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Glomerular protein sieving and implications for renal failure in Fanconi syndrome

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Background. Glomerular sieving coefficients (GSCs) of proteins have been measured extensively in animals but not humans. We have studied the proteinuria of Fanconi syndrome, a "knock-out" of renal tubular protein reabsorption, to estimate GSCs and detect potential contributors to development of renal failure.

Methods. Immunoassay of proteins and polypeptides in serum and urine of patients with early Dent's disease (mean GFR = 83 mL/min, range 60 to 101, $N = 5$), Lowe's syndrome ($N = 3$), and ADIF ($N = 2$) were used.

Results. Twenty-one proteins, ranging in mass from insulin (5.1 kD) and parathyroid hormone (PTH; 9.4 kD) to transferrin (78 kD) and intact IgG (160 kD), were present in Fanconi urine at >6 to 1000-fold normal. A simple model assuming complete "knock-out" of the reuptake of each protein filtered normally by the glomerulus was applied to protein excretion by Dent's patients. GSCs were estimated for 12 plasma proteins, including albumin ($7.7 \pm 0.9 \times 10^{-5}$) and IgG ($4.2 \pm 0.28 \times 10^{-5}$; mean ± SEM). We calculated the albumin concentration in normal glomerular filtrate to be 3.5 ± 0.41 mg/L (53 ± 6.4 nmol/L), consistent with studies in rat and dog.

Conclusions. To our knowledge, this study provides the first estimates of human in vivo GSCs. Our model explains why tubular proteinuria of Fanconi syndrome includes proteins of mass of albumin and above as well as low-molecular-weight proteins, and further characterizes the endocytic pathway(s) believed defective in these syndromes. High urinary concentrations of potentially bioactive hormones such as PTH, insulin, IGF-1 and the chemokine monocyte chemoattractant protein-1 (MCP-1), were found; their presence in tubular fluid may contribute to the hypercalciuria, interstitial fibrosis, and the progressive renal failure of Fanconi syndromes.

Key words: hypercalciuria, tubular proteinuria, Dent's disease, Lowe's syndrome, interstitial fibrosis, progressive renal failure, albumin, protein reabsorption.

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Although the sieving of plasma proteins by the glomerulus has been widely studied in animal models, little is known of the process in humans [1, 2]. The Fanconi syndrome, which includes a "knock-out" of renal tubular protein reabsorption causing "tubular" proteinuria, presents an opportunity for the estimation of the glomerular sieving coefficients (GSCs) of endogenous plasma proteins in humans. We also have investigated why the tubular proteinuria of the Fanconi syndrome consistently includes high concentrations of albumin, 65 kD and β_2-glycoprotein-I (β_2GI), 50 kD which sometimes exceed those of low-molecular-weight (LMW) proteins such as β_2-microglobulin (β_2m), 11.6 kD and retinol-binding protein (RBP), 21 kD [3–5]. In addition, we searched for proteins excreted in the urine of patients with the Fanconi syndrome that may be bioactive within the renal tubule and contribute to the progressive renal impairment commonly found in this disease [6, 7].

The renal Fanconi syndrome (Lignac-de Toni-Debré-Fanconi syndrome) consists of a generalized dysfunction of the proximal renal tubule with—in its full form—impaired proximal reabsorption of protein, amino acids, glucose, phosphate, urate and bicarbonate and rickets/osteomalacia [6, 8]. Dent's disease (*CLCN5* mutation), the oculocerebrorenal syndrome of Lowe (*OCRL* mutation) and autosomal-dominant idiopathic Fanconi syndrome (ADIF) are examples of inherited renal Fanconi syndromes. Although other features of the Fanconi syndrome vary in these disorders, there is a consistent defect of renal tubular protein reabsorption, a process largely localized to the proximal tubule [3, 6], and this defect causes tubular proteinuria.

Dent's disease is an X-linked disorder that presents clinically as nephrocalcinosis, nephrolithiasis, and eventual renal failure in affected males; females are usually

clinically unaffected or have a milder disorder [9–11]. The molecular basis of the disease is a defective CLC-5 chloride ion channel encoded by *CLCN5* [12–14]. In Lowe's syndrome, also an X-linked disorder, there is mental retardation, visual impairment, and chronic progressive renal impairment [15]; nephrocalcinosis also may be found in this disease [16]. The underlying defect in Lowe's syndrome is deficiency of phosphatidylinositol 4,5 bisphosphate 5-phosphatase due to mutation of *OCRL*. Patients with ADIF, for which the molecular basis is currently unknown, manifest progressive renal impairment and nephrocalcinosis [7].

