Journal of Chromatography, 569 (1991) 243–260
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5950

Review

Sodium dodecyl sulphate electrophoresis of urinary proteins

ADRIANA BIANCHI-BOSISIO*

Clinical Chemistry Laboratory, Ospedale "A. Uboldo", 20063 Cernusco sul Naviglio, Milan (Italy)

FILOMENA D'AGROSA and FEDERICO GABOARDI

Pediatric Department, Ospedale "A. Uboldo", 20063 Cernusco sul Naviglio, Milan (Italy)

and

ELISABETTA GIANAZZA and PIER GIORGIO RIGHETTI

Department of Biomedical Sciences and Technologies, University of Milan, Via Celoria 2, Milan 20133 (Italy)

(First received December 4th, 1990; revised manuscript received February 2nd, 1991)

ABSTRACT

The analysis of urinary proteins and their identification are discussed, particularly in regard to the technique of sodium dodecyl sulphate electrophoresis in polyacrylamide gradient gels. Urine collection, storage and preparation are evaluated, especially in regard to problems connected with concentration and dialysis of such samples. The instrumental approach to sodium dodecyl sulphate polyacrylamide gel electrophoresis represented by the Phast System appears to be particularly valuable in routine clinical analysis of urine specimens, since no sample pretreatment is required. The following types of proteinurias are evaluated: (a) orthostatic proteinurias; (b) post-renal proteinurias; (c) Bence–Jones proteinuria; (d) lower and upper urinary tract infection (cystitis and pyelonephritis) and (e) diabetes mellitus proteinurias.

CONTENTS

0378-4347/91/$03.50

LIST OF ABBREVIATIONS

α_1-M	α_1-Microglobulin
β_2-M	β_2-Microglobulin
B–J	Bence–Jones
Hb	Haemoglobin
HM_r	High molecular mass
IEF	Isoelectric focusing
IgA	Immunoglobulin A
IgG	Immunoglobulin G
2-ME	2-Mercaptoethanol
LM_r	Low molecular mass
M_r	Relative molecular mass
PAA	Polyacrylamide
PAGE	Polyacrylamide gel electrophoresis
SDS	Sodium dodecyl sulphate
T (%)	Total monomer concentration in a gel
T–H	Tamm–Horsfall protein

1. INTRODUCTION

Since the first observations by Cotugno [1] in 1770 that the urine of some patients would precipitate "albumen" upon heating, protein evaluation in urine has become part of the standard diagnostic tests. Proteinuria is usually assessed by qualitative assays based either on acid precipitation or on test strip procedures using the protein-error-of-indicator principle [2,3]. These tests, however, do not adequately fulfil the analytical requirements, since their low sensitivity and lack of specificity do not allow the recognition of some clinically relevant forms of proteinuria [4,5]. This is especially true for pre-renal (*i.e.* Bence–Jones proteinuria, myoglobinuria, haemoglobinuria) and tubular proteinurias as well as for the so-called microalbuminuria, all of which escape detection by the test strip procedure [3–5].

Many diagnostic protocols for the quantitation of total urinary proteins have

been introduced (precipitation [6], biuret reaction in many variants [7], Lowry procedure, light scattering [8], dye binding with Coomassie Brilliant Blue [9] or Ponceau-S [10], nephelometric evaluation after precipitation with trichloroacetic acid [11], etc.), but none is completely satisfactory. Comparisons of methods have been reported in the literature [12,13]. The specificity for different proteins is highly variable from one procedure to another [5,14]. No uniform standard is applied for the quantitation of urine proteins, and different components give different signals depending on the analytical method; thus it is not surprising that both the reference ranges and the results on control samples may differ considerably from laboratory to laboratory [14]. Since the determination of total protein does not satisfy the criteria for a reliable quantitative test [15] and since quantitative determinations are not by themselves adequate for assessing the actual mix of proteins, the diagnostic investigation of urinary components needs additional analytical tools.

On a quantitative basis, it is possible to make a broad classification of the different forms of proteinuria but not to differentiate precisely between glomerular and tubular protein losses. There is no clear-cut quantitative differentiation of the various forms of proteinuria, especially in the case of "tubular forms", which, by involving loss of low-molecular-mass proteins, may fall in the physiological range and therefore might not be recognized in time. Qualitative and quantitative changes in urinary proteins are clinically significant indicators of filtration and reabsorption in the nephron. For this reason a great variety of investigations of urinary proteins in health and disease have been developed using different separation techniques and methods for the immunological identification and quantitation of single proteins in urine [16].

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) has been favorably assessed in comparison with gel permeation chromatography and other electrophoretic methods as a technique likely to yield urinary protein distribution profiles consistent with a patient's clinical status [17–26]. However, the methods recommended in the past were often cumbersome and technically demanding, and as a result they were not widely used in the routine clinical laboratory.

Recently a semiautomated micro-PAGE apparatus was developed (the Phast System, Pharmacia, Uppsala, Sweden). The instrument combines a separation and a staining unit, controlled by a flexible microprocessor system. Quality-controlled [27], precast micropolyacrylamide (PAA) gradient gels of 8–25% T have proved capable of achieving highly reproducible separations of urinary proteins [28–30]. The method seems to satisfy clinical demands.

2. ANALYSIS OF URINARY PROTEINS AND THEIR IDENTIFICATION

Protein separation techniques including column gel chromatography, agarose gel electrophoresis, PAGE in different modifications and isoelectric focusing (IEF) have been reviewed in detail by Weber [31].

Molecular mass (M_r) analysis of urinary proteins by SDS-PAGE offers great potential for differentiating physiological, renal (glomerular and tubular), pre-renal and post-renal proteinurias. A great variety of SDS-PAGE methods has been developed. Vertical electrophoresis has been used both in homogeneous SDS PAA gel rods [32] or slabs [33] and in SDS gradient gel rods [34] or slabs [35]. Microelectrophoresis in gradient gels was carried out in capillaries [36,37] and slabs [38]. Finally, horizontal electrophoresis in thin [39] and ultra-thin layers [40–42] of SDS gradient gels was introduced, followed recently by semiautomated SDS-PAGE in Phast gradient gels [28–30].

According to our results on patients with various renal diseases micro-gradient slab PAGE fulfils the demands of methodological simplicity and reproducibility, high resolution in the low-molecular-mass (LM_r) and high-molecular-mass (HM_r) range, sensitivity, rapidity and economy in a nearly ideal manner.

Urinary proteins have mostly been stained by Coomassie Brilliant Blue [32–39] or with silver nitrate [40–45]. The silver staining technique installed in the semiautomated Phast system was derived from the method of Heukeshoven and Dernick [46].

Protein blotting, first described by Renart *et al.* [47] and Towbin *et al.* [48] in 1979, has become a powerful tool for identifying and characterizing proteins. The history, principles, methodological aspects and applications of protein blotting have been reviewed by Beisiegel [49]. Methods for blotting from PhastGel have been described [50–53].

