The Renal Response of Children to Acute Ammonium Chloride Acidosis

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Extract

The present observations were undertaken to establish the response of children to the acute administration of ammonium chloride, in order to provide a basis for identifying and interpreting abnormalities in the mechanisms involved in renal acid base regulation. Fifty-eight normal children aged 4 to 13 years were studied before and for 5 hours following administration of ammonium chloride. Group A, consisting of 23 children, received 75 mEq/m² body surface area or 2.75 mEq/kg body weight. Group B, consisting of 35 children, received 150 mEq/m², or 5.45 mEq/kg.

Values for serum electrolytes, urea, creatinine, osmolality and acid-base parameters in children in Groups A and B are shown in tables I and III. Urinary pH and rates of excretion of titratable acid and ammonium are shown in tables II and IV. The relations between blood tCO₂ and urinary concentration of H⁺, rate of excretion of titratable acid, and rate of excretion of ammonium for all subjects are shown in figures 1-3. Progressively lower values of pH were observed over the entire range of tCO₂ studied. During control periods, an inverse correlation was present between tCO₂ and rates of excretion of both titratable acid and ammonium. At levels of tCO₂ below 22.5 mmol/l, the rate of excretion of TA and ammonium became constant, with mean rates of excretion of 52 μEq/min/1.73 m² (range, ±2 SD, 33 to 71), and 73 μEq/min/1.73 m² (46 to 100), respectively.

These data are interpreted as demonstrating the importance of the blood tCO₂ or, more precisely, the concentration of bicarbonate in glomerular filtrate in urinary acidification mechanisms as well as in control of excretion of bicarbonate. Assessment of renal acidifying mechanisms requires administration of sufficient ammonium chloride or other acidifying substance to depress blood tCO₂ to levels well below the renal threshold.

The physiologic validity of the hydrogen ion clearance index must be questioned, since correcting rates of excretion of hydrogen ion for concentration of total CO₂ in blood presumes that there is a fixed relation between these two measurements. In figure 4, urinary excretion of total hydrogen ion is plotted as a function of blood tCO₂. Hydrogen ion excretion, negligible at tCO₂ levels of 26 or more, increases steadily as tCO₂ falls to values below the threshold. The rate of excretion then reaches maximal levels, despite further reduction in blood tCO₂. Thus, the hydrogen ion clearance index is valid only over the range of blood tCO₂ in which there is a linear relationship with rate of excretion of hydrogen ion. Renal acidification mechanisms should be evaluated with blood tCO₂ several millimoles below the threshold, assuring a maximal response and permitting direct comparison with normals. In subjects with a normal bicarbonate threshold, blood tCO₂ should be depressed at least to 17-18 mmol/l in infants and 20-21 mmol/l in older children. If an abnormal bicarbonate threshold is present the dose must be adjusted accordingly.

Speculation

These studies suggest that both the concentration of bicarbonate in glomerular filtrate and the level of the bicarbonate threshold play major roles in the regulation of renal excretion of hydrogen ion.

Introduction

Excretion of acid in urine is accomplished by renal tubular secretion of hydrogen ion causing reabsorption of filtered bicarbonate and urinary acidification, with formation of titratable acid and ammonium. Urinary acidification and formation of titratable acid and ammonium in children are considered here. Tubular reabsorption of bicarbonate and excretion of acid in infants have been the subject of a separate report [4].

In order to investigate the response of the kidney to metabolic acidosis, the capacity to excrete hydrogen ion in response to various acidifying agents, such as hydrochloric acid, ammonium chloride and calcium chloride, has been studied repeatedly in the past four or five decades [6, 8, 11]. Most tests have required prolonged administration of ammonium chloride [5, 25]. The complexity and duration of these procedures have precluded their use in routine clinical investigation, particularly in infants and children, in whom only a few studies have been reported [7, 9, 17, 28].

Although SARTORIUS, ROEMMELT and PITTS [29] had previously reported the renal response of a single subject to acute acidosis induced by a single dose of ammonium chloride, WRONG and DAVIES [3, 34] were the first to describe in detail the short ammonium chloride loading test. This test seemed especially suited to exploration of renal regulation of acid-base balance in infants and children. The procedure does not require hospitalization; it can be completed in the course of a single day, and a severe degree of acidosis is avoided. The present observations were undertaken to establish the response of children to the acute administration of ammonium chloride in order to provide a basis for identifying and interpreting abnormalities in the mechanisms involved in renal acid base regulation.

