RENAL FUNCTION IN THE PRETERM NEONATE AND THE NEWBORN RABBIT

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aan mijn vader

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CONTENTS page

ABBREVIATIONS

ANP	Atrial natriuretic peptide
AV	Artificial ventilation
AVP	Arginine vasopressin
BW	Birth weight
C_{H2O}	Free water clearance
C_{inulin}	Inulin clearance
C_{osmol}	Osmolar clearance
C_{PAH}	Clearance of para-aminohippurate
E_{PAH}	Extraction of para-aminohippurate
FE bicarbonate	Fractional bicarbonate excretion
FENa	Fractional sodium excretion
GA	Gestational age
GFR	Glomerular filtration rate
ID	Indomethacin
Kf	Ultrafiltration coefficient
MAP	Mean arterial pressure
PA	Postnatal age
pda	Persistent ductus arteriosus
PG	Prostaglandin(s)
PROM	Premature rupture of membranes
RBF	Renal blood flow
RDS	Respiratory distress syndrome
RVR	Renal vascular resistance
SV	Spontaneous ventilation
$T_{\frac{1}{2}}$	Plasma half life.

Chapter 1 INTRODUCTION

The transition from an aquatic environment in the fetus to an avian environment postnatally involves adaptations, which require the interactions of major organ systems such as the cardiovascular, pulmonary and also the renal system. Prematurity i.e. a birth before the 37^{th} week of gestation occurs in about 7% of deliveries. This means that about 13.500 neonates a year are born prematurely in the Netherlands.

Impairment of renal function of the preterm neonate might have serious consequences on postnatal adaptation. It has been shown that kidney function in preterm neonates differs from that in term infants. This is due both to anatomic and functional immaturity. Anatomic investigations performed in 1943 (Potter and Thierstein) and 1959 (McDonald and Emery) demonstrated that nefrogenesis continues until the 35^{th} week of gestation. Fetterman et al. (1965) showed that tubular maturation continues for a far longer period.

Data concerning development of renal function in neonates appeared during the last 40 years. Normal values for glomerular filtration rate (GFR) in term neonates were published by Dean and Mc Cance (1947). Barnett et al. (1948) established normal values for GFR in preterm neonates. GFR in preterm infants was low and increased after birth, demonstrating a limitation in glomerular function. Studies on tubular function in the neonate started in the same period. Dean and McCance (1949) were the first to study renal sodium excretion in young subjects. They found that term infants had a reduced ability to excrete sodium loads compared to later in life.

McCance and Widdowson (1953) analysed the constituents of urine in fetuses and in early human life. The first data about urine osmolality and maximal concentration capacity are from Heller (1944) and Smith et al. (1949). These studies have been supplemented by many others and provided evidence that tubular immaturity is present during the neonatal period and infancy, being more pronounced in the preterm neonate. The most prominent features of this immaturity in the preterm neonate are tubular sodium loss, a low maximal urine osmolality and a low serum bicarbonate level compared to the fullterm neonate.

Extrarenal problems in the preterm neonate can have major impact on renal function. The most well known example is respiratory distress syndrome (RDS). In two publications of the same group (Torrado et al., 1974; Guignard et al., 1976) a reduction in GFR is observed during RDS. These data are supported by Cort (1962) and Tulassay et al. (1979). In addition acidifying capacity of the kidneys is limited in infants during RDS (Torrado et al., 1974; Allen and Usher, 1971). Changes observed during RDS can originate from a number of factors, existing in a different degree. Hypoxemia, hypercapnia, acidosis and frequently a drop in systemic blood pressure can be present in this syndrome. The studies done so far have not revealed whether only one or more of these factors cause the reduced renal function.

The purpose of this thesis was to study renal functional changes related to extra-renal influences. Two studies were performed in the preterm neonate, the two other studies in the newborn rabbit. The following studies were done:

A. The effect of isolated hypercapnia on renal function in the newborn rabbit.

   Hypercapnia is assumed to be one of the main causes of decreased renal function in RDS. Studies about the effect of hypercapnia on renal function in neonates or neonatal animal models are however lacking.

B. The effect of acute acid-base derangements on renal bicarbonate handling in the newborn rabbit.

   Bicarbonate levels in neonates are low compared to infants and adults, suggesting a limitation in bicarbonate handling in the preterm infant. This study was undertaken to provide further insight in the mechanisms of and influences on renal bicarbonate handling during development.

The newborn rabbit was chosen as experimental model for several reasons. Firstly, nefrogenesis proceeds after birth and finishes after about 14 days of extrauterine life (Kazimierczak, 1963). This developmental stage resembles that of the preterm infant. Secondly, normal values of renal function have already been provided in this newborn animal under basal conditions (Cotting and Guignard, 1982) as well as during hypoxaemia (Valloton, 1984). These data allow better interpretation of our own experiments on respiratory acidosis. Thirdly, tubular perfusion studies in the

newborn rabbit demonstrate a limitation in bicarbonate reabsorptive capacity (Schwartz and Evans, 1983), while data on overall bicarbonate handling in this animal are lacking.

C. The development of glomerular function in the preterm neonate.
The GFR in the preterm neonate is known to increase with advancing gestational age and postnatal age. In this study we evaluated the changes in GFR in preterm neonates from 27-36 weeks with and without respiratory problems.

D. The effect of prenatal exposure to Indomethacin on renal function in preterm neonates during the first week of life.
Indomethacin is known to impair renal blood flow (RBF) and GFR in experimental animals and in neonates, exposed after birth. GFR was evaluated by the continuous inulin infusion method, in circumstances where the neonate was already exposed in utero.

A review of the relevant literature about renal functional development in the preterm human neonate precedes the experimental studies.

Chapter 2 reviews data from literature on glomerular function, tubular function and renal acid-base regulation in the preterm infant.

Chapter 3 deals with the effect of acute isolated hypercapnic acidosis on renal function in the newborn rabbit.

Chapter 4 is a study on bicarbonate handling in the newborn rabbit during acute changes in acid-base balance.

In chapter 5 the development of glomerular function in the preterm neonate and its relationship to gestational age and postnatal age is studied using the continuous inulin infusion technique.

Chapter 6 describes the effect of exposure to indomethacin during pregnancy on renal function in preterm neonates.

Chapter 7 reviews the results of the chapters 3 - 6 and discusses the relevant data from literature. In the final paragraph conclusions concerning clinical implications of these studies are presented.

Chapter 2 RENAL FUNCTION IN THE PRETERM NEONATE

2.A. DEVELOPMENT AND EVALUATION OF GLOMERULAR FILTRATION IN THE PRETERM NEONATE

2.A.1. Abstract

Glomerulogenesis in the human being finishes after 35-36 weeks of gestation. Changes in glomerular function after birth are the result of changes in net ultrafiltration pressure, ultrafiltration coefficient and Renal Blood Flow (RBF). The markedly lower RBF seems mainly responsible for the lowered glomerular filtration rate (GFR) in the neonate.

In this chapter we discuss the different methods applicated in the preterm newborn to evaluate changes in GFR. Only the classic inulin clearance as well as the continuous inulin infusion technique provide exact data, but are not easy to perform. In general pediatric practice the use of tables for "normal values" of serum creatinine is advised. Patient values can be compared to those "normal values".

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2.A.2. Introduction

Nefrogenesis in the human fetus begins in the middle of the third week of pregnancy with the appearance of the rudimentary, non-functioning pronephros. Although the nephrotomes hollow out and become vesicles, there is no formation of true nephrons. By the end of the fourth week the pronephric nephrotomes and the cranial portion of the pronephric system have been resorbed; the formation of the mesonephros begins. The mesonephros obliterates also and all but those portions that persist beyond fetal life have disappeared by the third month. An outgrowth of the mesonephric duct forms the ureteric buds, necessary for differentiation of the metanephros. The ureteric buds together with the metanephric system, appearing at about the fifth week of gestation develop into the final kidney.

The formation of glomeruli goes on up to the 35^{th} week of gestation and urine production starts as early as around the 9^{th} to 12^{th} week of gestation. This is the main source of amniotic fluid. Urine production increases during pregnancy. Mean hourly urine flow is about 12 ml at 32 weeks, increasing up to 28 ml at 40 weeks of gestation (Campbell et al., 1973). The fetal urine is hypotonic during pregnancy with sodium and chloride as major components. The amounts of creatinine and urea present in the urine reflect the development of glomerular filtration: the urine-to-plasma ratio of these substances is greater than 1 (McCance and Widdowson, 1953). However, only the placental barrier is responsible for the elimination of waste products.

Glomerular filtration depends on the net ultrafiltration pressure across the glomerular membrane, the ultrafiltration coefficient (Kf) and on the plasma flow through the glomerular capillaries.

The net ultrafiltration pressure is the resultant of differences in hydrostatic pressure along the glomerular membrane and the osmotic pressure of the non-filtered colloids. The ultrafiltration coefficient depends on the total capillary surface area and the permeability per unit of surface area. The plasma flow depends on systemic blood pressure, renal vascular resistance and the hematocrit.

Changes in GFR, present in neonates, will so depend on changes in one or more of the above mentioned factors. Animal experiments provide evidence for these changes. The development of glomerular ultrafiltration in the rat was extensively studied by Ichikawa et al. (1979). Equality between efferent osmotic pressure and transglomerular hydraulic pressure differences was usually achieved in immature rats (30-45 days old), indicating

that the ultrafiltration coefficient (Kf) is not the factor limiting GFR in these rats.

The mean value for transcapillary pressure differences, P, is slightly lower in immature than in adult rats, on average about 3 mm Hg. It is, therefore, the markedly lower glomerular plasma flow (79 $\pm$ 5 vs 136 $\pm$ 10 nl/min per gram kidney weight), which is responsible for the lower GFR in immature rats. We can conclude that the immature kidney has a high arteriolar resistance with a concomittant low plasma flow. Also other experimental studies indicate, that renal vascular resistance decreases postnatally and renal perfusion increases (Aperia and Herin, 1975; Gruskin et al., 1970; Spitzer and Edelmann, 1971). Systemic blood pressure in the human neonate increases rapidly (Versmold et al., 1981), facilitating an increase in renal plasma flow.

GFR in the human neonate is low compared to infants and adults. This low GFR depends probably on the presence of a high renal vascular resistance before delivery, decreasing thereafter, as is suggested by the above mentioned experimental data.

In addition to this development of renal function it is well known that also glomerular structures are still developing until the 35^{th} week of gestation.

2.A.3. Measurement of GFR

Many methods have been used to assess GFR in the preterm neonate. All have however their limitations, either the complexity of the method or the inaccuracy in establishing GFR.

1. Inulin clearance

   Inulin, a vegetable fructose polysaccharide with a molecular weight of about 5000 Dalton, is totally filtered even in the very young preterm neonate (Coulthard and Ruddock, 1983B) and may be considered as an ideal marker of glomerular filtration.

   The traditional inulin clearance requires intravenous infusion and correctly timed urine collection periods. A bolus injection of inulin precedes a continuous inulin infusion. An equilibration time of 60-90 minutes is needed, after which urine is collected, using 3-4 collection periods of 20-30 minutes each. In between the collection periods blood samples are taken to determine the inulin concentration. Inulin clea-

rance is calculated from urinary excretion divided by the inulin plasma concentration.

($C_{in} = \frac{U_{in} \cdot V}{P_{in}}$; C_{in} = inulin clearance; U_{in} = inulin urine concentration; V = urinary volume; P_{in} = inulin plasma concentration).

Bladder catheterisation is needed, which can easily lead to urethral damage especially in the very small neonate. A considerable risk for introducing infections exists. Intravenous infusion is necessary.

This technique has been used by different groups. Strauss et al. (1981) investigated GFR in healthy fullterm infants during the first 4 hours of extrauterine life. They found a GFR varying from 0.5 to 9 ml/min during the first hour of extrauterine life, declining to 0.8-3.7 ml/min after 4 hours. Guignard et al. (1975) measured GFR in 22 newborns with a postnatal age varying from 1-22 days, and found an increase in GFR from 10 ml/min per m^2 on day 1 to 30 ml/min per m^2 on day 12 postnatally. Only 2 neonates with a gestational age of less than 35 weeks were included in this study, so later on data of another 12 preterm neonates were added (Fawer et al., 1979A). An increase in GFR expressed in ml/min per m^2, which correlated with gestational age was found in the preterm neonates in contrast to term neonates. GFR in the latter group showed no rise with increasing gestational age. Postnatally a significant rise in GFR was present in all investigated infants (figure 1).

2. Creatinine clearance

The excretion of creatinine in the urine per time unit, divided by the plasma creatinine level is used as a measure for GFR in this method. An accurate urine collection is required. This is a difficult procedure, especially in the preterm female neonate. Urine loss due to leakage along collection bags occurs frequently. Moreover serum creatinine values are rapidly changing during the first days of life (Sertel and Scopes, 1973). Creatinine clearance is known to overestimate real GFR. The method is broadly applicated, while intravenous infusion is no prerogative in contrast to all other methods (Aperia et al., 1981A; Siegel and Oh, 1976; Arant, 1978). Aperia found a good correlation between inulin and creatinine clearance using a rather small number of patients (1981A). Siegel (1976) and Arant (1978) did not observe a postnatal increase in GFR using creatinine clearances, in contrast to the study of Fawer et al. (1979A).

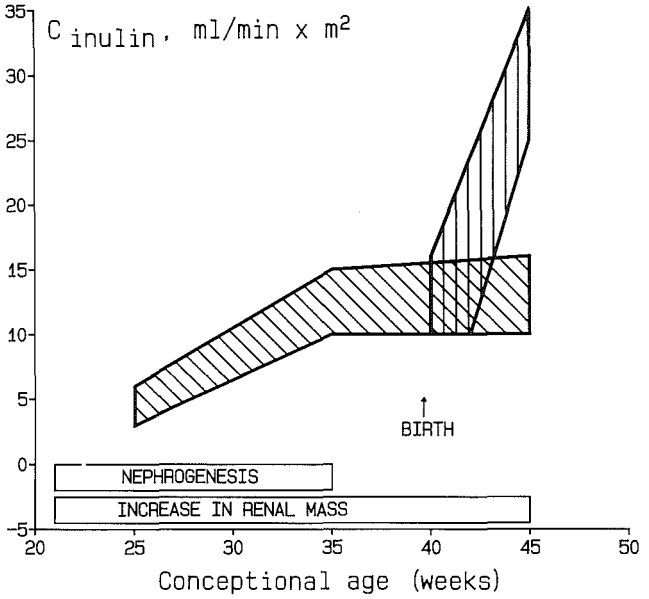

Figure I Maturation of GFR in relation to conceptional age (from Fawer et al., 1979A). Published with permission.

3. Inulin single injection technique.

In this technique inulin clearance is calculated using the plasma disappearance curve after one single dose of inulin. Calculations are made on the basis of the two-compartmental model proposed by Sapirstein et al. (1955). Repeated blood sampling is needed to calculate the inulin disappearance rate. No urine collection is needed.

Fawer et al. (1979B), demonstrated, that the use of this method leads to overestimation of GFR in term neonates during the first four months of life. The inaccuracy of calculated GFR may be rather large in preterm infants with their high and changing extracellular volume and their low GFR. Coulthard (1983A) demonstrated indeed that overestimation of GFR in the preterm neonate is a major disadvantage of the technique. The technique has been used by Broberger and Aperia (1978) in a study on the effect of respiratory distress on neonatal renal

function and by Catterton et al., (1980) in a study on the effect of indomethacin on GFR.

Svenningsen (1975) applied the method with polyfructosan, an inulinlike substance with a somewhat lower molecular weight. It is however unknown if polyfructosan is as reliable as inulin in the preterm neonate, while the disadvantages of the single injection technique remain present.

4. Continuous inulin infusion

During a constant inulin infusion a steady state can be reached, in which the inulin infusion rate equals urinary inulin excretion rate, assuming that no other way for inulin excretion exists. This means that inulin clearance can be calculated from the infused amount of inulin per time unit divided by the plasma inulin concentration ($C_{in} = \frac{I.R}{P_{in}} = \frac{U_{in} \cdot V}{P_{in}}$; C = inulin clearance; P_{in} = inulin plasmaconcentration; I = inulin concentration in infusion; R = infusion rate; U_{in} = inulin urine concentration; V = urinary volume). This method has for the first time been applicated to the preterm neonate by Leake et al. (1976), demonstrating stable inulin plasma levels after 78 minutes of continuous inulin infusion. Coulthard (1983A) demonstrated however that it takes at least 18 to 24 hours before steady state plasma concentrations are reached. We could confirm his data. The mean GFR in his study was 0.84 ml/min per kg. No correlation between GFR and postnatal age was found in his study.

5. Other methods No radioisotopic evaluation of GFR in preterm newborns has as yet been performed.

2.A.4. Conclusions

Many investigations on GFR in neonates have been performed. Unfortunately, the results of these studies vary and are frequently conflicting. Moreover GFR has been expressed in many different ways (ml/min, ml/min per kg, ml/min per m^2, ml/min per 1.73 m^2), which still increases confusion.

When GFR is expressed per body surface area, studies have to be used, in which a surprisingly small number of antropometric data on very young infants is included (Boys, 1935; Sendroy and Cecchini, 1954; Haycock et al., 1978). This may artificially increase or decrease the observed GFR. It

would be more logical to express GFR only in ml/min or in ml/min per kg (Coulthard and Hey, 1984).

The traditional inulin clearance and the continuous inulin infusion provide in our opinion the most reliable data on GFR. Both have however their restrictions. The traditional inulin clearance requires bladder catheterisation, the continuous infusion requires a fairly long infusion period. It seems therefore reasonable to reserve these techniques only for patients, in whom exact evaluation of GFR is of major clinical importance and for scientific studies. Repeated measurements of plasma creatinine values, using a reliable technique as for instance the Jaffé method will be sufficient in most clinical situations. The creatinine values obtained can be compared with "normal values" as reported in literature (Stonestreet and Oh, 1978; Feldman and Guignard, 1982; Rudd et al., 1983; Trompeter et al., 1983). In table I a useful example of normal values from the study of Rudd et al. (1983) is presented. Relative small changes in plasma creatinine values may reflect major changes in GFR.

PLASMA CREATININE VALUES IN THE FIRST MONTH OF LIFE

Gestation (weeks)	Postnatal age (days)						
	2	2	2	7	14	21	28
	All babies	Breathing Spontaneously	Ventilated	All babies			
28	116 ± 40 (n = 24)	108 ± 20 (n = 8)	121 ± 45 (n = 16)	84 ± 32 (n = 22)	72 ± 32 (n = 25)	60 ± 33 (n = 25)	58 ± 24 (n = 24)
29-32	104 ± 38 (n = 50)	100 ± 32 (n = 36)	115 ± 43 (n = 16)	83 ± 41 (n = 56)	69 ± 32 (n = 42)	59 ± 32 (n = 29)	52 ± 33 (n = 30)
33-36	93 ± 39 (n = 36)	94 ± 39 (n = 32)	87 ± 46 (n = 4)	68 ± 44 (n = 31)	55 ± 36 (n = 27)	50 ± 37 (n = 20)	35 ± 24 (n = 12)
37-42	75 ± 38 (n = 27)	75 ± 38 (n = 27)		50 ± 36 (n = 39)	38 ± 20 (n = 19)	35 ± 20 (n = 19)	30 ± 18 (n = 15)

Mean plasma creatinine μmol/l ± 2 SD.
Conversion SI to traditional units: 1 μmol/l ≈ 0.0113 mg/100 ml

Table I Plasma creatinine values during the first month of life. (from Rudd et al., 1983). Published with permission.

Chapter 2 RENAL FUNCTION IN THE PRETERM NEONATE

2.B. RENAL TUBULAR FUNCTION IN THE PRETERM NEONATE

2.B.1. Abstract

Renal tubular function and functional changes in the preterm neonate are discussed. The proximal tubule has a limited capacity to preserve sodium, leading to a daily sodium need of 3 to 5 meq/kg/day. The reabsorption of glucose, phosphate and aminoacids is also low compared to older children but increases quickly after birth. At the level of the distal tubule, a temporary insensitivity for aldosteron leads to a lowered sodium-potassium exchange. Concentration capacity is restricted with a maximal urine osmolality of about 360 mosm/liter. The kidneys are despite this immature tubular function capable to preserve their homeostatic function.

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2.B.2. Introduction

Anatomic investigations into dimensions and characteristics of the nephrons in the kidney at birth demonstrate that glomerular maturation precedes tubular maturation. Fetterman et al. (1965) demonstrated this in morfometric studies of subjects varying in age from 0 to 18 years. Glomerulogenesis finishes after 35-36 weeks of gestation, tubular maturation continues for a far longer period. These anatomic data indicate that glomerulotubular disbalance may exist in the preterm infant. In this chapter we review the literature on tubular function, functional development and adaptation after birth in the preterm neonate.

