THE RENAL EXCRETION OF HYDROGEN IONS IN INFANTS AND CHILDREN

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The ability of the kidneys to excrete an acid urine is often assessed by correlating plasma carbon dioxide and urine pH. A low plasma CO_2 should be accompanied by a low urine pH, provided that respiratory function is normal. Buchanan and Komrower (1958) consider that the renal excretion of hydrogen ions is normal in children if urine pH falls below 5.0, after a single dose of 0.1 g./kg. of ammonium chloride has been given to produce metabolic acidosis. Under similar conditions, urine pH falls to below 5.2 in normal adults (Wrong and Davies, 1959). However, Elkinton, Huth, Webster, and McCance (1960) found that urine pH was not sufficient to differentiate latent cases of renal tubular acidosis in adults after prolonged administration of NH_4Cl . These authors suggested a quantitative assessment of the renal response to an ammonium chloride load by the 'hydrogen ion clearance index' calculated as the product of the total H^+ excreted in the urine ($\text{mEq/min.}/1.73 \text{ sq. m.}$) and the plasma CO_2 content (mEq/l.) in subjects after a continuous NH_4Cl administration for 3-5 days. Abnormally low values of the index were considered to indicate renal acidosis. This index has not only proved a sensitive one in cases with latent renal tubular acidosis, but also useful in revealing the latent renal defect in otherwise normal relatives of patients with renal tubular acidosis, thus contributing to our knowledge of the genetics of the disease (Huth, Webster, and Elkinton, 1960).

In the present study the renal H^+ excretion on normal diet, as well as after the addition of NH_4Cl for several days, has been investigated in order to determine values for the hydrogen ion clearance index in normal infants and children. Results from similar investigations in children with renal tubular acidosis and their relatives will be reported later.

Subjects and Methods

Studies were carried out on 26 normal infants and children, mostly in-patients who had recovered from diseases

not associated with renal or metabolic disturbances. No subjects with abnormal respiratory function, abnormal plasma electrolyte or blood urea levels, or abnormal urines were included. Where necessary urea and endogenous creatinine clearances were estimated, and found to be normal for their age.

Since little is known about younger infants after the neonatal period, it was thought desirable to divide the results now obtained into two groups, one including those children aged 1 year or less, and the other including children over 1 year old. The younger age-group consists of 12 infants, 6 girls and 6 boys, whose ages range from 2 to 11½ months, 3 of whom were only tested while on normal diets, 8 while on normal diets and also after NH_4Cl had been added, and one only during NH_4Cl administration. In each case, the infants were fed on a reconstituted dried cows' milk, with suitable semi-solid foods, except for one aged 2 months who was on the milk mixture only. The older group consists of 14 children, 5 girls and 9 boys, aged from 1 year 1 month to 16 years: 6 were tested both on normal diets and after NH_4Cl had been added, and 8 only while NH_4Cl was being given.

Collection of Urine. Urine was collected for a period of four to nine hours (average about six hours) during the day, each collection being accurately timed. Older children were asked to empty their bladder directly into chemically clean bottles or pots. The urine was collected from male infants by using Paul's tubing strapped around the penis, and from females by the use of a metabolic bed. The specimens were refrigerated and at the end of the test they were pooled and analysed immediately for pH, titratable acidity (TA), titratable acidity less bicarbonate ($\text{TA} - \text{HCO}_3^-$) and ammonia. In most cases the pH of the individual specimens of each collection was also determined. Fluid intake was increased whenever possible in order to augment the urine flow, and thus minimize the error from incomplete emptying of the bladder. Incomplete collections due to leakage around the tube, or specimens contaminated with faeces, were discarded.

A blood sample for plasma CO_2 content, and in some cases for blood pH, was taken about the middle of the period during which urine was collected and two or three hours after a meal.

