NEPHROCALCINOSIS IN INFANTS
Incidence, risk factors, natural course and renal outcome in certain risk groups

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Abstract

The aim of the present work was to elucidate the incidence, associated risk factors and natural course of nephrocalcinosis (NC) in very low birth weight (VLBW) infants, and to evaluate renal function in affected infants during early childhood. The occurrence and course of NC in full-term infants receiving furosemide and in infants with congenital lactase deficiency were also studied.

A total of 129 VLBW infants were screened for NC by renal ultrasonography (US) at 2 and 6 weeks and 3 months, and ultrasonic follow-up was performed on the infants with NC at 6, 12, 18 and 24 months, and thereafter annually up to age 5-6 years or until ultrasonic resolution. NC was classified according to its pyramidal localisation and extent. Twenty VLBW children with neonatal NC and 20 control pairs without the condition were examined for renal function at 4.7 (SD 1.1) vs. 4.6 (0.9) years of age. Thirty-six full-term infants who had received furosemide treatment for congestive heart failure for at least 4 weeks and 36 control infants without any diuretic therapy were examined by renal US and by means of a random urine sample taken at a median age of 2.9 vs. 3.4 months. The case records of the 11 infants with congenital lactase deficiency were analysed for NC, and these children were re-evaluated at 2 to 10 years of age.

NC was detected in 26 out of the 129 VLBW infants (20%). The infants with NC were sicker and smaller than the unaffected ones and had more often received furosemide, dexamethasone and theophylline treatment. NC was peripheral in 14 cases (54%), scattered in 7 (27%) and extensive in 5 (19%). All the cases of peripheral NC showed resolution at 12 months, but abnormal renal findings were seen in 3 out of the 7 with scattered NC and 3 out of the 4 surviving children with extensive NC at 24 months, in 2 of whom the condition persisted at age 5-6 years. The children with neonatal NC showed increased urinary calcium and β2-microglobulin excretion as compared with the controls in early childhood, but there was no significant difference in distal tubular acidification capacity, nor in estimated creatinine clearance.

Five out of the 36 full-term infants receiving long-term furosemide had NC, but none of the controls. The daily dose of furosemide and the urinary calcium concentration were both higher in the infants with NC. Abnormal renal findings were still visible in two of the cases at 24 months of age. Hypercalcaemia was found in 7 out of 10 infants with congenital lactase deficiency tested at the time of diagnosis, and NC was seen in 5 of the 7 cases examined by renal ultrasonography. No constant dysfunction in calcium homeostasis was seen at re-evaluation, but nephrocalcinotic changes were observable in 3 out of the 11 children.

NC may complicate not only the course of VLBW infants, but also that of full-term infants with calciuric medication and diseases that involve hypercalcaemia. Some renal tubular dysfunction may result from NC in former preterm infants, but overall kidney function seems not to be seriously compromised in early childhood.

Keywords: calcium, furosemide, kidney function, ultrasonography
To my family
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[Signature]
Abbreviations

BW  birth weight
CI  confidence interval
CLCN5  renal-specific chloride channel
CT  computed tomography
FEK  fractional excretion of potassium
FENA  fractional excretion of sodium
GFR  glomerular filtration rate
IH  idiopathic hypercalciuria
NC  nephrocalcinosis
PTH  parathyroid hormone
TmP/GFR  maximal tubular reabsorption of phosphate per glomerular filtration rate
TPN  total parenteral nutrition
TRP  tubular reabsorption of phosphate
U-B PCO₂  urine minus blood carbon dioxide tension
VLBW  very low birth weight (=birth weight <1500 grams)
XLH  X-linked hypophosphataemic rickets
XLN  X-linked nephrolithiasis
List of original papers

This thesis is based on the following articles, which are referred to in the text by their Roman numerals I-IV:


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1. Introduction

Nephrocalcinosis (NC), i.e. microscopic calcium compounds precipitated in the renal tubules and parenchyma, could previously be detected only in its most severe forms by abdominal plain X-ray films (1-4), but the advent of more sensitive ultrasonographic methods in the early 1980's (5, 6) now enables it to be detected earlier and allows the factors and conditions associated with it to be investigated more thoroughly.

NC and nephrolithiasis were first described in a series of premature infants by Hufnagle et al. in 1982 (7). The renal calcifications were primarily associated solely with treatment involving furosemide, which has a considerable calciuric effect in addition to its diuretic properties. Although increased urinary calcium excretion is a confirmed risk factor, a multifactorial concept for the development of NC in preterm infants has gained increasing support (8, 9). The determination of a sole risk factor for NC is quite difficult in preterm infants, as they are often objects of neonatal intensive care. The changes of immature organs adapting to a new postnatal environment are mixed with metabolic and respiratory shifts related to various treatments such as fluids, electrolytes, nutrition, often numerous medications, and ventilatory support.

Although preterm infants are numerically the most significant group that suffer from NC, renal calcifications are found to associate with several other (and increasing number of new) diseases and conditions. A variety of abnormalities in mineral metabolism and urine composition due to certain diseases or treatments can result in NC, while in some cases the aetiology may remain obscure. Data on later kidney function in subjects with NC have previously showed variations from normality or slight tubular dysfunction to renal insufficiency, but the follow-up times have been limited especially in newly detected conditions.

This study was performed to investigate the incidence, risk factors, natural history and subsequent renal outcome related to NC in preterm infants, and to investigate the incidence and natural course of NC in full-term infants receiving long-term furosemide medication for congestive heart failure and the occurrence and causes of NC in infants with congenital lactase deficiency, an entity which belongs especially to Finnish disease heritage.
2. Review of the literature

2.1. Terminology

The two types of renal calcification located in the urinary tract are nephrocalcinosis and urolithiasis. Where urolithiasis is macroscopic calcifications (stones) in the urinary collecting system, nephrocalcinosis (NC) refers to accumulations of microscopic calcifications in the tubules, tubular epithelium or interstitial tissue of the kidney (10). In addition, the term nephrolithiasis (renal calculus) is used to describe stones within the renal pelvis with or without extension into the collection ducts or the upper ureter. In other words, where urolithiasis refers to one or more stones in the urinary tract, NC refers to a “starry sky” meaning many small stones (8).

2.2. Clinical, radiological and histological features of nephrocalcinosis

2.2.1. Clinical manifestations

NC and nephrolithiasis comprise two entities reflecting undesired precipitation of calcium compounds in the urinary system. In contrast with nephrolithiasis, which may manifest itself as severe abdominal pain cramps and gross haematuria, leading to prompt diagnostic examinations (11), NC is usually symptomless and is often diagnosed in connection of a screening ultrasonography performed on a known risk group, or even unexpectedly, without any prior suspicion of the condition (12). Although NC is usually clinically silent, it may sometimes be associated with haematuria and sepsis secondary to urinary tract infection (7, 13). Renal failure has been described as a presenting sign for NC, but it is rare in practise (1, 14-16).
2.2.2. Radiological imaging

The renal medullary pyramids are the sites typically involved in infants and children with NC, whereas the renal cortex is a relatively uncommon location for calcium precipitation (17). Until the use of renal ultrasonography, only the most severe cases of NC, those that were radiopaque enough to be seen in plain radiographs, were diagnosed (1-3). At ultrasonography, the change from the normal hypoechoic appearance of the renal pyramids (relative to the renal cortex) to distinctly increased echogenicity is typically a striking characteristic of medullary NC (18, 19). When relevant, computed tomography (CT) may be used to ensure that a hyperechogenicity actually reflects the presence of calcium compounds and is not caused by other factors (20-22). Many of the reports concerning NC in infants and children refer solely to abnormal hyperechogenicity of the renal pyramids, while some have adopted the strict criteria of Myracle et al. (23) that require the hyperechoic foci either to produce acoustic shadowing or to be apparently at least 3 mm in diameter in different planes. Unfortunately, most cases of renal calcifications in the series described by those authors represented nephrolithiasis and there was only one case of NC (23).

NC located in the cortical part of the kidney is rare in infants. A rim of cortical calcification is a typical manifestation of cortical NC (24). This type has been found in infants with primary hyperoxaluria, for example, and has also been related to acute renal cortical necrosis following severe renal ischaemia developing some weeks after the acute insult (3, 24, 25).

Differential diagnosis. Increased medullary echogenicity may be related to conditions other than NC. Transient medullary hyperechogenicity related to renal dysfunction in neonates was described by Avni et al. (26), and a similar increase in medullary echogenicity has since been connected with normal kidney function (27-29). The phenomenon is considered physiological and is more common in term than preterm neonates (29). The radiological changes last only a few days in most cases (27). It has been proposed that transient medullary echogenicity may represent brief tubular blockade caused by Tamm-Horsfall protein precipitation within the renal tubules (26, 28), but this has not been confirmed by others (27).

Other causes for non-nephrocalcinotic pyramidal changes include conditions such as abnormal architecture of the renal pyramids (medullary sponge kidney, autosomal recessive polycystic kidney disease, medullary fibrosis and sickle cell haemoglobinopathies), vascular congestion, certain infections (sepsis, candidosis and cytomegalovirus infection) and some other rare conditions (19, 21, 30-32).

Patterns of NC. On the grounds of a series of 24 children with various conditions that had led to renal calcification, Patriquin and Robitaille proposed patterns of renal medullary calcium deposition that they felt provided clinical evidence for the Anderson-Carr-Randall theory of kidney stone formation (33), which suggests that in the presence of abnormally high calcium excretion, microaggregates of calcium present in the normal kidney increase in size and rupture in the calyces to form calculi (34-36). The patterns of renal medullary calcium deposition recognised by Patriquin and Robitaille were as follows: 1. a faint hyperechogenic rim around the sides and tip of the pyramid, 2. a more intense echogenic rim with echoes faintly filling the entire pyramid, 3. intense echoes
throughout the pyramid, and 4. a solitary focus of echoes at the tip of the pyramid near the fornix. Several classifications which bear similarities to the patterns described by Patriquin and Robitaille and either grade the severity of NC or describe different patterns of it have since been employed in connection with X-linked hypophosphataemic rickets (XLH) and hypoparathyroid children treated with vitamin D preparations and/or phosphates. The association between the treatment measures of XLH and the grade of NC was found in the majority of the investigations (37-39), but not all (40). The ultrasonic patterns observed in children with various conditions associated with NC (30) have been similar to those described by Patriquin and Robitaille (33), but the concept of NC progressing through different stages has not been established in these children, nor have experiences with preterm infants been encouraging, either (41). Pope et al. used another type of classification, based on the number of renal pyramids affected, but this failed to predict later ultrasonic resolution of NC in preterm infants (42).

A recent study has indicated that the severity of NC can be interpreted reliably from an ultrasonographic grading scale (43), the intra-observer and inter-observer agreements being good in assessment of both the grade of NC in random sonographic films and the change of its grade in a longitudinal set-up (43).

