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REVIEW

URINARY PROTEIN ANALYSIS*

MICHAEL H. WEBER

Zentrum Innere Medizin der Universität Göttingen, Abteilung Nephrologie (head: Professor Dr. F. Scheler), Robert-Koch-Strasse 40, D-3400, Göttingen (F.R.G.)

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CONTENTS

*Dedicated to Professor V. Neuhoff, Max-Planck-Institut für experimentelle Medizin, Göttingen, F.R.G., on the occasion of his 60th birthday.

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LIST OF ABBREVIATIONS

BJ	Bence Jones
%C	Percentage bisacrylamide cross-linking
CBB	Coomassie Brilliant Blue
ELISA	Enzyme linked immunosorbent assay
FDP	Fibrin/fibrinogen degradation products
FPLC	Fast protein liquid chromatography
GBM	Glomerular basement membrane
GPP	Gel permeation chromatography
HMW	High molecular weight (mass)
HPLC	High-performance liquid chromatography
I.D.	Inner diameter
IEF	Isoelectric focusing
Ig	Immunoglobulin
IPG-DALT	Two-dimensional immobilized pH gradient IEF/SDS-PAGE
ISO-DALT	Two-dimensional IEF/SDS-PAGE
kDa	Kilodaltons
LMW	Low molecular weight (mass)
2-ME	2-Mercaptoethanol
MGGE	Micro gradient gel electrophoresis
M_r	Relative molecular mass
O.D.	Outer diameter
PAA	Polyacrylamide
PAGE	Polyacrylamide gel electrophoresis
SDS	Sodium dodecylsulphate
SRID	Single radial immunodiffusion
%T	Acrylamide concentration
Tris	Tris(hydroxymethyl)aminomethane
U/S	Urine/serum ratio

1. INTRODUCTION

Among the substances excreted in the urine, plasma proteins of high and low molecular mass play an important role as indicators of glomerular basement membrane integrity and tubular reabsorption capacity. Owing to a complex tubular recycling mechanism, the loss of plasma proteins into the urine is limited to the range 50–150 mg/day, although about 170 l of blood plasma are filtered through the glomerular filtration units.

These filtration units consist of four distinct anatomical entities. Their structural integrity is crucial for the maintenance of normal ultrafiltration. The fenestrated endothelial cell of the glomerular capillary restrains cellular components of the blood as well as several macromolecules from filtration. The function of the GBM as a charge-selective barrier for plasma proteins is dependent on anionic heparansulphate proteoglycans [1] localized in the lamina rara externa, whereas size selection takes place in the hydrated network of the lamina densa [2]. The epithelial side consists of the foot processes of the epithelial cells connected by the negatively charged slit membrane [3]. Finally, although not directly involved in the filtration process itself, the centrolobularly situated

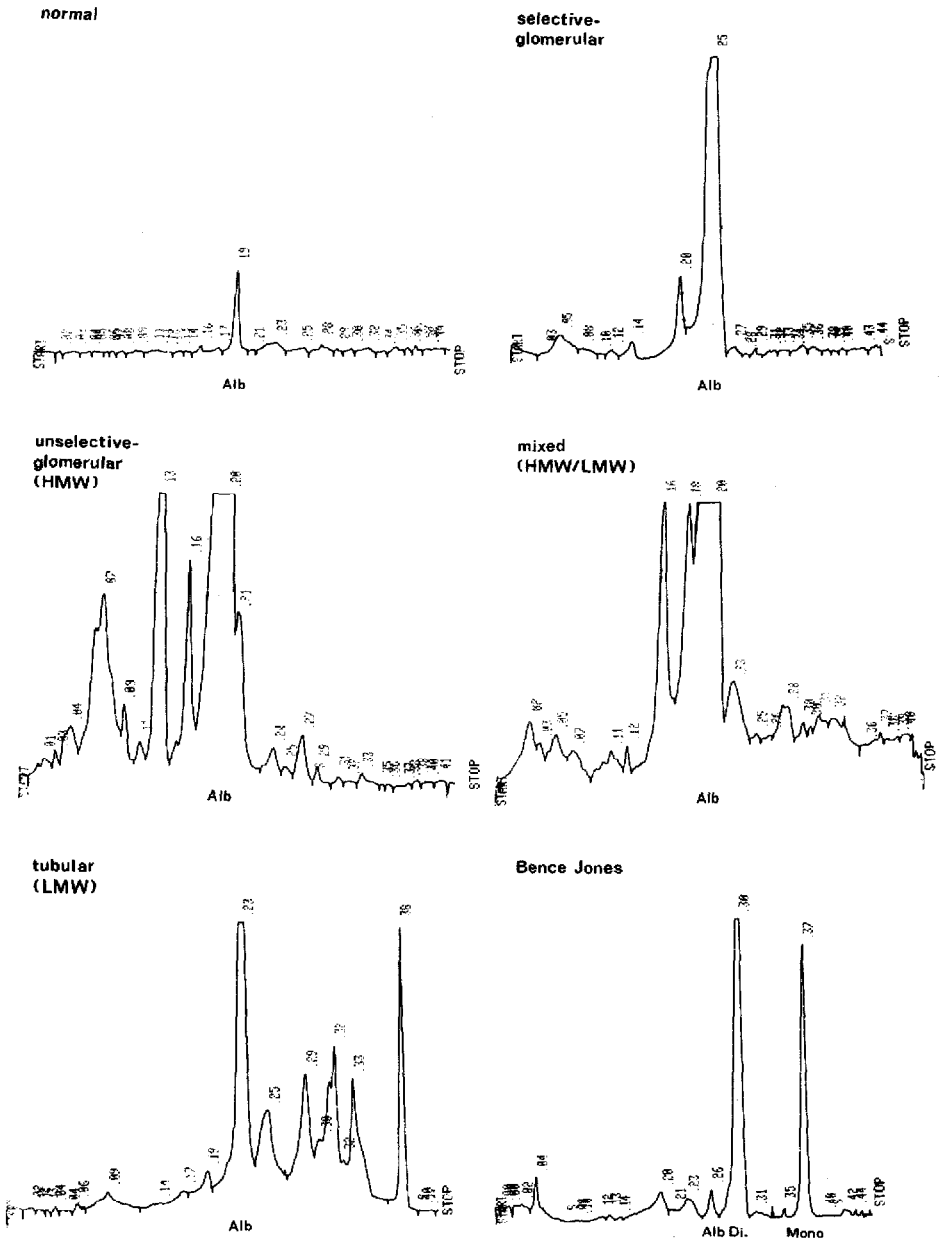

Fig. 1. Typical urinary protein patterns obtained by densitometry of urine specimens after 1D PAGE in SDS micro slab gradient gels and CBB G-250 staining [14]. HMW proteins are plotted on the left and LMW proteins on the right of the albumin peak (Alb.). First row: (left) physiological protein pattern (albumin and LMW proteins); (right) selective glomerular pattern (albumin and transferrin; M_r 65–90 kDa). Second row: (left) unselective glomerular pattern (HMW proteins; M_r 65–400 kDa); (right) mixed glomerulo-tubular pattern (HMW/LMW; M_r 10–400 kDa). Third row: (left) micromolecular tubular pattern (LMW proteins; M_r 10–70 kDa); (right) Bence Jones overflow proteinuria; two distinct peaks in the M_r range of 22 and 44 kDa (light chain monomer and dimer).

TABLE 1

PROTEINURIA IN RENAL DISEASES

Type of disease	Characteristics
Glomerular	Minimal changes glomerulonephritis Focal sclerosing glomerulonephritis Membranous glomerulonephritis Membranoproliferative glomerulonephritis Mesangiocapillary glomerulonephritis IgA nephropathy Endocapillary acute glomerulonephritis Hereditary glomerulopathies (Alport, nail–patella) Gold nephropathy
Tubulo-interstitial	Drug-induced tubulopathies (analgesics, aminoglycosides, cephalosporins, cyclosporin A, heroin) Heavy metals (Cd, Pb, Pl) Interstitial nephritis Pyelonephritis Acute renal failure (acute tubulonecrosis) Post-transplantation period Balkan endemic nephropathy Hereditary nephropathies (Fanconi)
Proteinuria in extra-renal diseases	Connective tissue diseases (Lupus erythematosus, Wegener, Henoch–Schönlein, Panarteriitis nodosa) Diabetic nephropathy Multiple myeloma, kidney amyloidosis Congestive heart failure Arterial hypertension Fever Infectious diseases (malaria, lues) Pregnancy-induced toxaemia

mesangial cells partake in the elimination and degradation of macromolecules [4].