TABLE 1
INFANTS AGED 2-11½ MONTHS: INDIVIDUAL RESULTS, MEANS, AND RANGE

No.	Sex	Age (mth.)	Diet	Blood		Urine*							H + Clearance Index
				Plasma CO ₂ (mEq/l.)	Whole Blood pH	pH		μEq/min./1.73 sq. m.				% NH ₄ ⁺ in NH ₄ ⁺ + TA	
						Range	Pooled Speci- mens	NH ₄ ⁺	TA	TA - HCO ₃ ⁻	Total H ⁺		
1	M	2	Normal Normal + NH ₄ Cl	19.0 18.3	7.36 7.35	5.7-6.3 5.0-5.2	5.8 5.0	18.7 26.3	35.3 40.8	— —	54.0 67.1	34.6 39.2	1.02 1.23
2	F	3½	Normal	21.5			5.1	11.7	18.7	—	30.4	38.5	0.65
3	M	3½ 4	Normal Normal + NH ₄ Cl	21.0 18.5		5.4-6.6 5.1-6.4	5.9 5.8	28.0 47.0	23.5 42.0	— —	51.5 89.0	45.4 53.0	1.08 1.65
4	F	4	Normal Normal + NH ₄ Cl	20.7 15.5	7.36	6.4-7.3 5.3-6.4	6.7 5.8	7.3 62.0	3.6 20.6	1.1 —	8.4 82.6	67.0 75.0	0.16 1.28
5	M	4 7	Normal Normal + NH ₄ Cl	19.5 20.0	7.45	5.6-6.4 4.7-5.3	5.9 4.9	34.5 100.0	33.7 72.0	— —	68.2 172.0	50.5 58.1	1.33 3.44
6	F	4½	Normal	19.0		5.2-5.5	5.3	17.9	22.1	—	40.0	44.8	0.76
7	M	6	Normal Normal + NH ₄ Cl	20.5 17.4	7.39 7.40	6.5-7.2 4.9-5.0	6.8 4.9	10.9 31.3	14.1 45.0	2.3 —	13.2 76.3	43.6 41.0	0.27 1.33
8	M	8	Normal	20.0	7.41	5.7-6.6	6.0	19.7	23.4	—	43.1	45.7	0.86
9	F	8	Normal Normal + NH ₄ Cl	19.0 11.0	7.44 7.46	5.5-6.8 4.8-5.0	6.1 4.9	14.3 70.0	29.5 42.8	— —	43.8 112.8	32.7 62.0	0.83 1.24
10	F	8½	Normal Normal + NH ₄ Cl	19.3 11.0	7.39 7.30	5.4-5.9 4.6-5.0	5.6 4.8	31.5 59.4	41.7 49.6	— —	73.2 109.0	43.0 54.4	1.41 1.20
11	F	9 9½	Normal Normal + NH ₄ Cl	19.5 18.0		5.0-5.2	5.3 5.1	30.3 44.0	35.2 25.5	— —	65.5 69.5	46.3 63.5	1.28 1.25
12	M	11½	Normal + NH ₄ Cl	20.0		5.2-5.3	5.2	50.0	44.5	—	94.5	53.0	1.89
Mean ..	..	..	Normal	19.9				20.4	25.5	—	44.7	46.3	0.88
Range ..	..	..	Normal	19.0- 21.5			5.1- 6.8	7.3- 34.5	3.6- 41.7	—	8.4- 73.2	32.7- 67.0	0.16- 1.41
Mean ..	..	..	Normal + NH ₄ Cl	16.6				54.4	42.5	—	97.0	55.8	1.61
Range ..	..	..	Normal + NH ₄ Cl	11.0- 20.0			4.8- 5.8	26.3- 100.0	20.6- 72.0	—	67.1- 172.0	39.2- 75.0	1.20- 3.44

TA = Titratable Acidity

* In all the Tables urinary excretions are expressed in μEq/min./1.73 sq. m. and not in mEq, as in text, because of the small amounts involved.

Hydrogen Ion Loading. The renal response to an added load of hydrogen ions was tested by giving NH₄Cl, usually for three days or in some cases for four or five days. Since the results in those few who had NH₄Cl for the longer period did not differ significantly from the mean values of the majority who had been given it for three days, all the results have been analysed together. The dose of NH₄Cl was from 6.0 to 9.0 g. (115-166 mEq)/day/1.73 sq. m., the actual dose being 1.0 to 7.5 g. day according to surface area. Each subject had the same amount daily, divided in three to four doses given with or after meals, to the younger children as a mixture in syrup, and to the older in uncoated capsules. No side-effects were observed apart from slight vomiting in three subjects on the second day of administration, but this was never severe enough to necessitate withdrawal of the NH₄Cl. A few of the older children were given the salt at home, but all came into hospital for the last day of the test. The urine was collected during the last day of NH₄Cl administration as described above, blood being taken two to three hours after a meal, when the subject had already taken the first of the three or four daily doses of NH₄Cl.