### 2.2.3. Histological features

In the original report produced by Hufnagle et al. in 1982 (7) considering preterm infants, all four kidney stones studied were composed of calcium oxalate and phosphate, and autopsies performed on four infants showed calcium deposits mainly interstitially in the renal medullary region with mild focal calcifications in the subcapsular cortex in one case (7). The sites of calcium deposits in histological specimens have varied between reports on NC in preterm and term infants, but intratubular location is mentioned in most of the reports (41, 44-46). In some cases with NC, histological examination has revealed considerable tubular loss and interstitial and periglomerular fibrosis in addition to intratubular calcium debris (6, 45, 47).

### 2.3. Pathophysiological aspects of renal calcification

In many cases of urolithiasis an additional renal ultrasonographic examination has revealed the coexistence of NC (9), and it seems reasonable to assume that NC and nephrolithiasis share basic pathophysiologic mechanisms. It is unclear, however, why some patients end up with one form of renal calcification and others with the other, or occasionally both (9).

The formation of a renal calcification is a complex process involving several variables, such as interaction of multiple ions, promoters and inhibitors of crystallisation and aggregation, urine volume and pH (11). The urine is normally supersaturated with
respect to calcium phosphates and calcium oxalate, but crystallisation occurs rarely in vivo because of the complex composition of urine.

The ionic activities of crystal-forming ions, and not simply their concentration, contribute to crystal formation (10). Ionic activity is the percentage of each ion available to combine in initiating crystal formation. The activity product, which is the propensity for crystal formation, is a function of ionic activity, inhibitors, and urine pH (10). Supersaturation is reached when the amounts of crystal-forming ions in the urine exceed what would be the solubility limit if urine were pure water (48). The concentration range above supersaturation, where crystallisation does not occur because of urinary inhibitors of crystallisation is called the metastable zone (11). The concentration of calcium oxalate in normal urine, for example, is four times higher than its solubility (48). Crystal formation actually occurs when the ion pairs exceed the formation product, the critical activity product above which spontaneous precipitation occurs (10).

The initial formation of a crystal is called nucleation. The process of nucleation is homogeneous when the earliest crystal nuclei form in pure solutions (49). In urine, however, nucleation is usually heterogeneous, that is, precipitation of ion pairs occurs on existing surfaces such as epithelial linings, red blood cells, cellular debris or other crystals (48). A heterogeneous nucleation process requires considerably less energy than homogeneous nucleation. Crystal aggregation and growth occur at lower ion pair concentrations than initial nucleation (17).

Promoters of crystal formation. Clinically the most important promoters of renal calcification, i.e. urinary compounds whose concentrations show a correlation with the risk of crystallisation, include calcium, oxalate and uric acid (10).

Hypercalciuria, which is a known risk factor for nephrolithiasis in both children and adults (50-52), may result secondarily from hypercalcaemia or be associated with other types of disturbances in calcium balance. Urinary calcium excretion is dependent on dietary calcium, net intestinal calcium absorption, the balance of bone formation and resorption, and renal processes such as glomerular filtration and tubular reabsorption (8).

Oxalate is the most insoluble physiological substance, and thus small changes in urinary concentration may greatly increase the relative supersaturation of calcium oxalate (8, 53). In addition to primary hyperoxaluria, which is a known disease entity, secondary hyperoxaluria has been associated with total parenteral nutrition, which may contain oxalate precursors in the protein load and ascorbic acid in the vitamin supplement (54, 55). Enhanced intestinal oxalate absorption may occur when free oxalate concentrations in the gut are increased. This may occur when on calcium-restricted diet or in the short gut syndrome (56).

Increased uric acid excretion may result in uric acid stones, and it has also been found to promote calcium oxalate stones in adults (57). The importance of uric acid in children and infants is unclear (58). Hyperuricosuria may derive from the diet, from overproduction or as a side effect of drug therapy (8).

Inhibitors of crystal formation. The known inhibitors of urine crystal formation include citrate, magnesium and certain macromolecules. Citrate is produced endogenously in the liver, freely filtered at the glomerulus and reabsorbed in part in the proximal tubule (59). It forms soluble complexes with calcium, thus reducing the amount of calcium available for the formation of insoluble calcium oxalate and calcium
phosphate crystals. The risk of renal calcification is therefore greater in states in which urinary citrate excretion is decreased, such as hypokalaemia, metabolic acidosis and certain diuretic therapies (8, 60). Magnesium is an inhibitor of crystal formation and growth. Hypomagnesaemia has also been found to associate with reduced urinary excretion of citrate (61, 62). Urine contains small amounts of macromolecules that may inhibit crystal formation, aggregation and growth, notably nephrocalcin, a vitamin K-dependent macromolecule that binds calcium and inhibits crystal formation, aggregation and growth (63), uropontin, which seems to block the growth of calcium oxalate crystals (64), and Tamm-Horsfall protein, which inhibits aggregation of the crystals (65).

Urine volume and acid-base changes. Urinary flow rate correlates inversely with the concentrations of urinary constituents and thus with the risk of crystal formation. Urinary volume is affected by fluid restriction, for example, and by any conditions associated with dehydration, such as diarrhoea (8). Alterations in urinary pH have marked effects on solubility products. Alkaline urine favours the crystallisation of calcium phosphates, while low urine pH reduces the solubility of uric acid (8). Systemic metabolic acidosis may increase the risk of renal calcification by increasing urinary calcium excretion (probably due to activated bone resorption) and by virtue of the fact that urinary citrate is decreased (59). Metabolic alkalosis, on the other hand, increases the urinary pH, thus increasing the formation products for calcium phosphates. Enhanced citrate excretion and some reduction in urinary calcium excretion may offset the latter effect of metabolic alkalosis, however (8, 59).

Dystrophic calcification of the kidneys is a separate type of mechanism that affects injured renal tissues, and may be associated with renal venous thrombosis (66) or renal cortical necrosis in neonates (24).

2.4. Clinical conditions associated with nephrocalcinosis

From a clinical point of view it is helpful to separate the clinical conditions associated with NC into roughly the following categories, on grounds of the main causes of NC: 1) hypercalcaemia (which also causes hypercalciuria in most cases); 2) hypercalciuria; 3) miscellaneous, including conditions characteristic of increases in urinary promotors (other than calcium) or conditions in which the causes of NC are unknown. In addition, two groups are presented separately for clarity. Iatrogenic conditions, i.e. conditions in which NC is associated principally with the treatment (or prevention) of a disease, and very low birth weight (VLBW) infants, in whom the aetiology for NC is apparently multifactorial. A summary of the many conditions associated with NC is presented in Table 1.
Table 1. Clinical conditions associated with nephrocalcinosis grouped by the main anticipated mechanism to predispose for the condition.

<table>
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<td>Wilson’s disease</td>
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2.4.1. Hypercalcaemia

Calcium balance is maintained by several hormonal systems that are normally efficient enough to keep the serum calcium level within a relatively narrow physiological range (2.20-2.70 mmol/l). The main organs involved in calcium balance are the gut, kidneys and bone, which are the target organs of parathyroid hormone (PTH) (67). PTH stimulates bone resorption and renal tubular reabsorption of calcium and also has a positive effect on calcium absorption from the gut by stimulating calcitriol production (67). The enhancement of intestinal absorption of both calcium and phosphate are the main effects of calcitriol (1,25-dihydroxycholecalciferol), which is the most active vitamin D metabolite in the human body. Calcitonin is produced by the parafollicular cells of the thyroid gland and its exact physiological role is unclear, but the net effect of elevated calcitonin levels is to lower the serum calcium level by reducing intestinal calcium absorption and inhibiting bone resorption and renal calcium reabsorption (68, 69).

In primary and secondary hyperparathyroidism, elevated serum PTH levels induce hypercalcaemia and often NC (70, 71). A milder form of familial neonatal hyperparathyroidism has been described in which hypercalcaemia resolves without surgery by two years of age but NC persists (72). NC has also been reported in children with juvenile primary hyperparathyroidism (15, 73).

Subcutaneous fat necrosis is a potentially fatal disorder in which asphyxiated infants develop firm subcutaneous nodules and possibly severe hypercalcaemia, hypercalciuria and NC some weeks after birth (74-76). It has been hypothesised that the inadequately elevated 1,25-dihydroxyvitamin D levels (and subsequent hypercalcaemia) associated with the condition may result from unregulated extrarenal 1,25-dihydroxyvitamin D production by macrophages situated in nodules of subcutaneous fat necrosis (77, 78).

Hypophosphatasia covers a group of autosomal recessive diseases characterised by defective skeletal mineralisation due to a deficiency in tissue-nonspecific alkaline phosphatase enzymatic activity (79). The severe infantile form is often fatal, entailing hypercalcaemia, failure to thrive, fractures, premature craniosynostosis and respiratory tract infections (80, 81). Defective mineralisation is associated with reduced calcium uptake by bone, resulting in hypercalcaemia, suppressed PTH levels, hypercalciuria and NC (80, 81).

Williams’ syndrome is a heritable syndrome characterised by typical facies, cardiovascular abnormalities and mental retardation (82). In the first years of life the syndrome may be associated with hypercalcaemia, which subsequently resolves by four years of age (83). NC is frequently associated with hypercalcaemia and has developed at two months of age at the earliest (84, 85). The specific cause of hypercalcaemia has remained unclear (83).

Idiopathic infantile hypercalcaemia involves hypercalcaemia, hypercalciuria and NC (86). The hypercalcaemia is presumed to be due to deficient stimulation of calcitonin and 24,25-dihydroxyvitamin D secretion and deficient suppression of PTH and 1,25-dihydroxyvitamin D secretion (10). It can be corrected within hours by means of calcitonin therapy, but a high-phosphate diet and thiazides are often needed to arrest hypercalciuria (86, 87).
Paraneoplastic phenomenon. Hypercalcaemia is associated with malignancies in adults, but is rare in children (88). Three young children with hypercalcaemia, hypercalciuria and NC related to sarcoidosis have been reported (89). Furthermore, severe hypercalcaemia and bilateral NC was reported in an infant with mesoblastic nephroma, in whom hypercalcaemia was anticipated to occur due to a humoral factor secreted by the tumour (90).