3. CLASSIFICATION OF PROTEINURIAS

Clinical and pathophysiological aspects of proteinuria have been reviewed in detail elsewhere [54–59]. The evaluation of the total spectrum of serum proteins excreted into urine under pathological conditions is of greater diagnostic value, and SDS-PAGE is the method of choice for simultaneous analysis of complete patterns of both HM_r and LM_r proteinuria in patients with kidney disease. Boesken [60] established a classification system for various kinds of glomerular and tubular selectivity as well as for some types of extrarenal proteinuria.

4. SAMPLE COLLECTION, STORAGE AND PREPARATION

Samples were obtained in accordance with the general rules for collection of urines for clinical–chemical analysis. Preferably fresh midstream morning samples, without pretreatment, were analyzed by SDS-PAGE in Phast gradient gels of 8–25% T (Phast system from Pharmacia-LKB). Samples stored for longer periods were frozen at $-20°C$ after previous addition of sodium azide (0.1 g/l) to prevent bacterial growth and after particulate removal by centrifugation at 1500 *g* for 10 min. The presence of blood, protein, glucose and the pH value of urine were assessed by dipsticks. Urinary proteins were quantified by the Coomassie

method [9]. For achieving optimal separation and detection, some aspects have to be considered with particular care, as follows.

4.1. Centrifugation

It has been reported by Marshall and Williams [61] that Tamm–Horsfall (T–H) mucoprotein (Synonym: uromucoid, M_r of monomers $94 \cdot 10^3$) and α_1-acid glycoprotein (M_r $43 \cdot 10^3$) are frequently lost by centrifugation. Shiba *et al.* [62] also pointed out the risk of discarding uromucoid by centrifugation. Therefore, centrifuging the urine as the first processing step, when possible, should be avoided.

4.2. Dialysis

Dialysis of urine specimens prior to SDS-PAGE is unnecessary. It has been demonstrated that dialysis may lead to some protein loss, *i.e.* polypeptides of M_r $< 10\,000$ [63].

4.3. Freezing and thawing

Freezing and thawing of urine specimens result in poor recovery of urinary proteins [64]. When deep-freezing of urine specimens cannot be avoided, adjustment to neutral pH should be carried out in order to reduce analytical errors resulting from loss of protein due to precipitation [65].

4.4. Concentration of urine

Micro-scale SDS-PAGE techniques do not require concentration of urine specimens as the macro methods did. We studied the effect of urine sample concentration using the Phast system and silver staining; fifteen urine samples with total protein content between 0.05 and 0.30 mg/ml were analyzed without any pretreatment and after a 25-fold concentration by Minicon concentrator (Amicon, Danvers, MA, USA). In only four out of fifteen cases were the urine protein patterns before and after concentration identical, while in seven cases we found a loss and in two cases a decrease of β_2-microglobulin in the concentrated urine compared with the untreated urine. In these cases other LM_r proteins were also less intense. In two cases the untreated urine gave a normal and, after concentration, a pathological (glomerular proteinuria) protein pattern. A loss of LM_r proteins during urine concentration by membrane filters has been observed by several workers [66–70], leading to an overestimation of the albumin fraction [19]. Alt and Maess [68] and Pesce *et al.* [19] showed that urine concentration with Amicon B-15 filters leads to losses of all sizes of proteins by adsorption to the filter membrane.

4.5. Dilution of urine

Urine samples with protein concentrations above 0.30 mg/ml need to be diluted for optimal separation. Otherwise a micromolecular proteinuria accompanying a glomerular proteinuria could be overlooked. This must be kept in mind when special questions have to be answered.

4.6. Treatment with 2-mercaptoethanol, urea and SDS

Because proteins with large molecular size cannot penetrate the gel, some authors used 2-mercaptoethanol (2-ME), a reducer, to dissociate oligomeric structures. This could lead to a misinterpretation of the protein patterns due to the splitting of oligomeric forms into a number of subunits [71,72]. According to Poortmans and Jeanloz [73], immunoglobulins (Ig)G and A together account for only about 10% of total urinary proteins; however under pathological conditions, when urinary immunoglobulin excretion increases, the use of 2-ME may result in an accumulation of their degradation products among the electrophoresed protein bands, which in turn may interfere with interpretation.

In the presence of SDS but under non-reducing conditions the quaternary structure of all major serum proteins is unaffected, except for the splitting of haemoglobin (Hb) into its monomers and dimers [60]. This improves the resolution of haematuric samples. In fact, on native pore gradient electrophoresis, Hb and albumin have almost identical migration. Balant *et al.* [18] and Cachera *et al.* [20] prevented reaggregation of urinary proteins during thermal denaturation in SDS by alkylating thiol groups with iodoacetamide.

4.7. Pattern reproducibility

In order to study the method reproducibility, R_F values of identical proteins were determined in ten different gels. The coefficient of variation was less than 10% for conventional gels [64] and between 3.56 and 6.76% in the case of 8–25% gradient Phast gels [53]. The reproducibility of conventional gels depended mainly on the skill and experience of technicians and varied among different workers. Calibration curves covering a wide range of molecular mass values revealed little difference among various Phast gels. The protein pattern and silver staining reproducibility seems to be satisfactory for the glomerular pattern, but the tubular profiles are less reproducible. Microproteins which are clearly detected in some gels are absent in others. Partly, these variations might result from uncontrolled fixing conditions and silver staining. The latter, in particular, is not as easy to perform as Coomassie Blue staining, and the results are less reproducible. In general, colour development in silver staining does not reach an end-point, but may continue rapidly, with concomitant darkening of the background. Experience and personal attendance are necessary to stop the reduction of silver ions at

the correct moment. Furthermore, protein staining in gels with low acrylamide concentration is faster than in the regions of high acrylamide concentration, which results in an uneven staining of background and protein zones [45]. It should also be remembered that silvering (like all photographic processes) is temperature-dependent: thus the Phast system, which offers a temperature-controlled environment during the staining process, allows a much better reproducibility when silver staining gels, as compared with manual staining procedures.