The surface area was calculated from the height and weight using the Du Bois and Du Bois (1916) nomograms.

Laboratory Methods.

PLASMA CO₂ CONTENT. Approximately 1 ml. capillary blood, obtained by finger- or heel-prick, was collected dropwise in heparinized 2 × ¾ in. test-tubes without paraffin oil. The plasma was then separated by centrifuging and kept for analysis within one to four hours. The CO₂ content was measured manometrically (Van Slyke and Neill, 1924).

BLOOD pH. This was determined on capillary blood collected in heparinized glass capillary tubes, both ends of which were sealed immediately after collection and examined within an hour after sampling. The pH was determined at 37° C. using a microcapillary glass electrode (Metrohm) and a pH meter with a Vibron electrometer (Electronic Instruments Ltd.).

URINE pH. This was determined at room temperature by external indicators using B.D.H. capillators.

AMMONIA. This was estimated by aeration and titration (Hawk, Oser, and Summerson, 1954).

The titratable acidity of the urine was determined by titration to pH 7.4 with N/20 NaOH. When the pH was greater than 6.2, titratable acidity minus bicarbonate (TA - HCO₃⁻) was measured (Albright and Reifstein, 1948).

CALCULATION.

The Hydrogen Ion Clearance Index = H⁺ excretion (mEq/min./1.73 sq. m.) × plasma CO₂ content (mEq/l.). Where H⁺ excretion = TA + NH₄⁺ - HCO₃⁻ each being expressed in mEq/min./1.73 sq. m.

Results

The individual findings for the younger age-group are shown in Table 1 and for the older in Table 2.

A comparison of the results in the two groups shows that both on normal diet and after NH₄Cl has been added, the plasma CO₂ was about 2 mEq/l. lower in the infants under 1 year. Urine pH was approximately the same in the two groups, as was the output of NH₄⁺, though after NH₄Cl, this was a little higher in the older group. However, the urine

of infants showed a higher titratable acidity than the children, both before and during NH₄Cl administration and therefore a smaller proportion of H⁺ was excreted as NH₄⁺.

It is interesting to note that under normal dietary conditions the total H⁺ excreted by the older age-group was about one-third less than the amount excreted by the infants. This may be explained by the fact that the diet of children contains less protein per kg. body weight than that of young infants who are being fed mainly on cows' milk, and the relatively large amount of phosphate in cows' milk also contributes to the excess of TA in the urine. After the acid load, the total H⁺ in the urine of both groups was the same but the proportion of TA remained higher in infants, the children compensating by an increase in ammonia production.

There is no marked difference between the two age-groups in the mean values of the H⁺ clearance index after NH₄Cl administration, though by exclusion of one exceptionally high index in the infant group a

