PAPERS AND ORIGINALS

Urinary Fibrin-Fibrinogen Degradation Products in Nephrotic Syndrome

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Summary

The urinary concentration of fibrin-fibrinogen degradation products (F.D.P.) was measured in 90 patients with proteinuria above 2 g/l and correlated with proteinuria, differential protein clearances, serum urea and creatinine, and renal biopsy findings.

There was a linear correlation ($r=0.7$; $P<0.001$) between the urinary F.D.P. excretion and the selectivity of the proteinuria such that patients with highly selective proteinuria excreted only small amounts of F.D.P. whereas those with non-selective proteinuria excreted much higher levels. There was a significant correlation between the urinary F.D.P. excretion and the urine:serum (U:S) ratio of IgG and the urinary IgG excretion but not with the U:S ratio or urinary excretion of albumin or transferrin. Sephadex G200 column chromatography of the concentrated urine in 26 cases showed that patients with highly selective proteinuria excreted predominantly F.D.P. of low molecular weight in the urine whereas those with non-selective proteinuria excreted mainly fibrinogen and products of high molecular weight.

Hence the type and quantity of F.D.P. in the urine are determined primarily by the differential filtration of fibrinogen and the various degradation products from the plasma through the glomerular basement membrane, which in turn is determined by the "pore size" of the basement membrane. In clinical nephrology measurement of the urinary F.D.P. level provides a rapid and convenient means of estimating the differential protein clearance.

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Introduction

Fibrin-fibrinogen degradation products (F.D.P.) have been found in the urine of most patients with glomerulonephritis (Naish *et al.*, 1970; Clarkson *et al.*, 1971; Briggs *et al.*, 1972). In such cases serum F.D.P. levels may be raised above normal (Briggs *et al.*, 1972) but are often within the normal range (Humair *et al.*, 1969; Preston *et al.*, 1971), and no correlation has been found between serum and urinary F.D.P. levels (Stiehm *et al.*, 1971; Briggs *et al.*, 1972).

The urinary F.D.P. level has been found to correlate with proteinuria by some workers (Briggs *et al.*, 1972; Shah *et al.*, 1972) but not by others (Naish *et al.*, 1970). Clarkson *et al.* (1971) observed a correlation between urinary F.D.P. levels and proteinuria in patients with minimal-change and membranous glomerulonephritis but not in those with proliferative glomerulonephritis, while Naish *et al.* (1974) correlated urinary F.D.P. and proteinuria when the patients were grouped (by means of immunofluorescent techniques) according to the extent and localization of the intraglomerular fibrin deposits. Naish *et al.* (1970) and Clarkson *et al.* (1971) found no correlation between urinary F.D.P. excretion and the selectivity of the proteinuria whereas Ekert *et al.* (1972) found a significant linear correlation such that patients with the least selective proteinuria had the highest levels of urinary F.D.P.

Briggs *et al.* (1972) reported that the range of urinary F.D.P. excretion in patients with renal disease was too great to be of diagnostic use, but Clarkson *et al.* (1971) found that the urinary F.D.P. level of patients with minimal-change, membranous, and inactive proliferative glomerulonephritis was less than 2 mg/l (2 μg/ml) whereas that of patients with active proliferative glomerulonephritis was more than 2 mg/l. Furthermore, in cases of active proliferative glomerulonephritis urinary F.D.P. excretion was closely related to the amount of intraglomerular fibrin as determined by electron microscopy.

A correlation between the urinary F.D.P. level and the blood urea was recorded by some workers (Stiehm *et al.*, 1971) but not by others (Naish *et al.*, 1970; Briggs *et al.*, 1972). Similarly conflicting reports exist as to the nature of the urinary degradation products. Nilsson (1971) found fibrinogen and the high molecular weight fragments FgX and FgY in the urine of patients with chronic nephritis, while Clarkson *et al.* (1971) found the low molecular weight fragments FgD and FgE in cases of proliferative glomerulonephritis and small amounts of

all fragments in cases of minimal and membranous glomerulonephritis.

