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Effect of NH_4Cl-induced metabolic acidosis on urinary calcium excretion in young infants

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Urinary calcium and net acid excretion as well as the acid-base parameters of the blood were determined before and after a NH_4Cl load applied in a single dose of 2.8 mEq/kg body weight.

In order to assess the effect of maturity and postnatal development, a study was made on *(a)* 47 newborn infants with a birth weight of 1000—4380 g on the 7th day; and *(b)* 11 prematures with a birth weight of 1000—1970 g, weekly for 6 consecutive weeks. The results were compared to those for infants of 3—11 months of age.

(i) At one week of age, NH_4Cl ingestion resulted in a significantly greater increase of metabolic acidosis in infants with a birth weight under 2000 g than in the larger ones. With increasing postnatal age the metabolic acidosis of premature infants increased to about the same extent in response to NH_4Cl load irrespective of the pre-loading level of acidosis or postnatal age.

(ii) Both the urinary NAE and UCaE of one week-old infants increased markedly with increasing birth weight and they were invariably augmented by the acid load.

NH_4Cl-induced NAE and UCaE were significantly higher in infants with a birth weight over 2500 g than in their smaller matches ($p < 0.025$).

(iii) In prematures, in spite of the postnatal development of renal capacity to excrete hydrogen ions, the NH_4Cl-induced UCaE remained unchanged, or expressed in per cents of the pre-loading level even a slight, statistically not significant decrease could be observed during the first six weeks of life.

(iv) It is suggested that the skeletal buffering measured as NH_4Cl-induced urinary calcium loss, may be an important defence mechanism against acidosis already in the early period of life. Low-birth-weight prematures are, however, compromised in face of an acid load by the limited buffer function of the bones.

Skeletal buffering has been shown to be an important defence mechanism against acidosis in the different forms of chronic renal diseases and in subjects given exogenous acid load. The chronic metabolic acidosis results in demineralization of the bones, increased urinary calcium loss and in a negative calcium balance [3, 17, 18, 19, 26].

This well-known relationship between the skeletal system and metabolic acidosis is somewhat complicated during the early weeks of life. The imbalance between metabolic acid production and the limit-

ed renal capacity to excrete hydrogen ions, particularly in low-birth-weight neonates, leads to late metabolic acidosis [13, 14]. Skeletal growth and calcium deposition in the bones liberates hydrogen ions, producing a further acid load on the already disturbed acid-base homeostasis [15]. The higher the retention rate of calcium the more pronounced the acidosis in small premature infants [6].

In the present study an attempt was made to investigate the role of the skeletal system in the control of acidosis, by measuring urinary calcium excretion (UCaE) in NH_4Cl-induced acidosis. The influence of birth weight and postnatal age on the NH_4Cl-induced changes in UCaE was also studied.

Material and Methods

Three groups of male infants were selected for the study.

Group I comprised 47 one-week-old newborn infants who were assigned according to their birth weight into the three categories, *(a)* 1000—1500 g (n: 13, mean: 1312 g); *(b)* 1500—2000 g (n: 22, mean: 1754 g); and *(c)* more than 2500 g (n: 12, mean: 2935 g). The corresponding mean gestational age was 30.6 (range: 28—33), 34.1 (range: 30—41) and 37.9 (range: 36—41) weeks, respectively.

Group II consisted of 11 premature infants with a mean birth weight of 1644 g (range: 1000—1970 g) and a mean gestational age of 32 (range: 29—35) weeks.

In all cases the pregnancy, the delivery and the perinatal course were unenventful. The birth weight of all infants, except for a small for dates pair of twins in group I/b (birth weight: 1850 and 1890 g, gestational age: 41 weeks), fell within the 10th and 90th percentiles according to the local chart [8].

The infants were fed appropriate cow's milk formulas by a round the clock feeding pattern at 2-hourly intervals until they weighed about 1500 g, and 3-hourly thereafter. Food intake was gradually increased in all infants to attain a calorie and fluid intake of 120—140 cal/kg and 180—200 ml/kg, respectively, by the end of the second week of life. 3000 IU vitamin D_3 was given on alternate days after 14 days of age.