5. SELECTED CLINICAL AND BIOMEDICAL APPLICATIONS

This section of the paper will be devoted to the analysis of some urinary protein patterns because they are particularly important indicators of different forms of proteinuria. Fig. 1 gives an example of some selected protein patterns

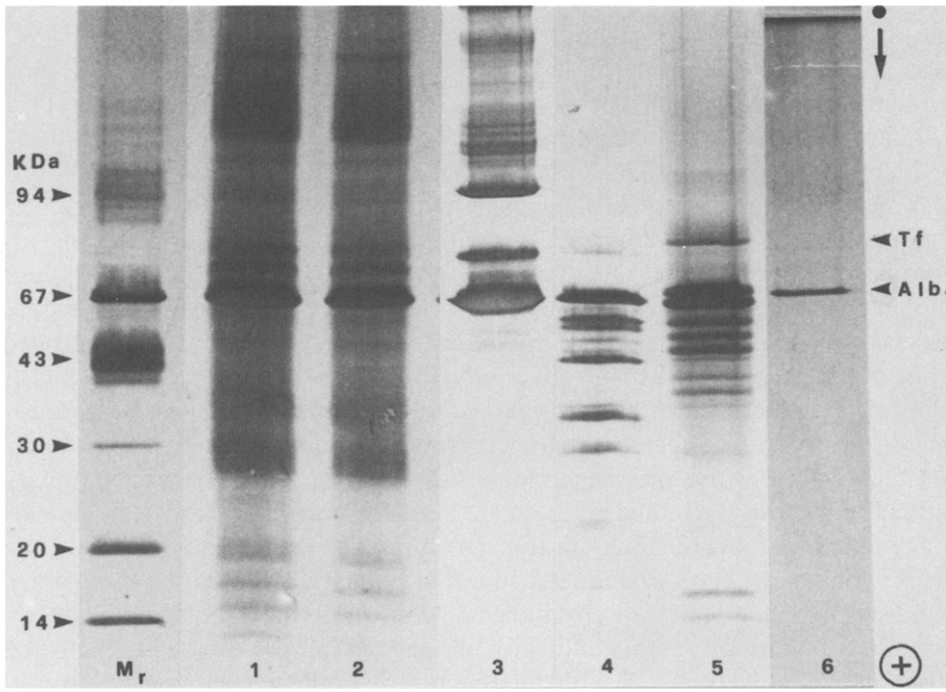

Fig. 1. Micro-SDS-PAGE with the Phast system of urinary proteins. Gel: 8–25% T. Running conditions: 30 min at 150 V, 6 mA, 1.8 W, 15°C. Sample load: 1 μl of untreated urine. Silver staining. Samples: 1 and 2, mixed glomerular–tubular pattern; 3, unselective glomerular pattern; 4 and 5, complete tubular pattern (with marked transferrin in No. 5); 6, physiological pattern; M_r, low-molecular-mass standards (Pharmacia) with phosphorylase B ($94 \cdot 10^3$), bovine serum albumin ($67 \cdot 10^3$), ovalbumin ($43 \cdot 10^3$), carbonic anhydrase ($30 \cdot 10^3$), soybean trypsin inhibitor ($20 \cdot 10^3$) and α-lactalbumin ($14 \cdot 10^3$). In this and all subsequent figures, the black dot and vertical arrow on the right side of the gel represent the application point and migration direction, respectively. The + sign at the bottom indicates gel polarity. Alb., albumin; Tf, transferrin.

characteristic of different pathological conditions. In the following, we will highlight different forms of proteinurias and emphasize their clinical significance.

5.1. Proteinuria in the normal range of total protein

Protein concentrations >0.10 mg/ml [70] or >0.15 mg/ml [74] have been defined as pathological. It is important to discriminate between mild disease conditions and the physiological state in patients at or just above the borderline. This can be particularly important in interstitial disorders, in tubular impairment or in slowly progressive systemic diseases such as diabetes, hypertension, lupus erythematosus, etc. Physiological and pathological proteinurias overlap at 40–400 mg of protein per 24 h. It has already been reported that even proteinuria in the normal range of total protein may show typical glomerular and/or tubular patterns [75], We studied protein patterns in selected individuals with an urinary protein concentration in the normal range and negative tests for blood and bacteria. Analyses were done using untreated, unconcentrated urine and SDS-PAGE in combination with silver staining. In 85% of the cases a clear physiological protein pattern was found, whereas 15% had a pathological protein pattern (11% tubular, 2% glomerular protein pattern and 2% vascular or mixed proteinuria).

Lison and co-workers [76,77] in their epidemiological studies found glomerular protein patterns in an unusually high frequency, more than 30%. The clinical significance of so many abnormal proteinurias in an unselected population is doubtful. A glomerular protein pattern with total protein in the normal range should be interpreted cautiously and further analytical and clinical studies will be necessary to make a reliable interpretation. In our experience, if concentrated urine is used, it is possible to introduce modifications of protein patterns: a higher number of glomerular patterns is found and LM_r species can be lost. Schiwara *et al.* [45] suggested that in cases of glomerular proteinurias with total protein in the normal range, sensitive enzyme immunoassay could be used to determine urinary albumin and transferrin and to calculate albumin/transferrin ratios. In glomerular protein patterns the albumin/transferrin ratio is <25. In any case an easily visible transferrin band is characteristic of a glomerular protein pattern. When glomerular protein patterns were studied on consecutive days, Schiwara *et al.* [45] observed a constant glomerular protein pattern in patients with increased albuminuria, while in people with an albuminuria in the normal range both constant and changing protein patterns were found.

5.2. Orthostatic (postural) proteinuria

This kind of proteinuria may appear while a person is standing and may be found in 15–20% of young men with proteinuria on routine urine analysis [78,79] as well as in some patients with resolving acute pyelonephritis or glomerulonephritis. The total protein excreted in 24 h is usually less than 1 g. Patients with

proteinuria high enough to show the nephrotic syndrome are an exception. If patients recovering from acute pyelonephritis or glomerulonephritis are excluded, the evaluation usually shows no evidence of significant renal disease. We evaluated five of these patients: proteinuria was found in all urine specimens collected in the upright position in four cases, but in the fifth patient proteinuria was not constant. We noticed an increased excretion of glomerular proteins (albumin, transferrin, IgG) while LM_r protein was not evident.

5.3. Post-renal proteinurias

5.3.1. Post-renal haematuria

Gross or microscopic haematuria is a frequent finding during urine analysis. It can be of renal or of lower urinary tract as well as of extrarenal origin. The patterns in post-renal haematuria are similar to those of blood haemolysate and they show the presence of all plasma proteins. The presence of blood modifies the urinary protein pattern evaluated by SDS-PAGE. Fig. 2 shows: (1) a physiolog-