Only minute amounts of high-molecular-weight (mass) (HMW; relative molecular mass, $M_r > 67$ kDa) plasma proteins are excreted in the urine. Low-molecular-weight (mass) proteins (LMW; $M_r \leqslant 67$ kDa) are filtered freely through the GMB and are reabsorbed nearly completely by the proximal tubular cells. Essentials of the reabsorption mechanisms for LMW proteins and the enzyme distribution along the nephron have been reviewed by Carone et al. [5] and Guder and Ross [6]. In addition to albumin (M_r 66.4 kDa), which marks the borderline between "glomerular' and "tubular" proteinuria, small amounts of LMW plasma proteins such as β_2-microglobulin, α_1-microglobulin and retinol-binding protein, in addition to proteins of glomerular and tubular origin such as GMB antigens, tubular enzymes, Tamm Horsfall mucoprotein and secretory immunoglobulin A appear in the urine [7,8].

Pathological proteinuria of the HMW type occurs when inflammatory and de-

generative processes of the glomerular filtration units (glomerulonephritis, glomerulosclerosis, kidney amyloidosis) lead to an increased passage of macromolecules into the ultrafiltrate. As agreed, the predominant excretion of proteins in the M_r range of albumin is termed "selective-glomerular", whereas the "unselective-glomerular" proteinuria of the nephrotic syndrome covers the whole M_r range of plasma proteins. Proximal tubular impairment, e.g., in interstitial nephritis, is accompanied by a reduction of the reabsorption capacity for LMW proteins resulting in a "tubular" type of proteinuria in the M_r range 10–70 kDa. In severe renal diseases such as rapidly progressive glomerulonephritis, a mixed glomerular and tubular pattern is frequently seen, indicating both loss of glomerular permselectivity and tubulo-interstitial involvement. Prerenal "overflow" proteinuria occurs when an abnormally high plasma level of LMW proteins [e.g., Bence Jones (BJ) proteins, myoglobin, haemoglobin] leads to an extreme concentration of this protein in the ultrafiltrate, exhausting the specific and unspecific reabsorption mechanisms for LMW proteins along the proximal tubules [9–14]. Typical examples of the protein patterns described above are demonstrated in Fig. 1.

Clinical and pathophysiological aspects of proteinuria have been reviewed in detail elsewhere [15–20]. A list of renal diseases in which the principal sign is proteinuria is given in Table 1.

2. ANALYSIS OF URINARY PROTEINS

For the investigation of urinary proteins in health and disease, several protein separation techniques, including column gel chromatography, agarose and polyacrylamide (PAA) gel electrophoresis (PAGE) and isoelectric focusing (IEF) have been employed, in addition to methods for the immunological identification and quantification of single proteins in urine.

2.1. Column gel chromatography

In the late 1960s, several prominent research groups applied Sephadex gel permeation chromatography (GPC) using different bead sizes (G-200, G-100 and G-75; Pharmacia LKB, Uppsala, Sweden) to the separation of urinary proteins, especially in cases of the nephrotic syndrome [21,22]. The basis of these studies was the conception of the glomerular basement membrane as an isoporous semipermeable membrane, acting as a molecular sieve for macromolecules: the higher the urine/serum (U/S) ratio of a given HMW protein, the lower is the so-called "selectivity" of the proteinuria. However, as was pointed out in an re-examination of various gel chromatographic techniques by Pesce et al. [23], the efficacy of Sephadex G-200 separation in a 0.1 *M* sodium chloride–0.03 *M* potassium phosphate buffer was low, with a marked overlap of the two main protein peaks (albumin and IgG) which could be quantitatively determined by immunological methods. Owing to the predominance of albumin in the urine of patients with the nephrotic syndrome, the urinary γ-globulin peak contained up to 50% albumin. Davis et al. [24], in a study of 29 patients, obtained a better resolution of the LMW proteins in tubular proteinuria using Sephadex G-75 and a pH 7.3 sodium

phosphate buffer, but reported a significant overlap of various LMW proteins. Owing to its time-consuming technique, its disappointingly low resolution compared with PAGE and the methodological errors in U/S calculations, conventional gel chromatography did not find general acceptance in the routine analysis of proteinuric diseases.

In 1982, the application of a new GPC procedure using silica gel microspheres (TSK GEL, type SW; Varian, Darmstadt, F.R.G.) was described by Ratge and Wisser [25]. They used a TSK GWSP and two TSK G 3000 SW columns in series and a 0.1 *M* sodium chloride–0.067 *M* phosphate buffer (pH 6.8). The elution profiles obtained by this high-performance liquid chromatographic (HPLC) method turned out to be superior to those in customary Sephadex gel chromatography and were approximately comparable to the sodium dodecylsulphate (SDS) PAGE patterns of the same urine samples. To remove excess amounts of albumin from urines, the authors recommended Blue Sepharose CL-6B (Pharmacia LKB).

Cooper and co-workers recently employed the fast protein liquid chromatographic (FPLC) system (Pharmacia LKB), using Mono Q anion exchanger on an HR 5/5 column for the analytical [26] and preparative [27] separation of urinary proteins. Having successfully optimized the separation buffer system [starting buffer, 6.25 m*M* Bis-Tris-propane (pH 7.5); limiting buffer, 6.25 m*M* Bis-Tris-propane (pH 9.5) + 0.35 m*M* sodium chloride] [28], the authors obtained very sharp and reproducible protein peaks, from which LMW proteins such as monoclonal light chains, α_1-microglobulin and β_2-microglobulin could be easily isolated using re-chromatography on a Mono Q 16/10 column after a desalting step (Sephadex G-25). Lysozyme was separated on a cation-exchanger (Mono S™ resin). From the viewpoint of clinical applicability, the FPLC system seems to possess a high potential for preparative purposes. On the other hand, the analytical pattern specificity of its elution profiles is surpassed by those achieved by modern SDS gradient PAGE. The initial investment in the separation hardware requires a multipe PAGE unit. However, a clinical laboratory in which the FPLC system is also routinely used for other purposes may employ this rapid separation system in the analytical separation of urinary proteins in lieu of PAGE.

2.2. Agarose gel electrophoresis

Owing to their physico-chemical properties, agarose gels proved to be of little value in the electrophoretic analysis of urinary proteins. Proteins are separated according to charge, resulting in several zones containing two to thirteen nearly equally charged polypeptides. Some of them are spread across more than two of the electrophoretic zones [29]. Although attempts have been made to differentiate between tubular and glomerular proteinuria in cases of chronic pyelonephritis using agarose gel electrophoresis [30], its applicability lies in the field of the immunological identification of monoclonal light chains (and other single proteins) by immunoelectrophoresis [31] and immunofixation electrophoresis [32].

2.3. Polyacrylamide gel electrophoresis

Owing to its nearly universal applicability for the separation of complex protein mixtures, PAGE has been widely propagated. At a very early point, even during the basic research of Raymond and Weintraub [33], Raymond [34], Ornstein [35], Davis [36], Bloemendal et al. [37], Shapiro et al. [38], Margolis and Kenrick [39], Maurer [40,41], Weber and Osborn [42], Weber et al. [43] and Laemmli [44] on (SDS) disc electrophoresis, a division into macro- and micro-scale PAGE techniques became obvious. The rapid development of both methodological lines led to several technical modifications to the separation of urinary proteins with PAGE systems. As it is beyond the scope of this review to consider theoretical aspects of the separation of proteins in PAA gels (for reviews see refs. 41 and 45–47), this paper is limited to a survey of the applicability of macro- and micro-PAGE techniques in the field of urine analysis and an assessment of their clinical relevance.

2.3.1. Macro-scale PAGE techniques

Waldmann et al. [48] were among the first to evaluate urinary protein excretion patterns using native homogeneous rod gels (63 mm × 5 mm diameter) of 7.5% T (acrylamide concentration), originally designed for the separation of serum proteins by Davis [36]. Urines had to be concentrated by ultrafiltration, and 200 μg of the protein concentrate were applied per gel. Electrophoresis was carried out in 0.1 *M* Tris–HCl buffer (pH 8.9) with 2–5 mA per tube with staining in 1% (w/v) Amido Black 10B in 7% (v/v) acetic acid–water and destaining in 7% (v/v) acetic acid–water. In addition, Waldmann et al. [48] performed SDS-PAGE according to Shapiro et al. [38] in rod gels of 5% T. In this system, urinary proteins were denatured and reduced prior to electrophoresis in 1% (w/v) SDS and 1% (v/v) 2-mercaptoethanol (2-ME) for 3 h. Very high urinary excretion rates of (immunologically measured) LMW proteins were found to correspond with a characteristic "tubular" protein pattern in patients with tubular disorders, in contrast to the HMW pattern (albumin, transferrin) of patients suffering from the nephrotic syndrome.

