Immunologically Reactive Equivalents of Fibrinogen in Sera and Urine of Children with Renal Disease

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Ekert, H., Barratt, T. M., Chantler, C., and Turner, M. W. (1972). *Archives of Disease in Childhood*, 47, 90. **Immunologically reactive equivalents of fibrinogen in sera and urine of children with renal disease.** Fibrin degradation products (FDP) were assayed by the tanned red cell haemagglutination inhibition technique in the sera and urine of children with renal disease, and their correlation with other parameters of proteinuria was examined. Urinary FDP were present in greater quantities in subjects with heavy and unselective proteinuria, and were not correlated with serum levels of FDP. FDP may derive from fibrinogen filtered through leaky glomeruli or from intravascular coagulation restricted to the kidney: the data presented are compatible with either hypothesis.

It is known that intravascular coagulation (IVC) and glomerular deposition of fibrin are involved in the pathogenesis of immunologically induced nephritis in the experimental animal (Vassalli and McCluskey, 1964, 1965), but in man the evidence is less secure. Intravascular coagulation has been demonstrated in patients with the haemolytic-uraemic syndrome (Sanchez Avalos *et al.*, 1970; Gilchrist *et al.*, 1969). Fibrin can be demonstrated by immunofluorescence in the glomeruli of some patients with various forms of glomerulonephritis (Koffler and Paronetto, 1965; McCluskey *et al.*, 1966). Immunologically reactive equivalents of fibrinogen (fibrin degradation products; FDP) have been shown in the sera of patients with various types of nephritis (Wardle and Taylor, 1968; Stiehm and Trygstad, 1969), though some of the methods used have been criticized (Ekert, 1970a, b). Heparin modifies experimental glomerulonephritis (Halpern *et al.*, 1965) and uncontrolled studies suggest that it has a beneficial effect in the haemolytic-uraemic syndrome (Gilchrist *et al.*, 1969), in anuric acute glomerulonephritis (Kincaid-Smith, Saker, and Fairley, 1968), and in some cases of persistent glomerulonephritis (Herdman *et al.*, 1970; Kincaid-Smith, Laver, and Fairley, 1970).

It seems possible that IVC confined to the kidney

might contribute to the pathogenesis of some forms of glomerulonephritis and that the presence of FDP in the urine might be a more sensitive index of glomerular fibrin deposition than serum FDP (Rayner *et al.*, 1969). FDP have been demonstrated in the urine of patients undergoing renal transplant rejection (Braun and Merrill, 1968; Antoine, Neveu, and Ward, 1969; Clarkson, Morton, and Cash, 1970b) and of patients with disseminated IVC (Cooiland *et al.*, 1968; Herschlein and Steichele, 1968), glomerulonephritis (Rayner *et al.*, 1969; Naish *et al.*, 1970; Vermeylen *et al.*, 1970), and acute ischaemic renal failure (Clarkson *et al.*, 1970a). However, explanations other than localized IVC could be invoked to account for FDP in the urine: the filtered load of FDP might be increased because of raised serum levels or increased glomerular permeability, or impairment of tubular protein reabsorption. Alternatively, urinary FDP might derive from fibrinogen filtered through a damaged glomerulus (Yatzidis, 1964) and degraded by fibrinolytic mechanisms normally present in urine. We have attempted to analyse this problem by assaying urinary FDP in children with a wide range of renal diseases, and relating it to other parameters of protein excretion.

Methods

Venous blood was drawn in plastic disposable syringes. The sample for estimation of albumin, creatinine, lysozyme, IgG, and C3 was anticoagulated with lithium sequestrene. For fibrinogen estimation,

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blood was anticoagulated with 3.8% trisodium citrate (1 vol anticoagulant: 9 vol blood) and platelet-poor plasma was prepared by centrifugation at 3500 g for 20 minutes. Blood for FDP estimation was collected into plain glass tubes containing EACA 1 mg/ml, and allowed to clot at 37 °C for 1 to 3 hours; the serum was separated by centrifugation, thrombin (10 NIH units) added, and incubated again at 37 °C for a further 30 minutes. Plasma and serum samples were stored at -20 °C until assay.

24-hour urine samples were collected into plastic containers to which thiomersalyl (20 mg) and aprotinin (500 units) had been added. An aliquot of 100 ml was removed and dialysed using 23/32 mm Visking tubing (Union Carbide Company, Chicago, Ill., U.S.A.) against cold running tap water (24 hr) and finally six changes of distilled water. The dialysed samples were then lyophilized and reconstituted before analysis in 2 ml phosphate-citrate buffer (Merskey, Lalezari, and Johnson, 1969). Albumin recovery from this fiftyfold concentration procedure was 70%.