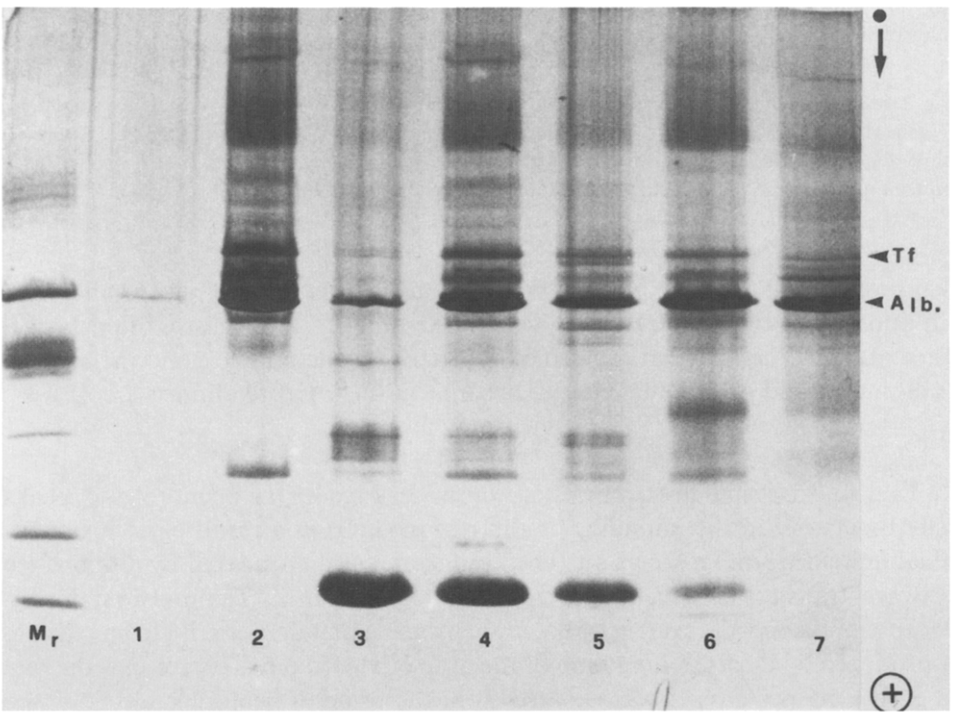

Fig. 2. Micro-SDS-PAGE with the Phast system of urinary proteins. Gel: 8–25% T. Running conditions: 30 min at 150 V, 2 mA, 1.8 W, 15°C. Sample load: 1 μl of untreated urine. Silver staining. Samples: 1, physiological pattern; 2, normal urine + serum; 3, normal urines + haemolysate; 4, normal urine + serum + haemolysate; 5–7, three cases of post-renal haematuria. All other conditions and symbols as in Fig. 1.

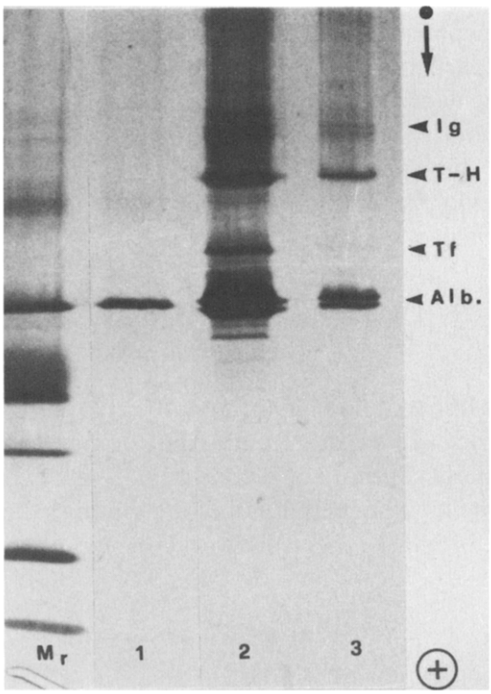

Fig. 3. Micro-SDS-PAGE with the Phast system of urinary proteins. Gel: 8–25% T. Running conditions: 30 min at 150 V, 6 mA, 1.8 W, 15°C. Sample load: 1 μl of untreated urine. Silver staining. Samples: 1, physiological pattern; 2, unselective glomerular pattern; 3, extrarenal proteinuria. T–H, Tamm–Horsfall protein; Ig, immunoglobulin. All other conditions and symbols as in Fig. 1.

ical proteinuria; (2) urine plus serum (dilution 1:500); (3) urine plus haemolysate (dilution 1:250); (4) urine, haemolysate and serum; (5–7) three cases of post-renal haematuria. When haematuric urines are analyzed, the presence and the concentration of blood must be taken into account in the interpretation.

5.3.2. Local excretion of proteins

Typical extrarenal proteinurias are shown in Fig. 3. One or more HM_r peaks may be present in the immunoglobulin region (IgG) as a result of a local post-renal immunoglobulin secretion. The local source of Ig is proved by discrepancy between transferrin (which is normal) and Ig clearance. Tamm–Horsfall mucoprotein, the most prevalent of the urine proteins that do not arise from plasma, is produced by the ascending limb of the loop of Henle and is excreted at the rate of 25–40 mg per day. It was isolated from the urine of healthy subjects by the method of Cvoriscec *et al.* [75]. It is a large protein (M_r several million) in the native state, but it dissociates into subunits (M_r 90 000–95 000) under the conditions used for electrophoresis [80]. It contains 28% carbohydrate and exhibits a pI of 3.5. In renal diseases that lead to a decrease in the number of tubules,

excretion of this protein is decreased accordingly. This is of limited diagnostic significance, but the measurement of this glycoprotein in serum seems to be an interesting indicator of intrarenal reflux. In diabetics both the carbohydrate composition and p*I* of Tamm–Horsfall glycoprotein are altered [4].

5.4. Bence–Jones proteinuria

Bence–Jones (B–J) proteinuria without signs of glomerular malfunction is a pre-renal proteinuria. Typical of the pattern of B–J proteinuria under non-reducing conditions are two prominent protein bands with distinct molecular masses (M_r 22 000 = light-chain monomers, M_r 44 000 = light-chain dimers) (see Fig. 4). Proteinuria results from tubular overload due to excessive synthesis and filtration of monoclonal Ig light chains. Under reducing conditions (2-ME) the dimer disappears so that only the light-chain monomer band (M_r 22 000) remains. This can be attributed to the cleavage of disulphide bridge-dependent polypeptide associations [81–83]. In addition, there is a shift in the mobility of the protein, which probably reflects an effect of the reducing medium. Some authors [45,84,85] have found an additional protein with M_r 15 000 due to "splitting" of B–J protein.

SDS-PAGE in the case of myeloma may be useful in monitoring the course of the disease and in identifying additional glomerular or tubular patterns as a renal complication of a B–J proteinuria. Fig. 4 shows serial dilutions of urinary proteins of two patients suffering from micromolecular myeloma with initial tubular complication (A) and from multiple myeloma (B). α_1-Microglobulin (α_1-M), as can be seen, is the first to appear when tubule is involved. Often, however, when the applied sample is too dilute, the α_1-M band can be too faint to be detected.

The nephrotoxicity of monoclonal light chains seems to depend on the type of light chain and on their p*I* value [84,85]. There is strong evidence that cationic free light chains are associated far more often with renal failure than anionic ones. IEF in microcapillary [86] or micro-slab gels [87] followed by immunofixation with monospecific antisera offers a new possibility for the determination of the p*I* of such proteins.

5.5. Lower and upper urinary tract infections: cystitis and pyelonephritis

Acute infections of the urinary tract can be subdivided into two general anatomic categories: lower-tract infection (urethritis, cystitis and prostatitis) and upper-tract infection (acute pyelonephritis). Chronic pyelonephritis refers to chronic interstitial nephritis which is believed to result from bacterial infection of the kidney.