Pires et al. [49] confirmed the usefulness of PAGE for the analysis of urinary proteins by demonstrating a satisfactory correlation between the separation results and clinical and morphological data for patients with renal diseases. Their method in 5% homogeneous rod gels was slightly different from the cited original description [50]; instead of SDS gels of 10% T in 275 mm × 6 mm I.D. cylindrical glass tubes, they used 75 mm × 6 mm diameter gel cylinders of 5% T. Urines were concentrated 10–200 fold by ultrafiltration in Visking tubes; 100–200 μg of this solution were applied per gel. Electrophoresis was carried out in 0.1 *M* sodium phosphate buffer (pH 7.2) containing 0.1% (v/v) SDS, with an applied voltage of about 3 V/cm gel. The gels were stained with Amido Black 10B in water–methanol–acetic acid (50:45:0.5, v/v/v). Using Pires et al.'s method, Virella and Lopes-Virella [51] compared SDS-PAGE with the determination of single LMW proteins (e.g., lysozyme) in tubular proteinuria [52].

Independently of Waldmann et al. [48], Pesce et al. [53] described a separa-

tion method for urinary proteins on homogeneous slab gels of 7% T and 5% C using a commercial Beckman Model R-113 PAA slab gel system (Beckman Instruments, Fullerton, CA, U.S.A.). Urine specimens were concentrated 50-fold and diluted to a final protein concentration of 10 mg/ml. Following incubation with SDS, the proteins were separated at 400 V for 45 min and stained with 0.125% (w/v) CBB R-250 in methanol–water–acetic acid (47.5:47.5:4.8; v/v/v). A linear relationship was found between the logarithm of the molecular mass of the proteins and their R_F values on the gel. In the presence of SDS, BJ light chains could be clearly separated into monomers and dimer aggregates. Later, Pesce et al. [54] employed a method for concentrating multiple urine samples simultaneously by using the Minicon ultrafiltration device and a modification of the SDS slab gel method. In a study on proteinuria following renal transplantation (therapeutic regimen: azathioprine–methylprednisolone), they subsequently observed a mixed LMW protein–albumin excretion in the immediate post-transplantation period, whereas acute tubular necrosis revealed an amplification of the tubular pattern. Using both Pesce et al.'s rod gel method and one-dimensional (1D) SDS slab gels, Fleming [56] successfully monitored patients with incomplete tubular proteinuria following the administration of tubulotoxic drugs.

Boesken et al. [10] applied 1D SDS-PAGE in homogeneous rod gels of 7.5% T in 63 mm × 7 mm I.D. glass tubes [40] to the differentiation of proteinuric diseases (Fig. 2). Urines were concentrated 100-fold by ultrafiltration and incubated with SDS–sample buffer. A 100-μl volume of this solution (containing 15–

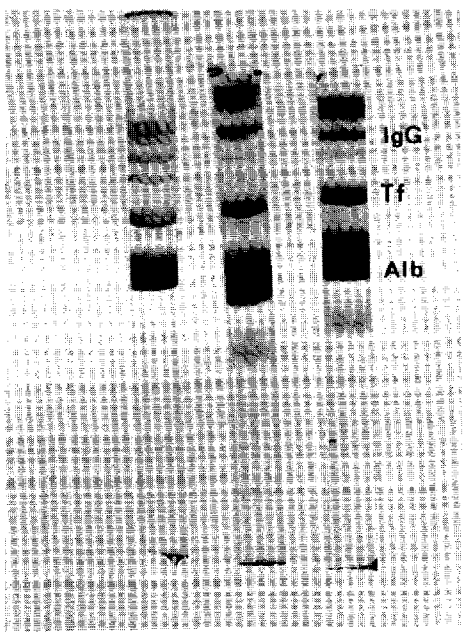

Fig. 2. Three urine specimens exhibiting unselective glomerular proteinuria as separated in SDS rod gels of 7.5% T and stained with Amido Black 10B according to the method of Boesken et al. [10]. Protein bands identified using immunoblotting are marked Alb (albumin), Tf (transferrin) and IgG (immunoglobulin G).

25 μg of protein) was loaded on to each of the gels. Following electrophoresis at 5–10 mA per tube, the gels were stained with 0.6% (w/v) Amido Black 10B in 7% (v/v) acetic acid in water. Using a Joyce-Loebl Chromoscan microzone densitometer, Boesken et al. [10] registered characteristic densitometric curves in inflammatory glomerular and tubular diseases and found typical glomerulotubular ratios (GTPR) for urinary proteins in various renal diseases. Boesken and co-workers' work on proteinuria over more than a decade included, among various other topics [57,58], investigations on tubular proteinuria [11], the differential diagnosis of nephropathies and the analysis of extrarenal forms of proteinuria [59–61] and the dimerization of albumin in nephrotic urines [62]. Doman et al. [63] described studies of albumin dimerization under steroid therapy. They suggested a chemical in vivo alteration of plasma albumin by steroids, whereas in vitro dimerization occurred only in the presence of hydrocortisone and an as yet poorly characterized "ultrafiltrate fraction" ($M_r < 0.7$ kDa).

Balant et al. [64], stimulated by the investigations of Waldmann's and Pesce's groups, extended those early investigations to a group of 92 patients with the intention of introducing SDS-PAGE into the clinical laboratory. They compared SDS-PAGE in cylindrical gels of 7.5% T [44] and native PAGE without SDS in gels of 5% T [36] with cellulose acetate electrophoresis, immunoelectrophoresis and column GPC, respectively. Prior to SDS-PAGE (200 V per gel), the concentrated urines (60 mg protein per ml) were diluted 1:40 in an application mixture containing 2.5% (w/v) SDS and 0.925% (w/v) iodoacetamide. Among the techniques evaluated, the results from the SDS-PAGE correlated best with the patients' clinical status. In 1980, Cachera et al. [65] varied the method of Balant et al. [64] by introducing an imidazole–phosphoric acid electrophoresis buffer in place of phosphate buffer for routine analysis.

Rautenstrauch [66] attained reproducible separations of urinary proteins in 7.5% slab gels in the presence of SDS on a horizontal electrophoresis device (Desaga, Heidelberg, F.R.G.). Depending on the results of a dipstick method for albumin, urines had to be concentrated 100–200 fold prior to the separation step. At that point, the method was not recommended for routine use. The same group [67] compared the diagnostic value of disc electrophoresis and the measurement of β-N-acetylglucosaminidase for the detection of renal tubular impairment; both methods were found to have equal diagnostic value. In 1979, Rautenstrauch [68] analysed urine samples from 50 patients with multiple myeloma and found monoclonal light chains in 44% of the cases. Brandhorst and Wetter [69] confirmed these results using homogeneous SDS slab gels (length 110 mm, diameter 5 mm) of 10% T according to Weber and Osborn [42]. Originally, Takeoka et al. [70] had reported the PAA gel disc electrophoresis of unconcentrated urines with special attention to BJ proteins. For molecular mass analysis of urinary proteins, a methodological variation of Weber et al.'s method of 1972 [43] was recommended by Lubega [71]. He used homogeneous SDS rod gels of 10% T in 75 mm × 6 mm I.D. glass tubes and an Amido Black 10B staining procedure.

Lison's group applied a variation of Maurer's recipe [40] (homogeneous 1D SDS slab gels of 7.5% T, 80 mm × 80 mm × 0.5 mm; CBB R-250 stain) [72] to the etiological diagnosis of chronic renal diseases [73,74]. Urines had to be con-

centrated 50-fold with Amicon B 15 membranes. About 500 μg of protein were applied per track. In their hands, HMW protein patterns obtained by SDS-PAGE had a high prognostic value in the early diagnosis of pregnancy-induced hypertension [75] and toxaemia in pregnancy [76].

The proteinuria of diabetic nephropathy was investigated by Lopes-Virella et al. [77] and Jones et al. [78], among others. The latter varied a method originally described by Smith et al. [79] and Walker and Pollard [80,81]. Electrophoresis in homogeneous PAA slab gels was performed on concentrated, non-denatured urine samples using a 7% separation gel and a 4% stacking gel. Instead of a continuous Tris–borate buffer (pH 9.5), they used a 0.37 *M* Tris–glycine buffer of the same pH. During the course of diabetic nephropathy, they observed a trend towards a more pronounced and less selective proteinuria as renal function was further impaired. Recently, Meier and Levitt [82] studied the mechanism of increased renal clearance of amylase during the course of acute pancreatitis. SDS slab gel PAGE (5% stacking gel, 10% separation gel; Fig. 3) [44] of urine speci-