Group III included 22 healthy male infants of 6817 g mean body weight (range: 4500—11750 g) and 6.9 months mean postnatal age (range: 3—11 months). Vitamin D_3 was given in a dose of either 3000 IU on every second day or 50 000 IU weekly after 2—4 weeks of age. No clininal, biochemical or radiological features of rickets were observed prior to the study. The babies were fed cow's milk formulas providing about 2.5 g protein and 100—120 cal/kg/day, respectively.

Procedures

Urinary calcium and net acid excretion as well as the acid-base parameters of the blood were determined before and after an NH_4Cl load applied in a single dose of 2.8 mEq/kg body weight. Urine was fractionally collected under toluene for a period of 12 hours. The specimens were refrigerated, pooled and analysed immediately for pH, titratable acidity, ammonia and within a few hours for calcium. The collection started at 10 p.m. for a control period of 12 hours. At 10 a.m. 2.8 mEq/kg NH_4Cl was given by mouth or stomach tube and urine collection continued for another 12 hours.

Arterial blood samples were obtained at the end of the control period and about 4 hours after NH_4Cl ingestion. The NH_4Cl loading test was performed on the 7th day

of life in group I, on the 7th day and later weekly for 6 consecutive weeks in group II and on one occasion in group III.

Analytical methods, calculations

Arterial blood acid-base status was determined by the method of Astrup [1]. Urinary pH was measured at 38°C with a Radiometer pH meter, titratable acidity according to Folin (end point of titration, pH 7.4), urinary ammonia according to McCullough [21] and urinary bicarbonate was calculated from the Gamble nomogram, using the corresponding pH value [9]. Urinary calcium was measured complexometrically [23].

Net acid excretion consisted of the sum of urinary titratable acid plus ammonium ion minus bicarbonate. Statistical analysis was performed by calculating the means and the standard errors. The p values presented were determined by Student's *t* test.

Results

Metabolic acidosis

To characterize the changes in metabolic acidosis, the negative base excess before and after NH_4Cl ingestion is shown in Fig. 1. In one-week-old newborns the base excess initially was similar irrespective of birth weight. All infants responded to the NH_4Cl loading by increasing the negative base excess (Fig. 1/a).

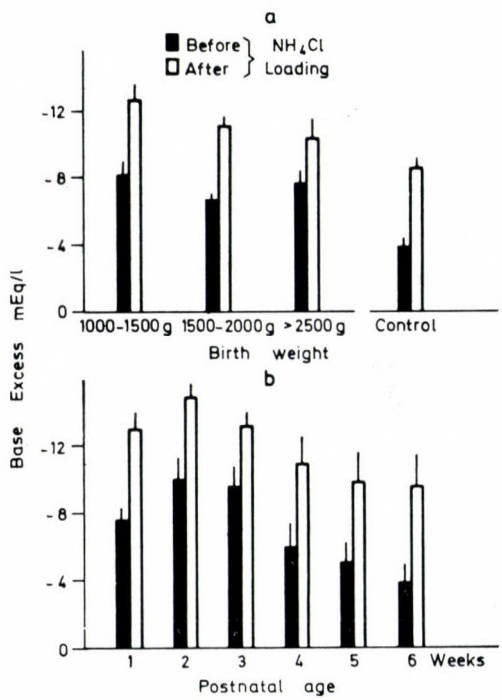

Fig. 1. Base excess in one-week-old newborn infants with different birth weights (a) and a group of prematures during the first six weeks of life (b), before and after 2.8 mEq/kg NH_4Cl administration. Vertical bars represent the standard errors of the means.