Tubular proteinuria, usually described as a predominantly LMW proteinuria, is found in the renal Fanconi syndrome [3, 6]. It is the most consistent laboratory finding among families affected by Dent's disease [11]. This proteinuria is believed to be due to a failure of endocytosis by the proximal tubule of proteins filtered by the glomerulus [13, 14]. Tubular proteinuria is present at an early age in Dent's disease and may be present at birth. It precedes significant renal glomerular failure by some 10 to 20 years [9]. How the ion-channel defect in Dent's disease and the phosphatidylinositol phosphatase deficiency in Lowe's syndrome cause tubular proteinuria and eventual renal failure in these disorders is unclear. In Dent's disease, a defect in normal trafficking of the giant proximal tubular endocytic receptor megalin has recently been proposed (abstract; Norden, *J Am Soc Nephrol* 11:93A, 2000) [13, 14].

Our findings show that the proteinuria of the Fanconi syndrome is more generalized than previously suspected. We present a unifying quantitative explanation for this, based on plasma concentrations and predominantly size-selective glomerular filtration, and offer the first estimates of human in vivo glomerular sieving coefficients for 12 plasma proteins. We also have found high concentrations of polypeptides, including hormones and a chemokine in urine from these patients, and speculate that tubular bioactivity of these peptides may play a role in the pathogenesis of progressive renal failure in the Fanconi syndrome.

METHODS

Patients

Clinical, laboratory, and molecular genetic features of the five affected male patients with Dent's disease whom we studied in detail have been described; these patients had relatively well-preserved creatinine clearances (Table 1): Patients C/II/2, total *CLCN5* deletion and F/II/1, W279X [9, 12]; a member of family 7.1/94, splice-site mutation with deletion of codons 132-241 [12] and cases 4/96, R34X [17] and 6/97, 1175-1176delGT; 346 amino acid deletion [18]. Each patient has a mutation in *CLCN5* associated with loss of function of the chloride channel, tubular proteinuria typical of the disease, and a creatinine clearance >60 mL/min. Creatinine clearance was either measured from a 24-hour collection of urine or calculated on the basis of serum creatinine, age, and weight [19]. A further three patients with a clinical, laboratory, and molecular genetic diagnosis of Dent's disease, but with creatinine clearances <45 mL/min, were also studied. The three patients with Lowe's syndrome have severe mental and growth retardation, visual impairment, and tubular proteinuria; two of the patients are brothers. The two patients with ADIF are father and son in a family described previously [7]. Creatinine clearances of patients studied are given in Table 1.

Specimen collection

Midstream random specimens of urine following at least 18 hours of sexual inactivity were refrigerated immediately, frozen within four hours at −80°C for up to two days, and transferred to liquid nitrogen for up to one year before analysis. Serum specimens were obtained from two of the five patients with Dent's disease and stored in the same way.

Immunoassays

The following methods were used for measurement of proteins and polypeptides in urine and serum (abbreviations as in Table 1): β_2m, IMx® analyzer (Abbott Diagnostics, Maidenhead, UK); RBP, [20]; TSH, FSH and LH AxSym® analyzer (Abbott Diagnostics); α_1m, TTR and albumin, Array® analyzer (Beckman Coulter, High Wycombe, UK); kappa and lambda immunoglobulin light chains, α_1AG, DBP and ZAG, radial immunodiffusion [21] using antibody from Dade-Behring (Milton Keynes, UK); β_2GI, [20]; TRF, BNII® analyzer Dade-Behring (Milton Keynes, UK); SHBG and GH, Immulite® analyzer (DPC Corp., Los Angeles, CA, USA); IgG, modification of immunoassay for β_2GI with use of anti-IgG antibodies supplied by Dako (Ely, UK) and purified urinary IgG calibrant from Scipac (Sittingbourne, UK); IGF-1 and PTH (intact), Advantage® analyzer (Nicholls Institute, Newport, UK); MCP-1 (ELISA using matched antibody pair, R&D Systems, Abingdon, UK); insulin was measured on both the Access® (Beckman, High Wycombe, UK) and DELFIA® (Perkin-Elmer, Beaconsfield, UK) analyzers. Results of protein and hormone excretion are expressed per mmol creatinine determined by a kinetic Jaffé method.