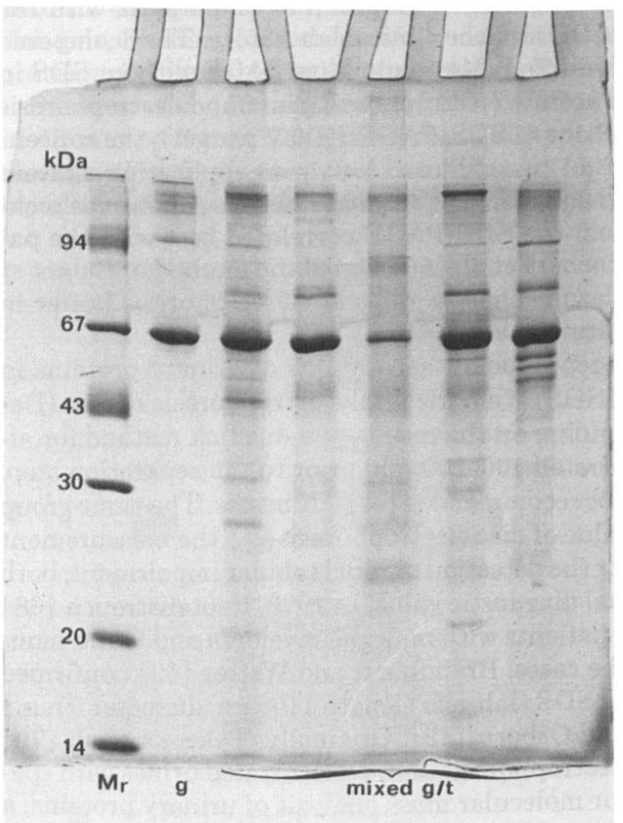

Fig. 3. SDS slab gel PAGE (5% stacking gel, 10% separation gel) according to the method of Meier and Levitt [82], stained with CBB R-250. M_r = LMW marker proteins (Pharmacia); g = unselective glomerular pattern; mixed g/t = mixed glomerulo-tubular patterns (urines from five different patients suffering from end-stage renal disease) (gift from Dr. N. Arold, Max-Planck-Institut für experimentelle Medizin, Göttingen, F.R.G.).

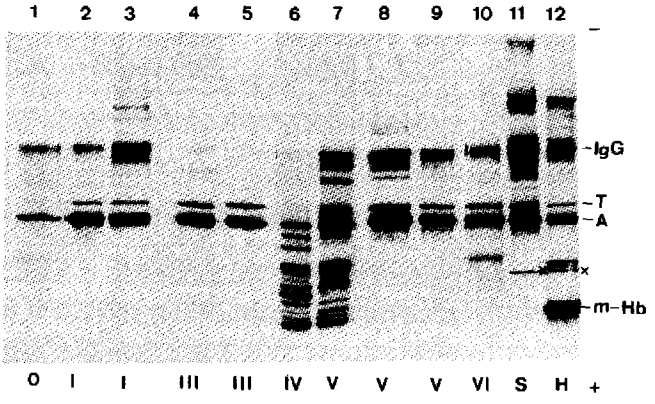

Fig. 4. 1D SDS ultra-thin-layer gradient slab PAGE of 4–22% T stained with silver, according to Schiwara et al. [92], exhibiting various types of renal proteinurias. (1) Physiological proteinuria with normal protein patttern (0); (2) and (3) unselective glomerular proteinuria (I); (4) and (5) selective glomerular proteinuria (III); (6) tubular proteinuria (IV); (7–9) unselective glomerular proteinuria + complete tubular proteinuria of differing degrees (V); (10) unselective glomerular proteinuria + incomplete tubular proteinuria (VI); (x) serum-specific microprotein; S = serum; H = haemolysate; IgG = immunoglobulin G; T = transferrin; A = albumin; m-Hb = monomeric haemoglobin. (From ref. 92, with permission.)

mens concentrated 17–22 fold was run on a Bio-Rad Model 220 electrophoresis cell (Bio-Rad Labs., Richmond, CA, U.S.A.) at 50 mA. The gels were stained overnight with a commercial Coomassie Brilliant Blue (CBB) method.

With the employment of a linear PAA gradient by Scherberich et al. [83], a crucial improvement in the separation qualities of urinary proteins on macroscale slab gels was achieved. The methodological variation was based on the work of Wright [84] and Margolis and Kenrick [85]. Using a horizontal flat gel chamber (Havanna equipment; Desaga), they established a PAA gel gradient of 3–20% T, applying a conventional gradient mixer cooled in an ice-bath. Unconcentrated 24-h urine specimens were centrifuged at 2000 *g* for 10 min prior to application to the gels [diluted 4:1 with 1% (w/v) SDS sample buffer, depending on the albumin content, measured with a dipstick method]. The gels were stained with 5% (w/v) CBB R-250 in ethanol (75%, v/v)–acetic acid (9:1, v/v). Using this technique, they obtained a uniformly high resolution of urinary proteins in the HMW and LMW range. In addition to pattern studies of several types of proteinuria, Scherberich et al. [86] investigated the influence of freezing and thawing on the recovery of certain urinary proteins. A simple routine method for 1D SDS electrophoresis in macro gradient slab gels (205 mm × 110 mm × 0.8 mm) was described in detail by Lapin et al. [87], using the Laemmli buffer system [44] and a recently developed CBB G-250 staining method in perchloric acid [88]. They successfully monitored urinary protein patterns in kidney-transplanted patients [89]. Kshirsagar and Wiggins [90] proceeded with the construction of a map of urinary proteins based on 1D PAGE in 80 mm × 70 mm × 0.4 mm SDS gradient slab gels, using silver staining for protein detection and the Western blot technique for the immunological identification of urinary proteins.

Instead of applying a linear PAA gradient, a discontinuous gradient consisting of layers of 18, 15, 11 and 6% T was poured. Unconcentrated urine samples were applied using the Agarose Drop technique developed earlier by the same group [91]. Electrophoresis was run using the Laemmli buffer system, at a constant current of 5 mA during stacking and 10 mA during separation. The positions of at least fifteen urinary proteins could be defined using the 1D PAGE system.

At present, horizontal ultra-thin-layer SDS pore gradient gel electrophoresis in 120 mm × 250 mm × 0.36 mm slab gels according to Schiwara et al. [92] seems to be one of the most promising PAGE systems for urine analysis on a macro-scale. Originally designed by Görg et al. [93], the method was soon adapted to the needs of routine laboratory diagnosis of proteinuria [94]. Combined with a sensitive silver staining technique [95], the 4–22% T gel gradient reveals a detailed urinary protein pattern covering the whole M_r range of plasma proteins excreted in glomerular and tubular diseases (Fig. 4). As urine specimens do not have to be concentrated, this macro method can be easily applied to the routine analysis of proteinuria. A variation of the described method was recently adapted by Schiwara [96] to the Midget Twin system (Pharmacia LKB).

2.3.2. Micro-scale PAGE techniques

Owing to the requirements of cell biology (e.g., neurochemistry), micro-scale adaptations of PAGE in rod and slab gels were developed very early in the history of PAGE.

2.3.2.1. Micro PAGE in capillary gels. Pun and Lombrozo [97], Grossbach [98], Hydén et al. [99] and Felgenhauer [100] were among the first to adapt the disc electrophoresis technique according to Ornstein [35] and Davis [36] to micro cells or micro capillaries. Neuhoff et al. [101,102] systematically optimized the method of Hydén et al. [99] for the separation of proteins of central nervous system cells in 5–10-μl capillaries using separation gels of 20–25% T and stacking gels of 5% T.

Homogeneous micro gels served as the basis for the first report, by Weise and Bockhorn [103], on the separation of urinary proteins for the early diagnosis of acute rejection crises following kidney transplantation. In their discussion, they indicated that micro-PAGE in capillaries was time-saving and did not require prior concentration of urine samples. However, not until after the introduction of micro-electrophoresis in gradient gels (MGGE) by Neuhoff's group [104,105] and the publication of a detailed handbook on micro methods in molecular biology [106] could micro-PAGE establish itself in the field of nephrology. Micro-PAA gradients (e.g., of 1–33% T and 2% C) in 5–10-μl capillaries are prepared by filling half of the capillary with buffered catalyst, then dipping the lower end of the capillary into the acrylamide–bisacrylamide stock solution. Owing to capillary action, the gel gradient is formed by a lancet-like entry of the PAA solution into the buffered catalyst. Micro gradient gels can be prepared batchwise and stored in the cold for several months.

We used MGGE in 1–33% capillary gels for the investigation of urinary fibrin–fibrinogen degradation products (FDP) as markers of disease activity in glomer-

ulonephritis [107] and for the differentiation of urinary protein patterns in patients suffering from various forms of glomerulonephritis (Fig. 5) [108]. Reichel et al. [108] pointed out that, owing to the continuous changes in the structure of the GBM during the course of glomerulonephritis, the pattern of proteinuria might not directly reflect the morphological type, but rather the stage of the inflammatory glomerular process. With MGGE, tubular proteinuria in 96% of the studied cases with acute pancreatitis could be demonstrated by Lankisch et al. [109], but this did not correlate well with the $C_{amylase}/C_{creatinine}$ ratio.