TABLE 2
CHILDREN AGED 1 YEAR AND 1 MONTH TO 16 YEARS: INDIVIDUAL RESULTS, MEANS, AND RANGE

No.	Sex	Age (yr.)	Diet	Blood		Urine							H + Clearance Index
				Plasma CO ₂ (mEq/l.)	Whole Blood pH	pH		μEq/min./1.73 sq. m.				% NH ₄ ⁺ in NH ₄ ⁺ + TA	
						Range	Pooled Speci- mens	NH ₄ ⁺	TA	TA - HCO ₃ ⁻	Total H ⁺		
1	F	1 1/12	Normal Normal + NH ₄ Cl	19.2 14.0	7.42 7.37	4.8-5.8	5.7 5.3	23.4 111.0	24.2 53.8	— —	47.6 164.8	49.3 67.5	0.92 2.30
2	F	1 4/12	Normal + NH ₄ Cl	16.0	7.42	5.4-5.7	5.6	59.4	26.5	—	85.9	69.2	1.38
3	F	1 10/12	Normal Normal + NH ₄ Cl	22.0 14.9	7.36 7.31	6.0-6.3 4.9-5.2	6.2 5.0	26.8 87.0	12.0 44.2	— —	38.8 131.2	69.0 66.3	0.85 1.96
4	M	2 6/12	Normal + NH ₄ Cl	21.0		5.1-5.2	5.1	50.0	25.5	—	75.5	66.2	1.58
5	M	3 4/12	Normal + NH ₄ Cl	16.8		5.2-5.2	5.2	53.0	30.0	—	83.0	64.0	1.40
6	M	4	Normal + NH ₄ Cl	18.5			4.8	65.0	33.3	—	98.3	66.0	1.82
7	M	4	Normal + NH ₄ Cl	17.0		4.9-5.1	5.0	71.8	26.0	—	97.8	73.4	1.66
8	F	4 8/12	Normal + NH ₄ Cl	18.5		5.0-5.7	5.3	70.0	30.0	—	100.0	70.0	1.85
9	M	5	Normal + NH ₄ Cl	20.0		5.1-5.2	5.1	45.7	27.0	—	72.7	63.0	1.45
10	F	5 3/12	Normal Normal + NH ₄ Cl	25.8 21.3			7.2 4.7	18.2 48.2	2.0 13.9	-7.9 —	10.3 62.1	90.0 77.6	0.26 1.32
11	M	5 9/12	Normal Normal + NH ₄ Cl	24.8 21.4	7.40 7.37	5.2-5.4	5.3 4.8	11.2 65.0	18.9 42.7	— —	30.1 107.7	37.2 60.4	0.74 2.3
12	M	8 3/12	Normal + NH ₄ Cl	17.5		4.6-5.6	4.8	47.6	24.0	—	71.6	66.5	1.25
13	M	14	Normal Normal + NH ₄ Cl	20.0 18.0	7.51 7.45	5.4-6.4 4.7-5.1	6.0 4.9	28.6 62.0	10.5 31.0	— —	39.1 93.0	73.2 66.7	0.78 1.68
14	M	16	Normal Normal + NH ₄ Cl	22.8 19.8	7.47 7.40	6.6-6.8 5.2-5.7	6.7 5.5	17.3 73.0	3.5 15.2	-8.3 —	9.0 88.2	83.1 83.0	0.20 1.74
Mean ..	..	..	Normal	22.4				20.9	11.9	—	29.2	64.0	0.63
Range ..	..	..	Normal	19.2-25.8			5.3-7.2	11.2-28.6	2.0-24.2	—	9.0-47.6	37.0-90.0	0.20-0.92
Mean ..	..	..	Normal + NH ₄ Cl	18.2				64.5	30.2	—	95.1	68.4	1.69
Range ..	..	..	Normal + NH ₄ Cl	14.0-21.4			4.7-5.6	45.7-111.0	13.9-53.8	—	62.1-164.8	61.0-83.0	1.25-2.30

TA = Titratable acidity

FIG. 1.—The relation of plasma CO_2 content to the rate of total hydrogen ion excretion in urine (infants and children). The interrupted line shows the limit of normal values.

FIG. 2.—The relation of plasma CO_2 content to urine pH (infants and children). The interrupted line shows the limit of normal values. Symbols as in Fig. 1.

FIG. 3.—The relation of ammonium ion excretion to the pH of urine (infants and children). The bold line is the mean and the interrupted lines represent the 95% confidence limits for adults (Wrong, 1961). Symbols as in Fig. 1.

lower mean index would have been obtained. This is due to lower plasma CO_2 values rather than lower total H^+ excreted in the urine.

The index in subjects receiving a free diet varied widely. It can be very low or even negative if the diet is alkaline, when the subject is excreting more HCO_3^- than NH_4^+ and TA together. In these cases the renal ability to excrete H^+ can be assessed by plotting the plasma CO_2 against total H^+ excreted in the urine. In Fig. 1 the results for both infants and children on normal diets and after NH_4Cl administration have been plotted in this way. The interrupted line joining the lower points of the graph indicates the lower limit of the values for the infants: the lower normal limit for children is slightly higher than the one for infants. Infants or children whose values fall below this line may be considered abnormal as far as renal ability to excrete H^+ is concerned. Fig. 2 similarly shows the relation between plasma CO_2 and urine pH . Fig. 3 shows the NH_4^+ output in relation to urinary pH and includes also the normal limits and the mean for adults given by Wrong (1961). Under normal dietary conditions the values for all our control subjects except two—one infant and one child—fell within the normal limits for adults, while after stressing with NH_4Cl , several of them exceeded the upper limits of normal for adults.