2.B.3. Glomerulotubular balance, β_2 microglobulin clearance and Na/K homeostasis

Differences between glomerular and tubular morfological development may probably lead to renal loss of solutes and water, especially in the very young newborn (glomerulotubular disbalance). Differences of dimensions of nephrons between newborns and adults are striking. Proximal tubuli are markedly underdeveloped relative to their corresponding glomeruli. This proximal convoluted tubule is the segment of the nephron, which undergoes most of the growth during development. Even in the proximal convoluted tubule differences in growth have been demonstrated. The luminal surface area, through which phosphate is cotransported with sodium, increases for instance several-fold more than the total length of the proximal tubule (Hay and Evans, 1979). Heterogeneity between individual nephrons at the same level of the cortex is also evident. Thereabove the dimensions of glomeruli and nephrons in the outer cortex of the immature kidney prove to be more markedly smaller than those in the inner cortex, compared with the adult kidney. Animal experiments show however that glomerulotubular balance exists in the newborn experimental animal (Kon et al., 1984).
The fractional reabsorption of beta-2-microglobulin, a small naturally occurring protein with a molecular weight of 11.800 Dalton is a good marker of proximal tubular function. Aperia et al. (1979A,1983B) demonstrated that this fractional reabsorption of beta-2-microglobulin increased during gestation reaching a level of 98% of the filtered amount after 35 weeks of gestation (figure 1). Thereafter the increase in beta-2-microglobulin reabsorption still continues, but in a much slower way until the 21st month of life (van Oort et al., 1980).

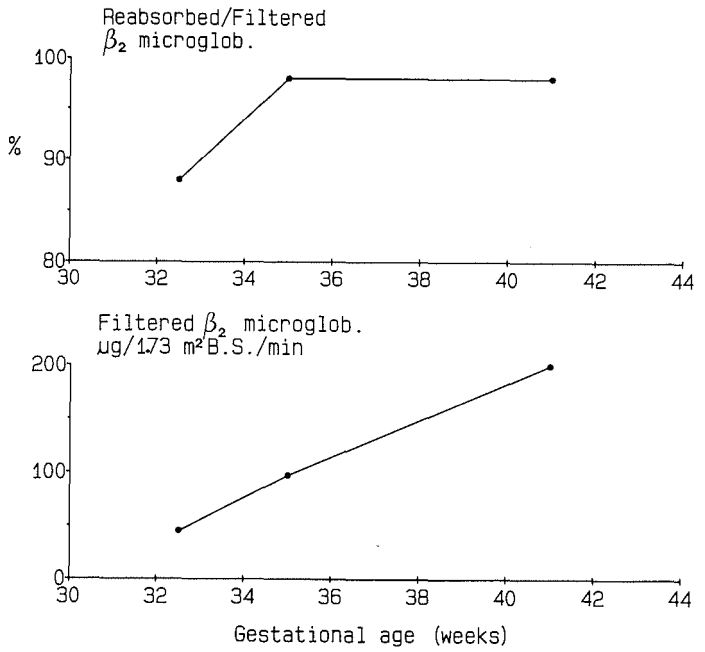

Figure I Fractionally reabsorbed and filtered beta-2-microglobulin in relation to gestational age in healthy 4-6 days old preterm and term infants. (from Aperia and Broberger, 1979A). Published with permission.

The value of this marker has wrongly been critisized by Engle and Arant (1983). They describe in a study on renal handling of beta-2-microglobulin in the human neonate a strong dependency of the excretion of this substance on urinary flow rate. However, they did not take a number of factors into consideration. Firstly urine flow is known to influence urine pH (Reid and Hills, 1965). Secondly the determination of beta-2-microglobulin in urine is already influenced by the urine pH in the bladder (Statius van Eps and Schardijn, 1983); a urine pH lower than 6 makes the determination unreliable. Thirdly correction of urine pH after micturation by alkalinisation before determining the levels of beta-2-microglobulin is not correct.

Sodium balance is influenced by the suboptimal glomerulotubular balance in the preterm neonate. Basal urinary sodium excretion in preterm infants is

higher than in full term infants. Sodium excretion is inversely related to gestational age and postnatal age (Al-Dahhan et al., 1983A; Siegel, 1982). The high sodium excretion is mainly due to an immaturity of the proximal tubules, the distal tubule being incapable to cope with the high sodium delivery. This can be deduced from experimental animal studies (Aperia and Elinder, 1981B) as well as from studies in neonates (Rodriguez-Soriano et al., 1983). A large fluid intake postnatally maintains extracellular volume expansion, already present at birth, and leads to limitations in sodium reabsorption. Lorentz et al. (1982) described a minimal sodium need of 3 meq per kg a day in preterm neonates with birth weights varying from 750 to 995 gram, in whom fluid intake was restricted: The infants received 70 ml per kg a day on the first day of life and input was gradually increased to 140 ml per kg a day on the fifth day of life. Al-Dahhan et al. (1983A) described a minimal sodium need of 5 meq per kg a day when fluid intake was higher; the infants in their study received about 100 ml per kg a day already on the first day of life. Also hormones may contribute to renal sodium loss. Aperia et al. (1979B) demonstrated that aldosteron excretion, being high in both preterm and fullterm neonates, does not correlate with the urinary sodium-potassium ratio in preterm neonates during the first week of life. Sodium- potassium ratio in the urine of preterm neonates is high, so a temporary unresponsiveness to aldosteron is supposed to be present. The role of other hormones remains to be defined. In recent studies high levels of atrial natriuretic peptide (ANP) have been found in preterm neonates especially in those with persistent ductus arteriosus (Lang et al., 1985; Andersson et al., 1987). The effect of ANP on renal salt wasting in these infants has to be elucidated. Also little or nothing is known about the influences of kallikrein, prostaglandins and substance P (a neurotransmitter with natriuretic properties) on renal salt handling in the preterm neonate. Not only salt depletion but also inappropriate secretion of arginin vasopressin (AVP) may cause hyponatriaemia in the preterm infant (Rees et al., 1984A). However, concentrating ability of the kidneys in preterm infants is rather limited, so the influence of AVP is surely less important compared to later in life. Medical closure of the persistent ductus arteriosus by indomethacin frequently results in the development of a dilution hyponatriaemia (Seyberth et al., 1983). This is related to the negative effect of indomethacin on water excretion by the kidneys.

Another possible cause for hyponatriaemia in the preterm infants may be chronic use of diuretics by pregnant women before delivery. An inefficient intestinal sodium absorption in the gastrointestinal tract in those infants is too small to play an important role in sodium homeostasis (Al Dahhan et al., 1983B).

The early hypernatriaemia, sometimes observed in immature infants is in general caused by their insensible water losses. It has also been described after intravenous administration of sodium, for instance, hypertonic sodiumbicarbonate during resuscitation.

Potassium regulation is strongly related to sodium regulation. Preterm infants are in a positive potassium balance under normal conditions. The described temporary unresponsiveness to Aldosteron (Aperia et al., 1979B) can even lead to hyperkaliaemia. In contrast also hypokaliaemia in critically ill preterm neonates due to renal potassium wasting is mentioned (Engle and Arant, 1984). A good regular control of potassium balance in the tiny neonate is important.

2.B.4. Renal water handling

The mechanisms involved in renal water conservation and excretion by the newborn kidneys are comparable to those in the mature kidney. Certain special limitations, unique to the immature infant, are however present.

Water conservation is determined by the GFR and the concentrating capacity of the kidneys. Glomerular filtration is low. The osmolar gradient in the renal medulla is low, probably due to the intrauterine existing polyuria. In addition the countercurrent multiplier system is less effective compared to the adult, as the loop of Henle is shorter and the NaCl supply to the loop is decreased, due to the low GFR. Insufficient AVP production in the preterm infant is improbable. Stimulation of AVP is possible in the very young infant and already demonstrated at 26 weeks of gestation (Rees et al., 1984B). AVP concentrations are high perinatally even in preterm infants (Pohjavuori and Fyhrquist, 1980). Concentrating ability of the preterm kidney is low, due to the above mentioned factors. Svenningsen and Aronson (1974B) described a urine osmolality of 359 mosm/kg after administration of exogenous DDAVP in preterm neonates with a gestational age varying from 30-35 weeks. Concentration capacity increases rapidly postnatally and after 4-6 weeks urine osmolality can reach values of 425-670 mosm/kg. A low maximal urine osmolality implicates that the minimal amount

of urine, necessary for eliminating the by the body produced solutes is high. Ten mosm per kg a day need to be excreted (Holliday and Segar, 1957). Maximal urine osmolality is about 360 mosm/kg (Svenningsen and Aronson, 1974B). So a minimal urine production of 27 ml/kg a day (about 1 ml/kg per hour) is required.
Water excretion depends on GFR and diluting capacity. The newborn can optimally dilute his urine and a urine osmolality between 30 and 50 mosm/kg H_2O can be reached (Aperia et al., 1983A; Coulthard and Hey, 1985). A water load of 200 ml/kg a day can be excreted without an increment in GFR and without evidence that maximal diuresis has been achieved; neither the osmolar excretion rate nor plasma osmolality changes during this high fluid intake. (Coulthard and Hey, 1985).

2.B.5. Acid-base balance

This subject is separately discussed in chapter 2C.

2.B.6. Glucose, Phosphate and aminoacid reabsorption

Reabsorption of glucose, phosphate and aminoacids occurs in the proximal tubule. Tudvad and Vesterdal described already in 1953 that renal transport capacity for glucose is limited in preterm infants. Glucosuria can be present when serum glucose concentration is as low as 5.5 mmol/L. Tubular reabsorptive capacity for glucose has more extensively been studied by Arant (1978). A glucose reabsorption of 92% of the filtered load is present in neonates with a gestational age of 30 weeks, increasing to 99.7% at 34 weeks of gestation, reflecting an increasing tubular transport capacity with gestational age. A significant increase in glucose excretion combined with sodium and solute excretion has been reported during parenteral feeding (Stonestreet et al., 1980).
Phosphaturia in the preterm neonate is described by Mc Crory et al. (1952). More recently Karlen et al. (1985) confirmed and further analysed this observation. Fractional phosphate excretion is high during the first week of life in the preterm infant compared to full term infants (20% vs 3%). After this first week urinary phosphate excretion decreases quickly and is even lower than observed in older children (Brodehl et al., 1982). This seems to be an adaptive response to increase and preserve phosphate stores. The data are supported by phosphate balance studies in young

growing rats (Caverzasio et al., 1982). Rapidly growing young rats have a higher phosphate reabsorption than older rats.
Aminoaciduria also occurs in preterm neonates (O'Brien and Butterfield, 1963). Brodehl and Gelissen (1968) investigated renal reabsorptive capacity for aminoacids in term infants and found limitations in reabsorption compared to later in life. Although studies in preterm neonates are lacking we assume that they probably have an even lower reabsorptive capacity.

2.B.7. Conclusions

Differences in tubular function exist between the preterm and fullterm neonate on proximal as well as on distal tubular level (table I). These differences depend on renal and extrarenal factors.
The most reliable marker for proximal tubular function is β_2 microglobulin. Studies on reabsorption of β_2 microglobulin reveale a quick maturation of proximal tubular reabsorptive capacity until the 35^{th} week of gestation. The capacity of the proximal tubules in reclaiming filtered sodium is limited, which cannot fully be compensated at distal tubular level. Also limitations in reabsorption of glucose, phosphate and aminoacids have been demonstrated with a quick increase of tubular transport capacity during the first weeks of life. A temporary unresponsiveness for aldosterone is present on the level of the distal tubule, leading to an elevated Na^+/K^+ ratio in the urine of preterm infants. Also a limitation in concentrating ability of the immature kidney is present. Extrarenal factors can affect renal tubular function. The high extracellular volume together with the high fluid intake is the most striking extrarenal factor. It stimulates natriuresis and diminishes tubular reabsorption of solutes.
Glomerulotubular balance for solutes like glucose and phosphate establishes itself quickly despite the presence of anatomically more mature glomeruli compared to the tubuli and despite the presence of an immature tubular function.
The high sodium loss in preterm infants as well as the pattern of maturation of beta-2-microglobulin reflects most likely glomerulotubular imbalance at the level of the proximal tubule (Aperia et al., 1983B; Arant, 1978).

TABLE I RENAL TUBULAR FUNCTION PARAMETERS IN THE NEONATE. (References see text).

Age	< 34 weeks	⩾ 34 weeks
H_2O balance maximal urineosmolality	359	> 360
Na balance Na need mmol/kg/24 u	3-5	1-2
minimal FENa %	2-3 %	1-2 %
Na/K ratio urine	6	2-2,5
Acid-base balance serum HCO^-_3 level mmol/l	> 14	18-22
acid-excretory capacity	decreased	decreased
Reabsorption phosphate	decreased the 1st week of life	normal (> 85 %)
glucose	94 % in the 1st week of life	normal (> 99 %)
amino-acids	decreased	decreased

Chapter 2 RENAL FUNCTION IN THE PRETERM NEONATE

2.C. MECHANISMS OF RENAL TUBULAR ACIDIFICATION

2.C.1. General aspects

Renal regulation of acid-base balance consists of reabsorption of filtered bicarbonate and net excretion of hydrogen ions in the form of either ammonium (NH_4^+) or acid buffer salts. The proximal tubule is responsible for the bulk of bicarbonate reabsorption, while the smaller amounts escaping proximal reabsorption are reabsorbed in more distal segments of the nephron. Net acid excretion occurs predominantly in the distal segments of the nephron. Giebisch (1986) described in a review article, gathering data from many experimental studies, the mechanisms responsible for bicarbonate reabsorption and net acid excretion in the human being. Active bicarbonate reabsorption on proximal tubular level depends on transepithelial hydrogen ion secretion. This hydrogen ion secretion occurs by means of a sodium-hydrogen ion exchange at the luminal side of tubular cells. Hydrogen ions secreted by the tubular cell couple with filtered bicarbonate to carbonic acid (H_2CO_3). Carbonic acid is converted to H_2O and CO_2 under influence of carbonic anhydrase present in the luminal wall of the proximal tubular cells and only a small amount diffuses back into the cell as H_2CO_3. A low permeability for HCO_3^- in the apical cell membrane is supposed to be present. Na^+-ions, which are transported in the cell via the sodium-hydrogen ion exchange are eliminated from the cell to the peritubular fluid by a sodium-potassium ATP-ase. Also bicarbonate is actively extruded across the basolateral membrane of proximal tubular cells together with sodium with a stoichiometry of three bicarbonate ions to one sodium ion (figure I). Passive reabsorption of bicarbonate does not substantially affect net bicarbonate reabsorption. It is mainly important when a large transcellular bicarbonate gradient exists.

In the distal tubule and in the collecting ducts active electrogenic hydrogen ion secretion is present. This transport is sodium independent in contrast to the described proximal sodium hydrogen ion exchange, but distal tubular sodium delivery is of importance for the maintenance of a lumen potential difference. Hydrogen ion secretion at the luminal tubular side depends on basolateral bicarbonate chloride exchange (figure II). So chloride concentration in the peritubular medium is crucial in this mechanism.

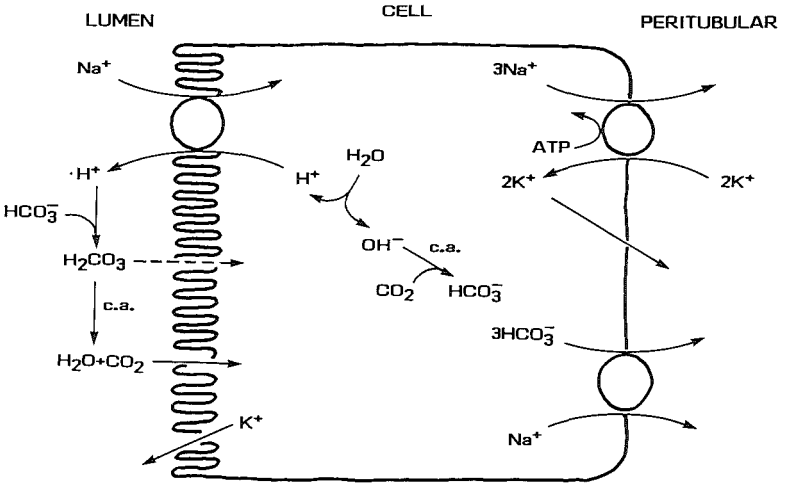

Figure I Model of proximal tubular cells showing the role of luminal Na^+-H^+ exchange in the process of bicarbonate reabsorption. (from Giebisch, 1986). Published with permission.

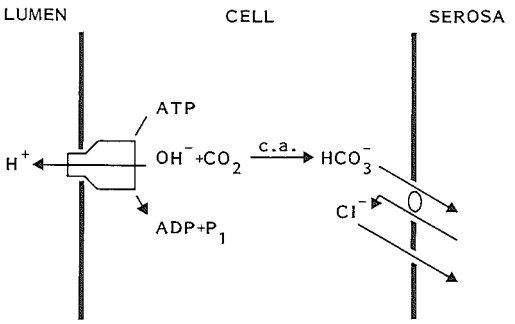

Figure II Model for hydrogen ion secretion by cells of the cortical and medullary collecting tubule. (from Giebisch, 1986). Published with permission.

The number of hydrogen pumps in the luminal membrane of the so called intercalated cells of the distal nephron can increase in conditions in which acidification is stimulated. These are the cells that are supposed to carry out net hydrogen excretion. Hydrogen ions secreted in the tubular fluid are excreted after binding to filtered organic acids especially HPO_4^{2-} and after binding with NH_3, which is generated in the renal tubuli from glutamine (figure III, Silbernagl and Scheller, 1986). The amount of free hydrogen ions appearing in the urine is neglectible.

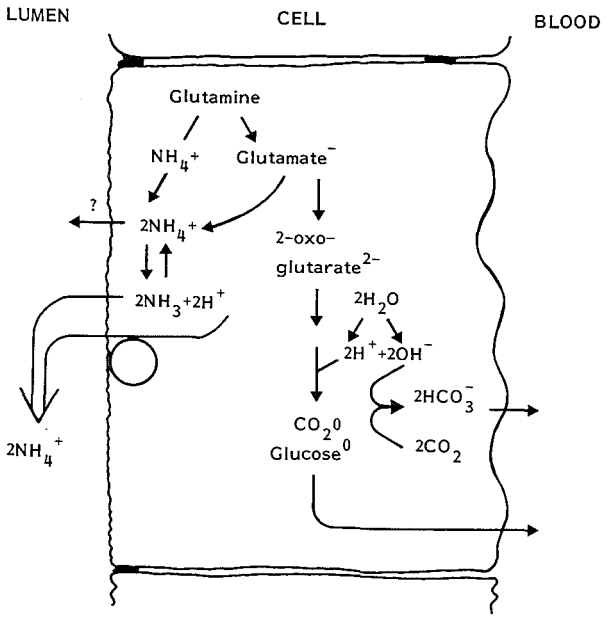

Figure III Proximal tubular ammoniagenesis from the main substrate glutamine. (from Silbernagl and Scheller, 1986). Published with permission.

Carbonic anhydrase is present in distal as well as in proximal tubular cells. Carbonic anhydrase is also present in the brush border membranes of proximal tubular cells, necessary for the conversion of H_2CO_3 to H_2O and CO_2.

2.C.2 Mechanisms of renal tubular acidification in the newborn

Newborn infants have lower serum bicarbonate levels than adults (Edelmann Jr. et al., 1967A). This is even more pronounced in the preterm neonate. Also metabolic acidosis is frequently observed in the premature infant (Svenningsen and Lindquist, 1973). These data suggest a limitation in renal acidifying capacity of newborns.

Clinical studies on this subject have been performed in two different ways. Firstly bicarbonate reabsorption studies were performed (Edelmann Jr. et al., 1967A; Tudvad et al., 1954; Svenningsen and Lindquist, 1973) in which the bicarbonate load of the glomeruli varied. Secondly renal tubular acid excretory capacity has been tested by means of acid loading with NH_4Cl (Edelmann Jr. et al., 1967A; Edelmann Jr. et al., 1967B; Kerpel-Fronius et al., 1970; Sulyok et al., 1972; Svenningsen and Lindquist, 1973; Svenningsen, 1974A).