2.4.2. Hypercalciuria

A common definition for hypercalciuria in childhood is urinary calcium excretion of more than 0.1 mmol/kg (4 mg/kg) per body weight per 24 hours (91, 92). As 24-hour urine collection may be laborious and unreliable especially in infants and younger children, attempts have been made to find reliable limits for normal and excessive calcium excretion measured in random urine samples. For screening hypercalciuria in paediatric patients, a fasting calcium-creatinine ratio of less than 0.6 mmol/mmol determined from a random urine sample has been regarded as normal (50, 93, 94). The correlation between these two methods has been found to be satisfactory (95, 96). There are many reports that describe normal and abnormal calcium excretion variables in different settings (96-104), but comparison between them is difficult because of differences in the size and selection of the populations, age distribution, the calcium, sodium and protein content of the diets, the timing of urinary collections in relation to fasting or meals, etc. Possible large intra-individual day-to-day changes in calcium excretion variables do not make the interpretation any easier (99). Some of the studies, but not all, show a significant negative correlation of age with calcium to creatinine ratios in children. Age dependence seems to be most obvious in the very first years of life (100, 105). Sargent et al. showed that the 95% percentile for the urinary calcium to creatinine ratio in infants up to 6 months of age was twice as high as that for the group aged 1½ to 6 years (2.42 vs. 1.18 mmol/mmol) (100).

Calcium in plasma exists in three fractions: protein-bound (40%), ionised (48%) and complexed (12%). Complexed calcium is that bound to various anions, such as phosphate, citrate and bicarbonate. Only the ionised and complexed fractions are filtered by the renal glomerulus. Normally about 98 to 99% of the filtered load of calcium is reabsorbed by the renal tubules, approximately 60 to 70% of this in the proximal convoluted tubule, about 20% between the late proximal and early distal tubule (primarily in the thick ascending loop of Henle), about 5 to 10% in the distal tubule and less than 5% in the collecting duct. Physiological regulation of urine calcium excretion occurs in the distal tubule.

Urinary calcium excretion results from several factors that involve the calcium balance of the human body. These comprise dietary calcium content and calcium absorption in the gut, the balance between bone formation and resorption in the skeleton, and filtration and reabsorption of calcium in the kidneys (106, 107). Excessive urinary calcium excretion, even with normal serum calcium, is an important risk factor for urolithiasis in both adults (93) and children (50).
Distal renal tubular acidosis is characterised by chronic metabolic acidosis resulting from the inability of the distal nephron to acidify urine below pH 5.5, the presence of hypercalciuria and low urinary citrate (108). Alkali therapy is used to relieve metabolic acidosis and it has also been shown to increase the urinary excretion of citrate (109, 110). The combination of high urine pH, hypercalciuria and low urine citrate favours the development of NC (111). In a large follow-up series of 28 children in an age range from 1 month to 13 years, NC was found in half of the patients, and nephrolithiasis, when present, was always associated with NC (112). In untreated patients the condition may progress to end-stage renal disease with extensive renal calcifications (112). In most severe forms of the disease NC has developed in the very first months after birth (113).

Bartter’s syndrome characteristically includes hyperplasia of the juxtaglomerular apparatus, hyperaldosteronism and hypokalaemic metabolic alkalosis (114). Hypercalciuria and NC may be present in more than 50% of cases, and in some, nephrocalcinotic changes may already be found in early infancy (115-117). NC has been pyramidal in most of the patients, but in some cases cortical changes have also been described (118). Elevated levels of urinary prostaglandin E2 and abnormalities in vitamin D metabolism have been found in Bartter’s syndrome infants with hypercalcaemia and NC (119). Treatment with indomethacin, an inhibitor of prostaglandin synthetase, has resulted in amelioration of both hypercalciuria and NC in many of these patients (120), but not in all (116). Hyperprostaglandin E syndrome, a rare neonatal variant of Bartter’s syndrome, is characterised by premature labour, congenital hypokalaemia, hypercalciuria and NC (121, 122). Fellman et al. have described an association between hyperprostaglandin E syndrome and hyperthyroidism (123).

Idiopathic hypercalciuria (IH) which has been reported in 2 to 6% of asymptomatic children (103), may be of familial nature, is more common in white than non-white populations, and carries a risk of developing renal calcifications (124, 125). In a sample of 28 children with IH, ultrasonography revealed medullary NC in 6 cases (126). Follow-up periods of 1 to 4 years for children with unexplained haematuria have revealed renal calcification or renal colic in 13 to 16% of those who had also exhibited hypercalciuria (125, 127). Urinary calcium excretion and calcium/magnesium ratios were significantly higher in the children with NC than in those without (126). Bone mineral density has later been observed to be lower in both children (128) and adults (129) with IH. Eggert et al. reported NC in three out of six siblings with IH (130). No generalised tubular function disturbance was seen in a series of 10 children aged 5-17 years with IH (131).

In Cushing’s syndrome increased endogenous cortisol production results in excess urinary calcium excretion. Medullary NC and osteoporosis have been observed in these infants (132). Autopsy confirmed the presence of calcium deposition in the renal tubules and the loop of Henle in one case, and calcium deposition was also seen in abdominal radiographs in another (132).

X-linked recessive nephrolithiasis. Frymoyer et al. described in 1991 a large kindred with a novel form of X-linked disease characterised by nephrolithiasis, variable proximal tubular dysfunction and progressive renal insufficiency (133). X-linked nephrolithiasis (XLN) is associated with mutations in the renal-specific chloride channel (CLCN5) gene, with at least 19 different mutations having been detected (134). Two
recent reports describe clinical features in five boys aged 2 to 7 years with XLN (135, 136). Characteristics of all of them were microscopic haematuria, proteinuria, hypercalciuria, medullary NC and a family history of kidney stones and/or renal failure. Three other hereditary diseases with hypercalciuria and renal calcifications (Dent's disease, X-linked hypophosphataemic rickets and idiopathic low-molecular weight proteinuria, hypercalciuria and NC in Japanese children) have been associated with mutations of the CLCN5 gene (134, 137, 138).

The oculocerebrorenal syndrome of Lowe, an X-linked recessive disorder characterised by congenital cataracts, developmental delay, poor growth and metabolic acidosis resulting from renal tubular dysfunction, was initially described in 1952 (139). In a series of 5 children, NC was found in four and hypercalciuria and crystalluria in all of them (140). NC was revealed already before the initiation of alkali therapy (140). Hypercalciuria was found to persist despite the correction of acidosis by means of alkali therapy and the restriction of calcium intake to a recommended daily allowance. The authors felt that the most likely explanation for this hypercalciuria was the decrease in renal tubular reabsorption of calcium as part of the more generalised tubular dysfunction related to the syndrome (140).

Cystinosis is a lysosomal storage disorder caused by impaired transport of cystine out of the cellular lysosomes. It has been proposed that the observed aminoaciduria, glycosuria, phosphaturia and hypercalciuria result from the dysfunction produced by cystine in proximal tubular cells (141). Signs of NC were found in 26 out of 41 (63%) cystinosis children aged from 2 months to 15 years and 5 of them also had renal stones (142). The presence of NC was verified by a CT scan in 4 of the cases. In addition to cystine-depleting cysteamine treatment, most patients receive supplemental phosphate, vitamin D and calcium, and hence it is difficult to assess whether NC results from the disease or the therapy, although both reports considering the issue found the role of supplementation in the development of NC to be a minor one (142, 143).

Familial hypomagnesaemia. Praga et al. reported on eight children and adolescents with familial hypomagnesaemia with related hypercalciuria and NC (144). Distal renal tubular acidosis is often associated with this condition (145, 146). Neither oral magnesium administration nor thiazide diuretics seem to normalise serum magnesium levels or urinary calcium excretion (144). The prognosis for renal function is poor, and end-stage renal failure often develops, with a subsequent need for dialysis or a renal allograft (144, 147).

Pseudohypoaldosteronism is caused by an apparent inability of the renal tubules to respond to mineralocorticoids, which leads to typical clinical findings such as hyponatraemia, hyperkalaemia, hyperreninaemia and increased aldosterone levels (148). Various degrees of clinical severity have been reported (149). Shalev et al. reported on four children aged 5 months to 5 years with the disease, all of whom manifested with hypercalciuria and all but the youngest one showed NC in ultrasonography (150). Indomethacin treatment has given a fair response in cases involving polyuria, hypercalciuria and hyponatraemia (150, 151).

Wilson’s disease. The underlying problem in Wilson’s disease is a severe impairment of biliary copper excretion, which leads to excessive intratubular accumulation of copper in the liver and subsequent overflow into other tissues such as the brain, cornea and kidneys. Hypercalciuria with either nephrolithiasis or NC is
commonly related to this condition (152), and they have also been observed as presenting signs of the disease (153, 154).

2.4.3. Miscellaneous

NC may be found in the absence of an increase in serum calcium level or its urinary excretion. An excess of other promoters of renal calcification may predispose the subject to this condition, for example, or else the cause of NC may remain obscure.

*Primary hyperoxaluria* is an autosomal recessive disorder characterised by increased urinary oxalate excretion, which promotes the precipitation of calcium oxalate crystals and consequently nephrolithiasis, NC and end-stage renal failure (155, 156). Hyperoxaluria may also be of a secondary type as in the short-bowel syndrome, in which enteric absorption of oxalate is increased due to the malabsorption of fatty acids (157). Renal calcification has been reported in a 6-month-old infant with short bowel syndrome (56).

Exocrine pancreatic insufficiency and bone marrow dysfunction are characteristic features of *Schwachman’s syndrome*, first described in 1964 (158). NC has been described in these patients first at autopsy (159) and later by renal ultrasonography (160).

Other diseases or conditions reported to have NC associated with them are *cerebrotendinous xanthomatosis* (161), *pyloric stenosis* (162), *amelogenesis imperfecta* (163), *glycogen storage disease type 1a* (164, 165), *autosomal dominant polycystic kidney disease* (166), *carbonic anhydrase II deficiency syndrome* (167), *hypothyroidism* (168), and *milk-alkali syndrome* (169).

2.4.4. Iatrogenic

**Furosemide.** There were two paediatric cases in the mid 1980’s in which furosemide medication may have influenced on the development of NC (12, 170). Furthermore, Shultz et al. mentioned in 1991 two cases of NC in non-premature infants treated with furosemide for heart failure (19). More recently, Alon et al. (46) have reported two cases of nephrolithiasis and three of NC in young children who had received furosemide for congestive heart failure. NC was detected by renal ultrasonography at ages 12 and 18 months in two of the cases and the third was revealed in autopsy. Some observations which associate NC with furosemide medication in adults have also been reported recently (171).

Furosemide is a highly potent diuretic agent that inhibits sodium and chloride reabsorption in the thick ascending limb of the Henle loop, thus provoking diuresis, natriuresis and (secondarily) urinary losses of potassium (172). It also prevents the passive reabsorption of calcium, which results in increased urinary calcium excretion (173). Experiences from animal experiments have suggested that the furosemide-
induced risk of the development of NC is not so much related to younger age but rather to the properties of the loop diuretic itself, and that most of the renal calcifications seem to occur quite soon after the initiation of furosemide treatment (174, 175).