Immunoblotting

To confirm the presence of intact IgG, urine samples were diluted in 62.5 mmol/L Tris chloride buffer, pH 6.8, and denatured by adding an equal volume of 10% sodium dodecyl sulfate (SDS), 20% glycerol, 0.003% bromophenol blue in the same buffer and heating at 40°C for 30 minutes. After electrophoresis on nonreducing 4

Table 1. Estimated glomerular sieving coefficients (GSCs) of proteins and protein excretion by patients with Dent's disease, Lowe's syndrome and autosomal-dominant idiopathic Fanconi syndrome (ADIF)

Protein	Abbv.	Molecular weight *kD*	GSC estimated from Dent's patients	Urinary excretion[a] *mg/mmol creatinine*				Normal plasma levels[a] *mg/L*[b]
				Dent's $N = 5$	Lowe's $N = 3$	ADIF $N = 2$	Normal $N = 8$	
β_2-microglobulin	β_2m	11.6	0.91 ± 0.14	12.8 ± 2.1	55 (40–73)	18.3 (4.4–32.2)	<0.05	1.3
Retinol-binding protein (free)	RBP	21	0.38 ± 0.057	21.4 ± 3.6	85 (68–93)	13.6 (5.1–22.1)	<0.017	5.8
Thyroid-stimulating hormone	TSH	28	0.067 ± 0.017	1.46 ± 0.35	2.4 (0.8–3.7)	0.99 (0.3–1.68)	<0.05	2
α_1-microglobulin	α_1m	31	0.092 ± 0.013	32 ± 4.6	38 (29–48)	21.2 (9.5–32.9)	<2	32
α_1-acid glycoprotein	α_1AG	40	$4.5 \pm 0.62 \times 10^{-4}$	3.75 ± 0.51	—	—	<0.2	770
Zinc-α_2-globulin	ZAG	41	$1.65 \pm 0.24 \times 10^{-3}$	2.59 ± 0.38	—	—	<0.2	140
β_2-glycoprotein I	β_2GI	50	$5.2 \pm 0.85 \times 10^{-3}$	8.5 ± 1.4	48 (37–56)	12.3 (6.1–18.5)	<0.03	150
Vitamin D binding protein	DBP	51.3	$2.0 \pm 0.55 \times 10^{-4}$	0.66 ± 0.17	—	—	<0.1	400
Transthryetin	TTR	55	$2.1 \pm 0.34 \times 10^{-4}$	0.68 ± 0.11	—	—	<0.1	300
Albumin	ALB	65.5	$7.7 \pm 0.9 \times 10^{-5}$	38 ± 4.4	40 (19–52)	13.8 (6.6–21)	<2.5	45000
Transferrin	TRF	78	$5.0 \pm 1.6 \times 10^{-5}$	1.45 ± 0.47	6.8 (1.9–15.5)	1.2 (0.6–1.8)	<0.19	2700
Immunoglobulin G	IgG	160	$4.2 \pm 0.28 \times 10^{-5}$	5.5 ± 0.36	6.1 (2.6–8.1)	5.0 (4.4–5.6)	<0.2	12000
Creatinine clearance[c] *mL/min*				83 (60–101)	33 (26–37)	58 (34–82)		

Data are mean ± SEM. Hyphens denote not measured.

[a] Normal plasma levels were obtained from the following literature sources:- β_2m and TSH, (Abbott IMx® and AxSym® datasheets); free RBP, [22]; α_1m, [37]; α_1AG, TTR, albumin, TRF and IgG, [26]; ZAG, [38]; β_2GI, [39]; DBP, [40]

[b] Except TSH, mIU/L

[c] Mean (range)

to 15% polyacrylamide gels (Bio-Rad, Hemel Hempstead, UK), proteins were transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were blocked with 3% albumin and probed with anti-human gamma chain $F(ab')_2$ conjugated to horseradish peroxidase (HRP; Sigma, Poole, UK; Cat. No. A2290), diluted 1:60,000 in 0.1% bovine serum albumin (BSA), 500 mmol/L sodium chloride, 0.1% Tween 20, in 20 mmol/L Tris, pH 7.5, and visualized with ECL+® reagent (Amersham Pharmacia, Milton Keynes, UK).

RESULTS

Measurements of protein excretion in random urine samples obtained from five affected male patients with Dent's disease are shown in Table 1. The five patients chosen for study were early in the course of their disease with relatively preserved glomerular filtration rates (GFR; mean 83 mL/min, range 60 to 101). The results demonstrate greatly elevated excretion of proteins ranging in molecular weight from 11.6 to 160 kD. Increased excretion of IgG measured by ELISA was confirmed by immunoblotting, which showed that all material reacting with anti-Ig-γ antibody had the mobility expected of intact Ig on nonreducing SDS electrophoresis.