Materials and Methods

Fifty-eight normal children, 4 to 13 years of age, were studied. All children were hospitalized for minor illnesses or for elective surgery. The latter were studied either before or at least five days after surgery, and no subject undergoing major surgery was included. Subjects with any type of renal disease or nonrenal disorder that might affect water and electrolyte or acid-base metabolism were excluded. All subjects were consider-

ed to be healthy children and fell within the tenth and seventy-fifth percentiles for height and weight. The investigations were performed with institutional approval. Informed parental consent was obtained in every case.

Studies were performed in the renal unit and were begun uniformly between 8 and 9 a.m. The patients remained recumbent during the test, but were allowed free movement in bed. Breakfast was permitted on the day of the test, and a usual lunch was given at the appropriate time. In order to ensure an adequate and suitably constant rate of urine flow, water was administered throughout the test at the rate of 50 ml/h/m². Urines were collected at hourly intervals by spontaneous voiding. Urine specimens were not collected under oil but were examined immediately for pH and titratable acid and were stored for other determinations in air-free containers.

Arterialized capillary blood samples were collected in heparinized tubes for determination of pH, P_{CO}₂ and total CO₂ content. Blood specimens for other determinations were obtained from superficial veins during free flow.

Following collection of two 30- to 60-minute control urines, samples of venous and capillary blood were also obtained. Ammonium chloride was given by mouth over the course of the next hour; it was taken either in gelatin capsules by the older children or dissolved in water flavored with lemon juice and sugar in the younger children. The ammonium chloride was well tolerated by most subjects, although a few vomited after receiving the first portion of the ammonium chloride. An equivalent amount of drug was given again, followed by the balance of the total dose, without further vomiting.

The dosage of ammonium chloride was as follows: Group A, consisting of 23 children, received 75 mEq/m² body surface area or 2.75 mEq/kg body weight. Group B, consisting of 35 children, received 150 mEq/m² or 5.45 mEq/kg.

Following administration of the ammonium chloride, five urine samples with approximately 60 minutes between samples were collected in subjects in Groups A and B, except that in some children in Group B, observations were discontinued after the third or fourth test hour. Blood samples were obtained three to four hours after the ingestion of ammonium chloride was completed.

Analytical procedures employed have been described previously [4, 26]. The hydrogen ion clearance index was calculated according to the formula proposed by ELKINTON, HUTH, WEBSTER and McCANCE [5]:

Hydrogen ion clearance index = $UV_{H^+} \times tCO_2$ where UV_{H^+} is the net rate of excretion of hydrogen ion, estimated as the sum of titratable acid and ammonium minus bicarbonate, and tCO_2 is the total CO_2 content of blood.

Results¹

Values for electrolytes, urea, creatinine, osmolality and acid-base parameters of serum in children of Groups A

¹ The following abbreviations are employed: total CO_2 or CO_2 content, tCO_2 ; partial pressure of CO_2 , P_{CO_2} ; base excess, BE; titratable acid, TA; ammonium, NH_4^+ .

Table I. Group A. Blood values before and three to four hours after administration of NH_4Cl , 75 mEq/m²

	pH	BE* mEq/l	tCO ₂ mmoles/l	P _{CO₂} mm Hg	Na	K	Cl	Ca	P	Urea	Osm	Creatinine
					mEq/l				mmoles/l		mOsm/l	mg/100 ml
Control												
mean	7.41	+0.06	25.1	39	141	4.9	103	5.0	1.6	5.4	280	0.60
range	7.35	-2.5	23.5	33	134	3.7	96	4.3	1.3	3.2	260	0.43
	7.48	+2.8	27.2	45	150	6.5	109	5.3	1.9	7.0	295	1.00
n	23	23	23	23	23	22	23	21	22	22	22	15
After NH_4Cl												
mean	7.36**	-3.86**	21.6**	38	141	4.9	108**	5.0	1.7	6.3**	282	0.62
range	7.29	-6.0	19.4	31	132	4.0	102	4.55	1.3	2.7	268	0.39
	7.45	-1.8	24.6	44	149	5.9	115	5.25	2.2	8.6	295	0.80
n	23	22	22	22	23	22	22	21	22	22	15	22

* BE: Base excess

** Significantly different from control ($p \leq 0.01$)

Table II. Group A. Urinary hydrogen excretion in hourly collections before and after administration of NH_4Cl , 75 mEq/m²