Acetazolamide is a carbonic anhydrase inhibitor which has been used either alone or in combination with furosemide for the treatment of posthaemorrhagic hydrocephalus (176, 177). By inhibiting bicarbonate reabsorption, acetazolamide induces urine alkalinisation and metabolic acidosis, the latter being known to increase urinary calcium excretion, while the former favours the precipitation of calcium phosphate. Consequently, NC has been a frequent side effect of combined acetazolamide and furosemide therapy in infants with hydrocephalus (176), and has also been reported to be associated with acetazolamide treatment alone (178).

Phosphate and vitamin D in X-linked hypophosphataemic rickets. XLH is a hereditary disease that is characterised both by hypophosphataemia and rachitic bone changes and also by high urinary phosphate excretion, normocalcaemia and growth retardation (179). The main defects involved in this condition are impaired tubular reabsorption of phosphate and blunted renal synthesis of 1,25-dihydroxyvitamin D3 (180). The conventional treatment used to alleviate the above-mentioned metabolic consequences consists of oral 1,25 dihydroxyvitamin D3 and phosphate supplements. NC is a frequent complication of therapy, however, as indicated in several reports (37, 40, 179-181). It seems that 1,25-dihydroxyvitamin D3 is sufficient in itself to cause NC in some patients (39), but the combination therapy with phosphate in particular reinforces the risk significantly (179). Significant associations have been found between NC and either the phosphate dose (37, 179, 180) or the duration of vitamin D treatment (38), but not in all studies (40). Most patients with XLH and NC but without evidence of secondary hyperparathyroidism exhibit normal or only slightly disturbed kidney function (37, 182). Impaired renal tubular acidification has been associated with NC in children with XLH, however, (38), and decreased serum bicarbonate, elevated serum chloride and increased urinary calcium excretion were found in the XLH children relative to those without NC (38). Renal failure in a 9-year-old boy with XLH and secondary hyperparathyroidism has also been described (14). The close monitoring of patients receiving combination therapy for XLH has been recommended in an attempt to avoid adverse effects of therapy (183, 184).

Hypervitaminosis D. Increased vitamin D intake may cause symptomatic hypercalcaemia, hypercalciuria and NC, resulting mainly from increased gut calcium absorption (185). Giant intermittent vitamin D doses as high as 600,000 IU were used for prophylaxis against vitamin D deficient rickets before it was recognised that a daily dose of 400 IU is most appropriate for preventing the condition (186, 187). Large doses of intramuscular vitamin D was used for treatment of preterm infants in 1950’s and 1960’s in Finland, and a fraction of this patient group developed severe NC, which lead some to end-stage renal failure in adolescence (Ollie Koskimies, personal communication 1999).

Infantile spasms with ACTH treatment. Adrenocorticotropic hormone treatment for infantile spasms and myoclonic epilepsy has been associated with both NC and osteoporosis (188, 189). The pathomechanism is obviously similar to that found in Cushing’s syndrome.
2.4.5. Very low birth weight infants

NC was first described in a preterm infant in 1978 by Cacciarelli et al. (5), who verified it with both plain abdominal radiography and renal ultrasonography. Subsequently, Hufnagle et al. reported a series of ten premature infants with renal calcifications who had been receiving long-term furosemide treatment, first for patent ductus arteriosus and later for chronic lung disease (7). Furosemide administration had been started at 2 mg/kg/day, but had been raised to 4 and 8 mg/kg/day in some cases. The presence of renal calcium deposits was confirmed by renal ultrasonography in all these infants and at autopsy in four of them (7). Several reports have since associated renal calcifications with furosemide therapy in preterm infants (47, 190-192). The coexistence of nephrolithiasis and cholelithiasis has also been reported (193, 194).

2.4.5.1. Incidence

Two prospective studies have given quite different incidence rates for renal calcifications in preterm infants of birth weight less than 1500 grams. Jacinto et al. (13) reported NC or nephrolithiasis in 20 out of 31 preterm infants (64%), whereas Short and Cooke screened 79 preterm infants of gestational age less than 32 weeks and noted that 21 of these (27%) developed renal calcifications, consisting of pyramidal NC in 17 cases and renal stones in four (195). Karłowicz et al. (196) found NC in 14 out of 50 infants (28%) with a birth weight of less than 1200g. It is significant that most of the infants had not received long-term furosemide (196). The incidences of NC among preterm infants treated with furosemide or not have ranged from 34% to 93% and 8% to 41%, respectively (8).

2.4.5.2. Risk factors

Since the first reports on preterm infants, NC has been attributed mainly or exclusively to treatment with furosemide, which has a considerable calciuric effect (7, 44, 47, 197). The prolonged half-life of the drug may further potentiate calciuric and other adverse effects in immature infants (198). Jacinto et al. were the first to describe NC in preterm infants without preceding furosemide treatment (13). Furthermore, Short and Cooke noted that although the cumulative dose of furosemide during the whole primary hospitalisation was greater in the infants with than without NC, no difference in the mean total furosemide dose existed at the time NC was detected (195). They thus suggested that long-term furosemide therapy is prescribed for infants who are already at risk of developing NC.

Several studies have indicated that the greatest risk of NC and renal stones exists among the smallest, most premature, most sick premature infants (13, 195). The
duration of oxygen treatment was the strongest clinical indicator of renal calcification in a multivariate analysis, as infants still requiring oxygen treatment at the age of 28 days had a 62% chance of having NC (195). The length of hospitalisation and duration of ventilation were also closely correlated with the risk of NC (195). Other associated factors included hypophosphataemia, hypercalcaemia and hypercreatininaemia (195).

Increased urinary calcium excretion is an apparent risk factor for developing NC, and there are other factors in addition to furosemide treatment that tend to promote calciuria in preterm infants. Calcium excretion has been shown to be increased when the phosphorus supply is inadequate for bone mineralisation. Vileisis demonstrated that an increase in the phosphorus content of the parenteral nutrition resulted in a decrease of urinary calcium excretion (199). During furosemide treatment, however, an increase in phosphorus was not an effective means of reducing calciuria (200). Preterm infants should receive calcium and phosphorus supplementation adequately to meet the needs of nutrition and mineralisation and to avoid disadvantages such as osteopenia and excess urinary excretion of minerals (201-204). Secondary hyperparathyroidism may also be related to increased calcium excretion during furosemide treatment (71, 200). Other factors that may aggravate hypercalciuria in preterm infants are chronic corticosteroid (205) and xanthine therapy, vitamin D treatment and prolonged immobility (8).

Increased urinary oxalate excretion may also favour the development of NC in preterm infants. The urinary oxalate concentration was increased in VLBW infants receiving total parenteral nutrition (TPN) by comparison with those receiving a glucose and electrolyte solution, and the increase was even more pronounced after the protein content of the TPN had been increased (55). As the oxalate precursors ascorbate and glycine are included in the TPN solution, it may contribute to the increased risk of NC. In another comparison of VLBW infant groups receiving TPN and a breast milk/clear fluid infusion, increased urinary calcium oxalate saturation as a result of TPN was combined with increased urinary calcium excretion and higher urinary oxalate/creatinine and calcium/citrate ratios, which may further compound the risk of developing NC (154).

Additional factors thought to contribute to renal calcification in VLBW infants include alkaline urine, diminished urine volume, increased urinary urate (58) and the reduction in urinary inhibitors of crystallisation such as magnesium, citrate and inorganic phosphates (8).

Genetic factors are also involved in the development of NC. Karlowicz et al. showed an increased risk of NC related to white ethnicity and to a positive family history of kidney stones (196).

2.4.5.3. Outcome

Adverse events. In their original report Hufnagle et al. noted that 6 out of the 10 infants with renal calcification had urinary tract infections and three of them had recurrent urinary tract infection with sepsis (7). Later microscopic and gross haematuria has been
associated with renal calcifications, and ureteral obstruction with acute renal failure has also been reported (44).

**Ultrasonic resolution.** Data on the ultrasonic resolution of NC in VLBW infants are limited and follow-up times are not long in most cases. Ezzedeen et al. (45) reported ultrasonic resolution in 4 out of 9 VLBW infants with NC at a mean age of 21 months (range 9-56), and in another report 12 out of 20 were still affected at about 16 months of age (44). In a more recent study, 5 children out of 11 had ultrasonic evidence of renal calcification at the age of 4-5 years (206).

**Kidney function.** The possible deleterious effects of prematurity-associated NC on kidney function later in life have not been conclusively established. Signs of compromised glomerular function have been found in some patients at ages of 1-4 years (45, 206, 207). Urinary calcium/creatinine ratio and β₂-microglobulin levels were normal in a small group of former preterm infants with NC examined at 12 months of age (208). The capacity of the distal tubule to secrete hydrogen ions, as measured by the oral acetazolamide test, was significantly lower at a mean age of 14 months in preterm infants with neonatal NC than in those without the condition (207), whereas Jones et al. failed to reveal any significant differences between infants with and without a history of NC regarding renal urine concentration capability and urinary calcium excretion after a calcium load test at age 4-5 years (206).

### 2.4.5.4. Prevention and treatment

One reasonable approach to lessening the risk of developing NC is to consider means of reducing urine calcium excretion. Previous studies have indicated that a relative deficiency of phosphate in the nutrition, for example, may result in increased calcium excretion, and that a decrease in urinary calcium is achieved in response to an increase in phosphate intake (199, 201).

In their original report, Hufnagle et al. mentioned that the addition of thiazide, which has a hypocalciuric effect, resulted in disappearance of the renal calcifications in four out of five infants, and no more cases were found after furosemide and thiazides were used in combination (7). Thiazide diuretics have been shown to be efficient in reducing calcium excretion in paediatric patients with hypercalciuria, but long-term benefits and disadvantages in particular are not well established (209-211). Thiazides have been shown to reduce calcium excretion in infants, but Atkinson et al. could not find any significant difference between treatment with either furosemide alone or in combination with hydrochlorothiazide and spironolactone (212). In a recent report, Campfield et al. found that the addition of thiazide did not reduce the calciiuric effect of furosemide, and speculated that as sodium and calcium excretion are closely linked, the use of a sodium supplement in premature infants may be sufficient to overcome the hypocalciuriic effect of thiazide (213). The avoidance of potassium depletion may also have a favourable effect on urinary calcium excretion (214, 215).

Administration of fish oils and other products containing essential fatty acids have had a protective effect against NC and nephrolithiasis in animal models (216-218), but
their advantage in human use has not been established (219). The role of inhibitors of crystal formation in the development of NC is unclear, although the administration of magnesium-potassium-citrate to adults with kidney stones has provided some promising results (219).
3. Purpose of the study

The ultimate aim of investigations concerning NC is naturally to be able to avoid this complication in the future. Before this objective can be reached, various diseases and clinical conditions with which NC is associated and various pathomechanisms operative in its development have to be examined. Research into the duration and possible harmful effects of NC is also of interest and value.