These many conflicting reports may in part be due to differences in the selection of cases for study and differences in the techniques used. Nevertheless, much controversy exists as to the nature, site of origin, and mechanism of clearance of urinary F.D.P. in renal disease. The present investigation was undertaken to clarify these points by studying patients with renal glomerular lesions causing proteinuria above 2 g/l (200 mg/100 ml).

Patients and Methods

Ninety patients with proteinuria above 2 g/l were studied. In 77 cases a precise histological diagnosis was made after percutaneous renal biopsy. Thirty-eight patients had proliferative glomerulonephritis, and of these 10 had membranoproliferative and six had focal proliferative glomerulonephritis and three had Henoch-Schönlein purpura. Minimal-change glomerulonephritis was seen in 16 further patients, membranous glomerulonephritis in 10, systemic lupus erythematosus in two, amyloidosis in six, renal vein thrombosis in three, and malignant hypertension in two. Biopsies were not performed in the remaining 13 patients.

Aliquots of consecutive 24-hour urine specimens were collected for three to 20 days from inpatients and either aliquots of four-hour collections or random samples were obtained from outpatients. The urinary F.D.P. level was determined on the unconcentrated urine by the tanned red cell haemagglutination inhibition immunoassay (Merskey *et al.*, 1969). The maximum sensitivity of this technique for fibrinogen was 0·3 mg/l. Urinary protein excretion was determined both by the biuret method and a quantitative salicylsulphonic acid method using a Unicam SP 1300 colorimeter. The differential protein clearance was measured by the technique of Mancini *et al.* (1965) by which the urinary and serum levels of albumin, transferrin, and IgG were determined by radial immunodiffusion in 1·5% agar gel buffered with 0·3-M phosphate. The agar gels were impregnated with the corresponding monospecific antiserum to each protein. The results were expressed as the urine:serum (U:S) ratio of IgG:transferrin and of IgG:albumin.

In 26 cases urine was concentrated tenfold to a hundredfold by pressure ultrafiltration in an Amicon chamber using an Amicon PM 10 membrane which is permeable only to molecules with molecular weights of less than 10 000. The 26 specimens were analysed by Sephadex G200 column chromatography. The analyses were carried out in 0·9% saline and alternate fractions were analysed for degradation products by tanned red cell haemagglutination inhibition immunoassay. The columns (100×2·5 cm) were characterized by means of a standard solution of blue dextran, IgG, albumin, and tryptophan using an SP 500 spectrophotometer and a Gilford 2000 multiple-sample absorbance recorder. Multiple control analyses were carried out using 1-ml volumes of solutions of (*a*) fibrinogen (Kabi grade L), (*b*) FgD and E, and (*c*) equal quantities of fibrinogen and FgD and E. All the solutions were made up in 0·9% saline to a final concentration of 1000 mg/l. In addition 1-ml volumes of these solutions were added to 1 ml of urine from a normal subject (without proteinuria) which had been concentrated one hundredfold and to 1 ml of urine from a patient with minimal-change glomerulonephritis in relapse which had been concentrated one hundredfold (F.D.P. of concentrate=10 mg/l). These analyses were carried out both to check the characterization of the columns and to confirm their ability to separate the various F.D.P. fragments in urine with heavy proteinuria which had been concentrated many times.

Results

The unconcentrated urine from 136 normal subjects did not contain detectable levels of F.D.P. In the 90 patients with the nephrotic syndrome a close correlation ($r=0·7$; $P<0·001$) was observed between the urinary F.D.P. excretion and the differential protein clearance whether measured as the U:S ratio of IgG:transferrin (fig. 1), or of IgG:albumin, such that patients with highly selective proteinuria excreted only small amounts of F.D.P. in the urine whereas those with non-selective proteinuria excreted much larger amounts of F.D.P.