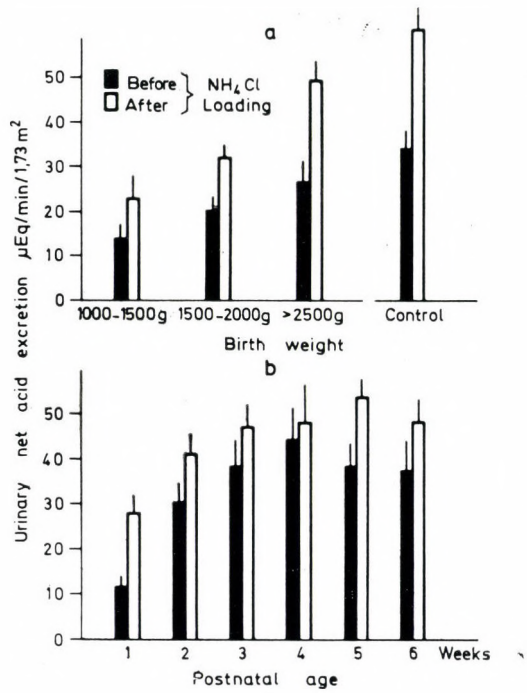

FIG. 2. Urinary NAE of one-week-old newborn infants with different birth weights (a) and a group of prematures during the first six weeks of life (b), before and after 2.8 mEq/kg NH_4Cl administration.

This increase was significantly higher in infants with a birth weight under 2000 g than in the larger ones ($p < 0.05$)

The postnatal development of metabolic acidosis in premature infants (group II) is seen in Fig. 1/b. The metabolic acidosis was the most pronounced in the second and third weeks of life and it fell to about 4 mEq/l by the end of the study. This value agreed well with that found for older infants. The NH_4Cl load resulted in an increase of acidosis of about the same degree irrespective of either the pre-loading base excess, or the postnatal age of the infants.

Urinary net acid excretion (NAE)

Urinary NAE of one-week-old infants markedly increased with increasing body weight and it was invariably augmented by the acid load. The NH_4Cl-induced H^+ excretion (Δ NAE) was significantly higher in infants with a birth weight over 2500 g than in their smaller matches (Fig. 2/a).

With increasing postnatal age the NAE of premature infants was steadily increasing and reached its maximum of about 45 μEq/min/1.73 m^2 at the end of the fourth week. Subsequently it was maintained at that level or even a slight, statistically

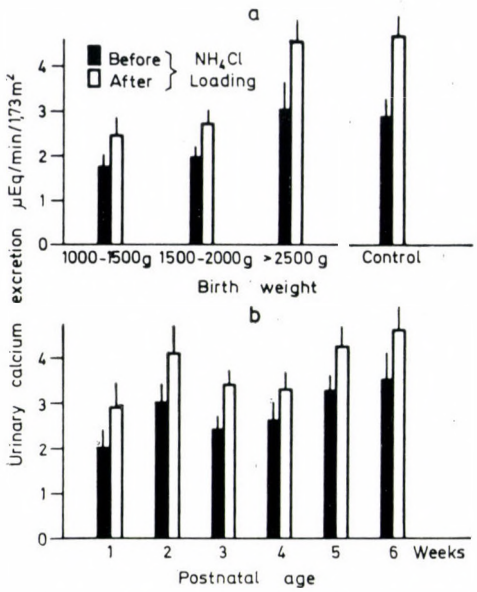

FIG. 3. UCaE of one-week-old newborn infants with different birth weights (a) and a group of prematures during the first six weeks of life (b), before and after 2.8 mEq/kg NH_4Cl administration.

insignificant, decrease could be observed. In response to NH_4Cl administration the NAE significantly rose, but the magnitude of this rise considerably changed with postnatal age. During the first four weeks of life Δ NAE gradually decreased to a value of 4 μEq/min/1.73 m^2 followed by an increase in the 5–6th weeks which was comparable to that found in the first week, but significantly lower than that observed in older infants (Fig. 2/b).

Urinary calcium excretion (UCaE)

As shown in Fig. 3/a, UCaE of the newborn infants markedly increased with increasing birth weight both before and after NH_4Cl loading, while the NH_4Cl administration resulted in a significantly higher rate of UCaE in infants with birth weight over 2500 g ($p < 0.025$).

UCaE of premature infants showed a clear tendency to rise during the study period of six weeks, although this trend did not reach significance and it was interrupted by a higher value found in the second week (Fig. 3/b). NH_4Cl ingestion enhanced the rate of UCaE in each postnatal age and paired t-testing showed the differences to be significant ($p < 0.025$).

