Discelectrophoretic Molecular Weight Analysis of Urinary Proteins
A Contribution to the Clinical Diagnostic Differentiation and the Pathophysiology of Proteinuria

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The protein handling within the nephron seems to depend largely on the molecular weight (MW) of proteins. Serum macromolecules are retained by the normal glomerular system while smaller microproteins are cleared according to their size and reabsorbed by the tubular cells by pinocytosis (1, 2). Damage to both the glomerular and tubular systems leads to protein losses in the urine (3). Quantitation of the total urinary protein permits the conclusion as to its glomerular origin only in the case of proteinurias of 3 g/24 h or more, while proteinurias of smaller extent as a more frequent event need further differentiation. For this purpose the sodium dodecyl sulfate polyacrylamide (SDS-PAA) electrophoresis - as compared to more time consuming gel chromatographies (3) — has proven to be a method for quick and reproducible separation of urinary proteins according to their molecular weight.

The purpose of analyzing about 600 individual urine samples by this method was: (1) Are different patterns of proteinuria correlated to diseases of various parts of the nephron as seen by histology?, and (2) which conclusions might be drawn by observing transitory and permanent proteinurias regarding the pathogenesis of proteinuria in man?

Materials and Methods

Detailed methods have been reported elsewhere (4).

Patients

24-hour urines were collected from patients of the medical clinic, medical policlinic (Prof. Dr. Kluthe) and pediatric clinic (Dr. Schinder) of the University of Freiburg. The histological diagnoses in most biopsied cases were done by Prof. Bohle, Tübingen, the rest by Prof. Thoenes, Marburg, and by the Institute of Pathology, Freiburg.

Supported by a grant (Bo 378/6) of the Deutsche Forschungsgemeinschaft.

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Results

Following glomerular damage macromolecular serum proteins are found in the urine. The polyacrylamide (PAA) gel exhibits about six bands G1 -G6 of different relative mobility (RM) (fig. 1A). This glomerular proteinuria may be found in a highly selective (MW 65,000130,000), moderately unselective (MW 65,000300,000) or unselective form (MW 65,0001,000,000), depending on the damage to the glomerular filter (4). The selectivity, however, in this study was not calculated by differential protein clearances, but roughly by the ratio transferrin:IgG in the urine or by comparing similar fractions in urine and serum PAA-gels (fig. 2).

Accordingly, the protein excretion of histologically verified tubulopathies resulting in an excretion of microproteins of MW < 70,000 was named tubular proteinuria (bands: albumin and T - T = MW 10,00070,000).

By precipitin reactions of monospecific antisera with protein containing PAA-gels, some of these bands could be identified: albumin (A); transferrin (G); IgG/A(G/), -2-macroglobulin and IgM (G), as well as -g1ycoprotein (MW 40,000) and monomer (T) and dimer (T) IgL chains. Furthermore, -2 microglobulin, retinol-binding protein and enzymes are known from the literature to be a part of tubular proteinuria.

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The prerenal proteinuria (fig. 1D) is caused by the over-production of a
single protein with a high renal clearance (e.g., IgL chains). Pathogenetically it is thought to be caused by saturation of a tubular reabsorption mechanism. Details on PAA-electrophoresis of paraproteinurias were reported by Virell et al. (5). Proteins derived from the lower urinary tract are the components of the post-renal proteinuria (fig. 1C). In the course of pyelonephritis immunoglobulins of different classes may be secreted locally into the urine. This type may be differentiated from glomerular proteinurias by low quantities of urinary proteins not involved in the inflammatory process, as, e.g., transferrin (compare fig. 1A +C).

Urinary proteins of various pathogenesis are found frequently in individual urine. They may be explained, for example, by tubular destruction in the course of chronic GN (fig.6B), secondary glomerular alterations in chronic pyelonephritis (PN), kidney damage by paraproteinurias ('myeloma kidney'), etc. For differentiation of these mixed proteinurias the calculation of the glomerulo-tubular protein ratio (GTPR = amount of proteins MW> 90,000:amount of proteins MW < 65,000) has proven to be a valuable tool (3). Mainly tubular diseases display a GTPR < 1.