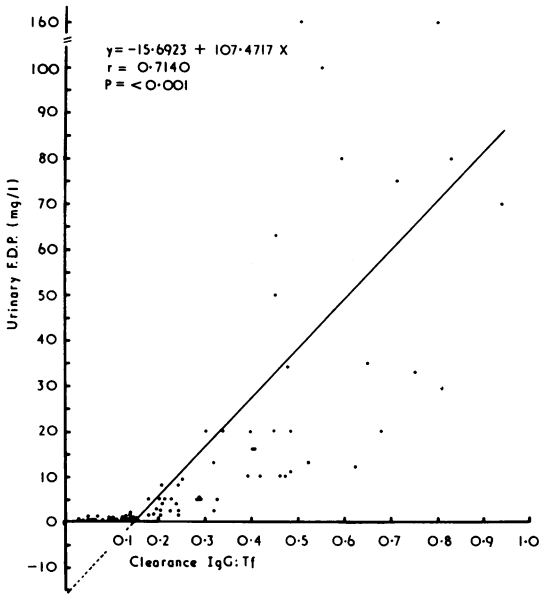

FIG. 1—Relationship between urinary F.D.P. excretion and differential protein clearance measured as urine: serum (U:S) ratio of IgG:transferrin (IgG:Tf).
Conversion: SI to Traditional Units—Urinary F.D.P.: 1mg/l=1 μg/ml.

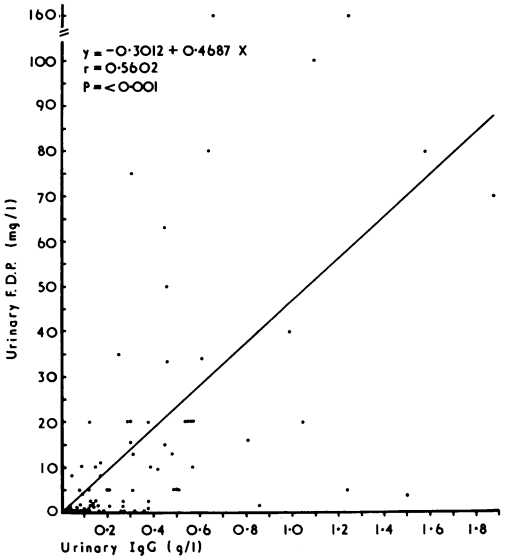

FIG. 2—Relationship between urinary F.D.P. excretion and urinary IgG excretion.
Conversion: SI to Traditional Units—IgG: 1 g/l=100 mg/100 ml.

Results of Sephadex G200 Column Chromatography of Urinary Concentrates of 26 Patients with Nephrotic Syndrome. Numbers in Parentheses are Percentages

Differential Protein Clearance	No. of Patients	Urinary F.D.P. (μg/ml)					
		Fg	FgX	FgY	FgD	FgE	Total
Highly selective (IgG:Tf <0·15) ..	8	0·005 (1·1)	0·066 (13·9)	0·11 (23·2)	0·26 (54·7)	0·034 (7·6)	0·475 (100)
Moderately selective (IgG:Tf 0·15-0·4)	7	0·65 (14·0)	1·06 (22·9)	1·23 (26·5)	1·33 (28·7)	0·37 (7·9)	4·64 (100)
Non-selective (IgG:Tf >0·4)	11	22·8 (36·1)	13·7 (21·7)	12·4 (19·6)	11·6 (18·4)	2·7 (4·2)	63·2 (100)

Fg = Fibrinogen.

A significant correlation ($P < 0.001$) was observed between the urinary F.D.P. excretion and the U:S ratio of IgG ($r = 0.4$) and the urinary IgG excretion ($r = 0.56$) (fig. 2) but not with the U:S ratio or urinary excretion of albumin or transferrin. There was no relation between the urinary F.D.P. excretion and the urinary protein excretion even when patients were grouped according to the selectivity of the proteinuria or the type of renal disease (fig. 3). A statistically significant correlation ($P < 0.001$), however, was observed between the urinary F.D.P. excretion and the serum urea ($r = 0.45$) and the serum creatinine ($r = 0.47$) (fig. 4).