The percentage of H^+ excreted as NH_4^+ is a useful index of ammonia production. Our lowest figure for this percentage is 32.7 in infants and 37 in children. McCance, Matheson, Gresham, and Elkinton (1960) consider that figures below 30% indicate impaired ammonia production. Two of the present subjects whose ratio of ammonia to urine pH was below the normal limits for adults (as shown in Fig. 3) also had a comparatively low percentage of H^+ excreted as NH_4^+ , but this increased in one of them, who was tested after NH_4Cl , from 37.2 to 60.4%.

Discussion

Though the tests were arranged so that the results could be compared with those of Elkinton *et al.* (1960) for adults, there were difficulties in doing this, and the findings are not exactly comparable. Difficulties in obtaining accurate 24-hour specimens were avoided by using short periods of urine collections that did not require admission to hospital overnight. A further advantage in this procedure is that the urine could be analysed without delay, thus minimizing the risks of changes due to bacterial activity. Rates of daily H^+ excretion calculated from a short period of the day are probably not representative of the average rates for the whole 24 hours,

measured by Elkinton *et al.* (1960), since urine passed at night tends to be more acid than that during the day. Furthermore, both the meals and the divided doses of NH_4Cl administered during the collection period may have resulted in rapid and brief changes in the blood chemistry and the urine acidity, but blood for analysis was always taken at the same time after meals to offset this possibility.

The normal capillary plasma CO_2 content in this study ranged from 19.25.8 mEq/l., which is lower than the range given by Singer and Hastings (1948) for arterial plasma in adults. The dropwise collection of blood, and the omission of paraffin oil in the collecting tube may have contributed to these lower values. In this laboratory, blood is no longer collected under oil because of the practical difficulties, one of which is always ensuring adequate mixing of drops of capillary blood under oil. Gambino (1961) has drawn attention to the fact that mineral oil may result in big losses of CO_2 due to its high solubility in oil. The routine method in this laboratory is to equilibrate the specimen with alveolar air only if it is not analysed within three to four hours after its collection, but this hardly ever needed to be done in the present investigation.

For comparison, the findings from our infant group have been tabulated together with those from infants aged 2-10 months recorded in the literature (Table 3). Unfortunately as the technique and methods of investigation among the different authors have differed, the results may not be entirely comparable. Thus, Gordon, McNamara, and Benjamin (1948) and McCance *et al.* (1960) analysed 24-hour urine specimens, while Fomon, Harris, and Jensen (1959) used 12-hour night specimens of urine. The latter authors analysed venous blood and this gave higher values of CO_2 content in their series than in ours. The H^+ output of our infants is higher than in the breast-fed infants reported by Fomon *et al.* and in the artificially fed infants by McCance *et al.*, but it is much lower than that of the infants fed on cows' milk investigated by Gordon *et al.* (1948) and Fomon *et al.* (1959). Low H^+ excretion is to be expected in breast-fed infants compared with those who are artificially fed, but it is difficult to explain the large differences observed among infants fed artificially (Table 3), unless their diets were quite different, especially with regard to protein consumption.

The output of H^+ by our normal infants (66 mEq/24 hr./1.73 sq. m.) under normal dietary conditions is approximately the same as by two infants, aged 8 months and 1 year, studied by Widdowson and McCance (1958) (if their output is calculated per 1.73 sq. m. surface area, assuming each was of

TABLE 3
EXCRETION OF HYDROGEN IONS IN NORMAL INFANTS ON NORMAL DIET AND AFTER
AMMONIUM CHLORIDE: PRESENT SERIES AND SOME PREVIOUSLY PUBLISHED FIGURES