Relationship between UCaE and NAE

Figure 4 compares the NH_4Cl-induced increase of urinary NAE and UCaE. It can be seen that in

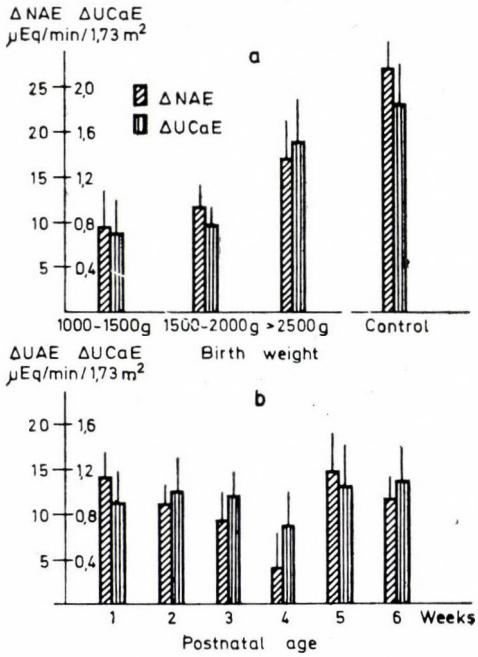

FIG. 4. NH_4Cl-induced urinary NAE and UCaE of one-week-old newborn infants with different birth weights (a) and a group of prematures during the first six weeks of life (b).

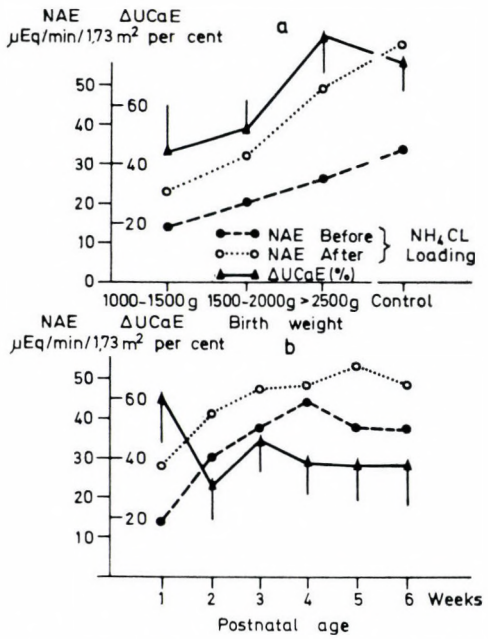

FIG. 5. Urinary NAE before and after NH_4Cl administration and the NH_4Cl-induced UCaE expressed in percentage of the basal value of one-week-old newborn infants (a) and a group of prematures during the first six weeks of life (b).

the first week of life both increased at a similar rate with increasing body weight (Fig. 4/a). The prematures of group II responded to NH_4Cl loading with about the same increase of UCaE irrespective of postnatal age, and their NAE gradually fell until the fourth week, followed by an increase in the further periods of study (Fig. 4/b).

In older infants both responses were parallel and significantly higher than that found for the premature infants ($p < 0.01$).

Urinary NAE both before and after NH_4Cl ingestion and the NH_4Cl induced increase of UCaE expressed as a percentage of pre-loading value are shown in Figure 5. It can be seen that the newborn infants with birth weights above 2500 g were able not only to excrete more hydrogen ions but also to increase the UCaE similar as the older infants (Fig. 5/a). In prematures, the percentage increase of UCaE in response to NH_4Cl load was most pronounced in the first week (60.8%), and with the increasing renal capacity to excrete hydrogen ions it decreased to 36% by the 5–6th postnatal week. This general declining trend was interrupted by a deep fall in the second week of life (Fig. 5/b).

Discussion

A complex relationship has been demonstrated between metabolic acidosis and calcium metabolism. Acute metabolic acidosis raises serum calcium concentration by directly releasing calcium from the bones and also by enhancing the PTH effect to mobilize calcium from the bones. In the kidney, acidosis directly inhibits tubular calcium reabsorption, but at the same time enhances the effect of PTH to increase it. These two opposite effects of acidosis on renal calcium handling result in an increased urinary calcium loss [4].