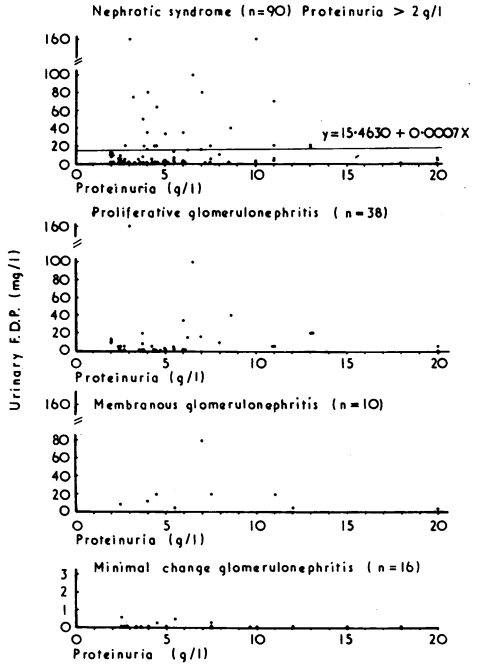

FIG. 3—Relationship between urinary F.D.P. excretion and proteinuria overall and within histological groups.
Conversion: SI to Traditional Units—Proteinuria 1 g/l = 100 mg/100 ml.

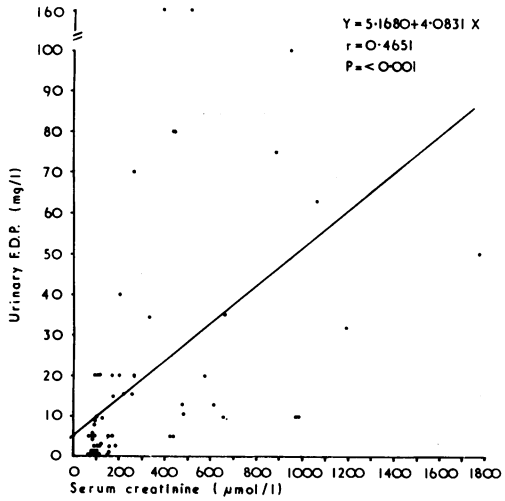

FIG. 4—Relationship between urinary F.D.P. excretion and serum creatinine.
Conversion: SI to Traditional Units—Serum creatinine: 1 μmol/ ≈ 0·0113 mg/100 ml.

In 26 cases G200 Sephadex column chromatography was carried out on urine concentrated 10-100 times by pressure ultrafiltration using an Amicon PM 10 membrane (see table). Patients with highly selective proteinuria (IgG:Tf <0·15) excreted only very small amounts of F.D.P. in the urine (mean = 0·5 mg/l) of which only 1% was fibrinogen, while 55% was FgD. Patients with moderately selective proteinuria excreted more F.D.P. in the urine, which consisted of 14% fibrinogen and about equal amounts of fragments FgX, Y, and D. Patients with non-selective proteinuria excreted much greater quantities of F.D.P. which consisted mainly of fibrinogen and progressively smaller amounts of the fragments X, Y, D, and E.

The urinary F.D.P. excretion of patients with minimal-change glomerulonephritis did not exceed 1 mg/l even when proteinuria was over 10 g/l. In marked contrast, a wide range of urinary F.D.P. excretion was observed in patients with other types of renal disease (fig. 5), and in each case the excretion correlated closely with the differential protein clearance.

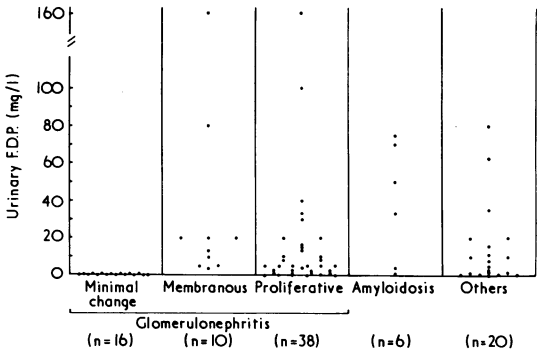

FIG. 5—Urinary F.D.P. levels in different diagnostic groups.