		Periods		1	2	1	2	3	4	5
				Control		After NH_4Cl				
pH	mean			6.46	6.51	5.33	5.16	5.11	5.15	5.11
	range			5.07	5.2	4.9	4.77	4.65	4.7	4.7
				7.7	7.43	6.05	5.9	5.85	5.95	5.9
	n			19	22	23	22	23	23	21
TA ¹	mean			15	16	41	46	48	49	51
	range			0-37	0-39	11-85	19-71	26-70	30-70	31-88
	n			20	21	23	22	21	21	22
NH_4^+ *	mean			21	27	57	60	65	63	68
	range			9-57	8-89	22-118	29-109	30-115	29-85	27-111
	n			19	20	22	21	21	21	22
UV_{H^+} *	mean			28	29	97	105	113	110	119
	range			0-74	0-80	33-203	51-175	56-174	59-148	60-168
	n			18	20	22	21	21	21	21

* $\mu\text{Eq/min/1.73 m}^2$

and B are shown in tables I and III. All control values fell within the normal range for children of this age. Following administration of ammonium chloride in Group A, pH, $t\text{CO}_2$ and BE in blood fell as expected with no significant change in PCO_2 . Concentrations of urea and chloride in serum rose but other electrolytes, osmolality and creatinine were unchanged. In Group B, a considerable drop in pH was noted, reaching a mean of 7.27. The changes in BE, $t\text{CO}_2$ and urea were

approximately double those noted in Group A with a similar rise in chloride. Other blood constituents measured were unchanged.

Urinary pH during control periods in both Groups A and B ranged from 5.1 to 7.7 (tables II and IV). Excretion of titratable acid ranged from 0 to 39 and ammonium from 8 to 89 $\mu\text{Eq}/\text{min}/1.73 \text{ m}^2$. Following administration of ammonium chloride, urinary pH decreased to values as low as 4.65 in Group A and 4.55

Table III. Group B. Blood values before and three to four hours after administration of NH_4Cl , 150 mEq/ m^2

	pH	BE mEq/l	$t\text{CO}_2$ mmoles/l	PCO_2 mm Hg	Na	K	Cl	Ca	P	Urea	Osm mOsm/l	Creatinine mg/100 ml
					mEq/l				mmoles/l			
Control												
mean	7.41	+0.2	25.7	39	138	4.5	103	4.7	1.55	5.5	277	0.66
range	7.38	-2.0	23.0	34	131	3.0	96	4.25	1.3	3.5	265	0.40
	7.51	+2.5	29.2	45	140	5.2	109	5.25	1.8	7.6	291	1.10
n	12	12	12	12	11	12	11	11	12	12	12	12
After NH_4Cl												
mean	7.27*	-9.1*	18.4*	38	138	4.7	109*	4.8	1.6	7.4*	290	0.63
range	7.19	-13.5	15.1	33	131	3.3	101	4.3	1.3	5.0	273	0.40
	7.36	-3.5	21.5	44	148	6.2	116	5.5	2.1	9.5	306	1.10
n	35	32	35	35	30	34	33	33	31	35	32	32

* Significantly different from control ($p \leq 0.01$)

Table IV. Group B. Urinary hydrogen ion excretion in hourly collections before and after administration of NH_4Cl , 150 mEq/ m^2

		Periods		1		2		1		2		3		4		5	
				Control				After NH_4Cl									
pH	mean			6.00		6.50		5.11		4.92		4.85		4.85		4.95	
	range			5.1		5.5		4.7		4.6		4.6		4.55		4.6	
				7.3		7.4		5.9		5.8		5.25		5.2		5.9	
	n			11		12		35		35		35		18		10	
TA*	mean			18		11		34		46		51		54		53	
	range			3-26		0-23		18-65		27-78		28-73		40-77		35-73	
	n			11		12		34		35		34		17		10	
NH_4^+ *	mean			24		24		66		73		74		79		80	
	range			15-35		13-45		38-129		33-136		42-105		58-106		62-89	
	n			11		12		34		35		34		17		10	
UV_{H^+} *	mean			31		14		100		119		125		133		133	
	range			0-67		0-68		61-168		78-181		91-157		100-157		115-155	
	n			11		12		34		35		34		17		10	

* $\mu\text{Eq}/\text{min}/1.73 \text{ m}^2$

Table V. Effect of administration of NH_4Cl on rates of excretion of urinary solutes

