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Renal functional maturation: renal handling of proteins by mature and immature newborns

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Abstract. Renal clearance of creatinine (C_{cr}), total protein excretion, urinary protein composition and renal clearance of albumin (C_{alb}) were measured and calculated in male premature and mature infants of gestational age 29–41 weeks and in mature infants 1 and 3 months of age. Total protein excretion decreased slightly but not significantly during maturation. The urinary protein composition changed significantly as the fraction of low molecular weight proteins decreased from 48% at a gestational age of 29–33 weeks to 24% in mature infants aged 3 months, the albumin fraction increased from 39%–46% and the proportion of higher molecular weight proteins increased from 12%–29%, respectively. C_{alb} decreased from 2.73–0.80 µl/min/1.73 m^2 in the presence of a rise in C_{cr}, resulting in a significant fall of the ratio C_{alb}/C_{cr} from 0.0137 in the youngest prematures to 0.00147 in 3-month-old mature infants.

Key words: Maturation of kidney function – Proteinuria

Introduction

The occurrence of protein in the urine of neonates and infants has been subject to numerous reports during at least 100 years. Early findings suggested a 41%–93% incidence of proteinuria in the first weeks of life [8, 11, 33]. More recent studies [9, 19, 20, 26] have found that protein excretion in infants and children aged 15 days to 16 years is in a range comparable to proteinuria found in adults [1]. Large variations in urinary protein excretion in infants seem to be common on comparing results in the literature, where values vary by several 100% [1, 6, 18, 19, 25].

The physiological mechanism of proteinuria seems to be well known from micropuncture studies in the rat kidney [3, 12, 13] and from clinical observations [14]. Proteins of normal mammalian urine are thought to be mainly plasma proteins that have been filtered at the glomerulus and subsequently escaped tubular reabsorption [3, 12, 13, 25]. Protein filtration depends on the glomerular filtration rate and protein reabsorption on tubular transport capacity, both parameters changing with altering glomerulo-tubular balance during the very early stages of life [2, 4, 15, 16, 27, 31, 32]. In this paper we have compared renal handling of different urinary proteins in seven groups of male immature and mature infants with different gestational and conceptional age. Results indicate that the fractional composition of urinary proteins changes significantly.

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Abbreviations: C_{cr} = clearance of creatinine; C_{alb} = clearance of albumin; GA = gestational age; GFR = glomerular filtration rate; HMW = high molecular weight; LMW = low molecular weight; A = albumin

Materials and methods

Seventy-three physiologically stable male newborn infants with gestational ages from 29–41 weeks, and birth weights from 1100–4190 g were studied after 3 days of life. The estimate of gestational age (GA) was made from menstrual history and the Dubowitz score [10]. These male infants were divided into five groups of premature and mature newborns according to their GA and into two groups of infants 1 and 3 months of age corresponding to a mean conceptional age (sum of gestational age + postnatal age) of 44 and 56 weeks. In addition, two groups of six female mature newborns and five 3-month-old female infants were studied. Accurately timed urine collections were obtained from each infant by means of an external collecting device applied over the external genitalia in a standard fashion. Collecting periods ranged from 6–24 h. Urine specimens were frozen and volumes per time were noted. Venous blood samples were drawn on the day of urine collection and serum was split for determination of creatinine and protein content. Urine samples were analysed for creatinine, total protein and protein pattern by electrophoresis procedure.

The endogenous creatinine clearance (C_{cr}) was taken as an estimate of glomerular filtration rate (GFR). "True" creatinine concentrations in blood and urine were determined by an automized micro-method [23] after eliminating non-creatinine chromogens. Urine was diluted 4–20 times.

Total protein in urine and diluted plasma was measured by a micromodification of the method of Lowry et al. [24], which showed a linear relationship over a range of 0.5–50 mg/100 ml of total protein concentration [3]. Differentiation of proteins and quantitative measurement of albumin were performed in 1.0 µl samples of unconcentrated urine and diluted plasma (51×) utilizing micro-polyacrylamide-gel electrophoresis with a continuous gradient from 4%–40% [30] contained in 5 µl capillary tubes. Proteins were separated according to their molecular size by passing through a mesh of polyacrylamide of continuously increasing density. After staining with FD and C Fast Green (Eastman Products), gels were

scanned quantitatively by a Joyce-Loebl microdensitometer with an integrator unit. Protein standards for the electrophoretic method were made from appropriate dilutions of human serum. Total protein concentration of serum standards was determined by the Kjeldahl method [18].

Numbers in tables are given as mean values and standard error (±); significance was calculated applying Student's *t*-test.

Results

Renal clearances of creatinine and albumin according to gestational or postnatal age are shown in Table 1. Table 2 summarized the qualitative pattern of urinary protein excretion.

