105).

Urine. pH 5.0, output 300–350 ml./24 hours; protein nil; culture sterile.

Renal function studies. GFR, calculated from creatinine and inulin clearances, was normal. Maximal concentrating capacity was clearly impaired. Urine osmolality after thirsting: 582 mOsm/kg. H_2O , after vasopressin: 513 mOsm/kg. H_2O . Reducing substances absent in urine.

TABLE I
Ammonium Chloride Loading Test

Dates	Serum		Urine			
	pH	Total CO ₂ mEq/l.	pH	TA	NH ₄ ⁺	(H ⁺)
				(μEq/min per 1.73 sq.m.)		
8.8.68	7.0	6	4.6	74	90	164
2.9.68	7.1	5.8	4.8	76	57	133
Normal values	7.19-7.36	15.1-18.5	4.6-5.1	40-77	40-80	80-160

Glucose reabsorption, no glucosuria at serum glucose of 180 mg./100 ml.; TmG 370 mg./min. 1.73 m². No abnormal aminoaciduria.

Calciuria: 4.9, 6.7, and 5.1 mg./kg. per 24 hours (normal up to 8 mg.). Phosphate reabsorption 80% (normal 75-95%).

NH₄Cl loading test: at serum total CO₂ level of 6 mEq/l., an acid urine was excreted (pH 4.6), containing normal amounts of TA and NH₄⁺ (serum and urine values three hours after acid loading are presented in Table I).

The hydrogen ion clearance index was abnormally low, $0.164 \times 6 = 0.984$ (normal range: 1.25-2.30).

Bicarbonate loading test.

(a) *Threshold.* During the HCO₃⁻ loading test, significant bicarbonaturia occurred once the serum total CO₂ rose above 10.3 mEq/l. (Fig. 2).

(b) *T_m HCO₃⁻.* This was determined twice. The low threshold made it difficult to attain high serum levels by the *standardized method* (Pitts *et al.*, 1949, and Edelmann *et al.*, 1967b).

Serum HCO₃⁻ levels attained by means of the

FIG. 2.—Relation between urinary pH and serum total CO₂.

standardized method ranged from 18.2 to 20.0 mEq/l.; the corresponding HCO₃⁻ reabsorption ranged from 0.93 to 1.09 mEq/100 ml. GF (Table I; Fig. 3).

In a second investigation (see methods) T_m was determined at serum HCO₃⁻ levels ranging from 25.0 to 27.0 mEq/l.; the corresponding HCO₃⁻ reabsorption ranged from 0.94 to 1.15 mEq/100 ml. GF. (Table I).

(c) *Acetazolamide effect.* The effect of acetazolamide was studied twice (Table II), after the administration of 12.5 and of 25 mg./kg.; neither was followed by significant change in HCO₃⁻ reabsorption.

Discussion

Our patient shows dwarfism associated with metabolic acidosis. Studies showed that there was a disturbance of renal acid-base regulation, involving lowered renal threshold for HCO₃⁻, combined with lowered maximum reabsorption capacity for

FIG. 3.—Bicarbonate reabsorption and excretion of the patient. The graph shows the HCO₃⁻ threshold at 10.5 mEq/l. and T_m at 1.1 mMol/100 ml. GF (normal values HCO₃⁻ threshold 24-26 mEq/l.; T_m 2.6-2.8 mMol/100 ml. GF).

TABLE II
Bicarbonate Reabsorption and Acetazolamide Effect

Period of Investigation	Minutes after Start of Infusion	Serum		Diuresis (ml./min.)	GFR (ml./min.)	Bicarbonate	
		HCO ₃ ⁻ (mEq/l.)	K			Reabsorption (mEq/100 ml. GF)	Excretion (ml. GF)
First study (see methods) NaHCO ₃ 0.45 mEq/min. . .	300-330	19.1	—	1	22.5	1.09	0.80
	331-361	20.0	—	1.17	23.4	0.93	1.00
	368-398	21.0	—	1.3	22.8	0.93	1.12
	399-429	23.0	—	1.4	23.8	1.06	1.24
Second study (see methods) NaHCO ₃ 0.45 mEq./min. . .	0		4.3				
	241-271	26.0		2.47	20.2	1.15	1.14
	272-302	27.0	3.5	2.33	18	0.94	1.70
	306						
	305-335	35.0		2.16	20.0	1.28	2.22
	335-366	33.0		2.16	18.7	1.15	2.05
	367-397	30.0	2.1	1.93	19	1.12	1.88

HCO₃⁻. As the serum HCO₃⁻ was below the threshold, the capacity for acid excretion was considered to be normal.