Discussion

These data indicate that the type and quantity of F.D.P. in the urine are determined mainly by the differential filtration of fibrinogen and the various products of fibrin-fibrinogen degradation from the plasma through the glomerular basement membrane (fig. 6). This differential filtration is determined by the pore size of the basement membrane. Thus patients with highly selective proteinuria (IgG:Tf <0·15) who excreted proteins of small molecular weight, mainly albumin (M.W. 70 000), in the urine also excreted small molecular weight F.D.P., mainly FgD (M.W. 80 000), while the high molecular weight F.D.P. and fibrinogen were largely prevented from passing through the glomerular filter. Gordon *et al.* (1973) have shown

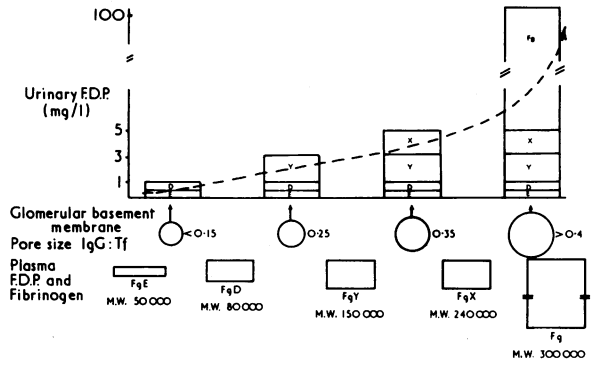

FIG. 6—Differential filtration of fibrinogen and F.D.P. from plasma through glomerular basement membrane (G.B.M.). Bottom line indicates molecular size, and blocks indicate approximate proportions of F.D.P. fragments and fibrinogen (Fg) in plasma. Circles indicate increasing pore size of G.B.M. expressed as clearance of IgG:transferrin (<0·15 — >0·4) as protein selectivity becomes impaired. Graph (top line) shows increasing urinary F.D.P. concentration due to filtration of progressively larger fragments as protein selectivity becomes impaired (G.B.M. pore size increases).

that the serum of normal people contains only 0·85 mg/l of FgD and 0·15 mg/l of FgE; thus only small amounts of F.D.P. appear in their urine. As the selectivity of the proteinuria becomes impaired (fig. 6) so increasing amounts of the larger molecular weight proteins appear in the urine. Corresponding with this change in selectivity (IgG:Tf 0·15-0·4) increasing amounts of FgY (M.W. 150 000) FgX (M.W. 240 000) and some fibrinogen (M.W. 300 000) appear in the urine, and with non-selective proteinuria (IgG:Tf >0·4) fibrinogen becomes the predominant urinary F.D.P. Though plasma fibrinogen levels are above 3 g/l whereas the other products are present only in milligram quantities its large molecular size permits only small amounts to pass through the glomerular basement membrane.

In cases of non-selective proteinuria, however, considerably more fibrinogen than any other product was present in the urine. The hypothesis that urinary F.D.P. originate in a process of differential filtration through a damaged glomerular basement membrane is supported by the work of Rayner *et al.* (1969), who infused purified F.D.P. fragments into normal animals and observed that only fragments D and E were excreted in the urine and of Bouma *et al.* (1971), who observed that the urine of patients undergoing thrombolytic therapy with streptokinase contained only fragments D and E. The high molecular weight fragments X and Y, however, have been found in the urine of patients with chronic glomerulonephritis (Hedner *et al.*, 1974), with acute renal failure, and after kidney transplantation (Bouma *et al.*, 1971), which are conditions characteristically associated with non-selective proteinuria. Furthermore, Wardle (1972/a, b) observed a good correlation between the urinary excretion of fibrinogen, determined by a chemical technique, and the urinary F.D.P. excretion and suggested that the presence of the high molecular weight fragments X and Y was associated with an impaired protein selectivity. The relation between urinary F.D.P. excretion and the selectivity of the proteinuria reported in this paper (fig. 1) corresponds very closely with that observed by Ekert *et al.* (1972) in a study of 24 children with the nephrotic syndrome.