Edelmann Jr. et al. (1967A) demonstrated in full term neonates that the presence of a low serum bicarbonate level is the resultant of a low bicarbonate threshold (defined as the serum bicarbonate level at which more than 0.02 mmol HCO_3^- per 100 ml GFR appears in the urine). This phenomenon remained present during the first year of life in the 6 children tested in his study. Confirmations of the data were provided by an earlier study of Tudvad et al. (1954) and later on by Svenningsen (1974A). The low bicarbonate threshold originates probably from the known heterogeneity of nephrons during the early stages of development and from proximal tubular immaturity. However, also extrarenal factors may influence renal threshold. Evidence for extrarenal factors is provided by animal experiments. The puppy as well as the fetal lamb both have a low bicarbonate threshold compared to the adult animal. The threshold can easily be increased by means of moderate extracellular volume contraction, thus leading to an increase in serum bicarbonate levels in those animals (Moore et al., 1972; Robillard et al., 1977). The study of Zilleruello et al. (1986) on fractional bicarbonate excretion in preterm infants during metabolic acidosis and respiratory acidosis is an indication that extrarenal factors have the same influence on bicarbonate reabsorption in the newborn. However, while no filtration studies were performed in this study, no definite conclusions on bicarbonate reabsorption can be drawn from their results.

In evaluating distal tubular acidification acid loading studies have been performed by Kerpel-Fronius et al. (1970) and Sulyok et al. (1972). They demonstrated, after loading preterm infants with ammonium chloride, a correlation of blood bicarbonate levels, urinary excretion of ammonia and of titrable acid with birth weight. The smaller premature infants tend to be more acidotic and have lower NH_4^+ and titrable acid excretion rates. The renal excretion of an acid load increases with both gestational and postnatal age.

So evidence is provided for tubular immaturity of the hydrogen ion excretory mechanisms. This is especially of importance in preterm infants receiving a high protein intake. Data about renal tubular acidification in the tiny premature infant remain however scarce.

Chapter 3 EFFECT OF HYPERCAPNIC ACIDOSIS ON RENAL FUNCTION IN THE NEWBORN RABBIT

3.1. Abstract

Anaesthetized mechanically-ventilated newborn rabbits were exposed to different degrees of hypercapnia. One hour of normocapnia was used as a control period. Renal function studies demonstrated an increase in renal vascular resistance with a concomitant decrease in effective renal plasma flow in all hypercapnic animals, combined with a less pronounced decrease in glomerular filtration rate. Filtration fraction rose significantly. A decrease in systemic blood pressure was only observed when the P_aCO_2 exceeded 100 mm Hg combined with an arterial pH below or equal to 7.10. We conclude that normoxemic hypercapnia in the newborn rabbit leads to an increase in renal vascular resistance and suggest that the renal vasoconstriction predominates at the level of the efferent arteriole.

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A.J. v.d. Heijden and J.P. Guignard.

3.2. Introduction

A decrease in glomerular filtration rate (GFR) and urine output has been described in newborn infants presenting with severe respiratory distress syndrome (1,2,3). The factors responsible for these disturbances include hypoxemia, hypercapnia, acidosis and a fall in systemic blood pressure. The effect of isolated acute hypercapnic acidosis has not been extensively studied in animals whose nephrogenesis has not yet been completed. Rosenberg et al. (4) observed a consistant but not significant decrease in renal blood flow (RBF) in lambs undergoing mild hypercapnia (P_aCO_2 60-70 mm Hg). In contrast, no changes were observed in fetal and adult sheep. Alward et al. (5) observed a decrease in RBF, a stable GFR and an increase in renal vascular resistance (RVR) in piglets exposed to combined hypercapnia (P_aCO_2 70 mm Hg) and hypoxemia (P_aO_2 35 mm Hg). In adult animals most studies have been performed in anaesthetised dogs, who frequently presented with a decrease in RBF and GFR when the P_aCO_2 exceeded 70 to 80 mm Hg (6,7,8). The purpose of the present study was to investigate the role of acute hypercapnia on renal function in the newborn rabbit before the end of nephrogenesis.

3.3. Materials and methods

Experiments were performed on 5 to 12-day-old New Zealand white rabbits (n=45), with a body weight varying from 81 to 214 g. The animals were anaesthetised with 25 mg/kg sodium pentobarbital 0.5% intraperitoneally. Additional small doses of pentobarbital were administered when needed throughout the experiment. After tracheotomy the animals were artificially ventilated (Harvard 683 Rodent Ventilator, Millis, M.A.). The respiratory rate was kept constant at 40/min and tidal volume was adjusted for age and weight. Body temperature, recorded by an intraesophageal thermometer was kept constant at 38.5°C, using a heating table and an infrared lamp. The femoral vein and artery were catheterized with polyethylene catheters (PE 10). Bladder catheterisation was performed for urine sampling. Arterial and ventilatory pressures were continuously measured, using Statham transducers and recorded on a multichannel recorder (Model 7B Polygrap Grass Instruments, Quincy, M.A.). The animals were paralysed for the duration of the experiment with tubocurarine (25 µg/kg), which was repeated as needed. Following surgery the animals received a priming dose of an inulin-PAH solution (100 and 1.25 mg/kg, respectively). Thereafter a

solution containing 50 g mannitol, 3 g inulin, 0.15 g PAH, 100 mmol NaCl and 5 mmol KCl/liter, was infused at a rate of 1 ml/100 g/h, to provide stable plasma levels of inulin (20-40 mg/100 ml) and PAH (0.2-1 mg/100 ml). $NaHCO_3$ was added to the solution in varying amounts (see below).
The experiments were started 90-120 min after surgery when urinary flow and blood pressure had stabilised. During the experiments timed urinary collections of 30 min each were obtained and arterial blood sampling was performed at the midpoint of alternate urinary collection periods (figure I). Clearances of inuline and PAH (C_{PAH}) were calculated from standard equations and used as estimates of GFR and effective renal plasma flow.

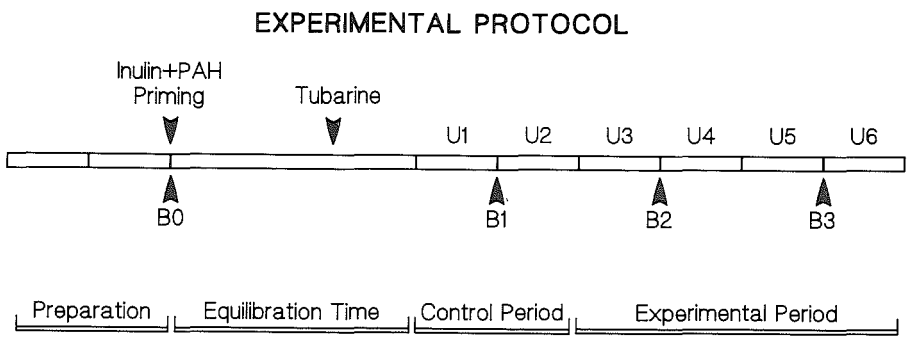

Figure I

The extraction of PAH was measured in a separate group of six hypercapnic newborn animals infused with 1 mmol $NaHCO_3$/ kg per h (P_aCO_2 = 96 $\pm$ 6 (SEM) mm Hg for 60 min) and compared to the value previously observed in a group of 14 normocapnic newborn rabbits (P_aCO_2 = 40 $\pm$ 2 (SEM) mm Hg) studied in this laboratory (9). The extraction was 0.55 $\pm$ 0.03 (SEM) in the normocapnic animals, and 0.56 $\pm$ 0.09 (SEM) in the hypercapnic animals. A value of 0.55 was subsequently used in the calculation of RBF, given by the formula (C_{PAH}/E_{PAH})/(1-hematocrit) and the filtration fraction (FF) as GFR/(C_{PAH}/E_{PAH}). RVR was calculated as mean arterial pressure (MAP)/RBF.

The following chemical methods were used for blood and urine analysis: Inulin and PAH by the Anthron-method and the Bratton-Marshall method respectively (Technicon Auto-analyser, Technicon Instruments Corporation, Terrytown, NY); gas-analysis under anaerobic conditions with a blood gas-analyser (pH/Blood-Gas- Analyser 168, Corning, Halstead, Essex, England).

The following experimental protocols were used. The first 2 urinary collection periods always served as controls:

Group I (n=8) and group II (n=7): normocapnia was maintained during 3 consecutive h.; the infusion delivered 0.5 (group I) and 1 (group II) mmol $NaHCO_3$/kg/h respectively.

Group III (n=8) hypercapnia: a P_aCO_2 of 100 mm Hg was obtained using a fixed gas mixture containing 13% CO_2, 40% O_2 and 47% N_2. $NaHCO_3$ was added to the infusion to deliver 0.5 mmol $NaHCO_3$/kg per h. Hypercapnia was introduced following the first control hour and was maintained for 2 h.

Group IV (n=8) hypercapnia: a P_aCO_2 of 100 mm Hg was similarly obtained and $NaHCO_3$ was added to the infusion to deliver 1 mmol $NaHCO_3$/kg per h. The protocol was the same as used in group III.

Group V (n=8) hypercapnia: a P_aCO_2 of 80 mm Hg was obtained, using a fixed gas mixture containing 8% CO_2, 40% O_2 and 52% N_2. The infusion delivered 0.5 mmol $NaHCO_3$/kg/h.

Data analysis: Because of the large interindividual and interlitter variations each animal was used as his own control. The changes between control and experimental periods have been evaluated by calculating the significance of the difference between their means and zero, using the t-test (10). In all cases a $p < 0.05$ was considered statistically significant.

3.4. Results

Groups I and II:

Infusion of 0.5 mmol and 1 mmol sodiumbicarbonate during 3 consecutive h. of normocapnia did not significantly influence blood pH, P_aCO_2 and MAP. Except for a significant rise in urinary flow rate in group II due to the higher solute load, renal function remained essentially stable.

Effect of hypercapnia with a P_aCO_2 of 100 mm Hg and a $NaHCO_3$ infusion rate of 0.5 mmol $NaHCO_3$/kg/h (group III).

The P_aCO_2 was increased from 39 mm Hg to 103 mm Hg in the 1st h. of hypercapnia and to 106 mm Hg in the 2nd h. The serum pH diminished abruptly from 7.48 to 7.10 during hypercapnia and remained stable for 2 hours. MAP decreased slightly, but significantly, from 33.5 to 30.5 mm Hg, and urinary flow rate from 0.057 to 0.043 ml/min.kg ($p<0.05$). There was no significant change in urinary output between the 1st and 2nd h. of hypercapnia. C_{PAH} fell from 6.42 to 3.86 ml/min.kg within the 1st h. of hypercapnia and to 3.33 ml/min.kg during the 2nd h. The clearance of inulin fell from 1.66 to 1.33 within 1 h. and to 1.22 ml/min.kg (table I) within the 2nd h.

Effect of hypercapnia with a P_aCO_2 of 100 mm Hg and a $NaHCO_3$ infusion rate of 1 mmol $NaHCO_3$/kg/h (Group IV).

The $NaHCO_3$ infusion rate was doubled in this group to partly blunt the decrease in serum pH while maintaining the same P_aCO_2. The increase in P_aCO_2 was similar to that present in the first group, from 39 mm Hg to 104 mm Hg. The decrease in serum pH was less pronounced, from 7.51 to 7.17. No decrease in MAP was observed and although urinary flow rate decreased from 0.064 to 0.058 ml/min.kg, the fall was not significant. However the decrease of C_{PAH} was similar from 5.91 to 4.14 in the 1st hypercapnic h. and to 3.07 ml/min.kg in the 2nd hypercapnic h. The decrease in GFR observed during the 1st h. of hypercapnia was not significant from 1.56 to 1.36 ml/min.kg, but later reached statistical significance ($p<0.05$) (table I).

Effect of hypercapnia with a P_aCO_2 of 80 mm Hg and a $NaHCO_3$ infusion rate of 0.5 mmol $NaHCO_3$/kg/h (Group V).

The P_aCO_2 increased from 35 to 78 mm Hg in the 1st and to 80 mm Hg in the 2nd h. of hypercapnia. Serum pH decreased from 7.51 to 7.20 and remained stable afterwards. MAP remained stable throughout the experiment and the observed decrease in urinary volume was not significant. A marked decrease in C_{PAH} from 7.45 to 5.16 during the 1st h. and to 4.43 ml/kg per min during the 2nd h. was also observed in this group. GFR declined from 2.03 to 1.57 and to 1.64 ml/min.kg in the 1st and 2nd h. respectively (table I).

The decrease in RBF is shown in table II, together with the concomitant increase in RVR. Filtration fraction increased in all hypercapnic groups.

TABLE I EFFECT OF HYPERCAPNIA ON BLOOD PH, MAP, AND RENAL FUNCTION*

		$PaCO_2$ (mm Hg)			Blood pH			MAP (mm Hg)			V (ml/min.kg)			C_{PAH} (ml/min.kg)			C_{inulin} (ml/min.kg)		
		C	1	2	C	1	2	C	1	2	C	1	2	C	1	2	C	1	2
I	Mean	42	42	38	7.48	7.45	7.49	31.3	30.3	29.6	0.071	0.075	0.081	5.32	4.79	4.70	1.71	1.55	1.55
	SEM	2.4	2.0	1.7	0.03	0.03	0.02	1.2	1.4	1.7	0.008	0.010	0.015	0.87	0.48	0.64	0.26	0.22	0.27
n = 8	p																		
II	Mean	37	37	36	7.51	7.52	7.52	35.5	34.5	35.0	0.054	0.064	0.079	6.58	6.88	6.43	1.68	1.69	1.74
	SEM	2.3	1.7	1.6	0.03	0.02	0.02	2.0	2.0	1.8	0.009	0.008	0.009	1.32	1.54	1.65	0.18	0.23	0.14
n = 7	p											†							
III	Mean	39	103	106	7.48	7.10	7.10	33.5	30.5	29.5	0.057	0.043	0.050	6.42	3.86	3.33	1.66	1.33	1.22
	SEM	2.4	1.3	1.8	0.01	0.01	0.01	1.0	1.3	1.3	0.007	0.005	0.009	0.67	0.45	0.61	0.14	0.14	0.24
n = 8	p		#	#		#	#		†	†		†			§	§		†	
IV	Mean	39	101	104	7.51	7.15	7.17	32.8	33.9	34.7	0.064	0.058	0.060	5.91	4.14	3.07	1.56	1.36	1.29
	SEM	1.7	1.5	3.2	0.02	0.01	0.01	1.2	1.8	2.0	0.006	0.005	0.003	0.39	0.30	0.34	0.12	0.07	0.11
n = 8	p		#	#		#	#,☆								†	#,★			†
V	Mean	35	78	80	7.51	7.20	7.21	33.7	32.6	32.9	0.079	0.056	0.070	7.45	5.16	4.43	2.03	1.57	1.64
	SEM	2.0	1.9	1.8	0.02	0.02	0.02	0.7	0.8	1.1	0.011	0.004	0.007	0.43	0.38	0.48	0.20	0.14	0.19
n = 8	p		#	#		#	#								§	§		†	

*V, urine flow rate; C_{inulin}, glomerular filtration rate; C, control period; 1, first period of hypercapnia; 2, second period of hypercapnia.

† Significant vs control < 0.05.

Significant vs control < 0.001.

§ Significant vs control < 0.01.

☆ Significant vs first period < 0.05.

★ Significant vs first period < 0.001.

TABLE II. VALUES OF RBF, RVR, AND FF BEFORE AND DURING HYPERCAPNIC ACIDOSIS*

	RBF	RBF (ml/min.kg)			RVR (mm Hg/ml/min.kg)			FF (%)		
		C	1	2	C	1	2	C	1	2
I	Mean	14.06	12.38	11.95	2.52	2.55	2.77	18.2	17.5	17.4
	SEM	2.30	1.21	1.64	0.31	0.18	0.38	1.0	1.0	0.9
n=8	p									
II	Mean	17.14	17.29	15.92	2.61	2.49	2.83	17.2	16.0	18.4
	SEM	3.36	3.68	3.93	0.50	0.44	0.46	2.7	2.4	2.4
n=7	p									
III	Mean	15.63	9.69	8.21	2.20	3.36	4.57	15.3	18.4	19.7
	SEM	1.12	1.14	1.51	0.26	0.41	0.92	1.3	1.7	0.8
n=8	p		§	§		†	†		†	§,☆
IV	Mean	15.40	10.63	7.99	2.17	3.29	4.70	14.6	18.3	23.7
	SEM	1.11	0.75	0.97	0.14	0.33	0.55	0.7	0.9	1.5
n=8	p		§	#,§		†	§,☆		§	#,**
V	Mean	19.36	13.20	11.53	1.76	2.54	3.12	14.8	17.0	20.4
	SEM	0.99	0.83	1.33	0.08	0.17	0.34	0.9	1.2	0.7
n=8	p		#	#		†	§		†	#,★

*FF, filtration fraction; C, control period; 1, first period of hypercapnia; 2, second period of hypercapnia.
A constant extraction factor of PAH of 55 % was used for the calculation of RBF.

† Significant vs control < 0.05.
\# Significant vs control < 0.001.
§ Significant vs first period < 0.01.
☆ Significant vs control < 0.05.
★ Significant vs first period < 0.01.
** Significant vs first period < 0.001

3.5. Discussion

The present study demonstrates that acute hypercapnia in the anaesthetised newborn rabbit is associated with major changes in renal function. Three different experimental protocols were used in order to establish this. The significant decrease in MAP in group III could be prevented by adding 0.5 mmol $NaHCO_3$/kg/h to the infusion in group IV. This may be due to the higher level of serum pH or to the expansion of extracellular volume by the hyperosmolar infusion. The effect of a fall in MAP was thus excluded in this group and was also absent in the other groups. A P_aCO_2 of 100 mm Hg with a serum pH of 7.15-7.17 (group IV) led to nearly the same changes as a P_aCO_2 of 80 mm Hg and a serum pH of 7.20 (group V). Only the GFR in group V seemed to stabilize. The observed effects can be due to the hypercapnia, the acidosis or to the combination of these factors, but remained present in all experiments thus supporting the consistency of the data. The most striking change is the decline in PAH clearance observed in all hypercapnic groups.

This could reflect a true decrease in RBF, or a decrease in PAH extraction in the hypercapnic animals. To exclude this second possibility, additional experiments were performed in newborn animals of the same age, undergoing hypercapnia for 60 min. The PAH extraction values were comparable to those observed in normocapnic animals, thus demonstrating that hypercapnia does not change PAH extraction, and that the drop in C_{PAH} corresponds to a true decrease in RBF. A 10% decrease in PAH extraction was observed by Anderson et al. during hypercapnic acidosis in dogs (11,12), but was apparently not found in other studies of the same group (13,14), nor was any change in PAH extraction during hypercapnia mentioned by Norman et al. (7) and by Berns et al. (15). This is in agreement with studies using either a sine wave electromagnetic flowmeter (6) or microspheres (4,5) for measuring RBF.

The increase in filtration fraction suggests that the vasoconstriction induced by hypercapnia predominates at the level of the efferent arteriole. The effects observed in our experiments are similar to the changes described in the adult dog by several authors (6,7,8). Rose et al. (14) discussed the validity of these results in dogs, arguing that the introduction of artificial ventilation and the use of pentobarbital could be responsible for the observed decrease in RBF, which they did not observe in their experiments on conscious dogs. Indeed Walker et al. (16) have clearly shown that pentobarbital can depress both RBF and GFR. However, in

the protocol used by Rose, the levels of P_aCO_2 were much lower than in our study, or in the studies mentioned above (6,7,8). It should also be noted that in another study the same group did not find a decrease in RBF in similar conditions despite the use of pentobarbital, at least when MAP was stable (13). That the decrease in RBF observed in our experiments is not due either to pentobarbital or to the artificial ventilation is demonstrated by the stability of the two control groups throughout the experiment. Several mechanisms may contribute to the decrease in RBF observed during hypercapnia. A major activation of the renin-angiotensin system has been found in neonates with respiratory distress syndrome (17) as well as in adults with acute hypercapnia (18). This has been confirmed in animal experiments by Kurtz and Zehr (19). A predominant effect of angiotensin II on the efferent arteriole of the rabbit kidney has been observed by Edwards (20), which may explain the increase in filtration fraction in our experiments.

The changes in RBF observed in our experiments can thus be due to a direct stimulation of the renin-angiotensin system by hypercapnia, or to a stimulation via the renal nerves as suggested by different authors (7,12,13,15, 20).

Arginine vasopressin (AVP) may be another important factor. An hyperosmolar infusion was used in our newborn rabbits. This may have stimulated AVP secretion, as has been demonstrated in the fetal sheep (21). The hypersecretion of AVP did certainly not influence renal hemodynamics or urinary volume in the two control groups. Hypercapnia per se also stimulates AVP secretion, as observed in the adult dog by Berns et al. (15). Thus a role for AVP in our experiments cannot be excluded.