		Na	K	Cl	Ca	P	Urea	Creatinine	Osmotically
		$\mu\text{Eq}/\text{min}/1.73 \text{ m}^2$				$\mu\text{moles}/\text{min}/1.73 \text{ m}^2$		$\mu\text{g}/\text{min}/1.73 \text{ m}^2$	active solute $\mu\text{Osm}/\text{min}/1.73 \text{ m}^2$
<i>Group A</i>									
Control	mean	88	113	107	10	19	350	691	644
(1-2)*	range	8-262	27-271	37-268	0.6-33	3-79	151-805	450-1062	361-1090
After NH ₄ Cl	mean	197	138	314	15	41	492	874	1142
(3-5)	range	22-433	23-399	97-602	2-29	14-98	231-855	440-1347	456-2111
<i>Group B</i>									
Control	mean	82	79	112	5	20	355	716	654
(1-2)	range	4-210	14-209	10-438	0.5-13	4-39	149-681	583-1169	317-1197
After NH ₄ Cl	mean	358	148	648	17	47	554	709	1580
(3-5)	range	100-588	49-246	245-1526	7-31	31-77	205-727	503-1136	980-2168

* Numbers in parentheses refer to hours during which urine was collected.

Fig. 1. Urinary $[\text{H}^+]$ as a function of blood $[\text{tCO}_2]$ in children. Two points are plotted for each subject: A control value derived from control blood and the mean of the two control urines (open symbols), and a post- NH_4Cl value derived from the test blood and the mean of the urines during the third, fourth and fifth hours (closed symbols). Subjects in group A are shown by circles, group B by triangles.

Control periods: $r = -0.47$, $p < 0.001$; after NH_4Cl : $[\text{H}^+] = 43348 - 1550 [\text{tCO}_2]$; $r = -0.59$, $p < 0.001$; $b = -1550$, $p < 0.001$. Note that this regression, although linear, is plotted here semilogarithmically.

Fig. 2. Excretion of titrateable acid as a function of blood $[\text{tCO}_2]$. Control periods: $r = -0.49$, $p < 0.01$; after NH_4Cl no regression is present. Shown are the mean and range (± 2 SD) for all values with $[\text{tCO}_2]$ below 22.5 mmoles/l. Symbols are the same as in figure 1.

Fig. 3. Excretion of ammonium as a function of blood $[\text{tCO}_2]$. Control periods: $r = -0.50$, $p < 0.001$. After NH_4Cl no regression is present. Shown are the mean and range (± 2 SD) for all values with $[\text{tCO}_2]$ below 22.5 mmoles/l. Symbols are the same as in figure 1.

in Group B. However, minimal values for some children in Group A were as high as 5.7, whereas all children in Group B achieved a urinary pH of 5.1 or less at some point during the test.

Titrateable acid excretion rose as urinary pH fell, reaching comparable levels in both groups, approximately $50 \mu\text{Eq}/\text{min}/1.73 \text{ m}^2$ from the 2nd to 5th test hours. Mean values for ammonium excretion in $\mu\text{Eq}/\text{min}/1.73 \text{ m}^2$ in Group A ranged from 60 to 68, as compared to 73 to 80 in Group B. Similarly, mean values for total hydrogen ion excretion in $\mu\text{Eq}/\text{min}/\text{m}^2$ in Group A ranged from 105 to 119 and in Group B from 119 to 133.

Along with the increased rates of excretion of TA and NH_4^+ , there was an increase in rate of excretion of all urinary solutes measured (table V).

The hydrogen ion clearance index, calculated with the mean values of the 3rd, 4th and 5th test hours, ranged in Group A from 1.36 to 4.12, with a mean of 2.66, and in Group B from 1.69 to 3.10, with a mean of 2.40.

The relation between tCO_2 of blood and urinary concentration of H^+ for all subjects in Groups A and B is shown in figure 1. Although levels of urinary pH usually considered low enough to indicate normal acidifying capacity were not produced until tCO_2 of blood fell well below threshold values, progressively lower values of pH were observed over the entire range of tCO_2 studied.

The relation between tCO_2 of blood and rate of excretion of titrateable acid is shown in figure 2. During control periods, an inverse correlation was present. At levels of tCO_2 below 22.5 mmol/l, the rate of excretion of TA became constant. The mean rate of excretion for all values with tCO_2 of blood below 22.5 mmol/l was $52 \mu\text{Eq}/\text{min}/1.73 \text{ m}^2$ with a range (± 2 SD) of 33 to 71.