The excretion of both albumin and α-1-microglobulin were evaluated in the five affected male patients with Dent's disease and relatively preserved glomerular filtration rates and three other Dent's patients with impaired GFR. There was no relationship between the amount of either protein excreted and GFR for the five patients in this group with GFR >45 mL/min. However, increased excretion, not exceeding 2.6-fold, of either one or both these proteins was present in the three patients with GFR <45 mL/min (data not shown). Impaired GFR and associated glomerular disease was therefore unlikely to affect excretion of protein by the patients with Dent's disease shown in Table 1. Protein excretion by three patients with Lowe's syndrome and two patients with ADIF was similar to those with Dent's disease, except that excretion of LMW proteins by patients with Lowe's syndrome was even greater (Table 1).

Calculation of glomerular sieving coefficients

Assuming that glomerular filtration is the only source of a protein in the urine of a patient with a Fanconi syndrome and that there are no losses from, or additions to, the filtrate, the glomerular filtrate concentration (GFC) of the protein is given by:

$$\text{GFC (mg/L)} = \frac{\text{(24-hour protein excretion, mg)}}{\text{(24-hour volume of glomerular filtrate, L)}} \quad \text{(Eq. 1)}$$

Random urine samples were used for measurements of protein excretion to minimize the effects of protein instability in urine and these are expressed as mg/mmol creatinine (Table 1). The 24-hour excretion for individual proteins was then calculated based on the 24-hour creatinine excretion by each patient. The 24-hour volume of glomerular filtrate was calculated as the 24-hour creatinine clearance. When these measurements are substituted into equation 1, this becomes:

$$\text{GFC (mg/L)} = \frac{\begin{pmatrix}\text{protein excretion,}\\ \text{mg/mmol}\end{pmatrix} \times \begin{pmatrix}\text{24-hour creatinine}\\ \text{excretion, mmol}\end{pmatrix}}{\text{(24-hour creatinine clearance, L)}} \quad \text{(Eq. 2)}$$