In conclusion, acute hypercapnic acidosis in the anaesthetised newborn rabbit leads to an increase in RVR, a decrease in RBF and, as a result of this, a decrease in GFR. We suggest that the renal disturbances observed in neonates with respiratory distress syndrome could be due, at least in part, to the effect of hypercapnic acidosis superimposed on the already known effects of hypoxemia.

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Chapter 4 BICARBONATE REABSORPTION BY THE NEWBORN RABBIT KIDNEY

4.1. Abstract

Bicarbonate reabsorption by the immature kidney in response to acute acid-base changes was assessed in 40 anaesthesised newborn rabbits before the end of nephrogenesis. The normal newborn rabbit (aged 5-12 days) is in a state of hypochloremic metabolic alkalosis (P_{HCO3^-} = 31.9 ± 0.6 mmol/l, P_{Cl^-} = 83.1 ± 1.0) and excretes a hypertonic (U_{osm} = 578 ± 41 mosm/kg H_2O) alkaline (U_{pH} = 7.40 ± 0.15) urine containing 50 ± 9 mmol/l Cl^- and 13.2 ± 3.8 mmol/l Na^+. The alkalosis is probably generated by an alkaline load originating from the mother's milk, and maintained by a state of chloride wasting and volume contraction.

In this alkaline model, bicarbonate reabsorption increases in response to an acute elevation of P_aCO_2, and is strongly dependent upon the bicarbonate filtered load during both acutely induced metabolic acidosis and alkalosis. The ability of the immature kidney to reclaim filtered bicarbonate in response to an elevation of the plasma carbon dioxide tension remains unlimited up to a P_aCO_2 of 110 mm Hg ($y = 22.4 + 0.13 \times r = 0.78$ $p<0.001$). The acid-base balance of the newborn rabbit is in sharp contrast with that of most animal species, and the renal handling of HCO_3^- does not show signs of tubular immaturity.

* Submitted for publication.

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4.2. Introduction

Newborn infants have lower plasma bicarbonate concentrations than adults (9,33,34). The concentrations are the lowest in very premature infants whose nephrogenesis is not yet complete (27). The low bicarbonate plasma concentration in neonates has been ascribed to renal tubular immaturity or to a state of relative volume expansion present early in life (27,30). Experimental studies in puppies (24), newborn rats(13) and lambs (26) as well as recent clinical studies (36) confirmed low bicarbonate threshold but suggested that it was not the result of tubular immaturity. The high body water and extracellular fluid volume present in the neonate (10) was thought to depress both sodium and bicarbonate renal tubular reabsorption. Tubular immaturity has not conclusively been ruled out however, and remains a possible cause of bicarbonate wasting in the neonate, as it is, in part, for sodium (1,32).

A recent study on the development of bicarbonate transport has been performed, using isolated perfused early proximal convoluted tubules of developing rabbits (28). It demonstrated that the absorption of bicarbonate in the newborn rabbit juxtamedullary proximal convoluted tubule was about 30-40% of that observed in the same segment of the mature nephron. This observation leads the authors to conclude that the low threshold for bicarbonate observed in newborn humans (9,27), rat (13) and dog (24) could be due, at least in part, to an immaturity in their renal capacity to reabsorb bicarbonate. Extrapolation of in-vitro studies in newborn rabbits to whole-kidney observations in other species may be hazardous.

The present study was thus performed to provide acid-base clearance data in the newborn rabbit, an animal whose nephrogenesis is not completed before the 2^{nd} to 3^{rd} week of life (20), and whose renal maturation shows close similarities to that of the human neonate (8,35).

4.3. Materials and methods

Experiments were performed on 5 to 12 day-old New Zealand white rabbits (n=40), with a body weight varying from 86 to 183 g. Babies were raised with their mothers, who were fed laboratory rabbit chow (Kanninchen-Würfel, Lactina Suisse, Puidoux, Switzerland). This chow provides proteins (14-16%), cellulose (19-22%), K (1.4%), Ca (0.91%), P (0.54%), Na (0.4%), Cl (0.33%), vitamins A, D, E, and oligoelements. The newborn animals were

anaesthetized with 25 mg/kg 0.5% sodium pentobarbital intraperitoneally. Additional small doses of pentobarbital were administered when needed throughout the experiment. After tracheotomy the animals were artificially ventilated (Harvard 683 Rodent Ventilator, Millis, MA, USA). The respiratory rate was kept constant at 40/min and tidal volume was adjusted for age and weight. Body temperature, recorded by an intraesophageal thermometer, was kept constant at 38.5° C, using a heating table and an infrared lamp. The femoral vein and artery were catheterized with polyethylene catheters (PE 10). Bladder catheterization was performed for urine sampling. The first urine and blood samples were obtained immediately after catheterization, in order to obtain basal values.

Arterial and ventilatory pressures were continuously measured, using Statham P23ID transducers and recorded on a multichannel recorder (Model 7B Polygraph, Grass Instruments, Quincy, MA, USA). The animals were paralyzed for the duration of the experiment with tubocurarine (25 µg/kg), which was repeated as needed. Following surgery the animals received a priming dose of an inulin solution (100 mg/kg). Thereafter a solution containing 3 g inulin and 5 mmol KCL per liter was infused at a rate of 1 ml/100 g/h to provide stable plasma levels of inulin (20-40 mg/100 ml). Mannitol, NaCl, $NaHCO_3$ and NH_4Cl were added to the infusion depending on the protocol that was used (see below).

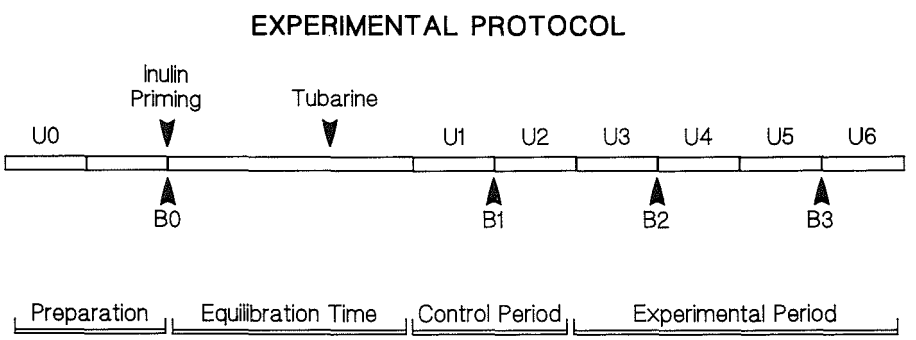

Figure I

The experiments were started 90-120 min after surgery, when urine flow rate and blood pressure had stabilized. During the experiment timed urinary collections of 30 min each were obtained and arterial blood sampling was performed at the midpoint of alternate urinary collection periods (figure I).

We previously demonstrated that blood pressure, heart rate and renal functions remain stable for up to 3 hours after equilibration in this animal preparation (8,17,35). The clearance of inulin was calculated from the standard equation and used as an estimate of GFR. The following chemical methods were used for blood and urinary analysis: inulin by the Anthron-method (Technicon Autoanalyzer, Technicon Instruments Corporation, Terrytown, NY, USA); Na and K by flame photometry (Flame Photometer 543, Instrument Laboratory, Inc., Lexington, Mass, USA); Cl by colorimetric electrotitration (Chloride Meter 920, Corning, Halstead, Essex, England), osmolality by vapour pressure osmometry (Wescor 5100c Vapour Pressure Osmometer, Wescor Inc., Logan, Utah, USA): gas-analysis under anaerobic conditions with a blood gas-analyser (pH/Blood-Gas Analyser 168, Corning, Halstead, Essex, England).

Filtered bicarbonate was taken as the product of the filtration rate and the plasma bicarbonate concentration without correction for the Donnan factor. Bicarbonate reabsorption was calculated from the filtered minus the excreted rate, and expressed in µmol per ml GFR. The following experimental protocols were used, where the first two urine collection periods always served as controls:

Group 1 (n=8) Normocapnia:

Normocapnia with a P_aCO_2 close to 40 mm Hg was maintained during 3 consecutive h. The infusion contained 100 mmol/l NaCl, 50 mmol/l $NaHCO_3$ and 50 g/l mannitol in addition to the other solutes described above.

Group 2 (n=8) Hypercapnia:

Following a normocapnic control period, hypercapnia with a P_aCO_2 close to 80 mm Hg was induced, using a gas mixture containing 8% CO_2, 40% O_2 and 52% N_2. Hypercapnia was maintained for 2 h. The infusion was the same as described for group 1.

Group 3 (n=8) Hypercapnia:

Hypercapnia with a P_aCO_2 close to 100 mm Hg was induced using a gas mixture containing 13% CO_2, 40% O_2 and 47% N_2. Time schedule was as in group 2. The infusion contained the same solutes as in group 1, except that the sodium bicarbonate concentration was doubled, in order to increase the amount of filtered bicarbonate available for reabsorption.

Group 4 (n=8) Metabolic acidosis:

Following the control period, a decrease in plasma HCO_3^- was induced by changing the solution, which contained 100 mmol/l NaCl, 50 mmol/l $NaHCO_3$ and 50 g/l mannitol to one in which the osmolar load of bicarbonate and NaCl was replaced by NH_4Cl. A bolus injection of 0.5 mmol/100 g body weight NH_4Cl was given at the onset of the acid infusion.

Group 5 (n=8) Metabolic alkalosis:

Following the control period, an increase in plasma HCO_3^- was induced by changing the solution which contained 100 mmol/l NaCl, 50 mmol/l $NaHCO_3$ and 50 g/l mannitol to one in which the osmolar load of NaCl and mannitol was replaced by $NaHCO_3$.

Data analysis.

All numerical data are expressed as mean $\pm$ SEM. Because of large inter-individual and interlitter variations each animal was used as his own control. The changes between control and experimental periods have been evaluated by calculating the significance of the difference between their means and zero, using the t-test. Regression lines were calculated by conventional statistical methods, as previously described (15,17). In all cases a $p<0.05$ was considered statistically significant.

4.4. Results

As shown in table I, the normal newborn rabbits were in a state of hypochloremic metabolic alkalosis at the onset of the experiment, with normal plasma sodium and slightly elevated potassium levels. These animals produced an alkaline urine with a high chloride and potassium content and a low sodium concentration.

Table I BLOOD AND URINE DATA IN THE NEWBORN RABBIT

		Plasma	Urine
pH		7.49 ± 0.02	7.40 ± 0.15
PCO_2	mmHg	35 ± 3.0	–
Bicarbonate	mmol/l	31.2 ± 0.5	50.0 ± 10.1
Sodium	mmol/l	138.0 ± 2	13.2 ± 3.8
Potassium	mmol/l	5.4 ± 0.2	52.4 ± 11.7
Chloride	mmol/l	83.1 ± 1.2	55.6 ± 0.15
Osmolality	mosm/kg H_2O	279 ± 2	578 ± 42

Mean arterial blood pressure, urine flow rate, GFR and acid-base parameters during the experiment in the different groups are summarized in table II. In all groups mean arterial blood pressure remained constant throughout the experiment.

Bicarbonate reabsorption during normocapnia (group 1)

In these animals infused with 0.5 mmol $NaHCO_3$/kg per h, the high plasma bicarbonate concentration present at the onset of the experiment was maintained constant. Bicarbonate excretion was substantial, with a fractional excretion varying from 9.5 to 11.3% and an urine pH close to 7.5. Bicarbonate reabsorption varied from 25.6 to 27.5 µmol/ml GFR, at plasma bicarbonate concentrations of 29.0 to 30.3 mmol/l.

Bicarbonate reabsorption during hypercapnic acidosis (groups 2 and 3)

Acute induction of severe hypercapnia resulted in a significant decrease in blood pH, and a marked increase in plasma bicarbonate concentration. The fractional excretion of bicarbonate and urine pH decreased slightly whereas bicarbonate reabsorption increased from 27.2 to 31.2 µmol/ml GFR when the P_aCO_2 was raised to 80 mm Hg, and from 29.0 to 36.7 µmol/ml GFR when it was raised to 104 mm Hg. A significant correlation was found between bicarbonate reabsorption and P_aCO_2 ($Y = 22.4 + 0.13\ x$, $r = 0.78$, $p < 0.001$) without evidence of a limit for bicarbonate reabsorption (figure II). Hypercapnic acidosis was associated with a decrease in GFR, urine flow rate remaining stable.

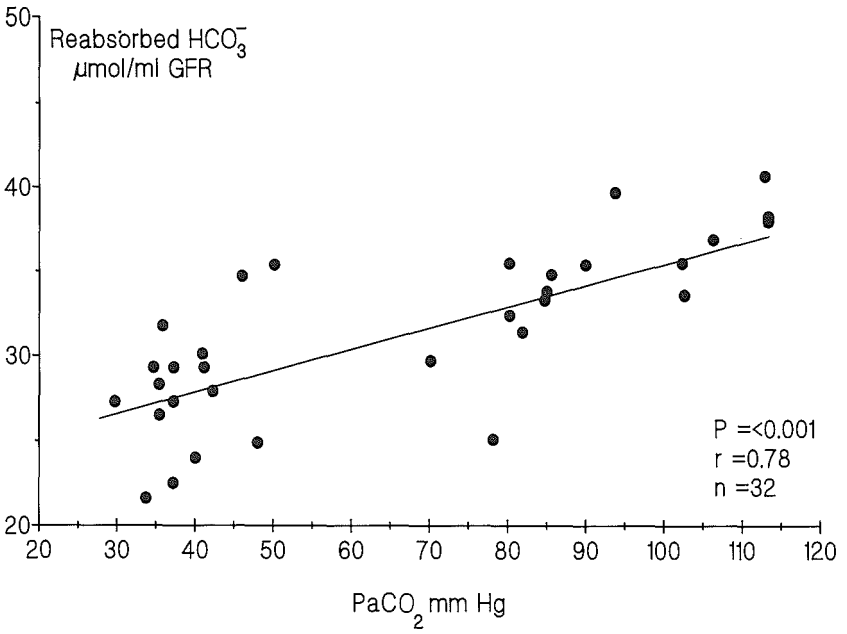

Figure II Bicarbonate reabsorption as a function of the plasma carbon dioxide tension.

Metabolic acidosis (group 4)

Infusion of NH_4Cl at a rate of 1.5 mmol/kg per h after a bolus injection of 0.05 mmol/kg induced a sharp decrease in plasma bicarbonate, from 29.3 to 18.7 mmol/l, associated with a significant decrease in bicarbonate reabsorption from 26.6 to 18.0 µmol/ml GFR and a non significant decrease in the fractional excretion of bicarbonate. Urine pH also decreased slightly, as did glomerular filtration rate. Urine flow rate remained stable.

Bicarbonate reabsorption during $NaHCO_3$ infusion (group 5)

Infusion of hypertonic bicarbonate at a rate of 3 mmol/kg per h. moderately increased plasma bicarbonate concentration, from 30.4 to 33.2 mmol/l. This was associated with a slight non significant increase in bicarbonate reabsorption and a non significant increase in bicarbonate fractional excretion. A slight transient decrease in GFR and urine flow rate was noted during the first hour of bicarbonate infusion.

Following the infusion of an acid or an alkaline load bicarbonate reabsorption could be measured at varying bicarbonate filtered loads.
A significant correlation was observed between filtered bicarbonate and reabsorbed bicarbonate and, as shown in figure III, between bicarbonate reabsorption expressed per unit GFR and plasma bicarbonate concentration ($Y = 1.05 + 0.9\ X$, $r = 0.99$, $p < 0.001$).

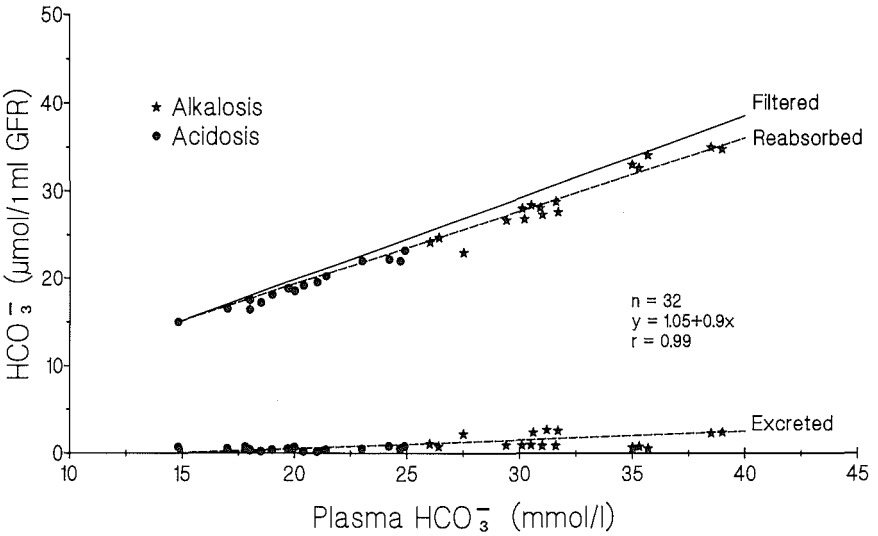

Figure III Bicarbonate reabsorption as a function of the plasma bicarbonate concentration.

TABLE II BLOOD AND RENAL FUNCTIONAL DATA IN THE EXPERIMENTAL GROUPS 1-5

Group	Period		Blood pH	Blood $PaCO_2$ mm Hg	Blood HCO_3^- mmol/l	MAP mm Hg	V ml/min.kg	GFR ml/min.kg	Urine pH	HCO_3^- reabsorption umol/ml GFR	Fe bicarbonate %
1 control group	C	mean	7.48	41.6	30.3	31.3	0.071	1.71	7.53	27.50	9.5
		SE	0.03	2.4	1.0	1.2	0.008	0.26	0.11	1.27	2.5
	I	mean	7.45	42.4	29.5	30.3	0.075	1.55	7.47	26.30	10.1
		SE	0.03	2.0	0.9	1.4	0.0010	0.22	0.15	0.88	3.1
	II	mean	7.49	37.9	29.0	29.6	0.081	1.55	7.49	25.65	11.3
		SE	0.02	1.7	0.78	1.7	0.0015	0.27	0.15	1.01	3.6
2 $PaCO_2$ 80 mm Hg	C	mean	7.51	35.0	28.1	33.7	0.079	2.03	7.40	27.27	6.0
		SE	0.02	2.0	1.1	0.7	0.011	0.20	0.15	1.41	1.6
	I	mean	7.20	78.0	30.8	32.6	0.056	1.57	7.06	30.19	4.3
		SE	0.02	1.9	0.9	0.8	0.004	0.14	0.15	1.28	1.2
		P	***	***	**			*	***	*	*
	II	mean	7.21	80.5	32.4	32.9	0.070	1.64	6.85	31.20	2.5
		SE	0.02	1.8	1.2	1.1	0.007	0.19	0.15	1.17	0.5
		P	***	***	*				***	***	*
3 $PaCO_2$ 100 mm Hg	C	mean	7.51	39.0	31.3	32.8	0.064	1.56	7.49	29.04	6.5
		SE	0.02	1.7	1.0	1.22	0.006	0.12	0.09	1.12	1.9
	I	mean	7.15	101.5	35.1	33.9	0.058	1.36	7.17	33.88	4.0
		SE	0.01	1.5	0.7	1.76	0.005	0.07	0.06	0.67	0.6
		P	***	***	**				***	***	
	II	mean	7.17	104.5	38.2	34.7	0.060	1.29	6.89	36.73	3.4
		SE	0.01	3.2	0.6	1.99	0.003	0.11	0.07	0.78	0.5
		P	***	***	***			*	***	***	

TABLE II BLOOD AND RENAL FUNCTIONAL DATA IN THE EXPERIMENTAL GROUPS 1-5 (continued)

Group	Period		Blood pH	Blood $PaCO_2$ mm Hg	Blood HCO_3^- mmol/l	MAP mm Hg	V ml/min.kg	GFR ml/min.kg	Urine pH	HCO_3^- reabsorption umol/ml GFR	FE bicarbonate %
4 metabolic acidosis	C	mean	7.49	38.3	29.3	35.4	0.068	1.91	7.47	26.56	5.7
		SE	0.02	2.3	0.9	1.4	0.008	0.17	0.12	1.44	1.7
	I	mean	7.35	38.7	21.7	36.3	0.070	1.51	7.25	20.85	5.9
		SE	0.02	0.9	1.0	1.8	0.007	0.17	0.09	0.88	1.2
		P	***		***			*	**	*	
	II	mean	7.33	35.1	18.7	35.3	0.082	1.48	7.09	18.07	4.3
		SE	0.02	2.1	0.8	1.7	0.009	0.16	0.09	0.77	0.7
		P	***		***			*	***	***	
5 metabolic alkalosis	C	mean	7.48	40.8	30.4	36.6	0.081	2.21	7.25	29.77	3.9
		SE	0.02	2.1	0.8	1.6	0.010	0.28	0.14	0.82	0.9
	I	mean	7.49	40.7	30.7	36.3	0.068	1.78	7.25	29.94	4.1
		SE	0.01	2.0	1.2	1.5	0.009	0.26	0.11	0.90	0.7
		P					*	*			
	II	mean	7.54	39.3	33.2	37.0	0.076	2.00	7.65	32.52	7.7
		SE	0.02	2.5	1.3	1.9	0.012	0.30	0.08	2.34	2.7
		P			***						

MAP = mean arterial pressure; V = urine flow rate; GFR = glomerular filtration rate; FE bicarbonate = fractional excretion of bicarbonate; C = control period; I = first experimental period; II = second experimental period; P* = significant vs control, $p < 0.05$; P** = significant vs control, $p < 0.01$; P*** = siginificant vs control, $p < 0.001$.