Creatinine clearance

In premature male infants of 29–33 weeks of GA the mean C_{cr} was 22.5 ml/min per 1.73 m^2 BSA. Values rose gradually with

Table 1. Creatinine clearance (C_{cr}) and renal albumin clearance (C_{alb}) in seven groups of infants of different gestational age or postnatal age

Age	Number and sex	C_{cr} (ml/min per 1.73 m^2)		C_{alb} (µl/min per 1.73 m^2)		C_{alb}/C_{cr} (%)	
29–33 weeks (gestational)	6 male	22.5 ± 12.7		2.73 ± 0.19		0.0137 ± 0.0122	
33–35 weeks (gestational)	8 male	23.0 ± 9.3		1.77 ± 0.15		0.00911 ± 0.00096	
35–37 weeks (gestational)	7 male	28.5 ± 10.7	***	1.28 ± 0.15	*	0.00369 ± 0.00576	**
37–39 weeks (gestational)	12 male	35.1 ± 16.4		1.17 ± 0.114		0.00256 ± 0.00027	
39–41 weeks (gestational)	17 male	34.1 ± 13.2	n.s.	0.907 ± 0.081		0.00265 ± 0.00208	n.s.
	6 female	35.3 ± 7.3		1.18 ± 0.62		0.00327 ± 0.00179	
1 month (postnatal)	11 male	37.8 ± 15.2	**	0.634 ± 0.180	n.s.	0.00202 ± 0.00304	n.s.
3 months (postnatal)	5 male	61.4 ± 18.4	n.s.	0.80 ± 0.171		0.00147 ± 0.00044	
	5 female	62.0 ± 27.0		0.856 ± 0.192		0.00138 ± 0.00053	

Results are given in means ± SEM
* $P < 0.0005$
** $P < 0.0025$
*** $P < 0.005$
n.s. Not significant

Table 2. Urinary protein excretion in seven groups of male infants of different gestational or postnatal age

Age	Total protein excretion (mg/24 h per 1.73 m^2)		LMW (%)		A (%)		LMW/A	HMW (%)	
29–33 weeks (gestational)	164 ± 91 (n = 6)		48.5 ± 4.2		39.0 ± 5.7		1.27 ± 0.29	12.5 ± 4.5	
33–35 weeks (gestational)	149 ± 108 (n = 8)		45.5 ± 6.5		40.5 ± 11.0		1.20 ± 0.38	13.5 ± 9.2	
35–37 weeks (gestational)	131 ± 88 (n = 7)	n.s.	45.0 ± 6.3	*	41.0 ± 4.3	***	1.19 ± 0.14	14.0 ± 2.9	***
37–39 weeks (gestational)	135 ± 105 (n = 12)		45.0 ± 6.2		41.5 ± 9.8		1.13 ± 0.19	12.5 ± 8.6	
39–41 weeks (gestational)	118 ± 101 (n = 17)		35.0 ± 4.0		48.0 ± 7.0		0.92 ± 0.25	16.0 ± 4.1	
1 month (postnatal)	115 ± 86 (n = 11)	n.s.	31.0 ± 3.0	***	49.0 ± 6.0	n.s.	0.76 ± 0.10	20.0 ± 2.1	**
3 months (postnatal)	98 ± 67 (n = 5)		24.0 ± 11.0		46.0 ± 6.0		0.62 ± 0.16	29.0 ± 13.0	

Proportions of low molecular weight proteins (LMW), albumin (A) and high molecular weight (HMW) protein excretion are given in percent of total protein excretion
* $P < 0.0005$
** $P < 0.001$
*** $P < 0.005$
**** $P < 0.05$
n.s. Not significant