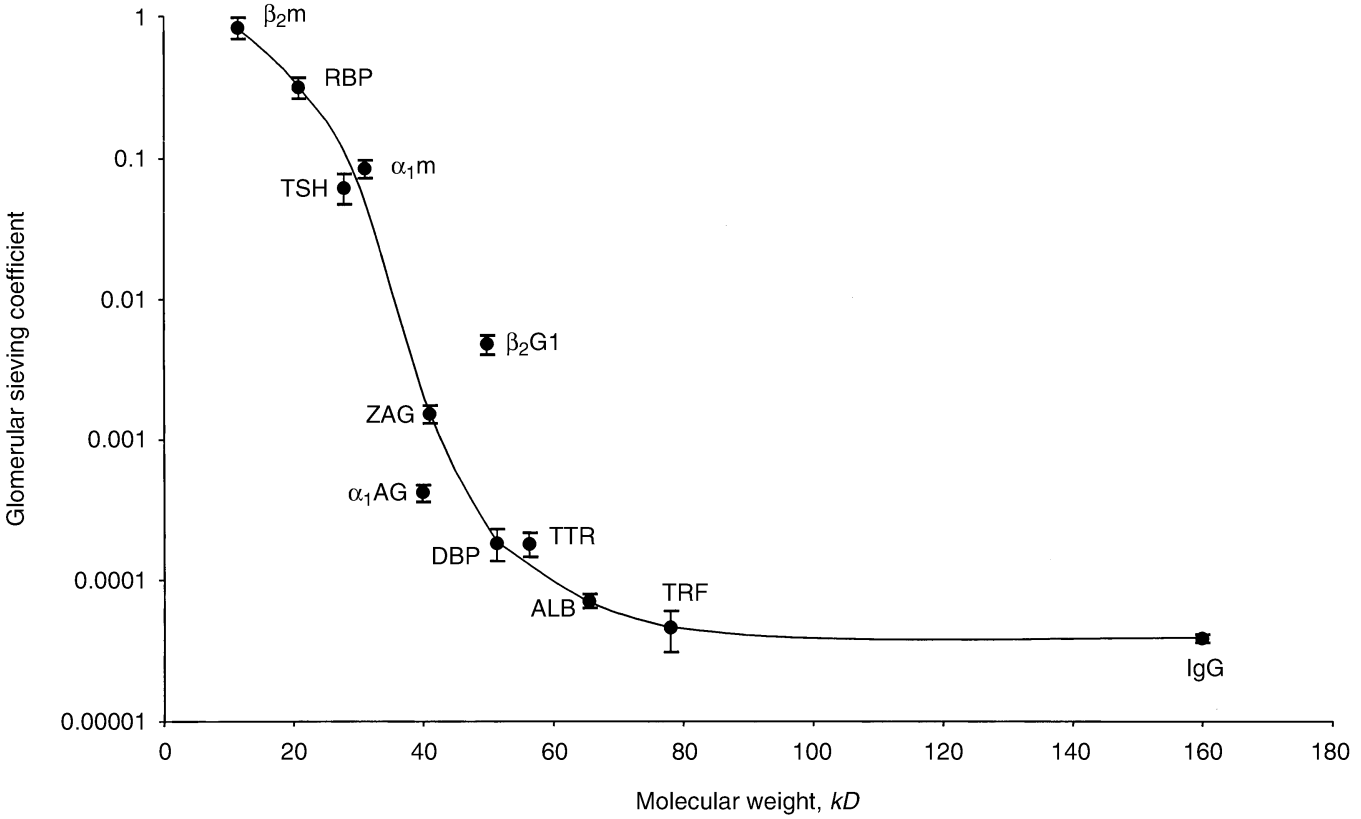

Fig. 1. Estimated glomerular sieving coefficients for 12 plasma proteins versus molecular weight. Data are from Table 1; mean (± SEM) of five determinations based on results from each of five patients with Dent's disease are shown. See **Molecular size, charge and glomerular sieving** for discussion of the anomalous behavior of α_1-acid glycoprotein (α_1AG) and β_2-glycoprotein-I (β_2GI). Abbreviations are: RBP, retinol-binding protein; α_2m, α_2-microglobulin; TSH, thyroid-stimulating hormone; ZAG, zinc-α_2-globulin; DBP, vitamin D-binding protein; TTR, transthyretin; ALB, albumin; TRF, transferrin; IgG, immunoglobulin G.

Then, by definition [2], we calculated:

$$\text{The glomerular sieving coefficient, GSC} = \frac{\text{GFC}}{\text{Plasma protein concentration}} \qquad \text{(Eq. 3)}$$

Any errors introduced by these assumptions are considered in the **Discussion** section. The glomerular sieving coefficients (GSCs) were estimated using the above model in the five patients with Dent's disease (Table 1), and Figure 1 shows the estimated GSC plotted against the molecular weight of each protein. Kappa and lambda immunoglobulin light chains, sex-hormone binding globulin and lysozyme could not be measured accurately in urine, either because of calibration bias or poor precision. Nevertheless, these four proteins were also present in at least tenfold increased concentrations over normal values (data not shown).

When hormone and chemokine excretion were measured (Table 2 and Fig. 2), greatly increased levels were found including those of several potentially bioactive proteins. Since the absolute concentrations of the hormones measured (Table 2) and of TSH (Table 1) were low, we looked for any effect of the analysis medium (urine vs. serum) on assay results. The apparent hormone levels were measured in the following mixtures using varying proportions of each component: serum and normal urine; serum and urine samples with high apparent levels of endogenous hormones and normal urine and urine samples with high apparent levels of endogenous hormone. This showed both over-recovery and under-recovery of up to 35% (IGF-1 and PTH, respectively) although generally not exceeding 20% (data not shown). Results in Table 2 are presented without adjustment for this apparent over-recovery and under-recovery. Insulin was measured by two assays based on different technologies and antibodies with almost identical results; measurements using the DELFIA® method only are given.

DISCUSSION

Our results for the 12 proteins shown in Table 1 show that the excretion of most proteins involved in tubular proteinuria is largely a function of plasma concentration and molecular size (Fig. 1). These measurements permit the first estimates of the in vivo GSCs in humans and