We studied 90 cases of urinary tract infection in children—50 cases of cystitis, 25 cases of acute pyelonephritis and 15 cases of chronic pyelonephritis—using the Phast system and silver staining. In cases of cystitis, 43 out of 50 (86%) showed

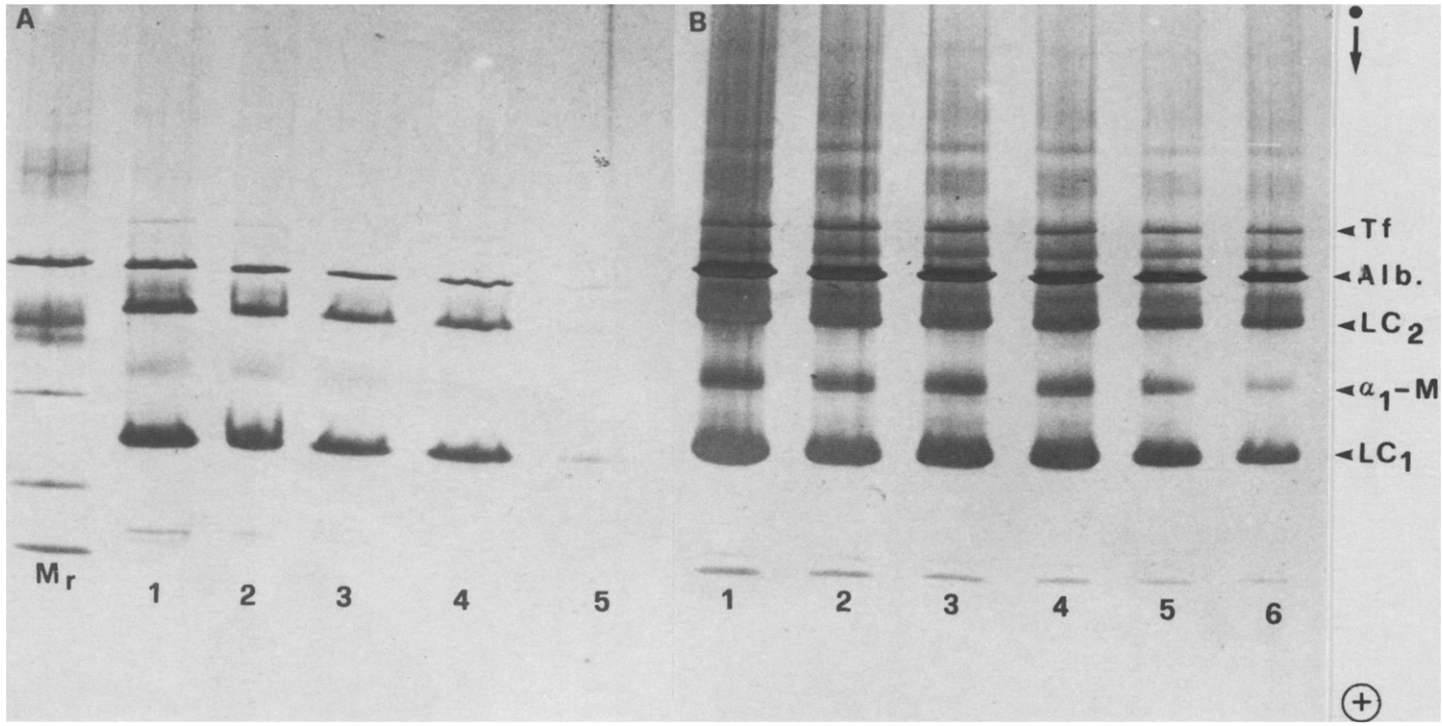

Fig. 4. Micro-SDS-PAGE with the Phast system of urinary proteins. Gel: 8–25% T. Running conditions: 30 min at 150 V, 6 mA, 1.8 W 15°C. Sample load: 1 μl of untreated urine. Silver staining. Samples: A, serial dilutions of Bence–Jones proteinuria with initial tubular impairment from a patient with micromolecular myeloma; B, serial dilutions of Bence–Jones proteinuria with glomerular and tubular involvement from a patient with multiple myeloma. LC_1, light-chain monomers; LC_2, light-chain dimers; α_1-M, $\alpha_1$microglobulin. All other conditions and symbols as in Fig. 1.

only a faint band of albumin on SDS-PAGE and no LM_r proteins thus excluding tubular involvement. In four cases proteins of M_r from 60 000 to 500 000 were present: in these cases haematuria was present as well and these proteins were probably derived from plasma. In two cases proteins of M_r 40 000–70 000 were evident without haematuria and finally, in one case, we noticed the presence of transferrin and albumin, *i.e.* a highly selective proteinuria. On the other hand, in acute pyelonephritis, disturbed reabsorption of LM_r proteins (M_r 10 000–67 000) clearly occurred in 70% of the cases and the increased incidence of LM_r proteinuria seems to be correlated with the severity of pyelonephritis. In all of these cases the level of α_1M in the urine, as measured by a nephelometric method, was above 10 mg/l (normal range from 3 to 8 mg/l). α_1-M is a glycoprotein of M_r 30 000–33 000 and, unlike β_2-microglobulin (β_2-M), has a great stability in the clinically important pH range (between 4 and 10) and its excretion is quite constant. In five cases the analysis of urinary proteins by SDS-PAGE revealed not only a tubular pattern (predominantly) but also the presence of albumin and transferrin. In four of these cases there was a massive vesicoureteral reflux due to anatomic abnormality of the urinary tract. After surgery, the urinary protein patterns resembled physiological proteinuria in three cases. SDS-PAGE monitoring of the course of pyelonephritis was carried out in almost all cases. Fig. 5 shows some examples of urine during the first 24 h after the appearance of symptoms. Complete micromolecular proteinuria is evident: in 50% of the cases, protein bands in the M_r 40 000–60 000 region are particularly marked. The most prominent band could reliably be identified by Western blotting and specific anibody as α_1-antitrypsin. The identification of α_1-acid glycoprotein was also possible by the same method: α_1-acid glycoprotein was increased as well but it appeared not as a sharp band but as an enlarged spot: this is probably because of the high carbohydrate content of this protein. After one or two days of treatment, the region of proteins of M_r 40 000–60 000 became less marked. During recovery, the most incline to subside are the proteins with M_r between 10 000 and 40 000 and at the end proteins with M_r 40 0000–60 000. Fig. 5 shows two cases of follow-up of two children with pyelonephritis.