A similar relation between tCO_2 of blood and rate of excretion of ammonium is shown in figure 3. During control periods, an inverse relation between these two measurements was observed. At levels of tCO_2 below 22.5 mmol/l, maximal rates of excretion of ammonium were noted. The mean value for rate of excretion of ammonium was $73 \mu\text{Eq}/\text{min}/1.73 \text{ m}^2$ with a range (± 2 SD) of 46 to 100.

Discussion

Regulation of Urinary Acidification and Excretion of Hydrogen Ion

Although studies of bicarbonate reabsorption and excretion in infants and adults have demonstrated the close relation between concentration of bicarbonate in blood and its excretion in urine [4, 19, 33], the effects of progressively lower concentrations of bicar-

bonate in blood on urinary acidification have not been completely investigated.

The dose of ammonium chloride initially chosen in the present studies was based on dosages employed in other reports [3, 10, 34] and was calculated to provide a maximal stimulus. After completion of the initial series in children (Group A), it was apparent that this goal had not been achieved. Several subjects appeared to excrete significantly less hydrogen ion than the remainder of the group, although the response of the group as a whole was comparable to values in children reported by others [15, 17].

On the basis of these results, a double dose of ammonium chloride was given in the second series of observations (Group B). In each of these subjects, urinary pH fell to 5.10 or less during the course of the study. The mean rates of excretion of titrateable acid were the same in Groups A and B, but rates of excretion of ammonium and total hydrogen ion in Group B were significantly greater.

These results are consistent with the usual interpretation that a larger dose of ammonium chloride acts as a greater stimulus for excretion of hydrogen ion by the kidney. However, when urinary acidification and rates of excretion of TA and NH_4 were examined as functions of tCO_2 of blood, it was apparent that the size of the dose of NH_4Cl played no role.

During control periods (figs. 1-3), when tCO_2 ranged from 23 to 29 mmol/l, significant correlations were present between tCO_2 of blood and urinary hydrogen ion concentration, rate of excretion of TA and rate of excretion of ammonium. Following ingestion of NH_4Cl , there was still a significant correlation between urinary hydrogen ion concentration and tCO_2 of blood. In contrast, when tCO_2 fell several millimoles below the threshold, excretion of hydrogen ion in the form of TA and NH_4 reached a plateau. Thus, the apparent difference observed in mean response of Groups A and B was due to an inadequate depression of tCO_2 in blood in six subjects of Group A; at comparable levels of tCO_2 , no differences in response were noted. Theoretically, the larger dose of NH_4Cl might have resulted in higher rates of excretion of ammonium due to increased production of glutamine and provision of additional substrate [2]. No such difference in rate of excretion of ammonium was noted, although the rate of excretion of urea in Group B was greater than that in Group A.

These data are interpreted as demonstrating the importance of the level of tCO_2 of blood, or more precisely, the concentration of bicarbonate in glomerular filtrate, in stimulating urinary acidification mechanisms as well as in control of excretion of bicarbonate [18-20]. The rate of excretion of titrateable acid depends upon availability of urinary buffer, particularly

phosphate, and urinary pH [30]. Acute changes in the rate of excretion of ammonium are dependent on changes in urinary pH and the rate of urine flow [12, 16]. When concentrations of bicarbonate in blood are above the renal plasma threshold, bicarbonate is incompletely reabsorbed; hydrogen ion secreted along the entire length of the nephron is utilized in bicarbonate reabsorption, and bicarbonate is excreted into the urine. At bicarbonate levels in blood just at the threshold, the urine is free of bicarbonate, but almost all hydrogen ion secreted is still utilized in bicarbonate reabsorption; urinary pH is lowered only slightly below that of plasma and little TA and NH_4^+ are present in urine. As bicarbonate concentration in blood falls below the threshold, more hydrogen ion appears in urine, but only when blood bicarbonate is well below threshold levels is sufficient tubular H^+ available to cause maximal urinary acidification and thus in turn formation of TA and NH_4^+ . Rate of excretion of hydrogen ion reaches a plateau, despite further depression of bicarbonate in blood, when urinary buffers are saturated and there is maximal diversion of tubular NH_4^+ into urine.

Although the present investigations suggest that the concentration of bicarbonate in the glomerular filtrate is the most important factor controlling renal excretion of hydrogen ion during acute metabolic acidosis, in other circumstances, variation in rate of excretion of hydrogen ion occurs in the apparent absence of changes in pH or bicarbonate of blood. However, this may be mediated through the same mechanism by a change in the bicarbonate threshold, similar to the effect on acid excretion of variation in the carbon dioxide tension of blood [1, 23, 24].