Hydrogen ion excretion, as expressed in terms of its clearance index, was defective. The hydrogen ion clearance index relates urinary acid excretion to serum total CO₂. Excretion of acid in response to acid loads however does not depend in the main on changes in plasma bicarbonate (Relman, 1969). This was illustrated by the absence of a linear relation between serum total CO₂ and acid excretion over the entire range of total CO₂ values (Edelman *et al.*, 1967c). The H⁺ clearance index cannot therefore be used to demonstrate impairment of acid excretion in conditions with lowered renal threshold of HCO₃⁻.

Our patient had no significant disturbance of glomerular filtration, nor any systemic disease, and her disorder should thus be classified as a primary bicarbonate wasting renal tubular acidosis.

To investigate the cause of the disturbed bicarbonate reabsorption, the effect of inhibition of renal carboanhydrase was studied by giving acetazolamide intravenously, after normalizing the HCO₃⁻ level and correcting the acidosis by continuous intravenous bicarbonate. There was no change in HCO₃⁻ reabsorption. Bicarbonate excretion increased in the first study period concomitant with the serum bicarbonate level and without a change in bicarbonate reabsorption. In the second period the increase in bicarbonate excretion and serum level was accompanied by a slight increase in bicarbonate reabsorption.

The effect of acetazolamide on bicarbonate reabsorption is dependent on several factors, such as dosage (Gordon *et al.*, 1960; Schwartz *et al.*, 1958), mode of administration, and degree of acidosis

(Kaye, 1955; Maren, 1956; Schwartz *et al.*, 1958; Webster *et al.*, 1960), and the nature of renal disorder (Kaye, 1955; Rodriguez Soriano *et al.*, 1967; Schwartz *et al.*, 1958; Yaffe, Craig, and Fellers, 1960). Our study was carried out at a blood pH of 7.40 and 7.44, a PCO₂ of 30 and 36 mm. Hg, and a serum total CO₂ of 20.0 and 35.0 mEq/l. The doses administered fall within the effective range indicated by Schwartz *et al.* (1958), and our patient had no gross reduction in nephron mass. The data obtained in the first part of the study are suggestive of the absence of an acetazolamide effect.

The slight increase in bicarbonate reabsorption in the second part of the study is difficult to interpret and the conditions of the experiment allow no firm conclusion about the presence or absence of an acetazolamide effect.

The absence of any decrease in reabsorption, however, does suggest that acetazolamide was without effect. This absence of an acetazolamide effect may be a consequence of a qualitative carboanhydrase deficiency. Proof of a quantitative deficiency would be obtainable only by an enzymatic study (Yaffe *et al.*, 1960).

The diagnosis of a renal carboanhydrase deficiency raises the question of carboanhydrase activity in other body cells; this is often studied by measuring the secretion of gastric acid following histamine stimulation (Hogben, 1960; Rodriguez Soriano *et al.*, 1967). We found that our patient was capable of secreting gastric acid after stimulation. However, little weight should be placed on this finding in relation to a possible deficiency of carboanhydrase in the gastric cells, as the effect of acetazolamide on gastric acid secretion is small (Powell *et al.*, 1962).

Primary bicarbonate-losing renal tubular acidosis

manifests itself clinically in retardation of growth. Association with ocular lesions has not been previously reported. Biochemically, the dominant feature is hyperchloraemic acidosis. The ability to produce acid urine is unimpeded in rate type RTA, which is further distinguished from gradient type RTA by the absence of abnormal calciuria and of nephrocalcinosis. The basic abnormality in these patients is a lowered renal threshold for HCO_3^- , some patients show a lowered Tm HCO_3^- as well. Once the serum HCO_3^- is brought below the threshold, they are capable of excreting acid urine and producing adequate amounts of TA and NH_4^+ .

The aetiology of the disease has so far remained obscure. Bicarbonate reabsorption involves three different mechanisms: (a) carboanhydrase dependent hydration of CO_2 ; (b) CO_2 -dependent, but non-catalysed hydration of CO_2 ; and (c) direct reabsorption of HCO_3^- as such (Gordon *et al.*, 1960; Hanley *et al.*, 1959; Rector, Carter, and Seldin, 1965; Schwartz *et al.*, 1958). The data collected in this study suggest a defect in the first mechanism as a probable cause of the disease in our patient.

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