The main purposes of the present study were:

1. to evaluate the incidence, associated risk factors and natural course of NC in VLBW infants,
2. to evaluate kidney function at 2-6 years of age in former VLBW infants with neonatal NC,
3. to evaluate the incidence and natural course of NC in full-term infants receiving long-term furosemide treatment for cardiac failure, and
4. to evaluate the occurrence and causes of NC in infants with congenital lactase deficiency
4. Patients and methods

4.1. Patients

The study populations consisted of four patient groups treated and followed up at Oulu University Hospital. The data on the four series are summarised in Table 2. All the investigations were approved by the Ethics Committee of Oulu University and conducted with informed parental consent.

VLBW infants (I). A total of 129 infants of birth weight less than 1500 grams and gestational age less than 34 weeks who were born in 1990-94 within the district primarily served by Oulu University Hospital were included in this series. A more detailed description of their birth weight distribution is shown in Table 2.

Children with prematurity-associated NC (II). Twenty case-control pairs were obtained by matching each VLBW infant with NC with the nearest VLBW child in the hospital birth record fulfilling three criteria: 1) differing in birth weight by less than 250 grams, 2) negative screening renal ultrasonographies for NC in the neonatal period, and 3) a postnatal age difference of less than 8 months as compared with the case at the time of kidney function examination. Fourteen of the case-control pairs were of same sex, but five female-male pairs and one male-female pair were also included. Seventeen of the cases and all of the controls also belonged to series I.

Infants treated with furosemide for congestive heart failure (III). A total of 36 full-term infants who visited the out-patient clinic of the Paediatric Cardiology Unit at Oulu University Hospital in 1995-96 and had received furosemide treatment for at least four weeks were included to this series (Table 2). In cases where furosemide medication had been stopped before the entry visit, discontinuation of the drug within the last four weeks was accepted. Thirty-six consecutive full-term infants of appropriate age referred to the same unit on account of suspected or proven congenital heart disease but not prescribed furosemide or other diuretics served as a control group.

Children with congenital lactase deficiency (IV). Series IV consisted of all the 11 children with proven congenital lactase deficiency born in Oulu University Hospital between 1982 and 1993.
Table 2. Summary of the patients in the series I-IV (values are given as mean (SD) or median (range), unless stated otherwise)

<table>
<thead>
<tr>
<th>Series</th>
<th>Number of patients</th>
<th>Birth weight, grams</th>
<th>Age at study</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Preterm infants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>&lt;750</td>
<td>2 and 6 weeks,</td>
<td>Infants with NC</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>750-999</td>
<td>and 3 months</td>
<td>up to 5-6 years, or</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>1000-1249</td>
<td></td>
<td>until resolution</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>1250-1499</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>129</td>
<td>&lt;1500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II Former preterm infants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>study group</td>
<td>20</td>
<td>905 (209)</td>
<td>4.7 (1.1) years</td>
<td></td>
</tr>
<tr>
<td>controls</td>
<td>20</td>
<td>957 (226)</td>
<td>4.6 (0.9) years</td>
<td></td>
</tr>
<tr>
<td>III Full-term infants with furosemide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>study group</td>
<td>36</td>
<td>3558 (656)</td>
<td>2.9 (1.1–8.0) months</td>
<td>Infants with NC up to 2 years or until resolution*</td>
</tr>
<tr>
<td>controls</td>
<td>36</td>
<td>3586 (432)</td>
<td>3.4 (1.1–8.4) months</td>
<td></td>
</tr>
<tr>
<td>IV Congenital lactase deficiency infants</td>
<td>11</td>
<td>3651 (756)</td>
<td>24 (6-88) days</td>
<td>Re-evaluation at age 2-10 years</td>
</tr>
</tbody>
</table>

* All infants with furosemide treatment were followed for NC until discontinuation of the drug
4.2. Methods

4.2.1. Ultrasonographic methods

*VLBW infants (I).* The renal ultrasound examinations for NC were performed by two paediatric radiologists with Toshiba Sonolayer SSA-270 equipment using 5 MHz and 7.5 MHz transducers. Renal ultrasonography was performed on the infants by 2 weeks of age and thereafter at 6±1 weeks and 3±1 months of age. When NC was detected, follow-up renal ultrasonographies were performed at 6, 12, 18 and 24 months of age and thereafter annually up to 5-6 years of age, or until resolution of NC.

The copy images were evaluated by two radiologists who were unaware of the clinical histories of the infants, and decisions were made together in borderline cases. The cases of NC were classified into peripheral, scattered and extensive types according to the extent and location of pyramidal abnormalities. The classification criteria are given in Table 3. The diagnosis and classification of NC were accepted only if ultrasonographic changes were seen in several pyramids in each kidney.

*Table 3. Classification of medullary nephrocalcinosis in preterm infants*

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Ultrasonographic finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral</td>
<td>Hyperechogenicity at the tip of the pyramid or hyperechoic rim around the pyramid</td>
</tr>
<tr>
<td>Scattered</td>
<td>Hyperechoic foci throughout the pyramid</td>
</tr>
<tr>
<td>Extensive</td>
<td>Intense hyperechogenicity filling the pyramid entirely</td>
</tr>
<tr>
<td>Stone</td>
<td>Solitary focus of echoes with acoustic shadowing at the tip of the pyramid</td>
</tr>
</tbody>
</table>

*Children with prematurity-associated NC (II).* Renal ultrasonographies were performed with the same device and transducers as in series I. All the images were interpreted by one paediatric radiologist, who was unaware of the patient history and clinical course. NC was defined here as the presence of medullary echogenic densities in the renal medullary pyramids. When signs of NC had been present at 2 years of age, the case was defined as being one of prolonged NC.
Infants treated with furosemide for congestive heart failure (III). The renal ultrasonographies for NC were performed on furosemide-treated infants and controls with the same device and transducers as in series I and II. Supine and prone images of both kidneys were obtained in longitudinal projections and hard copies were taken. These images were evaluated by one paediatric radiologist, who was unaware of the clinical histories of the infants.

In the cases in which NC was detected, renal ultrasonography was repeated at 3-6 month intervals up to 2 years of age or until ultrasonic resolution. The infants who did not have NC but continued to receive furosemide were monitored by renal ultrasonography until discontinuation of the diuretic.

Children with congenital lactase deficiency (IV). Renal ultrasonographies had been performed on each of the children with the same device and transducers as in series I, II and III.

4.2.2. Clinical and laboratory examinations

VLBW infants (I). Neonatal morbidity, duration of the need for assisted ventilation and supplemental oxygen, and number of days of acidosis and alkalosis were recorded. Similarly, the durations of treatment with furosemide, dexamethasone and theophylline during the primary stay in hospital were recorded and the cumulative doses of these drugs reached by 8 weeks of age were calculated. Calcium and phosphorus intakes were calculated at 4, 6 and 8 weeks of age, and serum calcium and phosphorus concentrations were measured before discharge, at a weight of 2000 g.

Children with prematurity-associated NC (II). The children came to the outpatient clinic after fasting for 3 to 5 hours. Serum for the analysis of creatinine, sodium, potassium, chloride, total and ionised calcium, phosphorus, albumin levels, alkaline phosphatase activity, osmolality and blood gas values was obtained by venipuncture. A second morning urine sample was analysed for pH, osmolality, minerals, electrolytes, creatinine and \( \beta_2 \)-microglobulin. Urinary calcium/creatinine, calcium/sodium and \( \beta_2 \)-microglobulin/creatinine ratios, creatinine clearance, fractional excretion of sodium (FENa) and potassium (FEK), tubular reabsorption of phosphate (TRP) and maximal tubular reabsorption of phosphate per glomerular filtration rate (TmP/GFR) were calculated from the baseline urine and blood sample data. The formulae used to calculate the kidney function parameters are given in Table 4. The serum and urine variables were analysed by routine laboratory methods.

Oral acetazolamide test. The children underwent the urine alkalisation test described by Alon et al. (220). After administration of an oral dose of acetazolamide (17 mg/kg), the first voided urine was discarded and the following specimens were drawn into a syringe immediately, freed of air and taken to the laboratory for immediate determinations of pH and carbon dioxide tension. At least three consecutive samples were examined, taken at intervals of about 30 to 60 minutes, and the test was terminated
Table 4. Formulae used to calculate different variables describing renal function

<table>
<thead>
<tr>
<th>Formula</th>
<th>Abbreviations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Estimated glomerular filtration rate from creatinine clearance (222):</td>
<td>GFR = glomerular filtration rate</td>
</tr>
<tr>
<td></td>
<td>expressed in ml/ minute/1.73 m²</td>
</tr>
<tr>
<td></td>
<td>k = a constant of proportionality</td>
</tr>
<tr>
<td></td>
<td>(0.55 in children aged 2–12 years)</td>
</tr>
<tr>
<td></td>
<td>L = body length in centimeters</td>
</tr>
<tr>
<td></td>
<td>P&lt;sub&gt;Cr&lt;/sub&gt; = plasma concentration of creatinine</td>
</tr>
<tr>
<td></td>
<td>in milligrams per deciliter</td>
</tr>
<tr>
<td>GFR = ( \frac{k \cdot L}{P&lt;sub&gt;Cr&lt;/sub&gt;} )</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Fractional excretion of compound ( x ) (FE&lt;sub&gt;x&lt;/sub&gt;):</td>
<td>U&lt;sub&gt;x&lt;/sub&gt; = urine concentration of ( x )</td>
</tr>
<tr>
<td></td>
<td>P&lt;sub&gt;x&lt;/sub&gt; = plasma concentration of ( x )</td>
</tr>
<tr>
<td></td>
<td>U&lt;sub&gt;Cr&lt;/sub&gt; = urine concentration of creatinine</td>
</tr>
<tr>
<td></td>
<td>P&lt;sub&gt;Cr&lt;/sub&gt; = plasma concentration of creatinine</td>
</tr>
<tr>
<td>FE&lt;sub&gt;x&lt;/sub&gt; = ( \frac{U&lt;sub&gt;x&lt;/sub&gt; \cdot P&lt;sub&gt;Cr&lt;/sub&gt;}{P&lt;sub&gt;x&lt;/sub&gt; \cdot U&lt;sub&gt;Cr&lt;/sub&gt;} )</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Tubular reabsorption of phosphate (TRP):</td>
<td>U&lt;sub&gt;p&lt;/sub&gt; = urine concentration of phosphate</td>
</tr>
<tr>
<td></td>
<td>P&lt;sub&gt;Cr&lt;/sub&gt; = plasma concentration of creatinine</td>
</tr>
<tr>
<td></td>
<td>U&lt;sub&gt;Cr&lt;/sub&gt; = urine concentration of creatinine</td>
</tr>
<tr>
<td></td>
<td>P&lt;sub&gt;p&lt;/sub&gt; = plasma concentration of phosphate</td>
</tr>
<tr>
<td>TRP (%) = 1 - ( \left( \frac{U&lt;sub&gt;p&lt;/sub&gt; \cdot P&lt;sub&gt;Cr&lt;/sub&gt;}{U&lt;sub&gt;Cr&lt;/sub&gt; \cdot P&lt;sub&gt;p&lt;/sub&gt;} \right) \times 100 )</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Maximal tubular reabsorption of phosphate per glomerular filtration</td>
<td>P&lt;sub&gt;p&lt;/sub&gt; = plasma concentration of phosphate</td>
</tr>
<tr>
<td>rate (( T_{m}P/GFR )) (241):</td>
<td>U&lt;sub&gt;p&lt;/sub&gt; = urine concentration of phosphate</td>
</tr>
<tr>
<td></td>
<td>P&lt;sub&gt;Cr&lt;/sub&gt; = plasma concentration of creatinine</td>
</tr>
<tr>
<td></td>
<td>U&lt;sub&gt;Cr&lt;/sub&gt; = urine concentration of creatinine</td>
</tr>
<tr>
<td>( T_{m}P/GFR = P_{p} - \frac{U_{p} \cdot P_{Cr}}{U_{Cr}} )</td>
<td></td>
</tr>
</tbody>
</table>
when at least two urine collections with pH above or equal to 7.50 were obtained. Venous blood gas values were determined between the second and third urine collections. The urine sample with the highest pH was used when calculating the urine minus blood carbon dioxide tension for the assessment of distal tubule hydrogen ion excretion capacity. A urine minus blood partial carbon dioxide tension difference (U-B PCO₂) of less than 20 mmHg (2.7 kPa), which has been established as indicating inappropriate hydrogen ion secretion in children from 5 years of age (220), and also seemed appropriate for children aged 1 to 2 years (207), was employed as a limit here. Blood gas values and urine carbon dioxide tension and pH were determined with a Ciba Corning 278 Blood Gas System (Ciba Corning Diagnostics Corp., USA).