The concept of a dynamic balance between the concentration of bicarbonate in the glomerular filtrate and the level of the bicarbonate threshold must be considered. A change in either will have a marked effect on the rate of delivery of bicarbonate into the distal tubule and, thus, on the rate of hydrogen ion excretion. An effect of potassium deficiency and extracellular volume expansion on proximal tubular reabsorption of bicarbonate has been demonstrated [13, 21]. Similarly, both the degree to which the kidney is stimulated to reabsorb sodium and the ratio of sodium to chloride in the glomerular filtrate influence the level of the threshold [31]. The change in bicarbonate threshold that occurs with a change in the rate of excretion of nonreabsorbable anions, such as sulfate [22], may explain part of the variation in hydrogen ion excretion that occurs with dietary changes [14].

Thus, the precise manner in which the kidney maintains systemic acid-base balance in health may be related to slight variation in the bicarbonate threshold, whereas in acutely induced metabolic acidosis with no

change in threshold, the primary factor appears to be the decreased concentration of bicarbonate in the glomerular filtrate. In chronic acidosis there is, in addition, an adaptation in tubular production of ammonia [29], which may relate to intracellular acidosis.

Clinical Assessment of Acidifying Capacity

It is apparent from these results that assessment of renal acidifying mechanisms requires administration of a substance which will produce maximal urinary acidification. This can be accomplished by administration of a nonreabsorbable anion, or, more physiologically, by administration of sufficient ammonium chloride or other acidifying substance to depress tCO_2 of blood to levels well below the renal threshold.

The results of the present studies, as well as those of WRONG and DAVIES [3, 34], indicate that the short test provides an adequate physiologic assessment of acidifying mechanisms, even though it does not assess ammonium adaptation during chronic acidosis. However, normal rates of excretion of ammonium during acute acidosis with deficient excretion of ammonium during chronic acidosis has not been described.

The hydrogen ion clearance index was proposed by ELKINTON and ASSOCIATES [5] in an attempt to relate rate of excretion of hydrogen ion in urine to the concentration of hydrogen ion in blood. Although this index has had widespread application, its physiologic validity must be questioned. Correcting rates of excretion of hydrogen ion for concentration of total CO_2 in blood presumes that there is a fixed relation between these two measurements. Examination of the data presented by ELKINTON *et al.* [5], however, reveals no regression of TA, NH_4^+ or total hydrogen ion with tCO_2 in blood following administration of NH_4Cl . In the present study (fig. 4), urinary excretion of total hydrogen ion is plotted as a function of blood tCO_2 .

Fig. 4. Net excretion of hydrogen ion as a function of blood $[\text{tCO}_2]$. Control periods: $r = -0.55$, $p < 0.001$. After NH_4Cl no regression is present. Shown are the mean and range (± 2 SD) for all values with $[\text{tCO}_2]$ below 22.5 mmoles/l. Symbols are the same as in figure 1.

Hydrogen ion excretion, negligible at $t\text{CO}_2$ levels of 26 mmoles/l or more increases steadily as $t\text{CO}_2$ falls to values below the threshold. The rate of excretion then reaches maximal levels, despite further reduction in $t\text{CO}_2$. Consequently, the hydrogen ion clearance index in a given subject would be much higher if measured at a $t\text{CO}_2$ in blood of 20 mmoles/l, for example, than if it were measured at 15 mmoles/l. This effect can be seen in comparing Group A with Group B. As a consequence of the lower values of $t\text{CO}_2$ produced in children in Group B, their hydrogen ion clearance index was lower, suggesting incorrectly that these subjects performed less well than those in Group A.

Thus, the hydrogen ion clearance index is valid only over the range of $t\text{CO}_2$ in blood in which there is a linear relation with rate of excretion of hydrogen ion, i.e. from the range of normal concentration down to levels around the threshold. Over this range of $t\text{CO}_2$, however, urinary hydrogen ion excretion is not maximal and the range of normal is great. Rather than using the hydrogen ion clearance index, therefore, acidification should be evaluated with $t\text{CO}_2$ in blood several millimoles below the threshold, assuring a maximal response and permitting direct comparison with normal subjects. It is obvious that arbitrary, 'standard' doses of ammonium chloride may be either too large or too small for this purpose. The proper dose for a given subject can be calculated on the basis of the CO_2 content of blood, assuming distribution of administered H^+ throughout body water. In subjects with a normal level of bicarbonate threshold, $t\text{CO}_2$ in blood should be depressed at least to 17–18 mmoles/l in infants [4] and 20–21 mmoles/l in older children. If an abnormal bicarbonate threshold is present the dose must be adjusted accordingly [26, 27].