Chronic pyelonephritis has been characterized as a combination of low LM_r protein pattern (tubular impairment) with one or more HM_r protein peaks in the Ig region [88]. These Ig peaks may be due to a local post-renal Ig secretion, where the secretory immune system (secretory IgA) plays an important role. The absence of glomerular lesions in these cases can be derived from the lack of a prominent transferrin peak. These findings were observed in four out of fifteen cases of chronic pyelonephritis followed by us, while in seven cases a purely tubular pattern and in four cases a mixed HM_r–LM_r pattern were present. These results are in agreement with the findings of other authors [38,89]. It is interesting to note that there are significant differences between chronic pyelonephritis and chronic glomerulonephritis. IgG and IgA from a higher percentage of the total immunoreactive urinary protein in pyelonephritis than in glomerulonephritis,

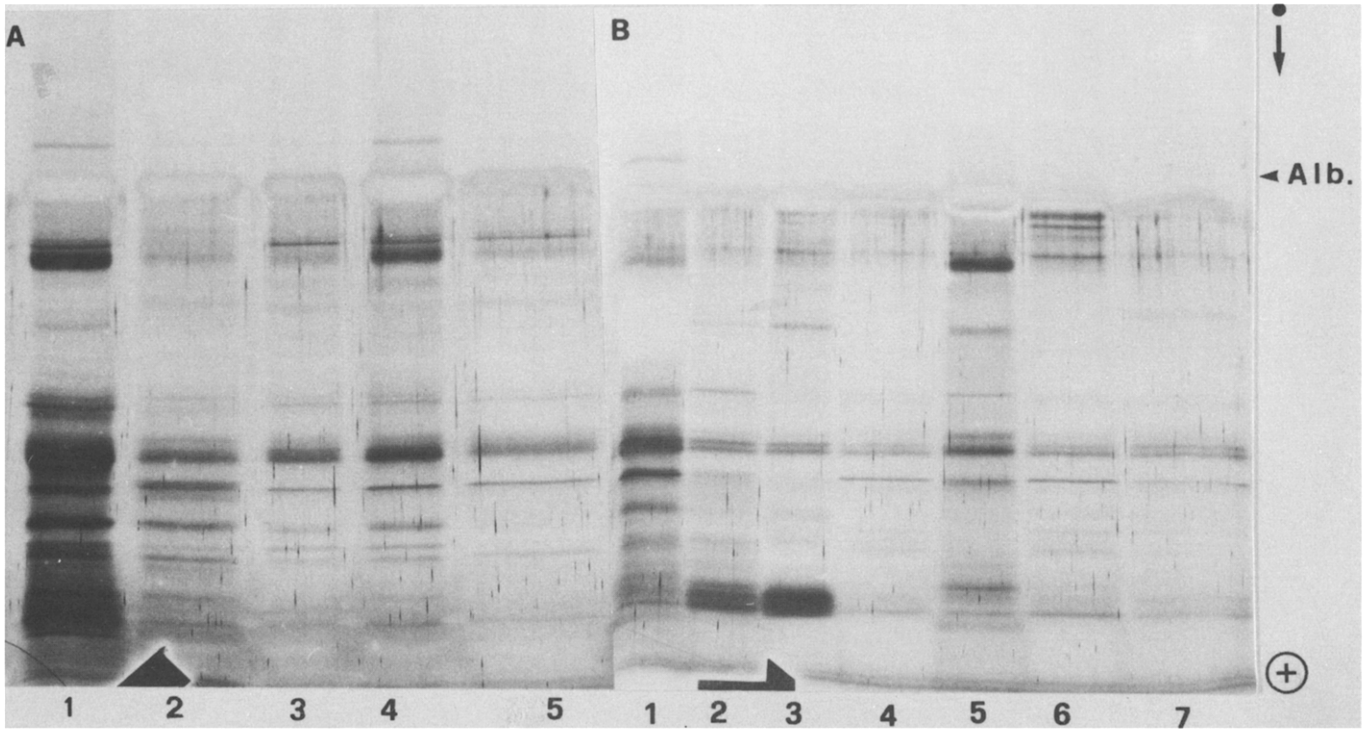

Fig. 5. Macro-SDS-PAGE of urinary proteins. Gel: 4–20% T. Running conditions: 3 h at 350 V, 50 mA. Sample load: up to 50 μl urine. A and B show urine samples from paediatric patients with pyelonephritis at various stages of follow-up. Series A: 1, acute phase; 2 and 3, urine taken at weekly intervals; 4, reinfection (acute phase); 5, one-week follow-up. Series B: 1, acute phase; 2–4, follow-up at weekly intervals (note blood contamination in 2 and 3, α- and β-globin chains at $16 \cdot 10^3$); 5, reinfection; 6 and 7, weekly follow-up. All symbols as in Fig. 1.

while the proportion of transferrin is higher in glomerulonephritis than in pyelonephritis.

5.6. Diabetes mellitus

About 30–40% of all patients with type 1 (insulin-dependent) diabetes mellitus will develop the clinical syndrome of diabetic nephropathy, characterized by persistent proteinuria (>0.3 g per 24 h), hypertension and deterioration of renal function. It invariably leads to terminal renal failure [90]. Manifest diabetic nephropathy is preceded by a phase in which albuminuria is abnormally increased but not yet detectable with routine methods. Microalbuminuria (30–300 mg per 24 h) is highly predictive of future development of manifest diabetic nephropathy [91]. In patients with microalbuminuria, strict metabolic control can delay the progression toward manifest diabetic nephropathy [92]. Some studies [93–96] have also shown increased concentration of enzymes or LM_r proteins in the urine of patients suffering from diabetic nephropathy. Increased excretion of albumin is generally assumed to result from changes in pore size and electrical charge of the glomerular basement membrane, whereas increased excretion of kidney enzymes or of LM_r proteins indicates tubular dysfunction. Some studies [97–99] have shown that the incidence of microalbuminuria is higher after exercise.

With SDS-PAGE, different protein patterns in urine from diabetic patients are observed depending on the stage of the disease. It is possible to find, in the early stage, only an increased albumin band or an incomplete tubular proteinuria, probably due to vascular impairment. Later on a selective glomerular proteinuria represents minimal-change lesions; degenerative glomerulopathies are revealed by the presence of a non-selective glomerular proteinuria. Finally, a mixed proteinuria consisting of non-selective glomerular and incomplete micromolecular-tubular proteinuria is characteristic of clinically manifest nephropathy with hypertension.

6. CONCLUSIONS

SDS gel slab electrophoresis, especially in the miniaturized Phast system, appears to be a most powerful tool for unambiguous determination of kidney malfunction, particularly in regard to urinary tract infection (cystitis and pyelonephritis). The technique is fast, simple, reliable and can be easily performed in a routine clinical laboratory, especially in view of the fact that precast gels are available and the instrumentation is highly automatic. Particularly attractive is the possibility of running urine without any priort treatment (*e.g.* desalting) or concentration step.

7. ACKNOWLEDGEMENTS

This work was supported in part by contracts from Consiglio Nazionale delle Ricerche (CNR, Rome, Italy), Progetti Finalizzati Biotecnologie e Biostrumentazione and Chimica Fine II and by Ministero della Pubblica Istruzione. We thank Pharmacia-LKB of Italy for lending the Phast system, which was used to perform the experiments shown in Figs. 1–4. In particular, we are grateful to Dr. R. Sandri for helping us assemble the instrument and for kind advice.