4.5. Discussion

A pronounced hypochloremic metabolic alkalosis was present in our newborn rabbits less than 2 weeks of age, as previously described in our laboratory (18,25). In an unpublished study (Schwartz and Zavilowitz, quoted in ref. 28), Schwartz et al. also found an elevated plasma bicarbonate concentration in their newborn rabbits during the first week of life and suggested that it was probably due to the presence of a substantial load of bicarbonate generating substances in mother's milk. Analysis of rabbit's milk, performed in our laboratory 3, 5 and 8 days after delivery, showed small amounts of bicarbonate in the milk, the concentrations varying from 1.8 to 4.4 mmol/l, with pH values between 6.72 and 7.00. The same milk contained substantial amounts of chloride (39-45 mmol/l), sodium (37-46 mmol/l), and potassium (40-54 mmol/l). The alkaline load in rabbit's milk appeared to consist of calcium carbonate compounds, potential generators of bicarbonate in the intestine (5). This was suggested by the very high calcium concentration of the milk (67, 90 and 114 mmol/l on day 3, 5 and 8 after delivery, respectively) and by the pattern of milk titration studies revealing a very high buffering capacity of the rabbit's milk, as compared to cow's milk (Grigoras O, Guignard JP, unpublished observation). An additional factor responsible for the generation of the alkalosis could be a state of extracellular volume contraction, which has been shown to induce relative bicarbonate regeneration (12). Indirect evidence for volume contraction in our newborn rabbits rests on the following findings: 1) the excretion of a hypertonic urine (mean U_{osm} = 578 mosm/kg H_2O); 2) a very low sodium fractional excretion rate (mean FENa at the onset of the experiment = 0.89%); 3) the occurrence of hypochloremia. Other mechanisms responsible for generating a metabolic alkalosis, such as an excess acid loss could not be demonstrated: neither vomiting, nor chloridorrhea was observed and early renal acid loss is unlikely, the animals always excreting an alkaline urine when they were tested.

The concomittance of volume contraction, sodium avidity and hypochloremia is probably responsible for the perpetuation of the metabolic alkalosis (7,16). Whether the low GFR present in these hypochloremic animals contributes to the maintenance of the alkalosis remains to be demonstrated (2,6, 12). The hypochloremia observed in our animals receiving milk with a high chloride content points to a defect in chloride intestinal absorption and/ or renal wasting. The occurrence of an elevated urine chloride concentra-

tion and of a relatively high fractional excretion of chloride (1.38 ± 0.25%) in comparison with sodium (0.89%), in the presence of marked hypochloremia (83 ± 1 mmol/l) suggests renal chloride wasting. The high rate of potassium urinary excretion is easily explained by the state of volume contraction, sodium avidity and chloride depletion, all leading to increased potassium secretion in the distal tubule. The maintenance of slightly elevated plasma potassium levels can, in turn, be explained by the high potassium content of the milk.

As a consequence of the metabolic alkalosis, bicarbonate concentration in the first urine sample collected at the onset of the experiment was high. It remained so during the control period of all animals with mean fractional bicarbonate excretion rates varying substantially, but always exceeding 3.9%. This is in agreement with the value of 4.8% observed by Schwartz and Zavilowitz (unpublished observations quoted in ref. 28). During our experiments however, the excretion of bicarbonate could have been increased by the infusion of mannitol, as the latter substance has been shown to depress bicarbonate reabsorption (25,31).

Bicarbonate reabsorption has been shown to be influenced by several factors, such as the plasma carbon dioxide tension (4,14,19,21,29), the bicarbonate filtered load (14,15,23,29), the state of extracellular volume and of effective arterial blood volume (21,22,24,26), the body's potassium stores and indirectly by the chloride concentration probably acting via its effect on GFR (12). Whether factors such as the P_aCO_2 and the bicarbonate filtered load also influence bicarbonate reabsorption in this alkalotic model was investigated by raising the P_aCO_2, or by changing the bicarbonate filtered load, either by infusing the animals with an acid or an alkaline load. The significant correlation between bicarbonate reabsorption and the P_aCO_2 in our newborn animals clearly indicates that bicarbonate reabsorption can be stimulated by raising the P_aCO_2. It is generally accepted that extracellular pH rather than blood P_aCO_2 regulates bicarbonate reabsorption (23) and that it is the alteration in intracellular pH, which is critical in determining bicarbonate reabsorption. Since induction of hypercapnia in our animals also significantly decreased blood pH and probably also intracellular pH, an independent effect of plasma P_aCO_2 and pH cannot be distinguished in the present study.

The increase in bicarbonate reabsorption induced by hypercapnia could also have a hemodynamic basis. In acute circumstances hypercapnia has been

shown to result in vasoconstriction, a fall in GFR (21) and a marked increase in bicarbonate reabsorption expressed per unit of GFR. A decrease in GFR was present in our hypercapnic animals and this decrease can well have contributed to the increase in bicarbonate reabsorption.
As previously demonstrated in several species, alterations in filtered bicarbonate result in proportionate changes in bicarbonate reabsorption (14,15,23,29). A tight relationship in the superficial proximal convoluted tubule of the rat between the filtered load of bicarbonate and bicarbonate reabsorption has been demonstrated by Cogan et al. (6) using micropuncture techniques. The same holds true for whole kidney bicarbonate reabsorption by the immature rabbit kidney. This is surprising considering the state of immaturity of superficial nephrons before the end of nephrogenesis. This may well be explained however by recent observations showing that in the rabbit (3,19), in contrast to the rat (11), bicarbonate reabsorption capacity is higher in deep nephrons than in superficial nephrons.
While experiments in dogs have provided persuasive evidence that at constant blood pH bicarbonate reabsorption is a function of filtered bicarbonate rather than of each of its two components (i.e. GFR and blood HCO_3^-) separately (23), the mechanism whereby bicarbonate reabsorption is stimulated by an increase in the filtered load awaits elucidation.

In conclusion a state of hypochloremic metabolic alkalosis exists in the newborn rabbit in the absence of hypokalemia. The factor(s) responsible for the generation of the metabolic alkalosis remain hypothetical. Its maintenance however is probably explained by a state of chloride wasting leading to hypochloremia and consequent volume contraction. Bicarbonate reabsorptive capacity is very efficient in this neonatal animal model and responds normally to the main factors influencing bicarbonate reabsorption, i.e. arterial PCO_2 and bicarbonate filtered load.

4.6. References

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Chapter 5 GLOMERULAR FILTRATION RATE IN THE PRETERM INFANT: THE RELATION TO GESTATIONAL AND POSTNATAL AGE

5.1. Abstract

In 41 preterm neonates with a gestational age (GA) varying from 27 to 36 weeks, glomerular filtration rate (GFR) was measured by means of the continuous inulin infusion technique. Reliability of the technique was confirmed. During postnatal development GFR was found to increase in two ways. Firstly, an increase with advancing gestational age, associated with the increase in body weight (BW) (GFR (ml/min) = 0.15 x GA - 3.20; r = 0.48; p = 0.0048). Secondly a postnatal increase, being independent from increment in BW. An increase in GFR (ml/min.kg) from 0.88 $\pm$ 0.23 to 1.18 $\pm$ 0.28 was observed between day 4 and day 11 postnatally ($p < 0.008$). This latter increase is probably associated with changes in renal hemodynamics. No significant influence of artificial ventilation on GFR could be demonstrated in preterm neonates.

* Submitted for publication.
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5.2. Introduction

Developmental changes in glomerular filtration (GFR) in neonates and especially in preterm neonates have been the subject of many studies. An increase of the GFR with gestational age (GA) has been described by most authors (1,4,10,14,20), although Aperia et al. (3) reported no increase. Furthermore data have been published indicating a rapid postnatal increase of GFR (3,14). This was, however, contradicted in other studies (1,10,20) (table I).

TABLE I THE EFFECT OF GESTATIONAL- AND POSTNATAL AGE ON THE DEVELOPMENT OF GLOMERULAR FILTRATION; DATA FROM LITERATURE

Author and reference		GFR markers	Effect of GA on GFR	Effect of PA on GFR
Al Dahhan et al	(1)	creatinine	↑	–
Aperia et al	(3)	creatinine	–	↑
Arant Jr.	(4)	creatinine	↑	–
Coulthard	(10)	inulin[a]	↑	–
Fawer et al	(14)	inulin[b]	↑	↑
Leake et al	(20)	inulin[c]	↑	–

GA: gestational age; PA: postnatal age; a: 24 hours continuous inulin infusion technique; b: traditional inulin clearance; c: 120 minutes continuous inulin infusion.

The aim of the present study is to establish the effect of GA on the development of GFR as well as the effect of postnatal age (PA). As the role of artificial ventilation on renal function is not completely defined, the effect of artificial ventilation on GFR was also studied. For these purposes, the GFR was measured in 41 preterm neonates using the continuous inulin infusion technique, reported to be a reliable technique also in the very young infant (8).

5.3. Materials and Methods

Patients

The GFR was measured in 41 preterm neonates, all admitted to the neonatal intensive care unit of the Sophia Children's Hospital. The GA varied between 27 and 36 weeks (mean 30.6 wks), the PA varied between 3 and 11 days. In 8 infants measured at day 4 or 5 the measurement was repeated at day 11 to determine the postnatal increase in GFR. The GA was estimated

from the mother's menstrual history and on physical assessment using the criteria of Dubowitz et al. (12). Birth weight varied from 810 to 2735 grams (mean 1384 grams). All infants were in stable clinical condition at the time of the study. Infants receiving nephrotoxic drugs were not included.

Relevant clinical data are summarized in table II. Fifteen neonates needed artificial ventilation.

GFR measurement

Inulin was administered as a glucose 10%-inulin solution containing 25 g inulin/l, at an infusion rate of 0.6 ml/kg.h. After 24 hours of infusion the inulin clearance (C_{in}) was calculated from the infusion rate (R), the inulin level in the infusate (I) and the plasma inulin level (P_{in}) with the following equation: ($C_{in} = \frac{I.R}{P_{in}}$).

The protocol was only performed when intravenous therapy was obligatory for clinical reasons. Blood sampling was performed, when possible, together with sampling for other laboratory data. Informed parental consent was obtained.

Inulin determination

Serum: At least 75 µl serum is deproteinized with an equal volume 0.6 N $HClO_4$ and centrifugated. The amount of endogenous glucose and fructose in serum is immediately measured (A_1). Then serum is incubated during 15 minutes at 70° C. During this incubation period inulin is converted into fructose (A_2). The difference between A_2 and A_1 is the amount of fructose originating from the acid hydrolysis of inulin.

The determination of the amount of fructose is performed by an enzymatic method (Boehringer Mannheim 716260) adapted to a Cobas Bio (Hoffman La Roche, Basel), in which the conversion of NADP to NADPH is measured (5). Serum blanc values of fructose-like substances were determined as soon as possible after deproteinisation. The recoveries of inulin at 200 mg/l and 500 mg/l were determined.

TABLE II CLINICAL DATA OF THE STUDY GROUP

Pat. nr	Gest. age weeks	Birth weight grams	Day of GFR measurement	Diagnosis**	Artificial ventilation	GFR ml/min
1	30.0	1930	4*	RDS	−	2.51
2	30.0	1010	4	RDS/p.d.a.	−	1.14
3	30.4	910	11	−	−	1.06
4	34.0	1940	4	RDS/icterus	−	1.80
5	29.0	1580	4*	RDS/asfyxia	+	0.77
6	29.0	1380	4	RDS/icterus	+	1.41
7	28.0	1250	4	icterus	−	1.63
8	29.0	1240	4	RDS/pneumonia/icterus	+	1.44
9	29.4	1330	4*	−	−	1.26
10	34.0	1280	4	pneumonia	−	1.28
11	27.0	970	5*	wet lung disease	+	0.87
12	29.4	1135	11	RDS/p.d.a.	+	0.43
13	34.0	2735	4	RDS/pneumonia	+	4.67
14	30.0	1210	4*	wet lung disease	−	1.10
15	30.0	1115	4*	RDS/p.d.a.	+	1.05
16	30.0	1050	9	−	−	1.10
17	28.0	1010	10	pneumonia	−	1.13
18	28.4	1100	4	RDS/pneumonia	+	1.12
19	28.0	1125	10	pneumonia/asfyxia	+	1.07
20	31.0	1365	11	RDS/pneumonia	+	2.00
21	36.0	2580	4	RDS	+	2.90
22	28.0	1100	4	RDS/p.d.a/pneumonia	+	1.02
23	31.0	1375	4	−	−	0.99
24	31.0	1920	4	PROM	−	1.98
25	27.0	900	5	RDS/p.d.a/pneumonia	+	0.68
26	27.0	810	5	RDS/pneumonia	+	0.59
27	34.0	1280	3	pneumonia	−	1.01
28	32.0	1030	5	−	−	1.09
29	33.0	1140	4	icterus	−	1.05
30	31.0	1670	7	icterus	−	1.26
31	32.0	1930	4*	wet lung disease/icterus	−	1.71
32	27.0	1080	7	−	−	1.08
33	33.0	1430	4*	icterus	−	1.01
34	33.0	970	4	pneumonia	−	0.84
35	34.5	2170	4	RDS/wet lung disease	−	2.13
36	30.0	990	4	icterus	−	0.97
37	30.4	1310	3	RDS/icterus	+	1.32
38	28.0	1050	3	RDS	−	1.28
39	32.0	1735	4	RDS/pneumonia	−	1.72
40	33.0	1985	3	RDS/icterus	−	1.77
41	33.0	1615	4	icterus	−	1.26

* GFR measurement was repeated on day 11.
** The diagnoses small for gestational age and prematurity are not mentioned in the diagnostic list.

RDS = respiratory distress syndrome; p.d.a. = persistent ductus arteriosus;
PROM = premature rupture of membranes.

Statistics

All values are expressed as mean ± SD. Differences between the mean values of groups of infants with or without artificial ventilation were tested using the Mann Withney test. The GFR values measured in the infants at day 4 and 11 were compared using the paired Student's t-test. A p level of less than 0.05 was considered as significant. The relationship between GA and GFR (ml/min or ml/min.kg) was calculated using linear regression.

5.4. Results

A. The reliability of the inulin determination.

The reliability of our test system was evaluated in various ways.

1) The hydrolysis step

The hydrolysis proved to be completed between 5 and 15 minutes at 70° C, an incubation time up to 60 minutes did not influence the results. Also glucose and fructose remained stable under these conditions. Although the hydrolysis at room temperature is slow, a few percents per hour, the blanc values were determined as soon as possible after deproteinization. These values varied between 1.6 and 48.4 mg/l "inulin" (average 19.3 mg/l, n=73) and are well above the noise of the analysis (4-5 mg/l). The recoveries at 200 and 500 mg/l levels were 101% ± 5.8% and 103% ± 4.4% respectively.

2) The infusion period

As a reliable inulin clearance depends strongly on a stable plasma concentration in an equilibrium situation, we controlled the validity of our infusion time. In 9 infants the infusion was continued for 30 hours and serum inulin concentrations were determined after 24 and 30 hours of infusion (table III). There was no statistically significant difference between the two sets of values, thus reflecting stable plasma concentrations.

TABLE III **INULIN PLASMA LEVELS IN 9 PATIENTS AFTER 24 AND 30 HOURS OF INULIN INFUSION (mg/liter)**

	24 hrs	30 hrs
1	324	393
2	337	346
3	345	318
4	279	285
5	291	248
6	356	349
7	272	302
8	355	372
9	184	175

B. Patients.

The effect of GA on GFR was evaluated using the data of 33 infants, their GFR being measured on day 3, 4 or 5. Although the interindividual variations in calculated GFR were rather large, we found a significant positive correlation ($r = 0.48$, $p = 0.0048$) between GFR (ml/min) and GA (weeks) (figure I). When GFR was expressed in ml/min.kg (figure II), there was no significant correlation between GFR and GA.

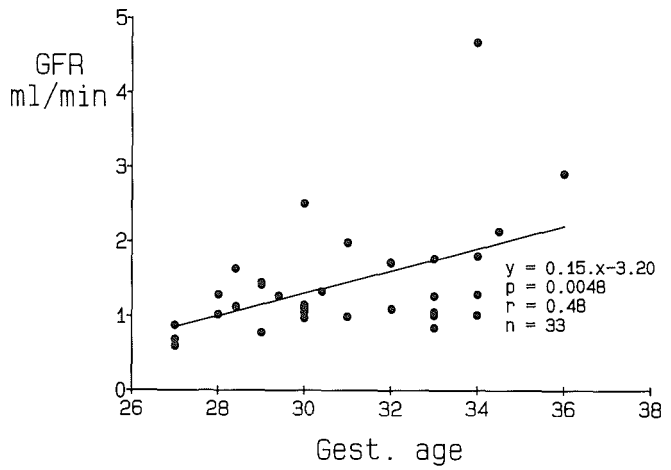

Figure I The relation between GFR (ml/min) and gestational age (weeks).

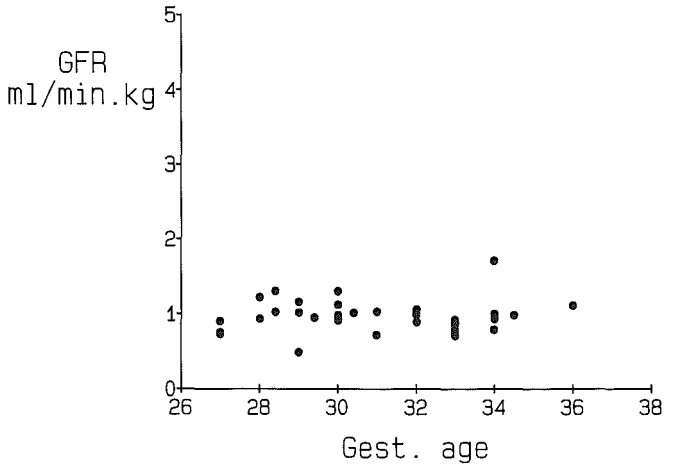

Figure II The relation between GFR (ml/min.kg) and gestational age (weeks).

In order to establish the effect of PA on GFR we repeated the GFR measurements on day 11 in 8 infants previously measured on day 4 or 5. A significant increase in GFR was present. The GFR increased from 1.28 ± 0.57 ml/min to 1.68 ± 0.57 ml/min. ($p < 0.007$). This increase was also present when GFR was expressed relative to body weight (0.88 ± 0.23 ml/min.kg to 1.18 ± 0.28 ml/min.kg; $p < 0.008$) (figure III).

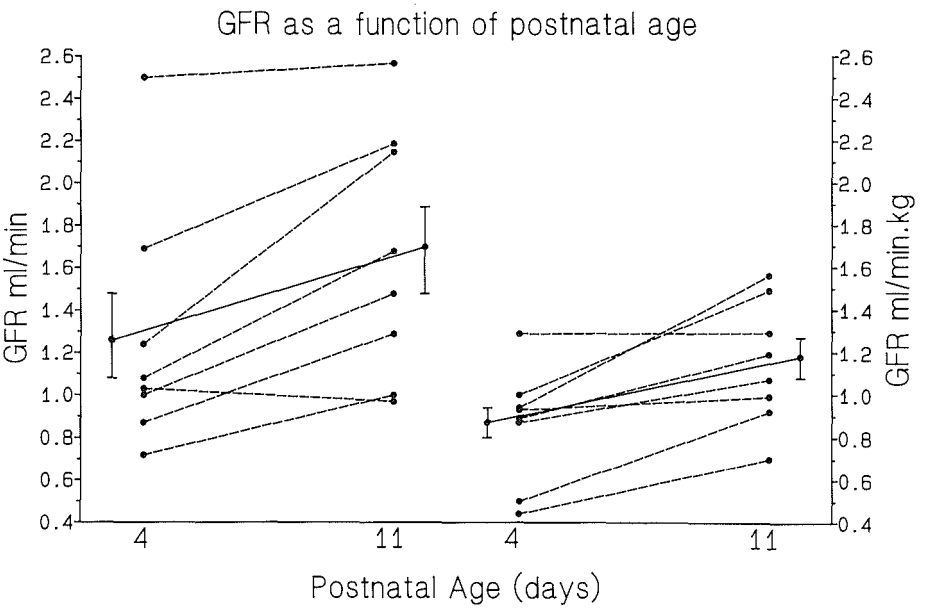

Figure III GFR as a function of postnatal age (⊢—⊣: mean values ± SEM).