Summary

Observations of the renal response of children to administration of ammonium chloride support the hypothesis that the concentration of bicarbonate in glomerular filtrate, together with the level of the bicarbonate threshold, plays a major role in the regulation of renal excretion of hydrogen ion. An inverse relation was demonstrated between the total CO_2 content of blood and the concentration of hydrogen ion in urine. Rates of excretion of titratable acid and ammonium increased as concentration of total CO_2 in blood fell below normal levels; both rates reached a plateau, however, when total CO_2 reached concentrations a few millimoles below the renal bicarbonate threshold.

These results indicate that the necessary condition for assessing maximal acidifying capacity during metabolic acidosis is that the blood bicarbonate concentra-

tion be several millimoles below the renal bicarbonate threshold; the use of a 'standardized' dose of ammonium chloride does not assure a maximum response. Evidence is presented that calculation of the hydrogen ion clearance index does not provide a physiologically meaningful assessment of urinary acidification.

References and Notes

1. BRAZEAU, P. and GILMAN, A.: Effect of plasma CO_2 tension on renal tubular reabsorption of bicarbonate. *Amer. J. Physiol.* 175: 33–38 (1953).
2. CANESSA-FISCHER, M.; SHALHOUB, R.; GLABMAN, S.; DEHAAS, J. and PITTS, R. F.: Effects of infusions of ammonia, amides, and amino acids on excretion of ammonia. *Amer. J. Physiol.* 204: 192–196 (1963).
3. DAVIES, H. E. F. and WRONG, O.: Acidity of urine and excretion of ammonium in renal disease. A preliminary report. *Lancet* ii: 625 (1957).
4. EDELMANN, C. M., Jr.; RODRIGUEZ SORIANO, J.; BOICHIS, H.; GRUSKIN, A. B. and ACOSTA, M. I.: Renal bicarbonate reabsorption and hydrogen ion excretion in normal infants. *J. clin. Invest.* 46: 1309–1317 (1967).
5. ELKINTON, J. R.; HUTH, E. J.; WEBSTER, G. D., Jr. and McCANCE, R. A.: The renal excretion of hydrogen ion in renal tubular acidosis. I. Quantitative assessment of the response to ammonium chloride as an acid load. *Amer. J. Med.* 29: 554–575 (1960).
6. FØLLING, A.: On the mechanism of ammonium chloride acidosis. *Acta med. scand.* 71: 221–279 (1929).
7. FOMON, S. J.; HARRIS, D. M. and JENSEN, R. L.: Acidification of the urine by infants fed human milk and whole cow's milk. *Pediatrics* 23: 113–119 (1959).
8. GAMBLE, J. L.; BLACKFAN, K. D. and HAMILTON, B.: A study of the diuretic action of acid producing salts. *J. clin. Invest.* 1: 359–388 (1925).
9. GORDON, H. H.; McNAMARA, H. and BENJAMIN, H. R.: The response of young infants to ingestion of ammonium chloride. *Pediatrics* 2: 290–302 (1948).
10. GREDER, G. et GUTTMANN, F. M.: Le test d'acidification urinaire au chlorure d'ammonium, complément à l'étude des fonctions rénales. *Helv. med. Acta* 29: 1–37 (1962).
11. HALDANE, J. B. S.: Experiments on the regulation of the blood's alkalinity. *J. Physiol.* 55: 265–275 (1921).
12. HILLS, A. G. and REID, E. L.: Renal ammonia balance. A kinetic treatment. *Nephron* 3: 221–256 (1966).
13. KUNAU, R.; FRICK, A.; RECTOR, F. C. and SELDIN,