Infants treated with furosemide for congestive heart failure (III). A venous blood sample was taken at entry for the determination of serum sodium, potassium, calcium, ionised calcium, phosphorus, chloride, alkaline phosphatase and creatinine and capillary blood gas values. A random urine sample was analysed for sodium, potassium, chloride, phosphorus, calcium, creatinine and pH. The urine and blood variables were analysed by routine laboratory methods. The urinary calcium/creatinine ratio, FENa, FEK, TRP and creatinine clearance were calculated from the data obtained at entry (Table 4). The average dose of furosemide/kg per day at entry was calculated. A random urine sample was obtained from the controls, but no blood sample was taken.

Children with congenital lactase deficiency (IV). The laboratory and clinical data of the infants at time of diagnosis were studied retrospectively The children were then called for re-evaluation at ages of 2 to 10 years to establish their current calcium homeostasis. A venous blood specimen was taken for the analysis of serum calcium, phosphate, creatinine, sodium, potassium, urate, alkaline phosphatase and osmolality, and a 24-hour urine specimen was collected for measuring of calcium, creatinine and protein. In the case of urinary incontinence, a random urine specimen was taken for analysis. In all cases, β₂-microglobulin was determined from a freshly voided random urine sample. The blood and urine analyses were performed by routine hospital laboratory methods.

4.2.3. Statistical methods

The data were recorded and analysed on a personal computer using SPSS for Windows (Statistical Package for Social Sciences, version 7.0) statistical software. Comparisons between the groups were analysed with Student's t-test for continuous variables with a normal distribution and the Mann-Whitney U test for those with a skewed distribution. Categorial variables were tested with the chi-square test or Fisher's exact test. The cases and controls in series IV were compared using Student's t-test for paired samples. A p value <0.05 was taken as indicating statistical significance.
5. Results

5.1. Nephrocalcinosis in very low birth weight infants (I, II)

5.1.1. Clinical findings (I)

*Incidence.* The overall incidence of NC in the VLBW infants was 20% (26/129), ranging from 50% and 33% in the two lower BW groups to 15% and 10% in the two higher ones (Figure 1).

*Risk factors.* The infants with NC were more premature and sicker than those without NC. Their condition was poorer at birth, they had more signs of acid-base instability, and respiratory problems were both more common and lasted longer (Table 5). The duration of furosemide, dexamethasone and theophylline treatment and that of parenteral nutrition were longer than in the non-affected infants. The mean cumulative doses of furosemide (18.8 vs. 5.0 mg; p < 0.001), dexamethasone (8.9 vs. 2.1 mg; p < 0.01) and theophylline (142.0 vs. 92.9 mg; p < 0.01) per kilogram of BW at two months of age were significantly higher in the infants who developed NC. An increased risk of NC was associated with the need for supplemental oxygen both at 28 days of age and at term (Table 5).

5.1.2. Classification (I)

Of the 26 cases with NC, 14 (54%) were classified as peripheral, 7 (27%) scattered and 5 (19%) extensive. All three patterns occurred in each BW category, except for the highest weight group, in which only peripheral and scattered forms were seen (Figure 1). The clinical condition of the infants exhibiting more extensive patterns of NC seemed to be poorer than that of the infants with the peripheral pattern. They needed assisted ventilation for longer and had more days of acidosis (peripheral vs. extensive pattern; p = 0.046 and p = 0.012, respectively) and their primary hospital stay lasted longer (peripheral vs. combined scattered and extensive patterns; p = 0.035). A trend for a
Table 5. Clinical characteristics of 129 VLBW infants with (NC +) or without (NC -) nephrocalcinosis during their primary hospital stay (numbers given are means (SD) unless stated otherwise).

<table>
<thead>
<tr>
<th>Variable</th>
<th>NC (+) (n = 26)</th>
<th>NC (-) (n=103)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, weeks</td>
<td>27.2 (2.1)</td>
<td>29.3 (2.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight, grams</td>
<td>993 (242)</td>
<td>1174 (238)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male/female</td>
<td>16/11</td>
<td>56/46</td>
<td>NS</td>
</tr>
<tr>
<td>Apgar score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-1 minute</td>
<td>4.3 (1.7)</td>
<td>5.8 (2.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>-5 minutes</td>
<td>5.7 (1.1)</td>
<td>6.9 (1.7)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>RDS, %</td>
<td>96 (25/26)</td>
<td>54 (56/103)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Need for supplemental O$_2$, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- at 28 days</td>
<td>92 (24/26)</td>
<td>37 (38/103)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>- at term</td>
<td>50 (13/26)</td>
<td>4 (4/103)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Acidosis (pH &lt;7.25) days†</td>
<td>6 [2, 11]</td>
<td>1 [0, 4]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alkalosis (pH &gt;7.50) days†</td>
<td>1 [0, 3]</td>
<td>0 [0, 1]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Assisted ventilation, days‡</td>
<td>33 [14, 47]</td>
<td>5 [1, 12]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oxygen therapy, days‡—</td>
<td>79 [47, 263]</td>
<td>16 [2, 36]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Furosemide therapy, days‡</td>
<td>36 [17, 110]</td>
<td>2 [1, 8]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dexamethasone therapy, days‡</td>
<td>28 [15, 73]</td>
<td>0 [0, 8]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Amiophylline therapy, days‡</td>
<td>78 [46, 118]</td>
<td>28 [5, 44]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Parenteral nutrition, days‡</td>
<td>14 [10, 22]</td>
<td>3 [0, 11]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Primary hospital stay, days‡</td>
<td>137 [87, 175]</td>
<td>57 [45, 81]</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

† numbers given are medians, with 25% and 75% quartiles in brackets; ‡ days on supplemental oxygen after discharge included; * chi square test; NS = not significant
Fig. 1. Frequency and patterns of nephrocalcinosis (NC) in each birth weight group of very low birth weight infants.

longer need for supplemental oxygen and more lengthy treatment with furosemide and theophylline was also observed (peripheral vs. combined scattered and extensive patterns; p = 0.064, p = 0.057 and p = 0.051, respectively).

5.1.3. Follow-up (I)

Adverse events. One infant in the peripheral pattern group and one in the extensive pattern group died of chronic lung disease after three months, but the remaining 24 infants were followed up until the age of 5-6 years or until resolution of NC. An episode of gross haematuria was seen in one of the infants with extensive NC at four years, and unilateral renal growth impairment of more than 2 SD below the normal occurred in another with the scattered pattern.

Ultrasonic resolution. Peripheral NC resolved by six months of age in nine out of 13 infants, and complete resolution was seen in the remaining four cases at 12 months. On the other hand, resolution was observed in only two out of the seven infants with scattered NC at 12 months and in none with extensive NC (Figure 2). Finally, only two children with extensive NC still had evidence of the condition at 5-6 years of age.
5.1.4. Kidney function (II)

Blood pressure and haematuria. Systolic and diastolic blood pressures did not differ between the former premature infants with NC and the controls. None of the children exhibited haematuria.

Serum variables. Serum chloride was lower in the children with NC, but no other differences in serum variables or blood gas variables were found.

Urinary variables. The calcium/creatinine and calcium/sodium ratios and $\beta_2$-microglobulin excretion were significantly higher in the children with a history of neonatal NC.

Acetazolamide test. Only one of the children with a history of NC had an abnormal response in the oral acetazolamide test, with a U-B PCO$_2$ of -0.3 mmHg, while the result was regarded as normal in the rest of the cases and in all of the controls. The case with reduced hydrogen ion excretion capacity did not belong to the group with prolonged NC.

Other kidney function variables. Creatinine clearance did not differ between the cases and controls, but 10 out of the 20 children in each group had a value below the reference range of 89-165 ml/min/1.73m$^2$ (221), although the latter was not based on
results obtained using the Schwartz formula (222). FENa, FEK, TRP and T\textsubscript{m}P/GFR did not differ between the two groups.

_Infants with prolonged NC._ NC was prolonged in 6 children, of whom 3 still had abnormal renal medullary echodensities at the time of the kidney function tests. Five out of these 6 infants had chronic lung disease and were still receiving supplemental oxygen at term age, while this was so in only one of the other 14 cases and one of the controls. The venous blood gas values of the 6 children and their matched controls were as follows: pH 7.38 vs. 7.40 (p = 0.492), carbon dioxide tension 5.9 vs. 5.1 kPa (p = 0.110), bicarbonate 26.3 vs. 23.5 mmol/l (p = 0.010) and base excess 1.2 vs. -0.4 mmol/l (p = 0.055). The serum chloride concentration was significantly lower in the cases than in their controls (103.5 vs. 107.3 mmol/l; p = 0.018) whereas serum chloride concentrations in the remaining 14 case-control pairs were very similar (106.7 vs. 107.2 mmol/l; p = 0.578). The serum ionised calcium concentration was above the upper reference value of 1.29 mmol/l for age in each of the three children with NC, but when the whole group of 6 children with prolonged NC was compared with their matched controls the difference was not significant (1.30 vs. 1.27 mmol/l; p = 0.263).