REFERENCES

1 D. Cotugno, *De Ischiade Nervosa Commentarious*, Graeffer Editor, Vienna, 1770.
2 D. Kutter, *Schnelltests in der Klinischen Diagnostik*. Urban and Schwarzenberg, Munich, Vienna, Baltimore, 2nd ed., 1983.
3 G. Weidemann and W. G. Guder, in H. Sarre and V. Gessler (Editors), *Nierenkrankheiten*, Thieme, Stuttgart, 9th ed., 1986, p. 59.
4 W. G. Guder and A. Heidland, *Clin. Chem. Clin. Biochem.*, 24 (1986) 611.
5 K. Lorentz and T. Weiss, *J. Clin. Chem. Clin. Biochem.*, 24 (1986) 309.
6 G. Esbach, *C.R. Soc. Biol.*, 1 (1874) 33.
7 A. C. Kibrick, *Clin. Chem.*, 4 (1958) 232.
8 S. Shahangian, P. I. Brown and K. O. Ash, *Am. J. Clin. Pathol.*, 81 (1984) 651.
9 J. A. Lott, V. A. Stephan and K. A. Pritchard, *Clin. Chem.*, 29 (1983) 1946.
10 M. A. Pesce and C. S. Strande, *Clin. Chem.*, 19 (1973) 1265.
11 H. Kirchherr and H. W. Schiwara, *J. Clin. Chem. Clin. Biochem.*, 24 (1985) 57.
12 B. A. Dilena, L. A. Penberthy and C. G. Fraser, *Clin. Chem.*, 29 (1983) 1533.
13 L. A. McEldeny, I. F. Tarbit and A. J. Cassells-Smith, *Clin. Chem.*, 28 (1982) 256.
14 W. Appel, in K.-Gv. Boroviczeny, R. Merten and U. P. Merten (Editors), *Qualitätssichering im Medizinischen Laboratorium*, Akademie-Verlag, Heidelberg, Berlin, 1987, p. 487.
15 D. Stamm, *Klin. Chem. Mitt.*, 17 (1985) 316.
16 E. A. Butler, F. V. Flynn, H. Harris and E. B. Robson, *Clin. Chim. Acta*, 6 (1961) 34.
17 A. J. Pesce, I. Boreisha and V. E. Pollak, *Clin. Chim. Acta*, 40 (1972) 27.
18 L. Balant, J. C. Mulli and J. Fabre, *Clin. Chim. Acta*, 54 (1974) 27.
19 A. J. Pesce, A. Hsu, C. Kornhauser, K. Sethi, B. S. Ooi and V. E. Pollak, *Clin. Chem.*, 22 (1976) 667.
20 C. Cachera, C. Mizon, J. C. Fruchart, J. Mizon and A. Tacquet, *Clin. Chem.*, 26 (1980) 1588.
21 J. Lubega, *Clin. Chim. Acta*, 128 (1983) 151.
22 R. C. Wiggins, B. Kshrisagar, R. C. Kelsch and B. S. Wilson, *Clin. Chim. Acta*, 149 (1985) 155.
23 B. Kshirsagar and R. C. Wiggins, *Clin. Chim. Acta*, 158 (1986) 13.
24 W. H. Boesken, *Urol. Ausg. B*, 17 (1977) 140.
25 J. E. Scherberich, *Biosci. Ber.*, 28 (1986) 213.
26 C. Spieker and A. E. Lison, in G. Lubec and A. Campese (Editors), *Advances in Non-Invasive Nephrology*, John Libbey, London, 1984, p. 195.
27 H. Olsson and S. Johansson, in M. J. Dunn (Editor), *Electrophoresis '86*, Verlag Chemie, Weinheim, 1986, p. 374.
28 K. S. Cheong, N. Arold, M. H. Weber and V. Neuhoff, in M. J. Dunn (Editor), *Electrophoresis '86*, Verlag Chemie, Weinheim, 1986, p. 336.
29 A. Lapin, F. Gabl, A. T. Endler and H. Kopsa, in B. J. Radola (Editor), *Elektrophorese Forum '86*, Technische Universität, Munich, 1986, p. 134.
30 R. Verwiebe and M. H. Weber, in B. J. Radola (Editor), *Elektrophorese Forum '87*, Technische Universität, Munich, 1987, p. 459.
31 M. H. Weber, *J. Chromatogr.*, 429 (1988) 315.