When GFR was expressed as a function of post-conceptional age (GA + PA) data were comparable to those in figure I and II. The GFR (ml/min) increased significantly (GFR = 0.151 x (GA+PN) -3.35; r = 0.48; p = 0.0015; n = 41). Again no increase was present when GFR was expressed relative to body weight.

To establish the effect of artificial ventilation on GFR in preterm neonates, we compared the GFR of those needing artificial ventilation (n=15) to the GFR in neonates breathing spontaneously (n = 26) (table IV). No significant difference in GA, body weight or in day of GFR measurement was present. The GFR, either absolute or relative to body weight, did not differ significantly between the two groups (0.98 ml/min.kg $\pm$ 0.16 vs 0.97 ml/min.kg $\pm$ 0.33).

TABLE IV **DIFFERENCES BETWEEN PATIENTS WITHOUT (I) AND WITH (II) ARTIFICIAL RESPIRATION (mean $\pm$ 1 SD)**

n	group I 26	group II 15	significance
gest. age wks	31.2 $\pm$ 2.09	29.5 $\pm$ 2.55	n.s.
birth weight grams	1396 $\pm$ 392	1363 $\pm$ 561	n.s.
day of GFR measurement	4.8 $\pm$ 2.1	5.5 $\pm$ 2.8	n.s.
GFR ml/min	1.35 $\pm$ 4.2	1.42 $\pm$ 1.09	n.s.
GFR ml/min.kg	0.98 $\pm$ 0.16	0.97 $\pm$ 0.33	n.s.

5.5. Discussion

The traditional inulin clearance, used for the first time in the early thirties (24,25) is considered to be the golden standard for determination of the GFR. Inulin is a fructose polysaccharide with a mean molecular weight of about 5000 Dalton, is not metabolised by the body and is completely filtered by the glomeruli. The technique has proven to be useful in adults, children, as well as in preterm neonates (8).

The measurement of creatinine clearance can be considered as an alternative for the inulin clearance. It depends on the success of urine collection and on bladder emptying. However serum creatinine levels during the first days of life are partly depending on maternal serum levels (22) and are rapidly changing. Moreover there is evidence from animal experiments that tubular reabsorption of creatinine is possible in the very young animal

(2,11). It is uncertain whether this latter finding applies to the preterm newborn.

The single injection technique for measuring GFR has been practised by Broberger using inulin (6) and Svenningsten using polyfructosan (27). Coulthard (9) however, demonstrated that the use of single injection techniques can lead to an overestimation of up to 10% of the real value of GFR. Only in a few patients trustworthy results can be obtained by this technique. These data are in accordance with those of Fawer et al. (13).

Continuous infusion without urine collection may be an alternative method to measure GFR. After an equilibration period the amount of infused inulin will be equal to the amount excreted by the kidneys. This equilibration time will depend on the plasma half life ($T_{\frac{1}{2}}$) and on the level of GFR itself. $T_{\frac{1}{2}}$ in neonates has been reported to be greater than 4 hours (9). This long $T_{\frac{1}{2}}$ in neonates can be attributed to the combination of a low GFR and the large extracellular fluid space, wherein inulin is distributed (15). After an infusion period of five times the $T_{\frac{1}{2}}$ the inulin plasma levels reach a value within 3% of the steady state concentration. This explains that an infusion for 24 hours is needed in preterm infants. A short interruption of the infusion requires again a fairly long infusion period before a stable plasma level is achieved.

Another complicating factor is the large fluctuation of serum blancs for inulin in neonates, due to variations in "fructose-like" substances. We found serum blanc values up to 50 mg/liter, independent of the exogenous administered inulin. This may lead to an inacceptable inaccuracy in calculation of GFR. By means of our stepwise procedure we were able to eliminate this problem, as is demonstrated in the recovery of 101% and 103% after addition of exogenous inulin to at random chosen plasma samples. We applicated the latter technique in our study.

Our data demonstrate that GFR rises during development in two different ways. In the first place there is an effect of increase in body weight as indicated by the correlation between GFR, expressed in ml/min, and GA (figure I) and the lack in correlation between GA and GFR expressed in ml/min.kg. In the second place there is a further increase in GFR postnatally (figure III). This increase appears independent of an increase in body weight. This latter finding, earlier demonstrated by Fawer et al. (14) was critisized by Coulthard (10). He considered the observed increase in GFR relative to body weight during early postnatal life as an artefact.

This was in his opinion related to a temporary decrease in body weight postnatally, while it was absent when GFR was expressed per kg of so called projected weight. This is the weight obtained by a parallel projection from the birth weight along the centiles of Gairdner and Pearson (16), as if the babies had continued to grow at a rate observed during intrauterine life. We found an increase in GFR of 30%; much more than can be expected to occur as a consequence of body weight changes. Body weight in the 8 neonates studied by us, was not significantly different at day 4 and day 11.

Also from animal experiments there is evidence that GFR is rising postnatally independent of an increase in body weight. This increase appears to depend on hemodynamic changes in the kidney. An increase in renal blood flow (RBF) has been described (17,26). More recently it was demonstrated in sheep that a postnatal increase in GFR without changes in RBF can occur, probably depending on intrarenal redistribution of blood flow resulting in a rise in glomerular plasma flow in outer cortical nephrons (23).

The GFR in patients with ventilatory support was not significantly different from that of infants without (table IV). This indicates that artificial ventilation had no significant effect on the level of GFR. However, 13 of the 26 neonates breathing spontaneously had respiratory problems, as for instance respiratory distress syndrome (RDS), known to decrease GFR (7,18,28). We compared the artificially ventilated newborns also to those without known respiratory diseases. Again no significant differences were found. This contrasts to the data of Leslie et al. (21), who did find a negative effect of artificial ventilation on glomerular filtration using creatinine clearance. In their study the reported values for creatinine clearance are surprisingly low for the infants needing artificial ventilation as well as for those breathing spontaneously. The observed changes in GFR in neonates with RDS and those needing artificial ventilation reported in the literature, are probably due to a combination of negative influences: hypoxemia, hypercapnia and variations in systemic blood pressure. We demonstrated for instance in the newborn rabbit model a significant decrease in GFR related to acute hypercapnic acidosis (19).

In conclusion we found that the 24 hours inulin infusion technique is sufficiently accurate to evaluate the development of GFR in the preterm infant. The GFR of the newborn rises in two different ways during development. Firstly, an increase during gestation, associated with body growth. Secondly, a postnatal increase independent of an increase in body weight. We were not able to demonstrate a significant effect of artificial ventilation upon the GFR in preterm infants.

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Chapter 6 RENAL FUNCTIONAL IMPAIRMENT IN PRETERM NEONATES RELATED TO INTRAUTERINE INDOMETHACIN EXPOSURE

6.1. Abstract

Renal function was measured during the first 4 days postnatally in 9 preterm neonates (gest. age 26.2 to 31 weeks) exposed to indomethacin (ID) during the last 2 days of pregnancy (group I). The data were compared to those obtained from 9 control neonates (gest. age 28 to 34.5 weeks) (group II). Five out of the 9 neonates in group I were markedly oedematous at birth, none in group II. Urine production in group I was low (32.2 $\pm$ 16.8 ml/kg.day on day 1 increasing to 68.6 $\pm$ 21.4 ml/kg.day on day 4) and differed significantly from group II (75.2 $\pm$ 26.8 ml/kg.day on day 1 increasing to 84.8 $\pm$ 20.9 ml/kg.day on day 4; $p < 0.001$). Fluid intake was adapted to urine production when necessary.

A continuous inulin infusion was started directly after admission and continued for 5 days. After at least 48 hours of inulin infusion renal function was evaluated for 3 consecutive days.

The values of the inulin clearance (C_{in}), serum creatinine (S_{creat}), urine osmolality (U_{osmol}), osmolar clearance (C_{osmol}) and free water clearance (C_{H2O}) were stable in both groups during the study period. C_{in} was lower in group I than in group II ($p < 0.001$), while S_{creat} was higher in group I than in group II ($p < 0.0001$). U_{osmol} was higher in group I ($p < 0.01$), while C_{osmol} and C_{H2O} were lower in group I ($p < 0.02$ respectively $p < 0.01$). Fractional sodium excretion (FENa) was not different between the groups. In conclusion a short course of ID therapy to pregnant women leads to a significant functional impairment of the kidneys in their offspring just after exposure. This requires adaptation of fluid intake.

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6.2. Introduction

Indomethacin (ID) has been used as an inhibitor of preterm labor in the pregnant woman since 1974 (1). Even recently it has been reported that short courses of this drug before 35 weeks of gestation are save and without any risk of side effects (2). However, side effects are well known. In the animal model intrauterine closure of the ductus arteriosus has been described (3,4), as well as a decrease in cerebral blood flow (5) and an arrest in fetal nefrogenesis (6). In the human newborn there are various reports about persistent fetal circulation (7,8,9), cardiac insufficiency (10) and irreversible renal insufficiency (11,12,13). Recently we also described an infant, who remained totally anuric after birth, while his mother has been treated with ID for 6 weeks (14). Furthermore, it has been reported that treatment of preterm newborns with ID for medical closure of the patent ductus arteriosus results in a temporary decrease in renal function (15,16,17,18). These observations prompted us to an evaluation of the renal function in preterm neonates, exposed to ID during the last days of intrauterine life.

6.3. Patients and methods

6.3.1 Patients

Renal function studies were performed in 20 preterm newborn infants born after intrauterine exposure to ID. Nine patients (group I) were selected for further analysis of renal function. Eleven infants were excluded because of perinatal asphyxia, unstable circulation, for having received nephrotoxic medication and because of discontinuation of the study due to technical reasons. Gestational age varied from 26.2 to 31 weeks (mean 28.1 weeks) and birth weight from 1000 to 1640 gram (mean 1290 gram) (group I). The patients were compared to 9 newborns (group II), whose mothers did not receive ID during pregnancy. Gestational age in this group varied from 28 to 34.5 weeks (mean 31.1 weeks) and birth weight from 920 to 2055 gram (mean 1503 gram). Blood pressure was monitored in all infants using an indwelling catheter in the radial or tibial artery or by means of an electronic blood pressure monitor (Dynamap, Kriticon). Blood pressure was within the normal limits for age in all children (19). Fluid intake consisted of a glucose-NaCl solution, providing 10 gr glucose/100 ml and 3-5 meq Na^+/kg body weight.day, while on the second day of life amino-acids

and a triglyceride emulsion were added. The amount of proteins and fat was increased during the first week of life from 1 gram up to 2½ gram per kg body weight. When possible, nasogastric tube feeding was started. Informed parental consent was obtained for the studies. Clinical data are summarised in table I for both groups.

Table I CLINICAL DATA OF THE STUDY GROUPS

	Gest. age	Birth weight	Diagnosis*	Ventilation	ID dosage** mg	ID levels ug/ml
Group I						
1	27.5	1060	RDS. PROM	SV	400	0.3
2	28	1400	PROM	SV	300	1.9
3	27.4	1100	RDS	AV	200	0.3
4	29	1260	PDA	AV	200	0.5
5	28.8	1405	RDS	SV	200	0.6
6	28.4	1500	RDS	AV	800	0.3
7	28	1250	PROM	SV	100	0.2
8	30	1640	RDS	SV	400	0.1
9	26.2	1100	RDS	AV	550	0.2
Group II						
1	34.5	2275	RDS	SV	--	
2	30	920	--	SV	--	
3	30.4	1230	RDS	AV	--	
4	28	1055	RDS	SV	--	
5	31.7	1745	RDS. PROM	AV	--	
6	32.7	2055	RDS	SV	--	
7	32.7	1745	RDS	SV	--	
8	30	1060	RDS	AV	--	
9	30	1450	RDS	AV	--	

RDS = Respiratory Distress Syndrome; PROM = Premature Rupture of Membranes;
SV = Spontaneous Ventilation; AV = Artificial Ventilation.
** doses of ID administered during the last 48 hours of pregnancy.
* the diagnoses small for gestational age and prematurity are not mentioned.

6.3.2. Methods

The experimental protocol is outlined in figure I.

Immediately after delivery of the neonates or after admission to the neonatal intensive care unit a blood sample was taken to measure the ID level. ID levels were measured by means of high pressure liquid chromatography.

An inulin glucose infusion with an inulin concentration of 25 g/l and an infusion rate of 0.6 ml/kg.h was started after admission, together with the other intravenous solutes and continued for a period of 5 days. Total urine production and fluid intake were evaluated after 1, 2, 3 and 4 days

of infusion; renal function after 2, 3 and 4 days of infusion.
A blood sample was taken daily to measure plasma levels of inulin, creatinine, sodium and osmolality. Urine was collected during 6 hour periods. Urinary volume, together with osmolality and sodium concentrations were determined. After about two weeks the measurement of S_{creat} levels in group 1 was repeated.

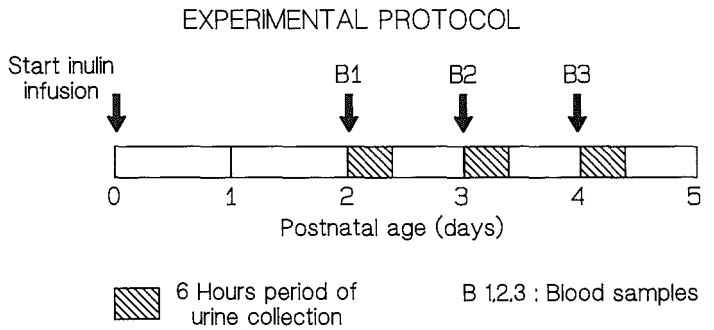

Figure I

Inulin concentrations in serum and urine were measured using an enzymatic method, adapted to a Cobaz-Bio Autoanalyser, serum creatinine was measured by an enzymatic method (Boehringer-Mannheim). C_{in} was used as a marker for GFR and calculated from the inulin concentration in the infusion (I), the infusion rate (R) and the plasma inulin concentration (P) ($C_{in} = \frac{i.R}{P_{in}}$) and expressed in ml/kg.min.
FENa, C_{osmol} and C_{H2O} were calculated from serum values and the values in the collected urine. Inulin was used as reference to calculate FENa.

6.3.3. Statistics

The paired t-test was used to compare the results obtained in the two patient groups on the various postnatal days. The two-sample t-test was used to compare the mean values of gestational age and body weight in the two groups. Multiple linear regression analyses with stepwise selection of the independent variables were performed to assess the best predictors of the variables used to quantitate glomerular and tubular function.

The independent variables included were the patient groups, gestational age, postnatal day and fluid intake. If a variable failed to meet entry requirement (probability of F to enter > 0.05), the procedure was terminated.

6.4. Results

Clinical data of the patients in group I were comparable to those in group II. Only gestational age was significantly different between the groups (28.1 ± 1.0 vs. 31.1 ± 2.0 weeks, $p < 0.01$). No differences in P_aCO_2, oxygen saturation or P_aO_2 between groups were present. In both groups 4 out of 9 neonates needed artificial ventilation during the study period. No significant differences in body weight were present (1290 ± 214 grams vs 1503 ± 478 grams) (table I).

Five of the nine infants in group I were oedematous during the study period. Ultimately two patients died, one in group I due to sepsis related to meconium peritonitis (patient nr. 6) and one in group II due to bilateral pneumothorax (patient nr. 8).

In group I ID levels varied from 0.1 to 1.9 µg/ml (table I). The data on fluid balance in both groups are summarised in table II. Fluid intake in group I was stable during the study period in contrast to group II.

TABLE II FLUID BALANCE DURING THE STUDY PERIOD IN NEONATES EXPOSED TO INDOMETHACIN (GROUP I) AND CONTROLS (GROUP II)

		Days of extrauterine life			
	Group	1	2	3	4
Fluid intake	I	95.9 ± 28.6	91.2 ± 18.9	96.3 ± 38.6	103.8 ± 29.7
	II	88.6 ± 17.5*	104.5 ± 16.0**	120.8 ± 27.8***	132.2 ± 28.0
Urine production	I	32.2 ± 16.8*	65.1 ± 29.5	62.3 ± 23.0	68.6 ± 21.4
	II	75.2 ± 26.8	78.2 ± 20.9	77.7 ± 26.9	84.8 ± 20.9

Muliple regression analysis revealed that the use of ID is the best predictor for a low urine volume ($p < 0.001$, $r = 0.41$) followed by postnatal age ($p < 0.001$, multiple $r = 0.50$).

* day 1: Significantly different vs day 2, 3 and 4 $p < 0.01$

** day 2: Significantly different vs day 3 and 4 $p < 0.01$

*** day 3: Significantly different vs day 4 $p < 0.05$

Intrauterine exposure to ID was found to be the best predictor for a low urine output among the variables included in the study (multiple r = 0.41). Postnatal age was second best. By adding postnatal age to the regression equation multiple r rose to 0.50, indicating that urine output increased with postnatal age. Other variables failed to meet entry requirements. In both groups parameters of glomerular function, i.e. C_{in} and S_{creat}, did not change during the study period. However group I had a significantly lower C_{in} compared to that of group II ($p < 0.002$, $r = 0.77$) and a significantly higher S_{creat} ($p < 0.0001$, $r = 0.70$). No correlation was found between the measured values and gestational age (table III). S_{creat} in group I after about 2 weeks was 55 µmol/L ± 16.

TABLE III GLOMERULAR FUNCTION PARAMETERS IN NEONATES EXPOSED TO INDOMETHACIN (GROUP I) AND CONTROLS (GROUP II)

		Days of extrauterine life		
	Group	2	3	4
Inulin clearance ml/min.kg	I	0.65 ± 0.15	0.63 ± 0.13	0.51 ± 0.13
	II	0.85 ± 0.15	0.89 ± 0.17	0.91 ± 0.12
Screatinine umol/l	I	103.9 ± 24.6	100.0 ± 22.8	103.7 ± 36.7
	II	56.2 ± 10.7	50.6 ± 12.6	50.7 ± 17.1

Multiple regression analysis revealed that inulin clearance correlates negatively with the exposure to ID ($p < 0.001$, $r = 0.77$). Serum creatinine values were higher in the ID treated infants.

Renal tubular functional parameters are summarised in table IV. None of the parameters changed during the study in the two groups. Multiple regression analysis demonstrated that the exposure to ID was the only predictor for a high U_{osmol} ($p < 0.01$, $r = 0.38$). C_{osmol} was less in group I compared to that in group II ($p < 0.02$, $r = 0.38$) as was C_{H2O} ($p < 0.01$, $r = 0.39$). Neither fluid intake nor postnatal age were significant predictors of the observed changes. FENa did not differ between the groups.

TABLE IV TUBULAR FUNCTION PARAMETERS IN NEONATES EXPOSED TO INDOMETHACIN (GROUP I) AND CONTROLS (GROUP II)

		Days of extrauterine life		
	Group	2	3	4
Urine osmol mosm/kg H_2O	I	295.1 ± 100.4	307.5 ± 116.6	316.0 ± 117.7
	II	220.0 ± 104.2	220.6 ± 77.4	238.0 ± 100.3
Cosmol ml/min.kg	I	0.0224 ± 0.10	0.037 ± 0.019	0.032 ± 0.017
	II	0.409 ± 0.18	0.040 ± 0.009	0.049 ± 0.016
C_{H_2O} ml/min.kg	I	0.0070 ± 0.010	−0.041 ± 0.015	0.0040 ± 0.013
	II	0.0149 ± 0.026	0.0201 ± 0.016	0.0172 ± 0.032
FENa %	I	4.0 ± 1.9	4.0 ± 2.6	3.2 ± 1.8
	II	4.8 ± 1.8	2.5 ± 1.6	2.8 ± 1.3

Multiple regression analysis revealed that the use of ID in group I was the only predictor of a high urine osmolality ($p < 0.01$, $r = 0.38$). C_{osmol} and C_{H_2O} were negatively influenced by the use of ID in Group I ($p < 0.02$, $r = 0.38$ and $p < 0.01$, $r = 0.39$ respectively).