In addition to metabolic acidosis, the UCaE influenced by other factors, even in healthy subjects, such as dietary calcium intake [16], calcium-phosphorus intake ratio [20, 32], fat composition of the diet [2, 11], skeletal size [16], hormones [25], vitamin D supply [24] and the maturity of the kidney [30].

In order to assess the effect of metabolic acidosis on UCaE, it was necessary to standardize the experimental conditions (similar nursing conditions, standard feeding pattern and vitamin D supply) and to keep constant the factors other than acidosis. This latter criterion was fulfilled by applying an NH_4Cl load at the end of the control period of 12 hours, followed by a test period of the same duration. In this way each subject served as his own control and the changes in UCaE were thought to be due solely to the NH_4Cl-induced metabolic acidosis.

On the basis of the well-established relationship between metabolic acidosis and UCaE [3, 17, 18, 19, 26], the acidosis-induced urinary calcium loss may be regarded as a reliable measurement of skeletel buffering.

It must, however, be taken into account that in response to acidosis calcium is released not only from the bones, but from the exchangeable calcium pool as a whole [27]. On the other hand, the bone also contains a very large quantity of sodium and in metabolic acidosis sodium is also released into the extracellular fluid in exchange for hydrogen ions [5].

The present results seem to suggest that in newborn infants the buffering function of the skeleton is increasing with increasing birth weight. This finding is consistent with earlier observations demonstrating that in the human fetus calcium accumulates at an increasing rate as pregnancy advances, particularly after the 28th week [28, 29].

In prematures, the postnatal calcium retention rate was found to be lower than that *in utero*, resulting in a cumulative calcium deficit which manifests with a marked bone demineralization at the age of 6–7 weeks [6, 7, 10, 22, 29]. This cumulative calcium deficit may be responsible for our findings showing that the acidosis induced UCaE in premature infants remains unchanged or, expressed in percents of the preloading level, even a slight, statistically not significant decline may be seen as the infants grow older.

The higher rate of UCaE in response to NH_4Cl ingestion in infants of higher birth weight might be due to the more advanced maturation of renal handling of calcium. The results obtained in prematures, where the acidosis induced UCaE remains unchanged in spite of the postnatal development of renal capacity to excrete calcium, provide, however, suggestive evidence that in NH_4Cl acidosis the increase of urinary calcium loss does not depend on renal maturity and it is mainly influenced by skeletal size and mineralization.

Most of the present data on the acid-base parameters of the blood and urinary NAE have been published previously [12, 31]. In this study the UCaE was related to them in an attempt to assess the relative importance of skeletal buffering in the control of acidosis.

Assuming a bone buffer equivalent quoted by Kildeberg of 20 mEq H^+/OH^- for each 1000 mg Ca^{++} laid down or reabsorbed from the skeleton [14], the observed changes in calcium excretion may be regarded to reflect an unimportant skeletal buffering relative to the urinary NAE.

In chronic NH_4Cl-induced acidosis, however, the quantity of retained acids was matched on an equivalent [17], or — as a result of carbonate-phosphate interchange within the apatite crystal — even less than equivalent basis, by negative calcium balance [18]. These data seem to suggest a higher bone buffer equivalent, consequently a more efficient skeletal buffering.

The present study does not provide sufficient quantitative information as to the role of the skeleton in the control of acidosis, therefore simultaneous hidrogen ion and calcium balance studies are needed to

describe quantitatively the relationship between metabolic acidosis and the skeletal system. Still, our findings can be interpreted as indicating that skeletal buffering may be an important defence mechanisms, against acidosis even in the early period of life, when the growth and mineralization of the skeleton has mainly been regarded as an additional factor to increase the endogenous acid production [6, 14, 15].

It is also suggested that low-birth-weight neonates are handicapped in maintaining the normal acid-base status not only by the limited renal capacity to excrete H^+, but also by the limited buffer function of the bones.

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