- D.W.: Effect of extracellular volume expansion, K^+ deficiency and PCO_2 on bicarbonate reabsorption in the rat kidney. *Clin. Res.* 14: 380 (1966).
14. LENNON, E.J.; LEMANN, J., Jr. and RELMAN, A.S.: The effect of phosphoprotein on acid balance in normal subjects. *J. clin. Invest.* 41: 637-645 (1962).
 15. LESTRADET, H.; CORREA, C.E.C. et BROYER, M.: L'exploration de la fonction d'acidification du tubule rénal par une épreuve courte utilisant une surcharge intraveineuse du chlorhydrate de L-Arginine. *Rev. franç. Etud. clin. biol.* 9: 885-892 (1964).
 16. ORLOFF, J. and BERLINER, R.W.: The mechanism of the excretion of ammonia in the dog. *J. clin. Invest.* 35: 223-235 (1956).
 17. PEONIDES, A.; LEVIN, B. and YOUNG, W.F.: The renal excretion of hydrogen ion in infants and children. *Arch. Dis. Childh.* 40: 33-39 (1965).
 18. PITTS, R.F.: Physiology of the kidney and body fluids, p. 163 (Year Book Medical Publishers, Chicago 1963).
 19. PITTS, R.F.; AYER, J.L. and SCHIESS, W.A.: The renal regulation of acid-base balance in man. III. The reabsorption and excretion of bicarbonate. *J. clin. Invest.* 28: 35-44 (1949).
 20. PITTS, R.F.; LOTSPEICH, W.D.; SCHIESS, W.A. and AYER, J.L.: The renal regulation of acid-base balance in man. I. The nature of the mechanism for acidifying the urine. *J. clin. Invest.* 27: 48-56 (1948).
 21. RECTOR, F.C., Jr.; BLOOMER, H.A. and SELDIN, D.W.: Effect of potassium deficiency on the reabsorption of bicarbonate in the proximal tubule of the rat kidney. *J. clin. Invest.* 43: 1976-1982 (1964).
 22. RECTOR, F.C., Jr. and SELDIN, D.W.: Influence of unrecabsorbed anions on renal threshold and T_m for bicarbonate. *Amer. J. Physiol.* 202: 313-324 (1962).
 23. RECTOR, F.C., Jr.; SELDIN, D.W.; ROBERTS, A.D., Jr. and SMITH, J.S.: The role of plasma CO_2 tension and carbonic anhydrase activity in the renal reabsorption of bicarbonate. *J. clin. Invest.* 39: 1706-1721 (1960).
 24. RELMAN, A.S.; ETSTEN, B. and SCHWARTZ, W.B.: The regulation of renal bicarbonate reabsorption by plasma carbon dioxide tension. *J. clin. Invest.* 32: 972-977 (1953).
 25. REYNOLDS, T.B.: Observations on the pathogenesis of renal tubular acidosis. *Amer. J. Med.* 25: 503-515 (1958).
 26. RODRIGUEZ SORIANO, J.; BOICHIS, H.; STARK, H. and EDELMANN, C.M., Jr.: Proximal renal tubular acidosis. A defect in bicarbonate reabsorption with normal urinary acidification. *Pediatr. Res.* 1: 81-98 (1967).
 27. RODRIGUEZ SORIANO, J.; BOICHIS, H. and EDELMANN, C.M., Jr.: Bicarbonate reabsorption and hydrogen ion excretion in children with renal tubular acidosis. *J. Pediat.* 71: 802-818 (1967).
 28. RUBIN, M.I.; CALCAGNO, P.L. and RUBEN, B.L.: Renal excretion of hydrogen ions. A defense against acidosis in premature infants. *J. Pediat.* 59: 848-860 (1961).
 29. SARTORIUS, O.W.; ROEMMELT, J.C. and PITTS, R.F.: Renal regulation of acid-base balance in man. IV. The nature of the renal compensations in ammonium chloride acidosis. *J. clin. Invest.* 28: 423-439 (1949).
 30. SCHIESS, W.A.; AYER, J.L.; LOTSPEICH, W.D. and PITTS, R.F.: The renal regulation of acid-base balance in man. II. Factors affecting the excretion of titratable acid by the normal human subject. *J. clin. Invest.* 27: 57-64 (1948).
 31. SCHWARTZ, W.B. and RELMAN, A.S.: Effects of electrolyte disorders on renal structure and function. *New Engl. J. Med.* 276: 383-389 and 452-458 (1967).
 32. SNEDECOR, G.W.: Statistical methods applied to experiments in agriculture and biology, 5th ed. (Iowa State University Press, Ames 1956).
 33. TUDVAD, F.; McNAMARA, H. and BARNETT, H.L.: Renal response of premature infants to administration of bicarbonate and potassium. *Pediatrics* 13: 4-16 (1954).
 34. WRONG, O. and DAVIES, H.E.F.: The excretion of acid in renal disease. *Quart. J. Med.* 28: 249-313 (1959).
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