Tubular Proteinurias
Permanent tubular proteinurias consisting of albumin and T- are seen in the course of chronic tubulopathies. One patient with Albright's syndrome excreted 200 mg/24 h, one suffering from renal tubular acidosis 800 mg/24 h of these microproteins. Fifty-two patients with chronic PN or interstitial nephritis (in 23 verified by histology) exhibited a tubular proteinuria (fig. 1B) with additional excretion of transferrin and IgG. Total protein excretion was 1002,200 mg/24 h. Urine analyses 12 months after the first one revealed differences of the amount of proteinuria but not of its pattern.

Tubular proteinuria AT of 5003,000 mg/24 h as transitory symptom (pattern as in fig. 3ac) could be detected in 19 cases (eight biopsies) of acute extrarenal kidney failure as early as in the oliguric phase. With clinical improvement during the polyuric phase the microproteins disappeared from the urine gradually, beginning with the smallest proteins T- = MW 10,000-40,000. A physiological protein pattern was restored within 14 months, the interval depending roughly on the grade of the disease.

During 1030 days following cadaveric kidney transplantation (31 patients) the same type of tubular proteinuria MW 70,000010,000 (2005,000 mg/24 h) (fig.3ac) was followed by a proteinuria (50200 mg/24 h) consisting of AT = MW 70,00040,000 (fig. 3c) in the case of well-functioning transplants. In contrast, however, to the acute renal failure this pattern persisted for the whole posttransplantation period of maximally 36 months, a physiological proteinuria in these patients was never observed. Eleven of 13 acute rejection crises
Fig. 3. Different forms of micromolecular 'tubular' proteinuria, as seen in a patient after kidney transplantation: (a) immediately after surgery a proteinuria MW 70,000–10,000 (A-T) was found. The smaller proteins T (MW 10,000–40,000) disappeared gradually (b, c) from the urine during the first three postoperative weeks. The pattern (c) A-T / persisted during the whole observation period despite normal transplant function in other patients. In this case an acute rejection crisis was accompanied by a similar tubular proteinuria (d—e), while the later chronic rejection gave a persisting tubular pattern. The pattern a—c followed by a physiological proteinuria is a symptom in acute kidney failure, too.

were accompanied again by a transient proteinuria A—T (fig. 3d—f), the chronic (interstitial) rejections (five patients, three biopsies) revealed the same pattern in a permanent form (fig. 3g-i).

The proteinuria MG 70,000–40,000 (fig. 3c) — as seen during certain stages of transient tubular damage - seems to be also a symptom in vascular nephrosclerosis. The different handling of macro- and micromolecular proteins by the nephron could be demonstrated in observing seven transplants treated with high doses of steroids for acute rejection. All proteins of MW >65,000 including the albumin disappeared from the urine, while the microproteins—in this case a sign for rejection crisis — remained (fig. 4). The gradual disappearance and later reappearance of urinary albumin was quantified by radial immunodiffusion.

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Fig. 4. Posttransplantation course of a patient treated with high doses of steroids for rejection. Note the disappearance and gradual reappearance of albumin (band A). The tubular proteins T are not affected by this treatment.

Glomerular Proteinurias

Macromolecular 'glomerular' proteinurias, too, could be detected permanently and transiently, the latter one being a symptom in heart failure, orthostasis, and in some cases of acute immune complex disease. Chronic glomerulopathies are accompanied by permanent glomerular proteinuria of varying degree: 46 patients suffering from mesangio-proliferative GN, as verified histologically, excreted urinary proteins of a glomerular pattern A—G/ (fig. 2 A; 5A). Markedly different from this pattern was the proteinuria of some children with minimal lesion nephritis and the nephrotic syndrome, excreting only small amounts of 7s-IgG, but up to 20 % of a protein MW ca. 130,000 (band G—3) (fig. 2C). With lower protein excretion the selectivity decreased in some of these samples due to a relatively greater IgG output (fig. SC, D). But not all patients with the nephro-
tic syndrome excreted this protein (fig. SD).

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Fig. 5. Changing patterns of acute glomerulonephritis: (A, B) in acute endocapillary GN proteinuria is reduced without changing its pattern, until the small protein excretion makes the additional physiological proteins visible, C, D, E: minimal change nephritis. After reduction of proteinuria either a physiological (C) or a more unselective glomerular proteinuria (D) may be seen.

This protein could not yet be identified. The band cut from the gel before staining did not react with monospecific antisera against IgG, transferrin, albumin, -I-c complement and fibrinogen, despite of a good precipitation of these antisera with their corresponding protein bands. A protein of identical RM was barely detectable in normal sera, but clearly present in hypoproteinemiac sera of patients whose urine contained G-3 (fig. 2C—F).