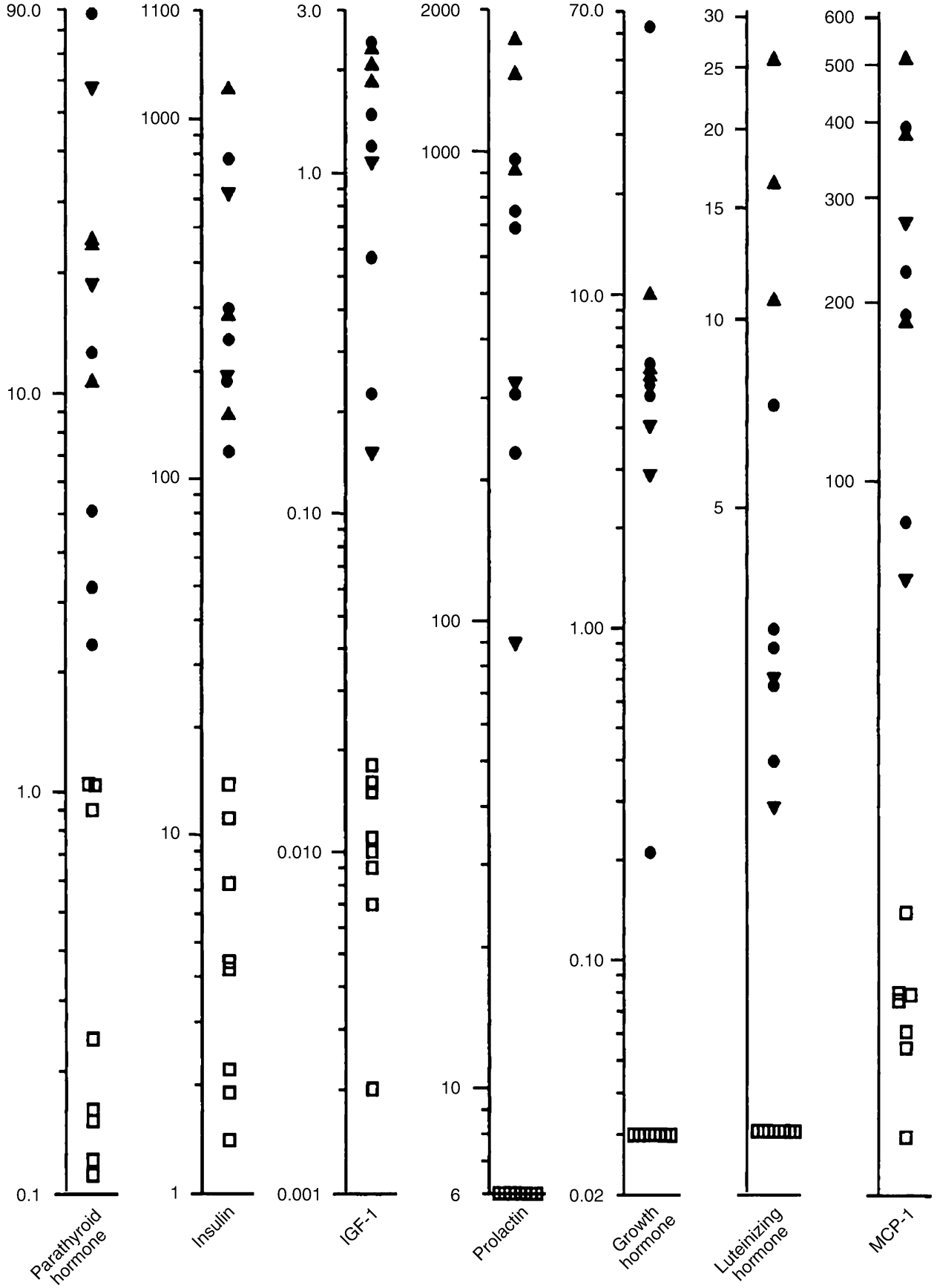

Fig. 2. Hormone and chemokine excretion in urine of patients with Dent's disease (●); Lowe's syndrome (▲); autosomal-dominant idiopathic Fanconi syndrome (ADIF) (▼), and normal individuals (□). Data are from Table 2. Excretion, in the following units, is expressed per mmol creatinine: parathyroid hormone, ng; insulin, pmol; IGF-1, nmol; prolactin, mIU; growth hormone, mIU; luteinizing hormone, mIU and MCP-1, ng.