Author	Group (No.)	Age (mth.)	Diet		Plasma CO ₂ Content (mEq/l.)	Urine		% of NH ₄ ⁺ in Total H ⁺ Excretion	H ⁺ Clearance Index
						μEq/min./1.73sq. m.			
						NH ₄ ⁺	TA		
Gordon <i>et al.</i> (1948)	Infants (3)	2-4	Cows' milk	Venous	26.2	31.5	52.7	37.0	2.19
Fomon <i>et al.</i> (1959)	Infants (4)	2-5½	Cows' milk + NH ₄ Cl	Venous	20.0	80.3	56.0	59.0	2.71
Fomon <i>et al.</i> (1959)	Infants (4)	3½-4½	Cows' milk + NH ₄ Cl	Venous	23.3	63.5	77.0	45.2	3.24
McCance <i>et al.</i> (1960)	Infants*	10	Human milk	Venous	17.5	118.0	82.0	59.0	3.60
			Human milk + NH ₄ Cl	Venous	25.6	15.8	6.2	70.8	<0.56
			Normal diet	Venous	15.5	48.9	13.6	79.8	0.97
			Human diet + NH ₄ Cl			+17.7	+14.4	55.0	
			Artificially fed			+37.2	+21.0	64.0	
Present Series	Infants (11)	2-11½	Artificially fed + NH ₄ Cl	Capillary	19.9	20.4	25.5	46.3	0.88
	(9)			Capillary	16.6	54.4	42.5	55.8	1.61

* The number of patients included is not stated. The series includes some infants studied by the authors, and others taken from published reports
† Calculated from authors' data.

TABLE 4
EFFECT OF NH₄CL ADMINISTRATION ON RENAL EXCRETION OF HYDROGEN IONS: NORMAL INFANTS AND CHILDREN (PRESENT SERIES) AND PREVIOUSLY PUBLISHED VALUES IN ADULTS

Author	Group (No.)	Age		Mean Plasma CO ₂ (mEq/l.)	Mean Total H ⁺ Excretion in Urine (μEq/min./1.73 sq. m.)	H ⁺ Clearance Index	
						Mean	Range
Present series	Infants (9)	2-11½ mth.	Capillary	16.6	97.0	1.61	1.20-3.44
Present series	Children (14)	13 mth.-16 yr.	Capillary	18.2	95.1	1.69	1.25-2.30
Elkinton <i>et al.</i> (1960)	Adults (17)		Venous (serum)	20.7	100.0	2.07	1.40-3.40

normal body weight and height for age) and by adults reported by Elkinton *et al.* (1960). All are much higher than those for infants on the first, second, and eighth day of life (Widdowson and McCance, 1958).

The response to NH₄Cl induced acidosis by both infants and children in our series (Table 4) is practically the same as that in adults (Elkinton *et al.*, 1960), though the clearance indices of adults are slightly higher owing to higher serum CO₂ levels. The present observations indicate that the kidneys of the children examined were able to cope as efficiently with the stress of H⁺ added to normal feeding as those of adults. Hatemi and McCance (1961), investigating the response of newborn infants to a single dose of NH₄Cl, found that H⁺ excretion was much less than in adults after a similar stress, the mean clearance indices in newborns being only 21% of their mean adult values. Our results now show that in the infant the specific renal function of acidifying the urine under stress has, presumably by a process of maturation, achieved adult level by about 3 months of age. Though the number of the younger infants examined in this series is not large enough to

suggest the earliest age at which this maturation occurs in any individual, the results can be used as a basis for the assessment of acidosis of renal origin with increasing confidence over the age of 3 months.

Summary

The renal ability to excrete hydrogen ions was assessed in 12 normal infants aged 2-11½ months and 14 children aged 1 year 1 month to 16 years on ordinary diets, and in most cases on the third day of continuous ammonium chloride administration. Titratable acidity and ammonium ion excretion in the urine and the carbon dioxide content in the plasma were determined within the same period, and from these values the hydrogen ion clearance index has been calculated for each child. The mean and range for the index in both age-groups, as well as the total hydrogen ions excreted/1.73 sq. m. surface area, were found to have reached adult levels.

It is considered that the values for these normal children are suitable to use as a standard that can be applied in assessing whether acidosis is, or is not, primarily of renal origin in children from the age of about 3 months.

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