Hrdwicke et l. (6) described Ig fragments of 48-55 Å radius in patients with MCN and focal sclerosis, resembling in size the protein G • Seven urines of three such patients (fig. 6A) contained the protein G in a significantly lower concentration compared with the cases of MCN (fig. 2C; 5C, D).

Four of five patients with membranous GN exhibiting a more unselective glomerular proteinuria with IgG/A and macroglobulin excretion revealed also protein G • A similar proteinuria, however more selective, could be seen in seven patients with membrane-proliferative GN. Beside an unselective glomerular proteinuria, six patients (nine urines) with proliferative sclerosing GN excreted 'tubular' microproteins, probably due to the advanced interstitial kidney damage of this group (fig. 6 B). One patient developing this disease was followed up for 2 years with SDS-PAA electrophoresis; he showed a G -proteinuria in the early nephrotic phase.

During the course of acute endocapillary GN (nine patients, 19 urines) an unselective glomerular proteinuria A—G/ without G or tubular microproteins
was detected (fig. 5 A, B). With clinical improvement the amount of proteins excreted decerased, its selectivity, however, was not altered. Experimental glomerulonephritis, caused by an immune complex or by anti-basement membrane antibodies, excreted serum proteins without protein G (daily samples of these experiments were provided generously by Dr. Bts-ford, Freiburg/Birmingham). From the very beginning these proteinurias appeared to be rather unselective despite varying total protein excretion. Regarding the G-3 proteinuria the observation of a GN recurring into a transplanted kidney allows more conclusions (fig. 7): following a typical transplantation course with tubular proteinuria the patient exhibited an unselective glomerular proteinuria of 23,000 mg/24 h (fig. 7, 11.10.), as seen usually in severe mesangio-proliferative GN (MesPGN). Due to increasing amounts of transferrin and protein G the total protein excretion increased to 45,000 mg/24 h,

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Fig. 7. Development of the proteinuria in a patient with recurrent GN into a transplanted kidney. The proteins were visualized by SDS-PAA electrophoresis and quantitated by immunodiffusion (except for protein G). The individual quantity is expressed as percentage of the total of seven proteins. Note the increase of transferrin and G-3 on day 15.11.73, while the amounts of larger proteins remain absolutely unaltered in the increased total proteinuria.

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the amounts of macroproteins (lgG, -A, -M, etc.) remained constant, as seen by radial immunodiffusion (fig. 7, 15.10.). As proteinuria decreased the IgG:G ratio reversed within 2 weeks, tests being made at 3-day intervals. Unselective glomerular proteinurias were detected in nine urines of six patients with kidney amyloidosis, two patients with cystinosis, four patients with the hemolytic-uremic syndrome, and in 42 patients with diabetic glomerulosclerosis.

Discussion

The SDS-PAA electrophoresis has proven to be an as easy and reliable method for molecular weight analyses of protein mixtures as the pathological urine. The advantage is its better resolution as compared to gel filtration; additionally, all proteins present in the urine are detected by this procedure, while by immunochemical methods only a few proteins are chosen. Quantitation by densitometry, however, is inferior to the result obtained by immunoprecipita-
tion, particularly for proteins present in relatively low amounts as IgM or -2 macroglobulin. A clear distinction could be obtained between glomerular and
tubular diseases, resulting in macro- (MW > 60,000) or micromolecular (MW 10,000 - 70,000) protein excretion (compare fig. 1A + B). In all cases a good correlation was obtained between the histological findings of glomerular and/or tubular alterations and urinary protein patterns.

The tubular proteinuria was detected as permanent symptom in hereditary (Albrights disease), inflammatory (pyelonephritis, interstitial nephritis, cyto-megaly) and degenerative (cystinosis) tubular or interstitial diseases. Following acute kidney failure and kidney transplantation microproteins were excreted transiently (fig. 3). Here with clinical improvement the microproteins gradually changed their relative concentrations, proteins MW 10,000 - 30,000 were the first to disappear from the urine (fig. 3 a — c, d— f). Obviously the tubules handle microproteins MW < 65,000 in a selective way; different cells or cellular functions might reabsorb proteins different in molecular weight as seen by the time-dependant recovery after acute tubular damage. The reabsorption capacity for proteins MW 40,000—60,000 is least restored. By L-chain survivals (7) the pathogenesis of tubular proteinurias was proven to be a failure of proximal tubular uptake. Our results permit conclusions only regarding the tubular handling of proteins of MW < 65,000. At least in the case of urinary albumin tubular secretion is excluded by its steroid-induced disappearance from tubular proteinurias in transplants (fig. 4).