5.2. **Nephrocalcinosis in infants treated with furosemide for cardiac failure (III)**

NC was found in 5 (4 males, 1 female) out of 36 infants (14%) with congestive heart failure, but in none of the controls. All the cases were detected at enrolment in the series, and no further cases were found during the ultrasonic follow-up of the infants who continued on furosemide treatment. No cases of nephrolithiasis were observed.

The infants in whom NC developed had been receiving significantly higher daily doses of furosemide than the unaffected ones. The urine calcium concentration was higher in the infants with congestive heart failure than in the controls and a similar trend was observed concerning the urine calcium/creatinine ratio, but these measures did not differ between the case infants with or without NC. Capillary blood pH was significantly higher in the infants with NC than without, but other blood gas values and serum calcium, ionised calcium, phosphorus, alkaline phosphatase, electrolytes and creatinine did not differ, nor did fractional excretion of sodium and potassium, creatinine clearance or tubular reabsorption of phosphate.

None of the five cases with NC had trisomy 21. NC was found before the cardiac operation in four out of the five infants, and ultrasonic resolution was observed in three cases at 12 months of age. The condition still persisted in the other two at 24 months, over 1 year after discontinuation of the diuretics.
5.3. Nephrocalcinosis in infants with congenital lactase deficiency (IV)

Clinical and laboratory findings. The mean age of the infants with congenital lactase deficiency at the time of diagnosis was 37 days. The patients were moderately dehydrated, eight of them weighing less than at birth, and they had a tendency for metabolic acidosis and hypernatraemia. PTH was determined in two patients, at levels of 10.4 and 7.1 ng/L, respectively (laboratory reference values 10-44 ng/L). Urinary 24-hour calcium excretion was 0.28 mmol/kg (11 mg/kg) in the only patient on whom collection had been performed at the time of diagnosis. Crystalluria was not observed in any of the patients.

Hypercalcaemia and nephrocalcinosis. Total serum calcium concentration was measured in 10 of the 11 infants on admission, and in seven of these the highest recorded level was above 2.70 mmol/L with a mean of 2.87 mmol/L. The serum ionised calcium concentration measured in one of the infants was 1.66 mmol/L at pH 7.4 (laboratory reference values 1.18-1.29 mmol/L). Five of the seven infants on whom renal ultrasonography was carried out had medullary echodensity and obvious NC. Hypercalcaemia ceased in all the infants less than a week after a lactose-free diet had been initiated and dehydration corrected.

Re-evaluation. After diagnosis all the children were kept on a lactose-free diet and their health, growth and overall development were uneventful. The mean serum calcium concentration at re-evaluation was 2.42 mmol/L (range 2.23-2.62 mmol/L) and mean serum creatinine was 41 µmol/L (range 24-62 µmol/L). The serum values for sodium, potassium, phosphate, urate, alkaline phosphatase and osmolality were also normal.

24-hour urine samples were collected from the eight continent children, and in only one of these did calcium excretion exceed 0.1 mmol/kg (4 mg/kg), the upper limit of normal values in children from 2 to 14 years of age (92). In the three youngest children, the molar calcium/creatinine ratio in random urine samples ranged from 0.05 to 0.34, i.e. they were well below 0.60 and were considered normal (102). Daily protein and β2-microglobulin concentration in the urine was normal in all cases.

NC was still seen in renal ultrasonography in two patients at the ages of four and five years, while in the other two who had medullary echodensity at diagnosis but had not been followed up until the time of the present study had complete resolution of NC at the ages of seven and ten years. In the one of the patients on whom renal ultrasonography was performed repeatedly at two months intervals, NC lasted up to the age of 6 months, and thereafter complete resolution took place. In one of the four children on whom renal ultrasonography had not been performed before, NC was revealed at the age of three years and had failed to improve one year later. NC was bilateral in every case.
6. Discussion

6.1. Diagnosis and differential diagnosis of nephrocalcinosis

NC is detected most sensitively by renal ultrasonography (6). Until the early 1980’s only the most extreme cases of NC and nephrolithiasis were seen by plain abdominal radiography, which was not accurate enough to verify milder cases of renal calcification (2, 223).

The ultrasonographic appearance of the medullary renal pyramids is normally hypoechoic relative to the renal cortex during the neonatal period, and other causes of abnormal hyperechogenicity in them such as papillary necrosis, vascular congestion, fungal infection or protein cast deposition in the renal tubules (19, 21) seem unlikely in preterm infants, as presented by series I. These other causes usually manifest themselves in the very first weeks of life, and no such findings were apparent in the first renal ultrasonography, performed by 2 weeks of age. Also, the furosemide-treated infants were not very seriously ill, so that the other causes of increased medullary echogenicities seemed unlikely in these, too. The infants with congenital lactase deficiency had hypercalcaemia (and thus hypercalciuria), which obviously predisposed them to NC. In both of the latter patient groups, however, a CT scan would have been a definitive method of ensuring that the hyperechogenic findings actually were related to abnormal calcium content in the renal medullary pyramids.

No signs of the many other disease entities with which NC has also been associated, such as X-linked hypophosphataemic rickets, medullary fibrosis and polycystic kidney disease, were noticed in any of the infants during follow-up (19, 21).
6.2. Nephrocalcinosis in VLBW infants

6.2.1. Incidence

Several reasons may be suggested for the somewhat lower overall incidence of NC seen in the series of VLBW infants than in previous reports. First, the incidence might have been greater if the series had been hospital-based instead of being population-based, as the infants referred to a university hospital from elsewhere tend to be more premature and more sick than average, and thus they have an increased risk of developing NC. Secondly, modes of therapy may have changed with time. Jacinto et al. (13) reported a 64% incidence of NC in VLBW infants who were receiving daily calcium supplementation of about 200-400 mg/kg, considerably more than the calcium intake of about 100 mg/kg/day in series I. Thirdly, surfactant treatment for respiratory distress syndrome was becoming more common at that time, possibly shortening the period of intensive care needed. Our figure is nevertheless quite close to the 27% incidence of NC reported by Short and Cooke in infants with <32 gestational weeks (195).

6.2.2. Risk factors

Concerning the role of furosemide medication before the detection of NC, our findings seem to contradict those of Short and Cooke (195), who showed that although the total dose of furosemide was greater in the infants that developed NC, the cumulative dose before the detection of calcifications was no higher than in the unaffected infants. In the present series, the cumulative dose of furosemide by the first eight weeks of life, i.e. the period during which most of the renal calcifications have developed (195, 196), was significantly higher in the infants who developed NC than in the unaffected ones. As we were not concerned primarily with the exact timing of NC but rather with screening and the later resolution of the condition, no definitive role can be established for furosemide medication in initiating NC in VLBW infants.

The notion that the risk of NC is greatest in the smallest, most premature and most sick infants was confirmed in this series, as the variables that point to more serious clinical condition in a neonate, such as parenteral nutrition, a prolonged demand for oxygen and assisted ventilation, more signs of acid-base imbalance and a need for furosemide and dexamethasone, were associated with an increased risk of NC (Table 5). It may be speculated, whether the disturbances in oxygenation, perfusion and acid-base balance, for example, make renal tissues more vulnerable to factors predisposing for the development of NC.
6.2.3. Classification

Although the classification proposed by Patriquin and Robitaille has been reported to be appropriate in connection with certain diseases associated with NC in older children (33, 37, 38), it has not been so convincing in prematurely born infants (41). The modified classification of pyramidal NC for preterm infants employed here seemed to be predictive in relation to the later ultrasonic resolution of the condition, although it fails to explain why the different distribution patterns have different rates of resolution. The fact that nephrocalcinotic changes seem to clear up more readily in the renal medullary tips than in the mid-pyramid areas might be related to solute concentrations, urine flow rate and pH, for example. On the other hand, it does seem that the peripheral type represents a milder form of NC with quicker ultrasonic resolution. These issues deserve further research.

6.2.4. Outcome

6.2.4.1. Ultrasonographic resolution of nephrocalcinosis

Present series described one population of preterm infants longitudinally, in that they were screened for NC in the neonatal period and followed up until ultrasonic resolution or up to 5-6 years of age. Although several studies have added to our knowledge of the timing of the ultrasonic resolution of NC in preterm infants (44, 45, 206, 207), no systematic investigation has previously been carried out into this. The results indicated that ultrasonographic recovery took place within the first year of life in more than half of all preterm-born infants with NC, whereas a quarter of those primarily affected had long-lasting renal changes, so that ultrasonic follow-up may be recommended. Renal ultrasonography carried out at 2-3 months of age seems sufficient for the routine screening of preterm infants for NC, while renal ultrasonography at 12 months of age would appear adequate for identifying individuals who have the greatest risk of developing long-lasting NC.

6.2.4.2. Kidney function

*Increased urinary calcium and β2-microglobulin excretion.* The finding of increased urinary calcium excretion is similar to that of Downing *et al.* in preterm infants at 1 to 2 years of age (207). In contrast with their results, which suggested that hypercalciuria was secondarily related to disturbances in renal sodium absorption, the results of series II nevertheless implied that not only was the urinary calcium/creatinine ratio increased in children with a history of NC, but the urinary calcium/sodium ratio was also higher. This tendency for actual hypercalciuria is an interesting finding. A primary propensity
for hypercalciuria during the neonatal period could be hypothesised as an additional risk factor for the development of NC, or else the finding may be explained by tubular dysfunction as a consequence of renal calcification. Interestingly, a report comparing XLH children: with and without NC found out that calcium excretion was significantly higher in those with NC (38). The study was retrospective, however, and the possible cause-effect relationships between NC and hypercalciuria remained unresolved (38). As decreased bone mineral density has been associated with idiopathic hypercalciuria in children (128) and with hypercalciuric nephrolithiasis in adults (129), this finding of increased calcium excretion in former preterm infants with a history of NC warrants more attention.

β₂-microglobulin is a low molecular weight protein found on the membrane of all nucleated mammalian cells. It is freely filtered by the glomerulus, but only little of it is excreted in the urine, as most is normally reabsorbed in the proximal tubule. The increased β₂-microglobulin excretion found in former VLBW weight infants with NC refers to disturbed functioning of the proximal tubule. In an earlier report, three preterm infants who had received long-term furosemide medication and had NC showed normal urinary β₂-microglobulin excretion at age one year (208), whereas Itami et al. reported increased urinary β₂-microglobulin in a full-term furosemide-treated infant with nephrolithiasis (224).