The contribution to the urinary F.D.P. level made by the renal tubules either by reabsorption or secretion of F.D.P. seems to be small because the urinary F.D.P. excretion of patients with primary renal tubular disorders—for example, renal tubular acidosis and cadmium nephropathy—has not exceeded 1·25 mg/l (Hall *et al.*, 1974) and the tubules have only a very limited capacity to reabsorb molecules in the molecular-weight range 50 000-300 000 (Blainey, 1967).

In our series the 16 patients with minimal-change lesions all had a highly selective proteinuria and excreted only very small amounts of F.D.P. in the urine whereas there was a wide range of differential protein clearances in the other histological groups and a correspondingly wide range of urinary F.D.P. levels. Thus when the F.D.P. level in the unconcentrated urine of a patient with the nephrotic syndrome is less than 1 mg/l the proteinuria will be of a highly selective type and the glomerular lesion is most likely to be of the minimal-change type. A urinary F.D.P. level greater than 1 mg/l does not provide a reliable guide to the histological diagnosis, but it does enable an estimate of the differential protein clearance to be made.

We disagree with others (Clarkson *et al.*, 1971; Briggs *et al.*, 1972; Naish *et al.*, 1974) in finding no correlation between the urinary F.D.P. excretion and the urinary protein excretion even when the patients were grouped according to the selectivity of the proteinuria or the type of renal disease. This discrepancy may be explained by the fact that all our patients had proteinuria above 3 g/day whereas in other series 40-50% of the patients had proteinuria of less than 3 g/day. The patients with low proteinuria had low or zero levels of F.D.P. in the urine which heavily weighted the correlation between urinary F.D.P. and proteinuria at its lower end.

Clarkson *et al.* (1971) found a close correlation between the urinary F.D.P. concentration and the extent of intraglomerular fibrin deposition (as determined by electron microscopy) in proliferative glomerulonephritis. They suggested that the degradation products were derived from the local breakdown of intraglomerular fibrin deposits. These findings may be explained also, however, by the differential filtration of fibrinogen and F.D.P. from the plasma through the damaged glomerular basement membrane. Proliferative glomerulonephritis without extensive intraglomerular fibrin deposits is often of an endothelial, mesangial, or focal type associated with a selective or moderately selective proteinuria. In such cases mainly products of small molecular weight are filtered so only small quantities of F.D.P. are present in the urine. The development of extensive intraglomerular fibrin deposits in proliferative glomerulonephritis, however, is associated with severe and progressive renal impairment and a non-selective proteinuria. Gross disorganization of the basement membrane which permits filtration of fibrinogen and high molecular weight F.D.P. results in high urinary F.D.P. levels. Thus the location, extent, and distribution of intraglomerular fibrin deposits correlate with the severity of the glomerular basement membrane damage, which in turn determines the type and quantity of F.D.P. and fibrinogen filtered and excreted into the urine. This association between severe glomerular basement membrane damage and non-selective proteinuria leads also to the significant positive correlation between the urinary F.D.P. level and the serum creatinine and serum urea that we observed.

Thus the urinary F.D.P. constitutes a family of molecules with a range of molecular weights from 50 000 to 300 000 which have a common immunological identity by which they can be detected quantitatively. As the pore size of the glomerular basement membrane increases so successively larger fragments are filtered from the plasma and excreted in the urine, each contributing an increment to the urinary F.D.P. level. As fibrinogen is present in the plasma in the greatest amounts, when the pores in the basement membrane are large enough to permit the filtration of fibrinogen it becomes the predominant urinary F.D.P. In this way the urinary F.D.P. level has a close correlation with the differential protein clearance in cases of renal glomerular damage sufficient to cause proteinuria greater than 2 g/l.

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