Table 2. Hormone and chemokine excretion in the urine of patients with Dent's disease, Lowe's syndrome and autosomal-dominant idiopathic Fanconi syndrome (ADIF)

Hormones and MCP-1		Molecular weight *kD*	Urinary excretion *mIU/mmol creatinine*[c]			
			Dent's[a] *N* = 5	Lowe's[b] *N* = 3	ADIF[b] *N* = 2	Normal[a] *N* = 8
Parathyroid hormone	PTH	9.4	22.2 ± 16.5	19.3 (10.7–24.1)	38 (18.8–57.6)	0.47 ± 0.2 ng/mmol
Insulin	INS	5.1	325 ± 116	545 (150–1202)	410 (195–625)	5.8 ± 1.6 pmol/mmol
Insulin-like growth factor 1	IGF-1	15[d]	1.2 ± 0.4	2.1 (1.8–2.4)	0.6 (0.15–1.08)	0.011 ± 0.002 nmol/mmol
Prolactin	PRL	23	586 ± 139	1360 (909–1719)	207 (90–324)	<6
Growth hormone	GH	22	15.9 ± 11.7	7.2 (5.7–10.0)	3.5 (2.9–4.1)	<0.03
Luteinizing hormone	LH	30	3.6 ± 0.9	17.5 (10.7–25.6)	2.2 (1.7–2.7)	<0.5
Monocyte chemoattractant protein 1	MCP-1	14	223 ± 64[e]	358 (185–510)	120 (68–171)	12.7 ± 1.2 ng/mmol[f]

[a] Mean ± SEM
[b] Mean (range)
[c] Except insulin, IGF-1, PTH and MCP-1
[d] Bound to IGF-BP3
[e] *N* = 4
[f] *N* = 7

cover almost a 10^5-fold range. The additional data of Table 2 and Figure 2 shows that several proteins of potentially high bioactivity are excreted in high concentration in the urine of patients with tubular proteinuria and, by implication, are present in tubular fluids at high concentration.

Calculation of glomerular sieving coefficients

Several assumptions are made in the calculation of the GSCs and errors in these will affect the final results differently. The effect of instability of proteins in urine was minimized by the use of rapidly processed random urine specimens. Conversely, any impact of disease on glomerular permeability might be expected to increase the estimated GSC, at least for less freely filtered molecules. No evidence was found for further increases in the excretion of α_1m and albumin until the creatinine clearance of patients with Dent's disease fell to <45 mL/min; measurements were specifically made early in the progression of renal impairment. Residual tubular protein uptake in Dent's disease, that is, an incomplete knock-out of tubular protein reabsorption, cannot be wholly excluded because the exact pathway(s) have not been defined. However the results for β_2m demonstrated a recovery in urine of 91 ± 14% of all β_2m filtered by the glomerulus; recovery is calculated as (GFC/plasma protein concentration) where GFC is found from equation 2 in the **Results** section using the data from Table 1. Since β_2m is probably freely filtered [2], this finding suggests that most tubular reuptake, at least for this protein, is disrupted; given the known instability of this protein in urine, the true recovery of β_2m is probably even higher [3]. The three diseases of diverse molecular pathology all appear to show similar increases in the excretion of each protein (Table 1) and therefore the simplest explanation is that there is a common loss of function of a single final pathway. The higher excretion of the low molecular weight proteins β_2m and RBP in the unselected patients with Lowe's syndrome may be related to lower glomerular filtration in these (Table 1), compared with the Dent's patients who were chosen for study because of relatively preserved GFR. Indeed, increased RBP excretion is found in patients with a variety of tubular and non-tubular diseases when GFR is reduced by more than 70% [22]. The approach used in Table 1 to estimate GSC cannot be used for the hormones and chemokines shown in Table 2, because of the wide physiological fluctuations in their plasma levels.

Comparison between human and animal results

Measurements of the glomerular filtrate concentration of albumin by micropuncture in rats and dogs have yielded a range from <1 to 50 mg/L. The reasons for this variability have been discussed in detail [2]. The approach here, using equation 2 (**Results** section), gives a value of 3.5 ± 0.41 mg/L (53±6.4 nmol/L) in humans, for this important pathophysiological measurement. There is controversy about the contribution of transcellular reabsorption to retrieve albumin from the glomerular filtrate [23–25]. It is not known how the molecular defect in the syndromes we have studied might affect any transcellular reabsorption, and some residual tubular reabsorption would mean an underestimation of the reported albumin GSC here. Nevertheless, even a twofold underestimate of true albumin filtration predicts a glomerular fluid concentration of albumin of only 7.0 mg/L (106 nmol/L), which is still toward the lower end of that determined by invasive animal studies.