Acetazolamide test. Testing the ability to increase urine carbon dioxide tension after proper alkalinisation is regarded as a sensitive means of revealing a defect in distal tubule proton secretory capacity. Urine alkalinisation may be achieved either by a bicarbonate load or by acetazolamide, the latter being more convenient for children as it entails better tolerance and shorter duration (220). As urine pH successfully increased to >7.5 in all the children tested, the test response showing intact acidification ability in all but one case with neonatal NC seems methodologically acceptable. Downing et al. nevertheless found a significantly lower U-B PCO₂ in the acetazolamide test in preterm infants with NC than in controls (207). This inconsistency may be explained first by the fact that the children had been examined at younger ages than the ones described here and the tubular damage caused by NC may have been repaired during subsequent growth of the infants. Secondly, the group represented a more severe manifestation of renal calcification, with 4 out of the 10 infants exhibiting renal lithiasis as compared with none in the present series. The importance of nephrolithiasis in causing tubular dysfunction is also supported by a report concerning children with idiopathic hypercalciuria, in which 4 out of 6 patients with renal lithiasis showed evidence of impaired distal tubular acidification but none of the 14 other children (225).

Creatinine clearance. Estimated creatinine clearance seemed not to be specifically disturbed by NC at the age considered here, although assessment of the results was admittedly hampered by the lack of a correct reference range. Furthermore, estimated creatinine clearance by no means provides an accurate in reflection of GFR (226). Especially when GFR is low, the Schwartz method may give false normal results, due to tubular creatinine excretion (227). Radioisotopic plasma clearance methods provide a more accurate means for the clinical measurement of GFR, but they involve the disadvantage of radiation exposure, although quite low. Decreased estimated creatinine clearance was nevertheless associated with NC in former preterm infants at 1-2 years of age (207), and creatinine clearances were considered low in some of the children in two
other reports (45, 206), but unfortunately no control groups were employed in the latter. The specific effects of NC on glomerular and tubular function may not be easy to distinguish, because preterm infants are exposed to several other events that can potentially affect renal function, such as hypoxaemia, acidaemia, hypovolaemia and nephrotoxic drugs during their neonatal intensive care treatment period.

Chronic lung disease. Where the association between a prolonged need for oxygen and an increased risk of developing NC was noted by Short and Cooke (195), the present results suggest that chronic lung disease in preterm neonates is associated not only with an increased risk of developing neonatal NC but also with a prolongation of the time elapsing before ultrasonic resolution. The present finding of a trend for compensated respiratory acidosis to be associated with prolonged NC may imply some role for acid-base imbalance in maintaining the condition. The decrease in serum chloride level observed in these children by comparison with their controls is probably a reflection of a secondary response to the increased bicarbonate concentration intended to maintain electroneutrality.

6.3. Nephrocalcinosis in infants treated with furosemide for cardiac failure

The incidence of NC in the full-term infants treated with furosemide was well below the figures of 34 to 93% reported in furosemide-treated VLBW infants (8). Human and animal studies nevertheless suggest that the development of NC does not always require hypercalciuria (42, 174, 175), but that alterations in urinary inhibitors of crystal formation, urinary excretion of oxalate and urine pH may also play a role (8, 92).

The risk of developing NC was correlated with the dose of furosemide, and although higher urine calcium excretion was associated with administration of this diuretic, the urinary calcium excretion variables were not sensitive in detecting those in the population who had developed NC. A possible explanation may be that the calciuric effect of the drug has marked fluctuations or may decrease over time. Other properties apart from the calciuric action of furosemide may be operative in the development of NC, e.g. the diuretic therapy may alter urinary levels of citrate and magnesium, which are known inhibitors of crystal formation in the urine.

The higher capillary blood pH observed in the infants who developed NC may be related to the higher furosemide dose received by these infants compared with the others who were on furosemide. The actual biological significance of this pH difference may be doubted, however, especially in view of the fact that bicarbonate and other blood-gas values did not differ between the infants with and without NC.

Although the true advantages of changes in diuretic treatment regimens for preventing and treating NC have not been well established, it has been recommended that furosemide treatment should be combined with or replaced by thiazide diuretics in infants (9). In contrast to the calciuric action of furosemide, thiazides increase renal reabsorption of calcium and thus reduce urinary calcium excretion. Hufnagle et al. observed a fall in urinary calcium excretion and the resolution of renal calcifications in
four out of five infants after thiazide had been added to the diuretic regimen (7). In a
more recent report, the addition of thiazide failed to reduce calcium excretion in preterm
infants initially receiving furosemide treatment, and the authors speculated that the
caliuric effect of the sodium supplementation given to many preterm infants would
have overwhelmed the hypocalciuric effect of the thiazide (213).

The period soon after initiation of furosemide therapy appears to be sensitive with
regard to developing NC. All the cases of NC in the present series were revealed within
a few months of furosemide treatment, and no more cases were seen during the later
course of the treatment. Early manifestation of NC after the initiation of furosemide
treatment has also been demonstrated in animal models (174, 175).

6.4. Nephrocalcinosis in infants with congenital lactase deficiency

6.4.1. Causes of hypercalcaemia

Several mechanisms may be active in promoting hypercalcaemia in infants with
congenital lactase deficiency before treatment. Hypernatraemic dehydration per se would
cause hypocalcaemia rather than hypercalcaemia, however (228). Hyperalbuminaemia
due to dehydration could raise the total serum calcium concentration, but all the infants
but one in this series showed a normal or slightly depressed level of serum albumin.

Metabolic acidosis, at least in a chronic situation, has a significant effect on calcium
homeostasis. The buffering of acid by bone salts is markedly enhanced during chronic
metabolic acidosis (229). Bone demineralisation results from the release of calcium
carbonate from bone to neutralize excess hydrogen ions, a process that can lead to
rickets and growth retardation in cases of distal renal tubular acidosis in children, and to
osteopaenia in adults (111). Most of the infants in this series had marked metabolic
acidosis, which may thus have had at least an additive effect in leading towards a hyper-
calcaemic state.

Hyperparathyroidism seems an unlikely cause of hypercalcaemia, as the PTH level
was low in both of the infants for whom it was determined at the time of diagnosis.

Lactose intake has been known to influence calcium metabolism ever since Greenwald
and Gross observed in 1929 that parathyroidectomized dogs fed on a diet containing
lactose no longer exhibited tetany (230). Several studies have indicated that dietary
lactose directly stimulates ileal calcium absorption (231, 232), and it also improves bone
mineralisation in rats (233). The calcium absorption enhancing effect of lactose is not
dependent on the presence or absence of vitamin D (234). The precise mechanism of the
lactose effect, although an object of considerable research, has remained unclear (235),
and the data available from human studies are somewhat conflicting. In normal infants,
however, Ziegler and Fomon found enhanced absorption of calcium with a diet
containing lactose (236), while the lactose effect seems to be milder in lactase-deficient
adults than in lactase tolerant ones (237-239), that is, the lactose effect would seem to be
dependent on normal intestinal lactase activity. In contrast, Pansu and Chapuy reported
improved calcium absorption in lactose-intolerant patients who ingested lactose (240). It may also be the case, that ageing has an altering impact on lactose-effect. Although it is not certain, enhanced ileal calcium absorption by non-hydrolyzed lactose, in addition to the effect of metabolic acidosis, offers a feasible explanation for hypercalcaemia in infants with congenital lactase deficiency.

6.4.2. Causes of nephrocalcinosis

Several factors predispose infants with congenital lactose deficiency to the development of NC before treatment. Enhanced urinary calcium excretion inevitably results from hypercalcaemia, while metabolic acidosis, in addition to enhancing bone resorption (229), causes a reduction in the urinary citrate concentration and consequently increases the risk of the formation of insoluble calcium oxalate and phosphate crystals (59). Dehydration, which was present in most of the infants at the time of diagnosis, results in oliguria, which makes the urine more concentrated and conditions more favourable for crystallization.

6.5. Clinical implications

There remains significant difficulty, especially considering preterm infants, in assessing means of reducing the risk of NC. It is not yet definitely known whether renal calcification in VLBW infants is the result of immaturity itself (e.g. a tendency for hypercalciuria), of the demands for care that infants require (e.g. nutrition), of the diseases that these infants develop (e.g. metabolic acidosis reduces urinary citrate, an inhibitor of crystal formation), of the consequences of their treatment (e.g. furosemide, dexamethasone, theophylline, aminoglycosides) or of any combination of the above. It seems reasonable to assume, however, that attention paid to prevention of the causes of hypercalciuria and disturbances in homeostasis may at least to some extent reduce the risk of this complication.

From a clinical point of view, knowing the lack of controlled trials in the field, some suggestions for reducing the risk of infants developing NC can be made. In an attempt to reduce urinary calcium excretion, phosphate and potassium depletion should be actively treated and avoided in preterm infants, while sodium supplementation should be used with caution. When clinically relevant, diuretic treatment with furosemide may be replaced or combined with thiazides in preterm infants and in infants with congestive heart failure. Repeated loose stools immediately after birth should alert the clinician to take into account the possibility of congenital lactase deficiency, in order to diagnose it quickly and prevent the development of dehydration, metabolic acidosis, hypercalcaemia and subsequent NC.

Ultrasonographic follow-up of infants with NC may be recommended annually for the first years of childhood until resolution. The follow-up of the children with prolonged
NC needs to be assessed *in casu*. Thiazide treatment may be considered in refractory cases, especially when furosemide therapy has continued for compelling reasons, or when marked calcium excretion has been established.
7. Conclusions

1. Approximately 20% of VLBW infants may develop NC during the first three months of life, the most premature and sickest infants exhibiting the greatest risk. Therapy with calciuric drugs such as furosemide carries an increased risk of NC but it may develop even without such treatment.

2. In about half of the affected infants the renal medullary changes are restricted and transient, but more extensive types may last several years. The classification of the medullary changes involved in NC used here seemed to serve well to predict the later ultrasonic resolution.

3. Some signs of renal tubular dysfunction, such as increased urinary excretion of calcium and \( \beta_2 \)-microglobulin, seem to be related to prematurity-associated NC in early childhood, but no glomerular function decline is specifically related to NC in prematurely born children.

4. The long-term furosemide treatment prescribed for full-term infants with congestive heart failure seems to entail a considerable risk of the development of NC. Despite discontinuation of furosemide medication, NC may persist for years in some patients.

5. Hypercalcaemia and NC seem to be frequent in infants with congenital lactase deficiency before treatment. Hypercalcaemia resolves itself in a few days after a lactose-free diet has been started and dehydration corrected, but NC may last several years in some patients. The mechanism of hypercalcaemia is not clear, but it may be related to metabolic acidosis and/or increased absorption of calcium induced by non-hydrolysed lactose in the gut.
8. References