Not only was MGGE in 1–5 μl capillaries consequently applied to micropuncture studies of glomerular protein filtration and tubular reabsorption in normal and nephritic rats by Galaske's group [110–113], but also to the study of the renal handling of proteins by newborn children [114]. Further miniaturization of MGGE for the analysis of nanolitre volumes of renal tubule fluid in 0.5 μl capillary tubes (PAA gradient, 4–27%) was recently descibed by Manz et al. [115]. In order to evaluate the predictive potential of MGGE, Burghard et al. [116] analysed urinary protein patterns before and after acute rejection crisis in transplanted children undergoing conventional azathioprine–prednisone therapy. Although an increase of LMW proteins was noted during the episodes, there was no change in protein patterns before other signs of rejection crisis. Alt et al. [117] investigated the renal protein excretion in chronic pyelonephritis of 25 patients using MGGE (5 μl capillaries, 4–40% T; Amido Black 10B stain; Joyce-Loebl microzone densitometer, type MK III-CS). In advanced stages of the disease

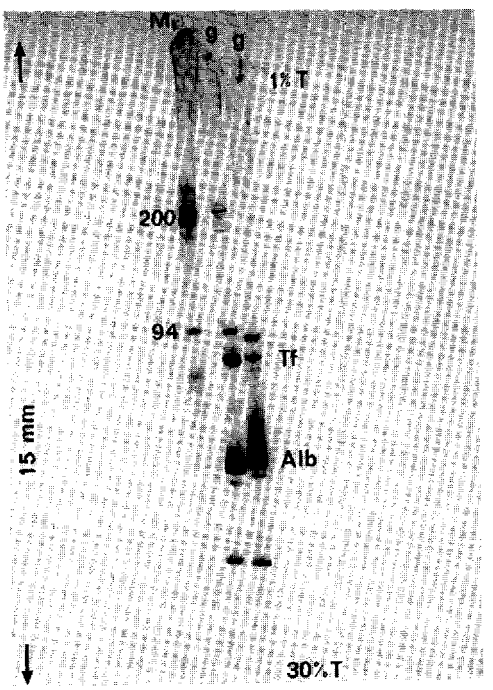

Fig. 5. Native micro gradient gel electrophoresis (1–30% T) in 10-μl capillaries according to the method of Reichel et al. [108]. M_r = HMW marker proteins; g = unselective glomerular proteinuria.

(creatinine clearance < 40 ml/min), an LMW protein pattern was observed, often associated with signs of increased glomerular permeability due to glomerulosclerotic alterations of the GBM. These results were later confirmed in an extended study on 103 patients suffering from various interstitial and tubular kidney diseases [12].

An interesting combination of the micro capillary and slab technique for the separation of urinary proteins was introduced by Leskovar and Kratzer [118]. They used 49 mm × 49 mm glass plates equipped with 28–40 longitudinal grooves covered with a slide-glass for the preparation of electrophoresis cells. As gradient gels can be cast batchwise with the aid of capillary action, the 40 capillary gels drawn from one cell exhibit an identical gradient. As was emphasized, numerous separation techniques including SDS-PAGE can be performed on unconcentrated urine specimens. Schmut et al. [119] employed a scaled-down version of the disc electrophoresis system according to Maurer [40] for the one- and two-dimensional analysis of body fluids with low protein contents such as normal urine. Interestingly, the gel dimensions, with an outer diameter of 2 mm and a length of 100 mm, were considerably larger than those of all other micro rod gels mentioned. However, as the term "micro" has never been defined precisely, the transition between "macro"- and "micro"-scale was always smooth.

2.3.2.2. Micro slab gels. The PAA slab gel technique, originally designed by Raymond [34] and further improved by Margolis and Kenrick [85], was soon adapted to the micro-scale by several groups [120–122]. Usually, homogeneous gels of 7–8% T on microscope slides in closed or open moulds were used. Micro slab electrophoresis equipment for the application of several different gel techniques (including PAA gradient gels) was described in detail by Maurer and Dati [123]. This method was later refined by Matsudaira and Burgess [124], who employed a 5–20% linear gradient separation gel of 82 mm × 102 mm × 0.5 mm using the buffer system of Laemmli. Other 1D micro techniques on slab gels were employed by Amos [125], Ogita and Markert [126] and Wiggins et al. [127], but none of these methods was applied to the separation of urinary proteins for diagnostic purposes in routine clinical applications.

Poehling and Neuhoff [128] comprehensively surveyed the spread of electrophoretic separations in micro slab gels, including 1D micro-PAGE in continuous and gradient gels and IEF and two-dimensional (2D) microelectrophoresis (the latter methods as a refinement of those of Rüchel [129]). From experience with the separation of urinary proteins by MGGE [108], Poehling and Neuhoff's micro slab technique [128] was soon adapted by us for the differential diagnosis of proteinuric diseases [130].

In contrast to the cited references, the dimensions of the glass plates for the preparation of the micro slab gel chambers are now 61 mm × 37 mm × 0.2 mm, and the gels (6–30% T) have a diameter of 0.5 mm (Fig. 6). Using a scaled-down, cooled gradient mixer, six gels can be cast simultaneously. Following polymerization, the gel block is removed from the casting chamber, the gel chambers are cut out and a stacking gel of 3% T is poured on top of the separation gels. Slots are formed with a 0.5 mm Teflon comb. Urine samples are used uncentrifuged and are mixed with 50 m*M* Tris–glycine buffer (380 m*M* glycine) (pH 8.4) containing 1% SDS to a final protein concentration of 0.05%. Electrophoresis is

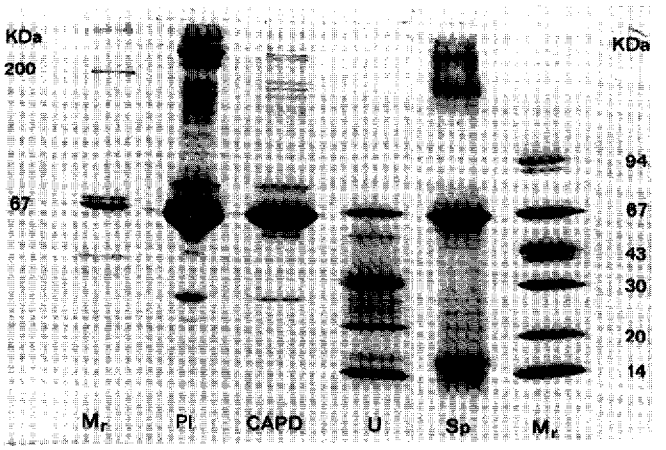

Fig. 6. 1D SDS micro gradient slab PAGE (6–30% T, stacking gel 3% T) [14], stained with improved CBB G-250 staining [88]. Comparative separation of four biological fluids from a patient on peritoneal dialysis: Pl = plasma; CAPD = peritoneal dialysate; U = urine (micromolecular tubular pattern); Sp = saliva; M_r = LMW and HMW marker proteins (Pharmacia). Note the correspondence of plasma and dialysate protein bands.

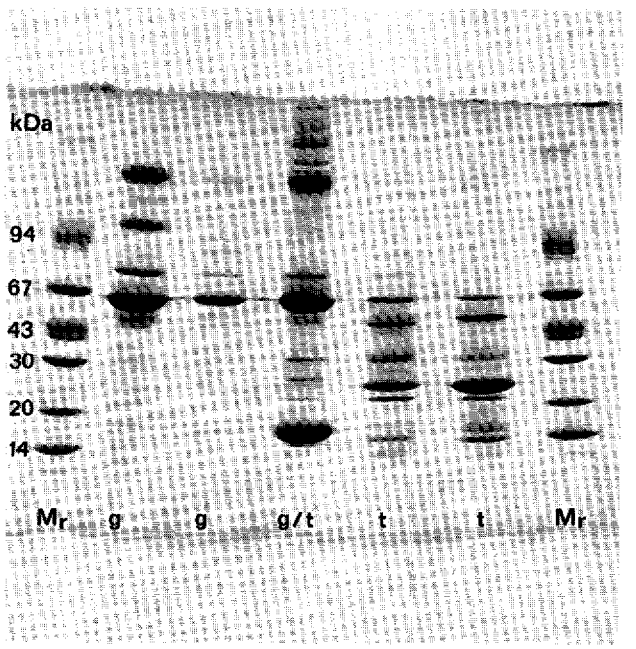

Fig. 7. 1D SDS micro gradient slab PAGE (6–30% T, stacking gel 3% T) [14], stained with improved CBB G-250 staining [88]. Renal proteinurias; g = unselective glomerular pattern of differing degrees; g/t = mixed glomerulotubular pattern; t = complete (micromolecular) tubular pattern; M_r = LMW marker proteins (Pharmacia).

carried out for 40 min in a modified Laemmli system, increasing the voltage stepwise from 60 to 100 V. The gels are stained for 1 h in a 0.01% (w/v) solution of CBB R-250 in 15% (v/v) acetic acid–water for first reading or with the intensi-

fied CBB G-250 staining in phosphoric acid as described by Neuhoff and Stamm [88].