6.5. Discussion

Our data indicate that intrauterine exposure to ID results in marked alterations in renal function during the first 5 days of postnatal life. Both GFR and urine production are suppressed. The decrease in urine production in group I necessitated a limitation in fluid intake at day 3 and 4 (table 2). The suppression of renal function is due to effect of ID on the developing kidney. Animal and human studies show, that ID passes slowly the placental barrier (20, 21). ID levels in the fetus increase and decrease slowly. In newborns ID levels at birth were equal to the maternal levels 5 hours after administration of ID (21). This suggests that the fetus is exposed to relatively high levels, when therapy to the mother is given several times a day during a prolonged period and that frequent drug administration can even lead to accumulation in the fetus. The half life of ID in preterm infants is longer than in adults. In adults a half life of 2.6 - 11.2 hours is present (22), in preterm infants half lifes varying from 11-20 h (23) up to 63 ± 38 h (24) are described.

Consequently the effect of ID given prenatally can continue for a considerable period postnatally. The levels of ID in our present study varied from 0.1 to 1.9 ug/ml, which is in the same range as observed when using ID for medical closure of the patent ductus arteriosus (25,26).

We used C_{in} for measuring GFR, as creatinine clearances during the first days of extrauterine life are not reliable (27). A disadvantage of the C_{in}, measured with a continuous inulin infusion is, that the GFR can only be calculated after a prolonged period of inulin infusion because of the known long equilibration time in the preterm infant (28). A small, but significant, difference in gestational age between the study groups is present. This is due to the fact that preterm labor in the referring hospital is nowadays generally treated with ID. Therefore few preterm babies are born without ID exposure. The C_{in} was expressed in ml/min per kg in order to minimize the effects of differences existing between the two groups. The difference in gestational age between both groups does probably not influence the results of the present study. In another study on 33 neonates with a gestational age varying from 27 to 36 weeks, in whom C_{in} was measured on day 3, 4 or 5, no correlation of the GFR per kg BW with gestational age between 27 and 34.5 weeks was observed (figure II, chapter 5). The data of Coulthard (29) concerning neonates with a comparable gestational age support these results. In our study groups no correlation existed between the measured values and gestational or postnatal age.
We assume that GFR increased to normal values after the observation period, since S_{creat} in all infants was normal for age after 2 weeks (30). C_{in} was not repeated at that time because of ethical and practical reasons. Intravenous infusion was not longer necessary in a number of patients and some were referred to another hospital. Also in the other 11 newborns exposed to ID but excluded from the statistical analysis no long-lasting effects on GFR were observed, as judged from the serum creatinine values. Renal tubular water handling was negatively influenced in the newborns exposed to ID. The observed effects on renal water handling are in accordance to those observed during medical treatment for a patent ductus arteriosus and can be attributed to either the low GFR or the improvement of concentrating capacity. Changes in FENa were not demonstrated in our study.
Although the effect on renal function in group I was temporary, we consider the use of this drug as controversial.

It is known from animal studies that differentiation processes in the maturing fetus are accompagnied by major changes in prostaglandin activity (31). The data of Novy et al. on intrauterine development of monkeys exposed to ID (6), demonstrating an arrest in nefrogenesis related to the use of ID, are in our opinion alarming. Total kidney volume in these monkey fetuses was reduced about 38% compared to non-exposed fetuses.
Furthermore, the effects of inhibition of prostaglandin synthesis on other organ systems cannot be neglected.
In the rabbit intrauterine closure of the ductus arteriosus can lead to intrauterine death (3,4), and in the newborn pig ID treatment leads to an important reduction in cerebral blood flow (5). In the newborn baby there are reports about reduction in cerebral blood flow velocities associated with ID treatment (32,33), persisting fetal circulation (7,8,9) and temporary insufficiency of the mitral valve (10). Itskowitz et al. (11) described three women in whom pregnancy ended with an oligohydramnios and perinatal death after exposure to ID for 4-6 days.
Cantor (12) reported transient anuria after 9 weeks of ID treatment for Still's disease of the mother. Veersema et al. (13) described a so called renal non functioning syndrome in a newborn related to 8 weeks ID treatment of the pregnant mother. We also described one newborn with the same symptoms (14) and we recently observed another infant remaining totally anuric associated with an intrauterine exposure of 9 weeks to ID.
It is known that ID impairs GFR in adults as well as in preterm newborns. In adults this phenomenon occurs especially in situations in which high levels of angiotensin II are present (34). In human preterm neonates an impairment of GFR has been observed during ID treatment of the patent ductus arteriosus (15,16,17,18). The underlying mechanism of the effect of ID upon GFR in adults, in neonates exposed after birth and in the newborns exposed during pregnancy is probably the same. Levels of angiotensin II are indeed high in newborns and decline after birth (35). Inhibition of prostaglandin synthesis blocks the antagonising effect of prostaglandins on angiotensin II induced vasoconstriction.
There is evidence from animal studies that renal vascular resistance is high and cortical perfusion low compared to later in life (36,37,38).
The inhibition of prostaglandin synthesis may so further increase renal vascular resistance, resulting in an impaired renal blood flow and a concomittantly reduced GFR. When ID is used for longer periods during

pregnancy a severe renal insufficiency can develop; sometimes irreversible functional damage occurs (11,12,13,14).

In conclusion, a significant suppression of glomerular function combined with a marked antidiuresis is present in neonates born after intrauterine exposure to ID. This has major implications on the fluid management of the newborns concerned and on the administration of drugs such as digoxin or nephrotoxic drugs. Although the alterations in renal function may be transient, we consider prolonged ID treatment during pregnancy as an important risk factor for the developing fetus.

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Chapter 7 GENERAL DISCUSSION

In chapter 3 the effect of acute hypercapnic acidosis on renal function in the newborn rabbit is investigated. Major changes in renal hemodynamics were observed. A dramatic fall in RBF was combined with a less pronounced decrease in GFR during acute hypercapnia leading to an increase in filtration fraction. We attributed this finding to an increase in renal vascular resistance, predominating at the level of the efferent arteriole. However, the increase in filtration fraction may also be related to intrarenal redistribution of blood flow, while differences in filtration fraction between surface and deep nephrons are shown to be present in other animal experiments (Seldin and Giebisch, 1985). The observations show that severe acute hypercapnic acidosis per se leads to vasoconstriction in the kidney. This is in agreement with data from experiments in the adult animal (Bersentes and Simmons, 1967; Norman et al., 1970; Farber et al., 1976).

The mechanisms contributing to the decrease in RBF were not investigated in this study, but it is of no doubt that hormonal influences are of major importance. Kurtz and Zehr (1978) demonstrated an increase in renin release during CO_2 retention in dogs, mediated via the hormonal catecholamine system and via the renal nerve pathway. These findings were confirmed by others with or without a decrease in MAP or RBF (Anderson et al., 1980, 1982; Rose et al., 1982). The observations of Edwards (1983) that the effect of Angiotensin II in rabbit kidneys predominates at the level of the efferent arteriole may explain the in our study observed increase in filtration fraction.

Denervation procedures were performed by different authors in studies aiming at defining the influence of renal nerves on renal hemodynamics during hypercapnia. Bersentes and Simmons (1967) found no improvement of RBF after denervation during hypercapnia in contrast to Norman et al. (1970) and Berns et al. (1979). The decrease in GFR found by Anderson et al. (1983) during hypercapnia was abolished by denervation. Other experiments have shown, that renal denervation can partially (Edwards, 1983) or totally (Anderson et al., 1980) normalise elevated renin levels. The changes in RBF, observed in our experiments can be due to a direct stimulation of the renin-angiotensin system by hypercapnia or to a stimulation via the renal nerves.

Arginin Vasopressin (AVP) is another, although less probable candidate. Hypercapnia per se stimulates AVP secretion as observed in the adult dog by Berns et al. (1979). Their study suggests that AVP may be one of the important factors causing renal vasoconstriction and antidiuresis in hypercapnia. On the other hand the influence of AVP may be less pronounced in the newborn animal, as suggested by the study of Robillard and Weitzmann (1980). Exogenous administration of AVP in the fetal sheep did not lead to renal vasoconstriction in contrast to observations in the adult animal. However a role for AVP in our experiments cannot be excluded.

Evidence for a role of catecholamines in the observed renal vasoconstriction is presented in the study of Bersentes and Simmons (1967). The vasoconstrictory response in their experiments was prevented by administration of phenoxybenzamine, an alpha blocking agent.

The effect of prostaglandins on changes in RBF during hypercapnic acidosis has not extensively been studied, but the study of Anderson et al. (1982) suggests an important role for prostaglandins in maintaining RBF during hypercapnic acidosis. They found in the anaesthetized dog, that pretreatment with indomethacin resulted in a significant decrease in RBF and GFR during hypercapnic acidosis. In animals without indomethacin pretreatment only a small decrease in GFR was noted.

In conclusion it seems that the negative effect of acute hypercapnic acidosis on renal hemodynamics is the resultant of imbalance between vasoconstrictive hormones (renin-angiotensin system, catecholamines, AVP) and vasodilator hormones as prostaglandins. In this respect these data are consistent with the observed changes in renal function related to the use of ID as inhibitor of preterm labor as described in chapter 6.

The effect of acute hypoxemia on renal function in the newborn rabbit has extensively been studied by Valloton (1984). In her experiments, in which newborn rabbits of the same age as in our study were used, P_aO_2 was diminished from normal values to 40 mm Hg. A significant decrease in urine volume combined with a decrease in RBF and GFR were noted. Filtration fraction remained stable. The calculated RVR rose. Other studies on hypoxemia in very young animals have been performed by Millard et al. (1979), Weissman and Clarke (1981) and Weissman et al. (1983) using the lamb, Rowe and Straus (1973) using the piglet and John et al. (1980) using the puppy. In all these studies changes in renal hemodynamics, i.e. a decrease in renal plasma flow and/or in GFR, were described.

Interestingly, one study has been performed in which the combined effect of hypoxemia and hypercapnia in a newborn animal (the piglet) was evaluated (Alward et al. 1978). A decrease in RBF together with a stable GFR, so an increase in filtration fraction was present in those experiments. In the adult dog Rose et al. demonstrated, that a combination of mild hypercapnia and hypoxemia led to a significant decrease in effective renal plasma flow and GFR, which could not be observed during hypercapnia or hypoxemia alone.

Also metabolic acidosis can alter renal hemodynamics. Robillard and Kisker (1987) studied the effects of severe lactic acidosis in the chronically catheterised fetal sheep model. They observed a significant decrease in RBF combined with an increase in RVR in these fetuses. Also blood flow decreased in the outer portion of the renal cortex during acidosis. pH changes were rather large in their experiments. The pH decreased from 7.37 to 6.95. Our own data on renal hemodynamics during metabolic acidosis in chapter 4 support these findings. We also observed a significant decrease in RBF and GFR in the newborn rabbit, associated with a decline in pH from 7.49 to 7.33 (table II, chapter 4). In the adult dog 2 studies have been performed. Bersentes and Simmons (1967) demonstrated a renal vasoconstriction during severe metabolic acidosis, when serum pH decreased more than 0.20; Farber et al. (1976) confirmed these results.

In conclusion we assume that our experiments in the newborn rabbit and data from the literature about hypercapnic acidosis, hypoxemia and metabolic acidosis indicate the background of changes in renal hemodynamics occurring in neonates with respiratory distress syndrome (Guignard et al., 1976; Cort, 1962; Tulassay et al., 1979).

In **chapter 4** the influence of acute acid-base changes on bicarbonate handling in the newborn rabbit was studied.

Data from literature demonstrate a low serum bicarbonate level in young animals compared to later in life. This low serum bicarbonate level can be related to renal proximal tubular immaturity as well as to extrarenal influences on bicarbonate handling. Evidence for proximal tubular immaturity is provided by studies in developing rats by Aperia (1987). She demonstrated that the Na^+/H^+ exchange in immature tubular cells of the rat is less efficient compared to that in more mature tubular cells. Bicarbonate reabsorption, in part depending on this Na^+/H^+ exchange may so be decreased in the developing kidney compared to later in life.

The fetal lamb has a low bicarbonate threshold compared to the adult ewe (Robillard et al., 1977), and the same applies to the newborn puppy (Moore et al., 1972). The low threshold for bicarbonate in these studies could easily be increased by a minor volume depletion. This relation between renal bicarbonate threshold and extracellular volume exists also in the adult animal, as documented by Kurtzmann (1970). The above mentioned observations suggest, that the low serum bicarbonate level present in the human newborn is in part associated with the high extracellular volume, present in the neonate (Friis Hansen, 1961), and does not only depend on a tubular immaturity in bicarbonate handling.

Kleinman (1978) demonstrated that tubular bicarbonate reabsorption mechanisms in the newborn dog were as efficient as those reported for the adult dog. Bicarbonate reabsorption increased as P_aCO_2 rose.

Schwartz and Evans (1983) found evidence in micropuncture studies in the newborn rabbit juxtamedullary proximal convoluted tubules for tubular immaturity in renal capacity to reabsorb bicarbonate. This prompted us to delineate bicarbonate handling of the kidneys in the whole neonatal rabbit model. The newborn rabbit is in a state of hypochloremic metabolic alkalosis compared to the adult rabbit, in sharp contrast to the described data from other species and also to data from human neonates. The origin of the alkalosis lies probably in three factors. Firstly we found evidence for renal chloride wasting in this young animal, suggesting a different tubular chloride handling. This can lead to the development and maintenance of a metabolic alkalosis, as is for instance known to be present in humans with Bartter's syndrome. Secondly, mother's milk of the rabbit provides a considerable alkaline load to the newborn rabbit. Thirdly, this newborn animal seems to be in a state of volume contraction. Indirect evidence for volume contraction rests on the presence of a high urine osmolality (mean U_{osmol} = 578 mosm/kg H_2O); a low FENa (0.89%) and the presence of hypochloremia (83 mmol/l). Volume contraction enhances bicarbonate reabsorption. Bicarbonate reabsorption studies in this chapter show that the capacity of the newborn rabbit's kidney to reclaim filtered bicarbonate is very efficient and responds normally to changes in P_aCO_2 and filtered bicarbonate load.

The study underlines the importance of evaluation of whole kidney function in animals before interpretating in vitro micropuncture studies to whole kidney observations in other species.

Our observations do not provide definite answers on questions about the presence or absence of immaturity in renal acid-base regulation in the human neonate. We may only conclude from our data as well as from the data of Robillard et al. (1977), Moore et al. (1972) and Kleinman (1978) that no convincing evidence exists to postulate limitations in bicarbonate reabsorptive capacity in neonatal animals before or just after the end of nefrogenesis. In contrast evidence is provided that extrarenal factors have a considerable impact on bicarbonate reabsorption. Further support for this statement is present in a study on renal bicarbonate handling in low birth weight infants by Zilleruello et al. (1986). They found a minimal or absent fractional bicarbonate excretion in the urine of preterm newborns, when blood pH was equal or less than 7.22 and P_aCO_2 exceeded 50 mm Hg. However, data on glomerular filtration are not presented, so no conclusions on bicarbonate reabsorption or bicarbonate threshold can be drawn from their data. The number of investigations in this study is also limited.

The observed impairment of renal acidification in newborns with severe RDS by Allen and Usher (1971) and Torrado et al. (1974) suggests a limitation in acid excretion in those infants. This may be due to an impaired capacity in excreting an acid load in the newborn mentioned by Hatemi and McCance (1961), who investigated the acidifying capacity in 7 days old newborns and by Kerpel Fronius et al. (1970) and Sulyok et al. (1972). More recent studies are lacking, while acid loading in newborns is nowadays not possible on medical ethical grounds.

Further evidence for a decrease in distal acidification during RDS is given by Androgué et al. (1983). They showed that acid-base regulation in the kidney of the anaesthetized dog during hypercapnia is different from the regulation under normal conditions. Proximal acidification increases, distal acidification decreases however. A decrease between pCO_2 in the urine and blood during respiratory acidosis under conditions, in which bicarbonaturia was maintained, was found. In conclusion we demonstrated that the rabbit, being in a state of hypochloremic metabolic alkalosis does show a perfect capacity in bicarbonate reabsorption of the kidney. This study, as well as the other mentioned studies, indicates that bicarbonate reabsorption in the neonate can be influenced by extra-renal factors in the same way as in the adult, despite the presence of proximal tubular immaturity.

In **chapter 5** the maturation of glomerular filtration after birth in preterm neonates is described. Studies on intrauterine renal hemodynamics in the human being are of course lacking. Animal experiments on intrauterine renal maturation have been performed in the intrauterine lamb. Rudolph and Heyman (1967) found low values for RBF in these fetuses compared to RBF values postnatally (Alexander, 1979). Studies on the circulation of the previable human fetus (Rudolph et al., 1971) have confirmed the presence of a low RBF during gestation (table I).

TABLE I PERINATAL 'RESTING' ORGAN BLOOD FLOW DISTRIBUTION (% cardiac output)

organ	fetal lamb term	neonatal lamb 1-2 days	human fetus first trimester
Brain	3	4	14
Heart	4	5	3
Extremity	16	10	11
Kidney	2	10	3
Liver	-	7	-
Intestines	5.5	32	11

A progressive rise in fetal umbilical arterial blood pressure in sheep and in man with increasing gestational age is present (Alexander et al 1958; Margolis and Orcutt, 1960). Animal studies on postnatal renal hemodynamics reveal major changes. A postnatal increase in RBF with a concomittant decrease in RVR has been found in the piglet (Gruskin et al., 1970; Spitzer and Edelmann, 1971). In sheep a postnatal increase in GFR without an increase in RBF, probably depending on intrarenal redistribution of blood resulting in a rise in blood flow through outer cortical nephrons has been observed by Nakamura et al (1987). This is in agreement with the findings of Aperia et al. (1977). They demonstrated that no significant changes in total renal blood flow existed between fetal and newborn lambs, immediately after cord clamping. This was accompagnied by an increase in intrarenal flow distribution to superficial nephrons in absolute terms (nl/min) and in relation to blood flow to the juxtamedullary glomeruli. Valloton (1984) described a decrease in RVR, an increase in RBF, combined with an increase in GFR in the newborn rabbit during the first two weeks of life. Systemic blood pressure rises in all studied animals during postnatal development.

In our study we evaluated changes in GFR during the first two weeks of life. GFR increases in two different ways. In the first place an effect of growth is present, demonstrated by a correlation between GFR expressed in ml per minute and gestational age. In the second place GFR increases postnatally. The data are in agreement with studies of Fawer et al. (1979A) and Aperia et al. (1981A). In full term infants the rise in GFR is paralleled by an increase in PAH clearance, indicating an increase in renal plasma flow (Guignard et al., 1975), accepting a constant PAH extraction in those infants, which is improbable. Whether the same applies to the preterm neonate is uncertain. The observed increase in PAH clearance, together with the progressive rise in blood pressure in the first day (Versmold et al., 1981) may be partly responsible for the postnatal maturation of GFR according to the animal experiments mentioned before.

We failed to demonstrate a negative influence of artificial ventilation on glomerular filtration. This is in sharp contrast to data from Leslie et al. (1986) in neonates and also to data from animal studies (Fewell and Norton, 1980; Tyler, 1983). The explanation for our contrasting results may be the selection of our patients. Only neonates with a stable clinical condition were admitted to our study. Leslie et al. (1986) found indeed that the decrease in GFR during artificial ventilation was accompagnied by a significant decreased blood pressure; Priebe et al. (1981) indicated that adequate intravascular volume plays a major role in maintaining a normal renal function during positive end-expiratory pressure ventilation. The low GFR found in other studies may probably be explained by disturbances in blood pressure, acid-base balance or hypoxemia and not by the effect of artificial ventilation per se.

The changes in renal hemodynamics occuring perinatally are probably related to actions of a variety of hormones and vasoactive substances such as catecholamines, bradykinin, histamin, the renin-angiotensin system, glucocorticoids, antidiuretic hormone, atrial natriuretic peptide, endogenous digoxin-like substance, prostaglandins. Evidence for hormonal influences is provided in a number of studies (Mott JC, 1975; Padbury et al., 1985; Leffler et al., 1985).

In **chapter 6** we evaluated the influence of intrauterine exposure to indomethacin, a cyclooxigenase inhibitor, on renal function in the neonate born premature despite this therapy.

Nadler and Brenner (1987) recently reviewed the synthesis and role of arachidonic acid metabolism in the kidney. The kidney is capable of synthetising products of all three families of compounds derived from arachidonic acid. Most prostaglandins (PG's) are believed to act only locally, so it is not surprising that their synthesis is compartimentalised, allowing specific actions on specific nephron processes. PGI_2 is the primary cyclooxigenase product in renal cortical arterioles; PGE_2 is present in many other parts of the nephron.

A number of stimuli for prostaglandin synthesis are known. Important are vasoactive hormones. Major vasoactive hormones as angiotensin II, vasopressin, bradykinin and norepinephrin can stimulate prostaglandin synthesis.