32 W. H. Boesken, K. Kopf and P. Schollmeyer, *Clin. Nephrol.*, 1 (1973) 311.
33 A. E. Lison and V. Herold, *Nieren Hochdruckkrankh.*, 11 (1982) 137.
34 B. Schulze, J. Reimer, G. Kopperschläger and B. Lemme, *Kinderärztl. Prax.*, 52 (1984) 88.
35 J. E. Scherberich, G. Soler and W. Schoeppe, in H. Peeters (Editor), *Protides of the Biological Fluids*, Vol. 33, Pergamon Press, Oxford, 1985, p. 533.
36 W. Reichel, D. I. Wolfrum, R. Klein and F. Scheler, *Klin. Wochenschr.*, 54 (1976) 19.
37 N. Manz, R. G. Galaske, J. Cho and J. B. Van Liew, *Electrophoresis*, 7 (1986) 183.
38 M. H. Weber, K. S. Cheong (Kim), K.-J. Schott and H. Neuhoff, *Electrophoresis*, 7 (1986) 134.
39 A. Lapin, E. O. Ogunyemi, H. Zyman, F. Gabl and J. Zazgornik, *J. Clin. Chem. Clin. Biochem.*, 23 (1985) 777.
40 H. W. Schiwara, J. Weser, W. Postel and A. Görg, in V. Neuhoff (Editor), *Electrophoresis '84*, Verlag, Chemie, Weinheim, 1984, p. 429.
41 A. Görg, W. Postel, J. Weser, H. W. Schiwara and W. H. Boesken, *Sci. Tools*, 32 (1985) 5.
42 H. W. Schiwara, T. Hebell, J. Weser, W. Postel and A. Görg, in H. Peeters (Editor), *Protides of the Biological Fluids*, Vol. 33, Pergamon Press, Oxford, 1985, p. 525.
43 J. G. Guevara, B. H. Herbert and B. A. Martin, *Electrophoresis*, 6 (1985) 613.
44 U. Wurster and J. H. H. Ehrich, *Nieren Hochdrukkrankh.*, 14 (1985) 491.
45 H. W. Schiwara, T. Hebell, H. Kirchherr, W. Postel, J. Weser and A. Görg, *Electrophoresis*, 7 (1986) 496.
46 J. Heukeshoven and R. Dernik, *Electrophoresis*, 6 (1985) 103.
47 J. Renart, J. Reiser and G. R. Stark, *Proc. Natl. Acad. Sci. U.S.A.*, 76 (1979) 3116.
48 H. Towbin, T. Staehlin and J. Gordon, *Proc. Natl. Acad. Sci. U.S.A.*, 76 (1979) 4350.
49 U. Beisiegel, *Electrophoresis*, 7 (1986) 1.
50 A. Edström and C. Jägersten, in M. J. Dunn (Editor), *Electrophoresis '86*, Verlag Chemie, Weinheim, 1986, p. 404.
51 C. Jägersten, A. Edström, B. Olsson and G. Jacobson, *Electrophoresis*, 9 (1988) 662.
52 W. Braun and R. Abraham, *Electrophoresis*, 10 (1989) 249.
53 J. E. Scherberich, P. Fischer, A. Bigalke, P. Stangl, G. B. Wolf, M. Haimere and W. Schoeppe, *Electrophoresis*, 10 (1989) 58.
54 Y. Manuel, J. P. Revillard and H. Betuel (Editors), *Proteins in Normal and Pathological Urine*, Karger, Basle, New York, 1970.
55 V. E. Pollak (Editor), *Nephron*, 13 (1974) 5.
56 L. Mingone (Editor), *Urinary proteins*, Karger, Basle, New York, 1981.
57 M. Weise (Editor), *Experimental and Clinical Aspects of Proteinuria*, Karger, Basle, New York, 1981.
58 E. P. Lillehoj and M. D. Poulik, *Exp. Pathol.*, 29 (1986) 1.
59 G. A. Kaysen, B. D. Myers, W. G. Couser, R. Rabkin and J. M. Felts, *Lab. Invest.*, 54 (1986) 479.
60 W. H. Boesken, in R. C. Allen and P. Arnaud (Editors), *Electrophoresis '81*, Walter de Gruyter, Berlin, 1981, p. 453.
61 T. Marshall and K. M. Williams, *Clin. Chem.*, 32 (1986) 2105.
62 K. S. Shiba, K. Kanamori, H. Cho, N. Furuhata, T. Harada, A. Shiba and M. Nakao, *Clin. Chim. Acta*, 172 (1988) 77.
63 T. Marshall, K. M. Williams and O. Vesterberg, *Electrophoresis*, 6 (1985) 47.
64 J. E. Scherberich, M. Wehrheim, H. G. Weidmann, W. Horatz, C. Wehrheim, W. Mondorf and W. Schoeppe, in G. Lubec and V. Campese (Editors), *Advances in Non-Invasive Nephrology*, John Libbey, London, 1985, p. 212.
65 J. C. Townsend, W. A. Sadler and G. M. Shanks, *Ann. Clin. Biochem.*, 24 (1987) 111.
66 F. Miyasato and V. E. Pollak, *J. Lab. Clin. Med.*, 67 (1966) 1036.
67 G. Lindstedt and P.A. Lundberg, *Clin. Chim. Acta*, 56 (1974) 125.
68 J. M. Alt and B. Maess, *Nieren Hochdrukkrankh.*, 14 (1985) 484.
69 A. E. Lison, E. Doedt and E. Meyer, *Clin. Nephrol.*, 32 (1989) 40.
70 J. H. H. Ehrich, H. G. Foellmer, F. Krull, H. J. Winnecken and U. Wurster, *I. Monatsschr. Kinderheilk.*, 132 (1984) 136.

71 L. Balant and J. Fabre, *Curr. Probl. Clin. Biochem.*, 9 (1979) 216.
72 T. Marshall, *Clin. Chem.*, 30 (1984) 475.
73 J. Poortmans and R. W. Jeanloz, *J. Clin. Invest.*, 47 (1968) 386.
74 W. H. Boesken, in H. Losse and E. Renner (Editors), *Kliniske Nephrologie*, Vol. 1. Thieme, Stuttgart, 1982, p. 136.
75 D. Cvoriscec, A. Stavljenïc and M. Radonic, *J Clin. Chem. Clin. Biochem.*, 23 (1985) 177.
76 A. E. Lison, L. Frommeyer, C. Spieker, A. Reisch and M. C. Kösters, *Forschungsbericht des Landes Nordrhein-Westfalen Nr. 3007*, Fachgruppe Medizin, Westdeutscher Verlag, Opladen, 1981, p. 3.
77 A. E. Lison, M. Rochell, J. Rakohl, P. Loermann and E. Meyer, *Nieren Hochdruckkrankh.*, 14 (1985) 515.
78 B. Antoine, A. Symvoulidis and M. Dardenne, *Nephron*, 6 (1969) 526.
79 R. R. Robinson, in Y. Manuel, J. P. Revillard and H. Bethe (Editors), *Proteins in Normal and Pathological Urine*, Karger, Basel, New York, 1970.
80 A. P. Fletcher, A. Neuberger and W. A. Ratcliffe, *Biochem. J.*, 120 (1970) 425.
81 A. Lapin, H. J. Zyman, J. Zazgornik and F. Gabl, in V. Neuhoff (Editor) *Electrophoresis '84*, Verlag Chemie, Weinheim, 1984, p. 506.
82 T. Marshall, *Clin. Chem.*, 30 (1984) 475.
83 R. Thorpe, C. R. Bird and M. Spitz, *J. Immunol. Methods*, 73 (1984) 259.
84 D. H. Clyne, A. J. Pesce and R. E. Thompson, *Kidney Int.*, 16 (1979) 345.
85 K. S. Cheong, M. H. Weber and V. Neuhof, in M. M. Galteau and G. Siest (Editors), *Recent Progresses in Two Dimensional Electrophoresis*, Presses Universitaires de Nancy, Nancy, 1986, p. 223.
86 G. Bispink and V. Neuhoff, *Hoppe Seyler's Z. Physiol. Chem.*, 357 (1976) 991.
87 H. M. Poehling and V. Neuhoff, *Electrophoresis*, 1 (1980) 90.
88 W. H. Boesken, *Contr. Nephrol.*, 1 (1975) 143.
89 J. M. Alt, H. Jänig, H. J. Schurek and H. Stolte, *Contr. Nephrol.*, 16 (1979) 37.
90 A. R. Andersen, J. S. Christiansen, J. K. Andersen, S. Kreiner and T. Deckert, *Diabetologia*, 25 (1983) 496.
91 C. E. Mogensen, *Kidney Int.*, 31 (1987) 673.
92 B. Feldt-Rasmussen, E. R. Mathiesen and T. Deckert, *Lancet*, ii (1986) 1300.
93 K. Shima, M. Hirota, M. Fukuda and A. Tanaka, *Clin. Chem.*, 32 (1986) 1818.
94 R. Beetham, A. Silver and A. Dawnay, *Clin. Chem.*, 33 (1987) 713.
95 P. H. Whiting, I. S. Ross and L. Borthwick, *Clin. Chim. Acta*, 97 (1979) 191.
96 E. N. Ellis, B. H. Brouhard, L. Lagrone and A. B. Travis, *Diabetes Care*, 6 (1983) 251.
97 G. C. Viberti, R. J. Jarret, M. McCartney and H. Keen, *Diabetologia*, 14 (1978) 293.
98 G. C. Viberti and M. J. Wiseman, *Diabetic Nephropathy*, 2 (1983) 22.
99 E. Vittinghus and C. E. Mogensen, *Scand. J. Clin. Lab. Invest.*, 41 (1981) 627.