As demonstrated by our investigations on unconcentrated urines from patients with various renal diseases, micro gradient slab PAGE fulfills the demands of methodological simplicity and reproducibility, high resolution in the LMW and HMW range, sensitivity and rapidity in a nearly ideal manner. Molecular mass analysis and immunological protein identification for laboratory investigations in addition to protein pattern differentiation and the monitoring of renal diseases can be achieved within a tenth of the time necessary for the corresponding macro methods (Fig. 7) [14].

Following the convincing results of the micro slab technique, a semi-automated micro-PAGE apparatus was recently developed (Phast System; Pharmacia LKB). The instrument combines a separation and a development unit, controlled by a flexible microprocessor system. Another innovation, quality-controlled [131], precast micro-PAA gels adhering to a rigid polyester film (43 mm × 50 mm × 0.45 mm) are available for various separation purposes (homogeneous PhastGels, PhastGels with PAA gradients, IEF PhastGels). SDS-PAGE in Phast gradient gels of 8–25% T has proved capable of achieving highly reproducible separations of urinary proteins (Fig. 8) [132–134] using either CBB R-250 or silver stain. For the immunological identification of proteins on PhastGels, unidirectional

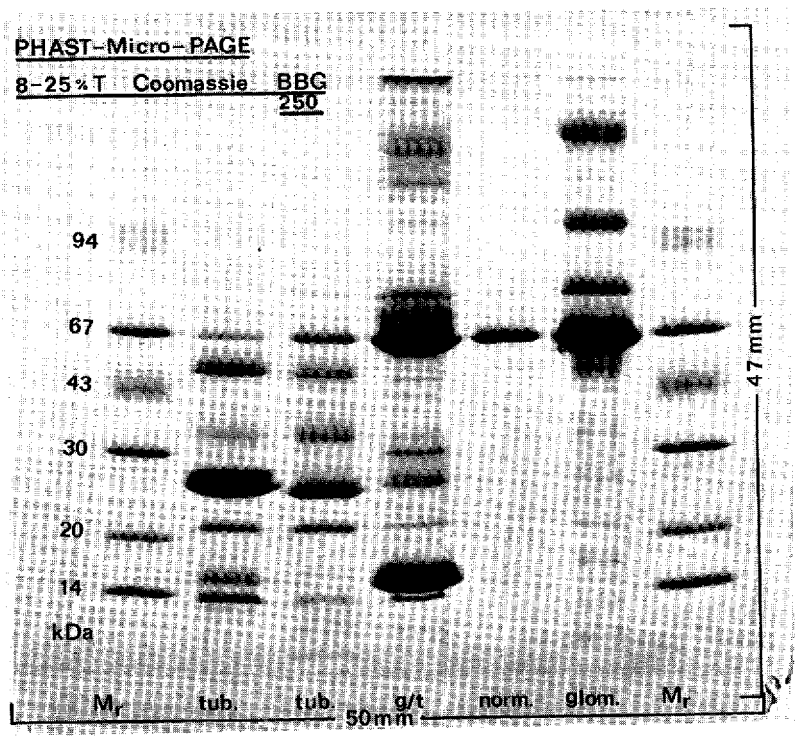

Fig. 8. PhastGel of 8–25% T, CBB G-250 staining. Renal proteinurias; tub. = complete tubular pattern; g/t = mixed glomerulotubular pattern; norm. = physiological pattern; glom. = unselective glomerular pattern; M_r = LMW marker proteins (Pharmacia).

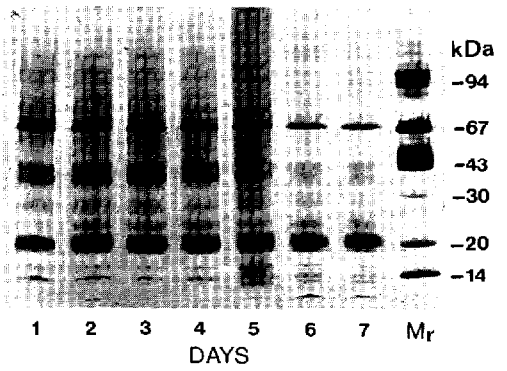

Fig. 9. Urinary proteins of a male tree shrew (*Tupaia belangeri*) separated on PhastGel (8–25% T) and stained with silver [136]. The specimens were collected daily from 8.00 to 10.00 a.m. over a period of one week. Note the male sex-associated protein in the M_r range 19–20 kDa. M_r = LMW marker proteins (Pharmacia).

diffusion blotting techniques have been developed [135]. Recently, 1D SDS-PAGE in gradient PhastGels of 8–25% T was successfully applied to studies of the physiological proteinuria of primates (Fig. 9) [136].

2.4. Isoelectric focusing

As protein separation by analytical IEF in PAA gels [137] results in very high resolutions compared with other biochemical separation techniques [138], this technique was early employed in the study of urinary proteins. Rotbol [139] analysed the urine from a patient suffering from pyelonephritis using rod gels of 6.5% T and a pH gradient of 3–10 with an increased ampholyte concentration of 2%. In this urine, he found distinct variations of "albumins" and "γ-globulins" compared with normal urine. A study of urinary proteins in tubular diseases by Vesterberg and Nise [140], using slab gels of 5% T with both wide and narrow pH ranges, revealed a much higher amount of polypeptides with low M_r such as β_2-microglobulin in patients exposed to cadmium than in patients with lupus glomerulonephritis or the nephrotic syndrome.

Charge heterogeneity of single urinary proteins has been of special interest in the investigation of glomerular filtration and tubular reabsorption mechanisms of plasma proteins. Following experimental animal studies on the nephrotoxicity of monoclonal light chains (BJ proteins) with a p*I* of 5.8 or greater [141], Coward et al. [142] confirmed a significant negative correlation between the p*I* of human BJ proteins excreted in the urine and endogenous creatinine clearance. Free light chains with p*I* values ranging from 4.5 to 8.2 were isolated from concentrated and desalted urine specimens using 15% agarose gels and were applied to pre-cast Ampholine PAA gel plates (PAG plates; Pharmacia LKB). From these and other investigations [143] there is strong evidence that cationic free light chains are associated far more often with renal failure than anionic ones. The effect of a cationic p*I* on renal handling of human albumin infused in rats was studied by Purtell et al. [144]; increasing the p*I* of the infused human albumin not only

caused an increase in the excretion of this albumin fraction, but also increased the permeability of the GBM to native endogenous albumin. In order to extend these studies on charge heterogeneity of human albumin to various renal and extra-renal diseases, Ghiggeri and co-workers [145–150] performed IEF under native and low denaturing conditions in ultra-thin (240 μm) PAA slab gels (130 mm $\times$ 130 mm) of 5–7% T and a non-linear pH range of 4–8. Among other items, the authors analysed glycosylated albumins in diabetes mellitus [145–147] and the alterations of molecular features of albumin in patients suffering from the nephrotic syndrome [148–150]. It can be concluded from these studies that several disease states lead to changes in the net charge of plasma proteins which are dealt with, consequently, in an altered manner by charged membranes such as the GBM.

2.5. Two-dimensional electrophoresis in macro and micro gels

It is an established fact that the highest resolution of complex protein mixtures such as urine can only be achieved by combining electrophoretic procedures utilizing, independently of each other, both the separation due to charge and molecular mass of the proteins. Based on the fundamental work on 2D electrophoresis of Smithies and Poulik [151], Dale and Latner [152], Macko and Stegemann [153], Stegemann [154], Domschke et al. [155] and Kaltschmidt and Wittmann [156], O'Farrell [157] reported the separation of more than 1000 polypeptides from *Escherichia coli* using a combination of IEF in cylindrical gels of 4% T in the first and the SDS gradient slab gel system of Laemmli in the second dimension [158]. Independently, Klose [159] published a detailed description of the 2D protein mapping of mouse tissues by combined IEF (rod gels of 4.8% T) and native slab PAGE according to Davis [36]. The introduction of a new silver staining method by Merrill and co-workers [160,161] added a hitherto unknown detection sensitivity for polypeptides to the high resolving power of 2D electrophoresis. O'Farrell et al.'s 2D system [162] was later called "BASO-DALT", in contrast to the "ISO-DALT" of Anderson and Anderson [163], where "ISO" represents isoelectric focusing in the first dimension and "DALT" (Daltons) SDS slab PAGE in the second dimension. Under denaturing conditions, Anderson et al. [164] could distinguish more than 250 urinary constituents using a CBB staining procedure.