The major renal effects of cyclooxigenase metabolites are an effect on renal hemodynamics and an effect on water and sodium handling.

1. The effect on renal hemodynamics

Infusion of PGE_2 and PGI_2 in renal arteries leads to a reduced vascular resistance and increased renal blood flow in a number of species. Also the distribution of renal blood flow is influenced by PG's.

Juxta-medullary blood flow increases more than superficial cortical blood flow during PG infusion. PG's are important modulators of renal blood flow, especially under circumstances, wherein increases in circulating and intrarenal vasoconstrictive factors are present. They attenuate vasoconstrictive effects of these factors. Edwards (1985) demonstrated for instance in microperfusion studies in segments of arterioles of the rabbit that norepinephrin induced vasoconstriction could be attenuated by arachidonic acid perfusion. This effect could be blocked by addition of cyclooxigenase inhibitors.

Futhermore interactions between PG's and angiotensin II in the regulation of renal vascular resistance are of major importance. PG's stimulate renin release by juxtaglomerular cells, but counteract renal vasoconstrictory effects of angiotensin II (figure I).

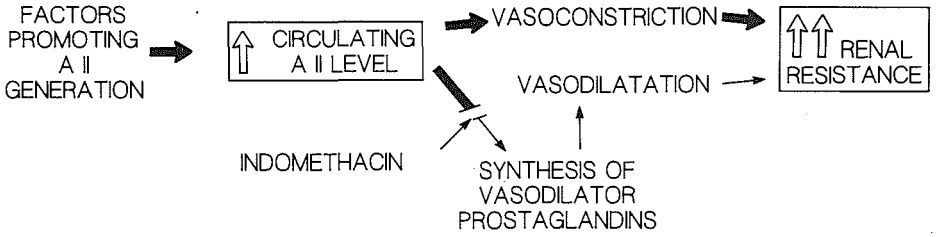

Figure I In the presence of indomethacin high circulating AII levels cause exagerated renal vasoconstriction because prostaglandin-induced vasodilator forces are lacking.
(from Nadler and Brenner, 1987; published with permission).

Also interactions with the effects of the kallikrein-kinin system are described, i.e., the vasodilatatory effect of this system may be augmented by PG's. The enhancement of renal blood flow related to vasopressin activity is also dependent on intact renal prostaglandin synthesis.
Most studies demonstrated that the effect of PG's on GFR is the same as the effect on RBF, so PG's serve to maintain GFR in the presence of neural or humoral factors, that would otherwise reduce filtration.

2. The effect on renal salt and water handling

Renal arterial infusion of arachidonic acid or its metabolites is generally associated with an increase in the excretion of sodium. It is probable that PG's, especially PGE_2, are important modulators of salt transport in the medullary thick ascending limb of Henle, i.e., they reduce sodium transport in the thick ascending limb. This effect of PG's together with an inhibitory effect on AVP stimulated increases in the osmotic water permeability of collecting ducts has also a physiologic role in the regulation of urine concentrating ability.
Studies on fetal prostaglandin metabolism in the kidney were performed by Pace-Asciak (1976), demonstrating an increase in renal prostaglandin activity with advancing gestational age. Prostaglandins may play an important role in regulation of fetal growth and morfogenesis. The increase in renal prostaglandin catabolic activity noted by Pace-Asciak during advancing

gestation parallels major renal morfological events. Novy (1978) mentioned that indomethacin administration to pregnant monkeys led to an arrest in nefrogenesis in their fetuses. Kidney growth was severely impaired in these fetuses.

Several case reports concerning oligohydramnios and irreversible anuria after delivery in neonates due to intrauterine exposure to ID drew attention to the adverse effects on renal function in the human fetus (Itzkovitz et al., 1980; Cantor et al., 1980; Veersema et al., 1983; v.d. Heijden et al., 1986). In our study we demonstrated an impairment in renal function in preterm neonates during the first days of life after intrauterine exposure to ID during a relative short period. The observation of a reduced GFR resembles that observed in adults. ID can decrease renal function in adults especially in situations in which renal and systemic vasoconstriction occurs, for instance, congestive heart failure, liver cirrhosis and nephrotic syndrome (Walshe and Venuto, 1979; Dunn, 1984; Arisz et al., 1976). The same applies probably for the human fetus and newborn. Levels of angiotensin II are high in newborns and decline after birth (Fiseliers et al., 1983).

ID can so disturb the balance between the different vasoactive hormones leading to an increase in renal vascular resistance. In a study on the effect of ID on renal function in neonatal rabbits a dramatic increase in renal vascular resistance was observed, confirming this hypothesis (Duarte-Silva et al., 1986). The depressing effect on renal function that we observed was reversible. However, this still has important implications on medical management of those often critically ill neonates during the first week of life. Adjustment of fluid intake and dosages of different drugs as for example aminoglycosides and digoxin seems to be necessary. Our observation of fetal anuria in two newborns after intrauterine exposure to ID during 6 and 9 weeks respectively confirms the case histories already described. Longlasting ID therapy during pregnancy presents a high risk for the fetus.

In the context of the described changes in renal hemodynamics, the overall adaptation of renal function in the newborn is astonishing. This has already been mentioned by Mc Crory in 1972, stating: "Our clinical experience attests to the admirable way in which the young infant's kidney regulates his water and electrolyte balance in the varied circumstances normally encountered in the postnatal period".

The more knowledge is obtained, the more this statement is confirmed. Even in the very preterm neonate glomerulotubular balance established itself quickly despite a difference in morphological maturation between glomeruli and tubuli pointing to glomerular preponderance. In clearance and micropuncture studies in the newborn guinea pig Kaskel et al. (1987) beautifully demonstrated that glomerulotubular balance during the early postnatal life is made possible by a high permeability of the proximal tubuli, which compensates for existing low net reabsorptive pressures in the tubuli. In the more mature animal, in which hydraulic conductance diminishes (Larsson and Horsten, 1976), an increase in active transport mechanisms for sodium is postulated (Kaskel et al., 1987). In the developing rat maintenance of glomerulotubular balance appears to be due to a combination of factors. Glomerular plasma flow rises, the ultrafiltration coefficient increases. Concurrently peritubular capillary reabsorption increases, presumably reflecting an enlargement of peritubular capillary reabsorption surface area (Kon et al., 1984). These data are probably the physiological basis for the existence of glomerulotubular balance in the immature animal and the quick establishment of glomerulotubular balance in the preterm neonate (Aperia et al., 1983).

7.1. Clinical implications of the study

The purpose of the studies performed was to analyse renal functional adaptation to extrauterine life and renal functional changes due to a number of extrarenal influences.

The study in **chapter 3** on hypercapnic acidosis together with the data in **chapter 5** on changes in glomerular function in the neonate leads to the following statements.

A Renal functional adaptation to extrauterine life is excellent even in the tiny premature neonate. However, GFR is low and negative influences on renal hemodynamics can lead to important decreases in glomerular function.

B Extreme hypercapnic acidosis is proven to have a negative impact on RBF and GFR in the newborn rabbit. Hypercapnia may aggravate renal functional decrease due to other factors in preterm neonates. Maintenance of an adequate ventilation and a pH within the normal range

therefore has a positive influence on renal function. A critical and frequent control of renal function is needed in neonates with severe respiratory distress permitting adaptations in fluid and drug regimes.

The study on acid-base regulation in the newborn rabbit **(chapter 4)** leads to the following statements.

A Micropuncture studies in animal models are of extreme importance to elucidate physiological mechanisms. Complementary investigations in the whole animal model are however needed before results of micropuncture studies lead to interpretations of mechanisms in the human being.

B Bicarbonate handling of the immature kidney responds to extrarenal influences in the same way as that of the mature kidney despite the presence of proximal tubular immaturity.

C We suggest that a maintenance dose of sodium bicarbonate in the tiny premature infant may be beneficial considering the known sodium loss on proximal tubular level and the limitations of the kidneys of these infants in excreting an acid load.

The study on the influence of indomethacin in **chapter 6** leads to the following statements.

A Longlasting indomethacin treatment of pregnant women bears an unacceptable risk for the fetus considering the data on oligohydramnios and irreversible anuria post partum in such infants.

B When preterm infants are born shortly after ID exposure in utero glomerular function is restricted indicating that adaptations in drug dosages are needed.

C When preterm infants are born shortly after ID exposure in utero the renal capacity in excreting a water load is restricted indicating that adaptations in fluid intake are needed.

D A case control study concerning other side effects of intrauterine indomethacin exposure is needed, as we now know the list of complications of indomethacin administration postnatally: a reduction in cerebral blood flow velocity, gastrointestinal bleeding and the risk of intracerebral hemorrhage.

7.2. Summary

Nephrogenesis in the human being proceeds until the 35th week of gestation. The anatomic immaturity of the kidneys in preterm neonates concurs with a functional immaturity on glomerular as well as on tubular level. The studies in this thesis are performed in order to analyse the effect of extrarenal influences on renal function in the developing kidney. Studies are in part performed in newborn rabbits before the end of nephrogenesis, and in part in preterm human neonates.

Chapter 1 describes the backgrounds and the questions posed in this investigation. The questions may be summarised as follows:

1. What is the effect of isolated hypercapnic acidosis on renal hemodynamics in the newborn rabbit?
2. What is the capacity of the immature kidney of the newborn rabbit to reclaim filtered bicarbonate during acute acid-base changes?
3. How does glomerular function develop in the preterm neonate?
4. How does the immature kidney of the preterm human newborn respond to exposure to indomethacin during pregnancy?

Chapter 2 summarises data on renal functional development from literature. The development of glomerular function, tubular function and acid-base regulation are separately discussed.

Chapter 3 is a study on the effect of isolated hypercapnia on renal hemodynamics in the newborn rabbit. Acute hypercapnia with a P_aCO_2 of 80 mm Hg and 100 mm Hg leads to an increase in renal vascular resistance, combined with a decrease in effective renal plasma flow. Renal vasoconstriction due to hypercapnia predominates at the level of the efferent arteriole leading to an increase in filtration fraction. The results suggest that the renal disturbances observed in neonates with respiratory distress may, at least in part, be due to the effect of hypercapnic acidosis superimposed on the already known effects of hypoxemia.

Chapter 4 is a study on renal bicarbonate handling in the newborn rabbit during acute acid-base changes. The normal newborn rabbit is, in contrast to observations in other species, in a hypochloremic metabolic alkalotic state compared to the adult rabbit. The alkalosis is probably generated by an alkaline load contained in the rabbit's mother's milk and maintained by a state of renal tubular chloride wasting and volume contraction.
Bicarbonate reabsorption proves to be strongly dependent on the bicarbonate filtered load during acute metabolic alkalosis and acidosis. A linear positive relationship exists between increments in P_aCO_2 and bicarbonate reabsorption. The immature kidney in this animal shows an adequate response to changes in the bicarbonate filtered load and P_aCO_2.

Chapter 5 describes the normal development of glomerular filtration rate in 41 preterm neonates, using the continuous inulin infusion technique. The reliability of this method for establishing glomerular filtration rate was evaluated. The technical procedure as well as the duration of the inulin infusion proves to be adequate. The in this study investigated preterm neonates demonstrate an increase in glomerular filtration rate in two different ways. An increase is present, closely depending on an increase in body weight and an increase is present related to postnatal age independent on body growth, probably associated with changes in renal hemodynamics. In this study no differences in glomerular filtration rate between neonates with or without artificial ventilation can be demonstrated.

Chapter 6 is a study on the effect of recent intrauterine exposure to indomethacin on renal function in the preterm neonate just after delivery. Nine preterm neonates exposed to indomethacin during the last two days of pregnancy are compared with 9 neonates not exposed to this drug. Renal functional evaluation is performed using a continuous inulin infusion for 5 consecutive days. Exposed neonates are markedly more oedematous than controls. Renal functional evaluation reveals that glomerular filtration is significantly decreased in exposed neonates compared to controls. Also a significant decrease in water excretory capacity is present during the study period. Renal functional impairment is temporary. This study underlines the need for critical evaluation of renal function and fluid balance in neonates intrauterine exposed to indomethacin, even after a short

period of treatment. Treatment of pregnant women for longer periods may be harmful for renal functional development in the fetus. It may also cause renal failure.

Chapter 7 is a general discussion reviewing the results of the previous studies as well as data from literature on the same issues. Mechanisms that may be related to the observations in the studies are highlighted. The role of vasoactive hormones is discussed; especially the role of prostaglandins on renal function during maturation is reviewed in order to explain the observed effects of indomethacin on renal function described in chapter 6. Considering our observations it is concluded that the overall adaptation of renal function of the immature kidney is good. Finally clinical implications of the studies are proposed.

7.3. Samenvatting

De nefrogenese van de mens eindigt omstreeks de 35ste zwangerschapsweek. De zodoende aanwezige anatomische onrijpheid van de nieren bij preterme pasgeborenen gaat samen met een functionele onrijpheid. De in dit proefschrift beschreven studies werden verricht om de reactie van de zich ontwikkelende nieren op extrarenale veranderingen te bestuderen. De onderzoeken werden deels verricht bij het pasgeboren konijn voor de beëindiging van de nefrogenese; deels bij preterme pasgeborenen.

In **hoofdstuk** 1 wordt een kort overzicht gegeven van de achtergrond van het onderzoek. Vervolgens worden de in het proefschrift gestelde vragen opgesomd, namelijk:

1) Wat is het effect van geïsoleerde respiratoire acidose op de hemodynamiek in de nier bij het pasgeboren konijn?
2) In hoeverre zijn de nieren van het pasgeboren konijn in staat tot bicarbonaatreabsorptie tijdens acute veranderingen van het zuur-base evenwicht?
3) Hoe ontwikkelt zich de glomerulaire functie bij de preterme pasgeborene?
4) Hoe is de reactie van de nieren van de preterme pasgeborene op intrauterine blootstelling aan indomethacine?

In **hoofdstuk** 2 wordt een literatuuroverzicht gegeven van de nierfunctieontwikkeling bij de preterme pasgeborene. De ontwikkeling van glomerulaire functie, tubulaire functie en de renale zuur-base regulerende mechanismen worden afzonderlijk behandeld.

In **hoofdstuk** 3 wordt het effect van geisoleerde respiratoire acidose op de hemodynamiek van de nier bij het pasgeboren konijn beschreven. Acute respiratoire acidose met een P_aCO_2 van 80 mm Hg en 100 mm Hg resulteert in een toename van de renale vaatweerstand gecombineerd met een afname van de effectieve renale plasmaflow. De optredende renale vasoconstrictie is het meest uitgesproken op het niveau van de efferente arteriolen, hetgeen leidt tot een toename van de filtratiefractie. Uit de resultaten van deze studie blijkt, dat de veranderingen in nierfunctie, welke worden waargeno-

men bij pasgeborenen met hyaliene membranen ziekte mede het gevolg kunnen zijn van de hierbij aanwezige respiratoire acidose.

In **hoofdstuk 4** wordt de bicarbonaat reabsorptiecapaciteit van de nieren bij het pasgeboren konijn beschreven. In vergelijking met het volwassen konijn bestaat er een hypochloraemische metabole alkalose bij het pasgeboren konijn. Deze alkalose lijkt te berusten op een combinatie van factoren. Ten eerste zijn er aanwijzingen voor een hoog aanbod van alkali in de moedermelk van het konijn. Ten tweede is er sprake van tubulair chloorverlies. Ten derde is het pasgeboren konijn in een staat van volumecontractie. Beide laatste factoren onderhouden een eenmaal bestaande alkalose.
De hoeveelheid bicarbonaat, welke tijdens metabole alkalose en acidose geresorbeerd wordt, is in hoge mate afhankelijk van de hoeveelheid gefiltreerd bicarbonaat.
Er bestaat een lineaire positieve relatie tussen een toename van P_aCO_2 en de bicarbonaatreabsorptie. De onrijpe nier van dit pasgeboren proefdier reageert adequaat op veranderingen in de hoeveelheid gefiltreerd bicarbonaat en in de P_aCO_2. Uit de studie blijkt, dat de immaturiteit van de nieren, althans bij het jonge konijn, in bovenbeschreven omstandigheden geen beperkende factor is voor bicarbonaatreabsorptie.

In **hoofdstuk 5** wordt de normale ontwikkeling van de glomerulusfiltratie van de preterme neonaat behandeld. De glomerulaire filtratiesnelheid wordt bij 41 neonaten d.m.v. de continue inuline infusie vastgelegd. Zowel de technische procedure van de inulinebepaling als de toegepaste infusieduur zijn voldoende voor een betrouwbare vaststelling van de glomerulusfiltratie. De glomerulusfiltratie van de in deze studie onderzochte pasgeborenen neemt na de geboorte op 2 manieren toe: In de eerste plaats is er een toename aanwezig, welke nauw samenhangt met een toename in lichaamsgewicht. In de tweede plaats bestaat er een toename, welke samenhangt met de postnatale leeftijd onafhankelijk van lichaamsgroei. Deze laatste stijging berust vermoedelijk op veranderingen van de hemodynamische verhoudingen in de nier. In deze studie kan geen relatie tussen de glomerulusfiltratie en beademing van de pasgeborenen worden aangetoond.

In **hoofdstuk** 6 wordt het effect van recente kortdurende intrauterine blootstelling aan indomethacine op de nierfunctie van preterme neonaten onderzocht. Negen kinderen, geboren na recente indomethacine toediening worden vergeleken met 9 kinderen zonder deze therapie. De nierfunctie wordt geëvalueerd gedurende de eerste 5 extrauterine dagen d.m.v. continue inuline infusie. Vijf behandelde pasgeborenen hadden klinisch manifest oedeem. De glomerulaire filtratiesnelheid in deze groep is significant verlaagd in vergelijking met de controlegroep. Ook de waterklaring en de osmolaire klaring zijn significant lager in de behandelde groep in vergelijking met de controle groep. De effecten op de nierfunctie lijken van tijdelijke aard te zijn.
Uit de studie blijkt het belang van nierfunctiebewaking en een goede controle van de vochtbalans bij pasgeborenen, waarvan de moeders recent indomethacine toegediend kregen tijdens de zwangerschap, zelfs indien dit een kortdurende therapie is. Langdurige indomethacinebehandeling van zwangeren is mogelijk blijvend schadelijk voor de nierfunctieontwikkeling van de foetus.

In **hoofdstuk** 7 worden de resultaten van de studies evenals relevante literatuurgegevens besproken. De mechanismen, welke verband houden met de beschreven bevindingen worden toegelicht. De rol van vasoactieve hormonen, met name van prostaglandines op de nierfunctie en functieontwikkeling bij de pasgeborene wordt besproken. In het licht van de gegevens uit onze studies en de gegevens uit de literatuur blijkt, dat de aanpassingsmogelijkheden van de nier ondanks de zowel morphologische als functionele onrijpheid bij de preterme neonaat uitstekend zijn. Tenslotte worden klinische consequenties uit de verichtte studies getrokken.

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CURRICULUM VITAE

De schrijver van dit proefschrift werd op 19 mei 1950 te Hengelo (O) geboren. In 1968 behaalde hij het diploma gymnasium-β aan het gemeentelijk gymnasium te Hengelo (O). In datzelfde jaar werd de studie medicijnen aangevangen aan de Rijksuniversiteit te Groningen.

Van 1970 tot 1972 was hij student-assistent in het laboratorium voor vegetatieve fysiologie in Groningen (hoofd: prof. dr. W.G. Zijlstra). Het artsdiploma werd in februari 1975 behaald. Na 1 jaar militaire dienst was hij gedurende 9 maanden werkzaam op de afdeling kindergeneeskunde van het Diaconessenhuis te Groningen (Dr. N.J. Jansonius en Dr. H.A. Polman, kinderartsen). Van 1977 tot 1981 specialiseerde hij zich in de kindergeneeskunde in het Sophia Kinderziekenhuis te Rotterdam (hoofd: prof. dr. H.K.A. Visser). Op 1 januari 1981 werd hij als kinderarts in het specialistenregister ingeschreven. Vanaf 1981 is hij werkzaam als wetenschappelijk hoofdmedewerker op de afdeling kindernefrologie van het Sophia Kinderziekenhuis.

In 1984 verbleef hij in Lausanne (Zwitserland) na het verkrijgen van een research fellowship van de Sophia Stichting Wetenschappelijk onderzoek. Aldaar werd gedurende 1 jaar dierexperimenteel onderzoek verricht in het "Laboratoire de Nephrologie Pediatrie" (hoofd: prof. dr. J.P. Guignard) van het Centre Hôpitalier Universitaire Vaudois. Hij is getrouwd met Janny van Eerden en heeft drie kinderen: Gert-Jan, Marijke en Thijs.

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