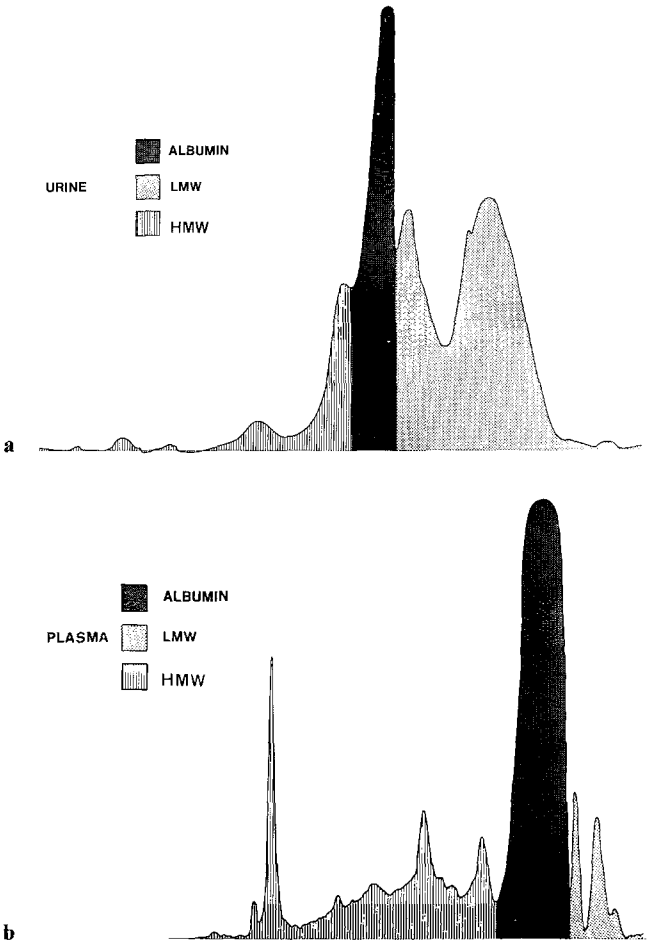

Fig. 1a, b. Gradient gel electrophoresis pattern of urine **a** and plasma **b** of a male mature newborn. Plasma was diluted 100×, but urine was unconcentrated. Migration direction is from left to right

increasing GA to approach 34.1 ml/min per 1.73 m^2 in male mature newborns and reached 61.4 ml/min per 1.73 m^2 in male infants aged 3 months. There was no significant difference in C_{cr} between male and female mature newborns and 3-month-old infants of both sexes.

Total protein excretion

Mean protein excretion decreased with maturation from 164 in the youngest group to 98 mg/24 h per 1.73 m^2 in 3-month-old infants, but there was no significant difference between any of the groups due to large variations (Table 2).

Urine protein composition

The composition of urinary proteins in male infants is presented in Table 2. Proteins are divided into three main groups: albumin (A) as a reference protein, proteins with molecular weight higher than A (HMW) and proteins with molecular weights lower than A (LMW). In all groups of premature but not in mature infants, LMW proteins constitute the major fraction of urinary proteins. Differences in LMW protein excretion are significant between the youngest premature and mature infants and between mature newborns and infants aged 3 months. The urinary A fraction increased from values between 39% and 41% in premature infants to 48% in mature newborns. Correspondingly, the mean ratio LMW/A in urine fell from 1.27–0.92 (Table 2). Concomittantly the fraction of HMW proteins rose from 12.5%–16% in mature infants and to 29% in 3-month-old infants. The electrophoretic pattern of proteins excreted in the urine of a mature infant is shown in Fig. 1a. The relative preponderance of LMW proteins in urine is evident and is in contrast to the pattern in plasma, where only two pre-albumin bands can be detected (Fig. 1b).

Clearance and reabsorption of A (Table 1)

There was a significant decrease of A clearance from the youngest premature infants to mature newborns. However, no further decrease occurred in mature infants after birth. There were no significant sex differences in mature newborns and older infants. The ratio C_{alb}/C_{cr} decreased rapidly with growing gestational age, but only slightly in mature infants after birth. For a logarithmic scale a significant relationship was found between C_{alb} and C_{cr} ($r = 0.76$ for $y = 0.00693 - 0.00161 \ln x$).

Discussion

It is generally accepted that glomerular permeability to macromolecules decreases with maturation [17, 20, 22, 28, 34], whereas tubular reabsorption capacity increases. This is reflected by the decreasing clearance of A with maturation. The exponentially decreasing clearance ratio C_{alb}/C_{cr} (Table 1) may indicate both increasing glomerular filtration rate during maturation [16, 32] and decreasing excretion of A due to rising tubular protein reabsorption [29].

Clearance ratios C_{alb}/C_{cr} found in this study are comparable to experimental data in young rats, where 0.004% [13] and 0.0015% [12] were found for C_{alb}/C_{inulin}. They are also comparable to data from Barratt et al. [5, 6], who found C_{alb}/C_{cr} of 0.0011% in neonates with a further decrease to 0.00033% in adults.

During maturation there is a relative decrease in the LMW protein fraction in final urine, while the HMW proteins and A show a relative increase. This implies that either relatively more globulins are filtered and/or less reabsorbed, or that relatively less LMW proteins are filtered and/or more reabsorbed. Since the reabsorption mechanism seems to be the same for all proteins [7, 12, 13], increasing plasma levels of globulins and relatively decreasing plasma levels of LMW proteins can explain only partially the change in urinary protein spectrum [6].

The decreasing ratio of LMW/A in urine with age is in agreement with the findings of Miltenyi in older children [26] and those of Karlsson and Hellsing, who found a relatively high excretion of β_2 microglobulin in neonates [19]. Similarly, Barrett and Crawford found a 2.6 times higher renal clearance of lysozyme (MW 15000) in newborns than in adults [6].

It is possible, that the variations in the pattern of protein excretion during maturation observed by us are related to changes of the sieving coefficients for different proteins. Sieving coefficients (ψ) are known to change with chemical and ultrastructural composition [17, 22, 34] and with electrical charge of the glomerular basement membrane, endothelial pore size and epithelial slit areas [21]. They can only be measured experimentally by micropuncture of Bowman's capsular space and determination of protein concentration in ultrafil-

trate. Therefore, for differentiation between increasing tubular reabsorption capacity and glomerular filtration rate during maturation, experimental studies in immature animals suitable for micropuncture might be worthwhile.

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