Edwards and co-workers [165,166] continued with the 2D mapping and identification of major urinary polypeptides in pathological and normal urine specimens. Patterns of urinary proteins in patients with rheumatoid arthritis were analysed by Clark et al. [167]. Practical advice for the use of 2D ISO-DALT gels in the clinical laboratory, including the separation of urinary proteins, was given by Tracy et al. [168], based on their experience with serum proteins [169,170]. In order to extend further the potential of non-invasive diagnosis of renal diseases, Bauer et al. [171] compared proteins of pathological urines with water-soluble kidney preparations and serum. They were able to distinguish at least four groups of proteins (serum contaminants, tissue antigens, filtered LMW proteins and protein breakdown products) on 2D slab gels stained with CBB and silver.

Several attempts have been made to overcome troublesome sample manipula-

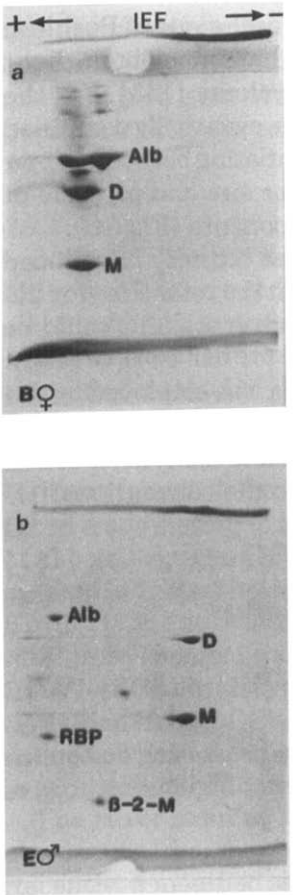

Fig. 10. Micro 2D electrophoresis of two patients suffering from multiple myeloma with Bence Jones proteinuria (BJP) of differing tubulotoxicity [143]. First dimension, IEF in 10-μl capillary gels of 4.8% T, pH 3-10; second dimension, SDS-PAGE in micro slab gels of 15% T; CBB G-250 stain; immunoblotting. (a) Patient B, female, 53 years, normal renal function: BJP lambda-type, monomer and dimer, p*I* 5.1. (b) Patient E, male, 65 years, progressive renal failure: BJP kappa-type, monomer and dimer, p*I* 7.7; concomitant LMW proteinuria. Alb = albumin; β-2-M = β_2-microglobulin; RBP = retinol-binding protein; M = light chain monomer; D = light chain dimer.

tion steps such as concentration, desalting and denaturing of urine specimens. By using ultrasensitive silver staining and a simplified 2D technique [172], Marshall et al. [173] detected over 600 polypeptides in unconcentrated, unprocessed urine from workers occupationally exposed to cadmium. Guevara et al. [174] recommended acidified acetone extraction for the separation of urinary proteins from constituents of the urine interfering with both IEF and SDS-PAGE, whereas Gianazza et al. [175] succeeded in the direct loading of unprocessed urines on to IEF gels with immobilized pH gradients (IPG-DALT [93]) for both 1D and 2D electrophoresis.

Medium- and micro-scale adaptations of the 2D technique were developed and optimized by Klose [176], Rüchel [129], Poehling and Neuhoff [128] and Man-

abe and co-workers [177,178]. We employed the micro 2D technique of Poehling and Neuhoff [128] for the identification of monoclonal immunoglobulin light chains in the urine of patients suffering from multiple myeloma [143]. For the clinical practitioner, 2D micro slab PAGE of BJ proteinuria, eventually combined with a rapid micro Ouchterlony technique [106] for differentiating between kappa and lambda chains, provides important information on the size and p*I* value of BJ proteins and on the accompanying LMW protein components (Fig. 10).

A micro modification of IPG-DALT electrophoresis was recently introduced by Görg et al. [179], utilizing the horizontal Phast system: the total time for 2D electrophoresis including IPG in the first dimension and silver staining could be reduced to 3 h (the macro version requires 12–18 h solely for IEF in IPG gels!). Another micro 2D variation using pre-cast PhastGel media was employed by Jägersten and Eriksson [180]. Although these modified Phast techniques cannot be definitively judged at present with regard to urinary protein analysis, it seems that they might add a new dimension of great promise to the conventional 1D PAGE systems. With respect to the densitometric analysis of protein spots in 2D gels, the mechanical high-performance 2D gel scanner of Kronberg et al. [181] seems to have solved some of the difficulties in data acquisition for digital images, at least for biomedical research purposes [182].

Taking into account the diversity of the 2D electrophoresis methods [e.g., ISO-DALT, IPG-DALT and BASO-DALT, systems consisting solely of (SDS)-PAGE in both directions, medium- and micro-scale variations, ISO-DALT and IPG-DALT on Phast system], the differences in urinary protein processing and staining and the various spot analysis devices, it is difficult, if not impossible, to give one system preference at present.

3. URINARY PROTEIN IDENTIFICATION

3.1. Protein staining techniques

3.1.1. Coomassie Brilliant Blue

At present there are two accepted methods of staining urinary proteins which are both sensitive and reproducible, namely the CBB R-250 and G-250 dyes [183] and silver. A CBB staining procedure of equal quality to silver was, however, first possible following the systematic analysis of the nature of protein staining in PAA gels with colloidal CBB dyes by Neuhoff and co-workers [88,184]. We now use the intensified CBB G-250 stain in phosphoric acid [0.1% (w/v) purified CBB G-250 in 2% (w/v) phosphoric acid plus 6% (w/v) ammonium sulphate] after prefixation in 12% (w/v) trichloroacetic acid (TCA) for 1 h for the analysis of urinary protein patterns in 1D micro slab gradient gels [134,136]. Even without any destaining step, intensive staining of protein bands (approximately three times more intense than after staining with CBB R-250 in methanol–water–acetic acid) can be achieved on a clear background. Through extension of the staining time (up to 24 h), it is possible to increase the sensitivity of this method to a detection limit of 0.7 ng of bovine serum albumin per mm^2 gel [184]. For storage,

the protein–dye complexes are fixed in 20% (w/v) ammonium sulphate. Lapin et al. [87] evaluated the above-mentioned staining techniques for use on macro gradient slab gels. With their system, 0.1% (w/v) CBB R-250 in 3.5% (w/v) perchloric acid was capable of detecting proteinuria at concentrations as low as 20 mg/l.

3.1.2. Silver

Although burdened by considerable difficulties with regard to both reproducibility and background staining [185], silver staining has been employed in the electrophoretic analysis of urinary proteins owing to its unique gain in sensitivity, which approaches nearly 100 times that of conventional CBB R-250 staining. The simplified method of Heukeshoven and Dernick [95] was successfully applied by Schiwara et al. [92] to the differentiation of physiological and pathological proteinurias of low protein concentration. They pointed out that silver staining helps in identifying tubular microproteins such as α_1-microglobulin, retinol-binding protein and β_2-microglobulin through the geometry and typical coloration [186] of the protein band. Recently, Hempelmann and Kaminsky [187] described a method for obtaining long-term colour stability after silver staining. Wurster and Ehrich [188] critically evaluated the silver staining of urinary proteins. For certain proteins, an equal loading with silver ions can only be achieved by special, time-consuming procedures. The comparison of silver-stained gels with conventionally stained gels requires substantial experience, as the multiplicity of bands may easily lead to an over-interpretation of urinary protein patterns which are, in fact, not pathologically altered.

The silver staining technique installed in the semi-automated Phast system was derived from the above-mentioned method of Heukeshoven and Dernick [95]. Recently, a modified silver staining method with low background tailored to the requirements of urinary protein analysis was presented by Bülles [189].

3.2. Immunoblotting

The electrophoretic transfer of proteins from PAA gels to nitrocellulose sheets and their subsequent detection by immunological procedures (immunoblotting), as described by Towbin et al. [190], has been widely applied in biomedical research [191]. The history, principles, methodological aspects and applications of protein blotting have recently been reviewed in detail by Beisiegel [192]. Using Western blotting, a map of urinary proteins following the electrophoretic separation in 1D gradient gels was obtained by Kshirsagar and Wiggins [90]. In the analysis of urinary proteins in cases of tubular impairment, the interpretation of immunoblots prepared with commercial antisera may be difficult because of artifacts due to the presence of large amounts of albumin and immunoglobulin fragments. Perini et al. [193] were able to reduce these artifacts by treating the blots with 2-ME and SDS. By this procedure, the affinity of albumin and IgG for specific antibodies frequently present in impure antisera could be lowered without noticeably denaturing the blotted proteins. Through the use of special glass-fibre membranes instead of nitrocellulose sheets, problems with the transfer and analysis of very-low-concentration proteins can be better managed [194]. For the use

on PhastGels adhering to polyester foils, a unidirectional diffusion blotting technique was introduced by Edström and Jägersten [135].