Molecular size, charge, and glomerular sieving

Not unexpectedly, Figure 1 shows several deviations from a monotonic curve. Excretion of α_1-acid glycoprotein and β_2-glycoprotein I appear anomalously low and high respectively (Fig. 1). Alpha$_1$-acid glycoprotein has

an unusually low isoelectric point, pI = 2.7, [26] whereas in contrast the pI of β_2GI is unusually high, pI = 6.4 to 8.2 for nine major isoforms in urine [4, 5]. The isoelectric points of most of the other proteins studied, except for IgG, are all in the range from about 4.0 to 6.0 [26]. An effect of extremes of charge on GSC is therefore apparent for those proteins with GSCs that neither approach one nor are very low (Table 1 and Fig. 1). This is consistent with studies of the glomerular filtration of dextrans in humans, which suggest that fixed, negatively charged components of the capillary wall relatively hinder filtration of more negatively charged proteins [27]. Tables 1 and 2 show that before development of marked renal failure, the commonly used LMW protein markers (β_2m, RBP, and α_1m) account for about one half of the proteinuria in the Fanconi syndrome, albumin for some 30% and the remainder comprises a diverse group of proteins.

Implications for specificity of the endocytic pathway

Receptor-mediated endocytosis is believed to underlie protein transport from the proximal tubular lumen and defects of this pathway have been studied in Dent's disease [abstract; Norden et al, *J Am Soc Nephrol* (in press)] [13, 14]. The exact structure of the receptor(s) is unclear, although both megalin and cubilin are almost certainly involved [13, 14, 28–31]. In vitro receptor-binding studies of a variety of proteins to megalin and cubilin are entirely consistent with our results (abstract; Christensen, *J Am Soc Nephrol* 11:49A, 2000) [4, 28, 30, 32, 33]. However, the putative receptor(s) for several important potential ligands described here, remain to be identified: These include thyroid-stimulating hormone, immunoglobulin G and monocyte chemoattractant protein-1. Although our findings do not delineate which pathways are defective in Dent's disease, they suggest that the normal endocytic pathway can process an even wider array of ligands than described so far.

Pathophysiological role of tubular fluid

Progressive renal failure is a very common feature of the Fanconi syndrome [6, 7]. There is evidence from animal studies that bioactivity of tubular fluid may contribute to the progression of renal disease [34], and our results demonstrate that a number of potentially bioactive proteins are excreted in large amounts by patients with Dent's disease, Lowe's syndrome, and ADIF. The high levels of excretion in urine imply that tubular fluid distal to the earliest part of the proximal tubule is perfused with high concentrations of these potentially bioactive proteins. Recent studies indicate that parathyroid hormone receptors are expressed on apical surfaces of tubular epithelium and that activation of these apical receptors by PTH down-regulates the type IIa NaPi cotransporter [35]. This might lead to phosphaturia, enhanced 1α-hydroxylation and either increased or inappropriately elevated serum 1,25$(OH)_2$-vitamin D levels leading to enhanced intestinal calcium absorption and thus explaining the hypercalciuria of Dent's disease [13]. Elevated urinary PTH levels have also recently been reported in a mouse model of Dent's disease [13]. It is notable that the levels we have found in the urine of patients with Dent's disease, expressed as a multiple of normal, are frequently higher than those reported in that mouse model (Table 2).

Monocyte chemoattractant protein-1 in tubular fluid may promote tubulointerstitial fibrosis [36] and the potential tubular fluid concentrations calculated from the results of Table 1 are well within the range of those known to have biological activity. Increased urinary MCP-1 is found in patients with active renal vasculitis and persistence of this increase is associated with progression to end-stage renal failure (abstract; Tam, *J Am Soc Nephrol* 11:99A, 2000). The relative contributions of any local renal production and glomerular filtration to the amount excreted in these conditions are unclear. An important role for MCP-1 in macrophage-mediated tubular injury role has been found by the study mice deficient in this chemokine [36]. Similarly, bioactive IGF-1, which has been shown by micropuncture studies to occur in the tubular fluids of nephrotic rats [34], might contribute to the tubulointerstitial lesions of patients with the renal Fanconi syndrome.

Progressive renal failure has been described as a feature of most forms of the Fanconi syndrome [6, 7]. The explanation for this renal failure may lie in progressive tubular and interstitial damage caused by the high levels of potentially bioactive hormones and MCP-1 identified (Table 2 and Fig. 2).

Our findings provide a unifying quantitative explanation for the excretion of large amounts of higher molecular weight proteins in addition to the more freely filtered LMW proteins in patients with the renal Fanconi syndrome. Results of glomerular sieving coefficients estimated here are both self-consistent (Table 1 and Fig. 1) and consistent with much animal work. Further molecular studies are needed to account for the versatility and efficiency of the proximal tubular endocytic uptake system and to assess the pathophysiological consequences of the probable high hormone and chemokine content of tubular fluids in the Fanconi syndrome.

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