Regarding the tubular handling of serum proteins MW > 65,000 possibly filtered by the normal glomerulus some controversy exists. While calculating the albumin clearance its tubular load was estimated to be as high as 30 g/24 h more, recent micropuncture studies in rats reported (8, 9) concentrations of 0.51 mg/100 ml, i.e., about 2 g/24 h or less in man. As seen in our study, the tubular proteinuria of acute kidney failure does not contain more than 30 % 'glomerular' proteins, i.e., not more than 1 g/24 h. Assuming an unselective tubular damage this finding favours the concept of a glomerular system being rather impermeable for proteins MW >65,000.

The quantity of the tubular proteinuria depends on the acuity of the disease, chronic pyelonephritis excreting hardly more than 2,000 mg/24 h, acute kidney failure up to 6,000 mg/24 h. This might depend, too, on the different reduction of glomerular filtration rate.

Alterations of the glomerular system lead to macromolecular glomerular proteinurias of different composition. Based on the concept of protein clearances different glomerular selection of serum proteins is thought to be due to various histological types of glomerular damage. This, however, hardly could explain the clinical observation that the heavy proteinuria of minimal change nephritis (MCN) exhibit the highest grade of selectivity. Our results might indicate...
a different pathogenesis of this latter selective proteinuria. MesPGN, acute
GN and experimental GN lead to a moderately unselective proteinuria, while
hyperacute GN, membranous GN and degenerative membrane alterations reveal
a higher percentage of urinary IgG, being rather independant from the amount
of proteins excreted. In children with MCN, frequently a proteinuria consisting
of MW 65,000-130,000 was observed, while IgG over-represented in the serum
could hardly be detected in the urine. This indicates a rather sharp decrease in
permeability for proteins above MW 130,000, which cannot be explained by
MW-dependant, linearly degressing clearances. Keeping in mind the difference
between protein and PVP glomerular clearances (10) and the low albumin content
of the proximal tubule (8, 9) one might speculate that the podocytes
physiologically retain these protein MW 65,000-130,000, filtered according to
their radius by the membrane. The MCN proteinuria might result from podocyte
damage, the more unselective one from diseases of the membrane and the
mesangial system. Electron microscopic observations of damaged podocytes with
'holes' (11, 12) might support this concept.

Hypothetically, the renal protein handling could be seen as a cascade system,
where proteins MW 10,000-130,000 are filtered by the basement membrane
according to their radius and kept back by different cellular mechanisms
depending also from the MW: podocytes retain proteins MW 65,000-130,000,
while different populations of tubular cells reabsorb microproteins MW
60,000-40,000, 40,000-30,000, etc. Proteins above MW 160,000 do not penetrate
the membrane except for states of disease. The observed steroid effect in
transplant proteinuria proves that different cells are involved. The proteinuria in
one individual might be explained by damaging only one (acute GN; MCN;
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Albright) or several of these mechanisms. For clinical practice SDS-PAA urine
analyses may distinguish:
— chronic glomerulonephritis from chronic pyelonephritis;
— the different diabetic nephropathies;
— some cases of minimal change disease from proliferative and degenerative
glomerular diseases;
— the uncomplicated posttransplantation course from (interstitial) rejection
crises and from glomerular diseases (recurrent GN, glomerular rejection disease);
— the persisting small glomerular proteinuria after acute GN from protein-
urias becoming physiological.

Summary

The SDS polyacrylamide gelelectrophoresis (SDS-PAA) as used in this study has proven
to be an excellent tool to differentiate urinary proteins qualitatively and quantitatively, since the proteins are differentiated exclusively according to their molecular radius. Selectivity was estimated by the ratio transferrin:IgG. Some of the proteins were identified by specific antisera. For clinical use SDS-PAA may distinguish: chronic glomerulonephritis from chronic pyelonephritis; the different diabetic nephropathies; some cases of minimal change nephritis from proliferative and degenerative glomerular diseases; the uncomplicated posttransplantation course from (interstitial) rejection crises and from glomerular diseases (recurrent GN, glomerular rejection disease), and the persisting small glomerular proteinuria after acute glomerulonephritis from proteinurias becoming physiological.

References


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