4. TECHNICAL ASPECTS

The analysis of urinary proteins is carried out for at least three different purposes: (1) for the clinical practitioner, 1D electrophoretic separation patterns provide information concerning the level of impairment in renal diseases (prerenal, renal-glomerular and -tubular, post-renal) and are of use in the non-invasive monitoring of therapeutic measures and the side-effects of drugs; (2) in biomedical research, combined electrophoretic procedures such as ISO-DALT, IPG-DALT and micro 2D electrophoresis are necessary for the investigation of the comlete range of polypeptides excreted in defined renal and extrarenal diseases; and (3) identification and characterization of urinary proteins by single radial immunodiffusion (SRID), ELISA techniques and IEF help us to understand their influence on the pathophysiology of glomerular filtration and tubular reabsorption processes. The quantitative determination of selected indicator proteins such as albumin [195], α_1-microglobulin [196] and β_2-microglobulin [197] may precede the more expensive PAGE methods.

In the last fifteen years, 1D PAGE has been widely used for urinary protein analysis in clinical medicine (Table 2). To achieve optimal separation and detection results, some aspects have to be considered with particular care, as follows.

(1) Sample preparation. Urines should not be centrifuged, as some proteins such as Tamm Horsfall mucoprotein (M_r of the monomers 94 kDa) and α_1-acid glycoprotein (M_r 43 kDa) may be diminished or even lost altogether [198]. A loss of LMW proteins during urine concentration by membrane filters has been observed by several workers [199–201], leading to an overestimation of the albumin fraction [54]. Scherberich et al. [83] reported on the poor recovery of urinary proteins after freezing and thawing of urine specimens. If deep-freezing of urine specimens cannot be avoided, adjustment to neutral pH should be carried out in order to reduce analytical errors resulting from the removal of protein-containing precipitates [202]. Decomposition of polypeptides into numerous subunits by 2-ME, urea and high concentrations of SDS may lead to a misinterpretation of protein patterns and should therefore be omitted in 1D PAGE [203]. Using native micro gradient capillary PAGE, Reichel et al. [108], Alt et al. [117] and others elegantly bypassed the problem of producing artifacts by denaturing sample processing. In their PAGE systems, Balant et al. [64] and Cachera et al. [65] stabilized urinary proteins against thermal denaturation in the presence of SDS by alkylating thiol groups with iodoacetamide.

(2) Buffers. The influence of various buffer systems containing SDS on the quality of the electrophoretic separation of urinary proteins was investigated by Wartha et al. [204]. Using a homogeneous slab gel system of 12% T and a set of standard proteins (Serva, Heidelberg, F.R.G.) in the M_r range 25–167 kDa, they compared the imidazole–phosphate buffer of Cachera et al. [65], the Tris–HCl buffer of Laemmli [44], the Tris–glycine buffer of Pesce et al. [53] and the phosphate buffer of Weber and Osborn [42]. The discontinuous Laemmli system of-

fered the best results with respect to migration, detection capability and detection limits of protein bands and also the shortest separation time of all the buffers tested.

(3) PAGE system. As the rapid analysis of the actual pattern of a patient's

TABLE 2

GEL CHARACTERISTICS AND URINE SAMPLE PROCESSING IN VARIOUS 1D PAGE SYSTEMS

Reference		Gel					Sample		
		Size	Type	Structure	%T	Stain*	Concn.**	SDS	2-ME***
Waldmann et al., 1972 [48]	(a)	Macro	Rod	Homogeneous	7.5	CBB	+	0	0
	(b)	Macro	Rod	Homogeneous	5	CBB	+	+	+
Virella and Lopes-Virella, 1977 [51]		Macro	Rod	Homogeneous	7.5	CBB	+	0	0
Boesken et al., 1973 [10]		Macro	Rod	Homogeneous	7.5	A10B	+	+	0
Doman et al., 1980 [63]		Macro	Rod	Homogeneous	7.5	A10B	+	+	0
Balant et al., 1974 [64]	(a)	Macro	Rod	Homogeneous	5	CBB	+	0	0
	(b)	Macro	Rod	Homogeneous	7.5	CBB	+	+	0
Lubega, 1983 [71]		Macro	Rod	Homogeneous	10	A10B	+	+	0
Cachera et al., 1980 [65]		Macro	Rod	Homogeneous	7.5	CBB	+	+	0
Pires et al., 1975 [49]		Macro	Rod	Homogeneous	5	A10B	+	+	0
Jones et al., 1980 [78]		Macro	Slab	Homogeneous	7	CBB	+	0	0
Meier and Levitt, 1986 [82]		Macro	Slab	Homogeneous	10	CBB	+	+	0
Rautenstrauch, 1977 [66]		Macro	Slab	Homogeneous	7.5	A10B	+	0	0
Brandhorst and Wetter, 1980 [69]		Macro	Slab	Homogeneous	10	CBB	+	+	0
Pesce et al., 1972 [53]		Macro	Slab	Homogeneous	7	CBB	+	+	(+)
Sethi et al., 1977 [55]		Macro	Slab	Homogeneous	7	CBB	+	+	0
Fleming, 1984 [56]		Macro	Slab	Homogeneous	7	CBB	+	+	0
Lison et al., 1980 [73]		Macro	Slab	Homogeneous	7.5	CBB	+	+	0
Scherberich et al., 1985 [83]		Macro	Slab	Gradient	3-20	CBB	0	+	0
Schiwara et al., 1984 [94]		Macro	Slab	Gradient	4-22.5	S	0	+	0
Kshirsagar and Wiggins, 1986 [90]		Macro	Slab	Gradient	6-18	S	0	+	Urea
Weise and Bockhorn, 1972 [103]		Micro	Capillary	Homogeneous	20	A10B	0	0	0
Reichel et al., 1976 [108]		Micro	Capillary	Gradient	1-33	A10B	0	0	0
Alt et al., 1983 [12]		Micro	Capillary	Gradient	4-40	A10B	0	0	0
Burghard et al., 1986 [116]		Micro	Capillary	Gradient	4-40	FG	0	0	0

(Continued on p. 338)

TABLE 2 (continued)

Reference	Gel					Sample		
	Size	Type	Structure	%T	Stain*	Concn.**	SDS	2-ME***
Weber et al., 1986 [14]	Micro	Slab	Gradient	6-30	ICBB	0	+	0
Cheong et al., 1986 [132]	Phast	Slab	Gradient	8-25	CBB	0	+	0
Lapin et al., 1986 [133]	Phast	Slab	Gradient	8-25	CBB/S	0	+	0
Weber and Fuchs, 1988 [136]	Phast	Slab	Gradient	8-25	CBB/S	0	+	0

*CBB = Coomassie Brilliant Blue; ICBB = improved Coomassie Brilliant Blue; A10B = Amido Black 10B; S = silver; FG = Fast Green FD/C.
**Concn. = concentration step required.
***2-ME = 2-mercaptoethanol.

proteinuria may help in avoiding invasive diagnostic procedures such as kidney biopsy (although it cannot replace the morphological diagnosis), the PAGE system used for clinical monitoring has to be adapted to the special needs of urinary protein analysis. With reference to 1D PAGE systems, the micro-scale modifications are superior to the corresponding macro versions with respect to rapidity, sensitivity and economy [128], and further their resolution is as high as that of the macro-scale techniques. Using micro gradient SDS-PAGE systems up to 30% T, even small urine samples (1–2 μl) with low protein contents (0.5%) can be analysed in a very short time (1 h), resulting in sharp bands throughout the entire range of plasma proteins excreted in the urine (10–400 kDa) [14]. In our opinion, micro-PAGE in SDS gradient slab gels fulfils the criteria and clinical demands mentioned above in a manner as yet unexcelled.

5. SUMMARY

The differentiation and analysis of urinary proteins has substantially contributed to our knowledge of physiological and pathophysiological processes during glomerular filtration and tubular catabolism of plasma constituents. By use of high-resolution biochemical separation techniques, several urinary polypeptides could be identified as plasma proteins, tissue antigens, tubular enzymes and protein breakdown products. With regard to clinical application, the separation results of conventional gel chromatography and agarose electrophoresis were surpassed by fast protein liquid chromatography and polyacrylamide electrophoresis in one- and two-dimensional systems. In contrast to early one-dimensional polyacrylamide gel electrophoresis (PAGE) methods using homogeneous gels on a macro scale, modern gradient slab gels achieve better resolution over the entire relative molecular mass range of urinary proteins. For clinical demands, the use of micro-scale gradient gels, either laboratory-made or pre-cast, together with an improved Coomassie Brillant Blue staining, offers several advantages, including

rapidity, sensitivity and economy. Isoelectric focusing and two-dimensional PAGE, combined with sensitive silver staining and immunoblotting methods, have proved to be valuable tools for the identification and characterization of urinary proteins in defined renal and extra-renal diseases. The quantitative determination of urinary indicator proteins such as albumin and α_1- and β_2-microglobulin can be regarded as a reasonable complement to the pattern diagnosis, especially in the long-term course of renal diseases.

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