FDP in serum (S_{FDP}), unconcentrated urine (U_{FDP}), and concentrated urine (U'_{FDP}) were assayed by the tanned red cell haemagglutination inhibition technique (TRCHI) (Merskey *et al.*, 1969). Doubling dilutions on lucite plates were performed using a Cornwall automatic syringe. Human group O red cells coated with fibrinogen and a 1/8000 dilution of antifibrinogen antiserum (Wellcome Laboratories, Beckenham, Kent) were used. The antiserum was shown to be mono-specific by immunoelectrophoresis against human plasma. Results are expressed as the logarithm (base 2) of the reciprocal of doubling dilution titres. The observed range for S_{FDP} in hospitalized children with unrelated disease was <1-4. Pooled normal human urine was shown not to interfere with the TRCHI assay.

Recovery experiments on the effect of concentration of urine samples on the FDP showed that the titre observed in the concentrated urine almost invariably exceeded that in the unconcentrated urine by a factor greater than that which could be attributed to the degree of concentration alone (see Table II). Dilution of the concentrated urine to its original volume resulted in FDP titres similar to those observed in the unconcentrated urine, and so the effect cannot be attributed to removal of an inhibitor of the TRCHI assay by dialysis or the generation of more immunoreactive material during the concentrating procedure. High concentrations of albumin in the urine concentrates may have contributed to this effect (C. Merskey, A. J. Johnson, and P. Lalezari, 1971, personal communication) and systematic inaccuracies of doubling dilution techniques have been described (Merskey *et al.*, 1971, personal communication). We have no satisfactory explanation of this phenomenon, but since there is a significant rank order correlation between the data on concentrated and unconcentrated urine specimens (Spearman; $P < 0.01$) we have used the data from concentrated specimens and analysed them by non-parametric statistical techniques.

The methods of estimating the clearances of albumin (Barratt, McLaine, and Soothill, 1970) (C_{alb}), creati-

nine (Barratt *et al.*, 1970) (C_{creat}), and lysozyme (Barratt and Crawford, 1970) (C_{lys}), and of the plasma C3 (Ngu and Soothill, 1969) and fibrinogen (Jacobsson, 1955) concentrations are described elsewhere. Urinary fibrinogen was measured by the method of Yatzidis (1964). IgG was measured by the single radial gel diffusion method (Mancini, Carbonara, and Heremans, 1965), and selectivity of proteinuria (C_{IgG}/C_{alb}) was calculated when proteinuria was moderate or heavy ($C_{alb}/C_{creat} > 0.2 \times 10^{-3}$).

Patients

Thirty-one children with renal disease and 6 healthy adults were studied. Details of clinical features and renal function are given in Table I.

(i) **Nephrotic syndrome.** Twelve children had heavy proteinuria ($C_{alb}/C_{creat} > 1.0 \times 10^{-3}$). Six responded to steroid therapy: of these, 5 had highly selective proteinuria ($C_{IgG}/C_{alb} < 20\%$) and those investigated had no abnormality of renal histology on light microscopy. One (Case 5) is included in this group, even though at the time of study his glomerular leak did not reach nephrotic proportions, because of the similarity of renal histology and protein selectivity. The other 6 children were steroid resistant (prednisolone 2 mg/kg per day for 4 weeks) or presumed to be so (Case 12; congenital nephrotic syndrome); 4 had poorly selective proteinuria ($C_{IgG}/C_{alb} > 20\%$); and 3 had proliferative changes on renal biopsy.

(ii) **Acute nephritic syndrome.** Three children had acute nephritis: haematuria, proteinuria, oedema, and hypertension of less than 3 weeks' duration. In 2, the illness followed a streptococcal throat infection, but in one (Case 13) no evidence of a streptococcal aetiology was discovered. All had low C3 levels.

(iii) **Persistent glomerulonephritis.** Twelve children had moderate proteinuria ($C_{alb}/C_{creat} > 0.01 \times 10^{-3}$) for more than 6 months and proliferative changes on renal biopsy. In three the disease followed an episode of acute nephritis, though a streptococcal aetiology was not demonstrated in any. Four cases were associated with Henoch-Schönlein purpura; one of these cases (Case 24) has been included in this group even though renal biopsy had not been performed.

(iv) **Recurrent haematuria.** Two children had recurrent haematuria with little or no increase in albumin excretion, normal cystoscopy, and normal renal biopsy.

(v) **Fanconi syndrome.** One child had cystinosis with multiple tubular dysfunction and a tubular pattern of proteinuria ($C_{lys}/C_{creat} > 150 \times 10^{-3}$).

(vi) **Haemolytic-uraemic syndrome.** One child was studied with the characteristic syndrome of haemolysis and fragmented erythrocytes, thrombocytopenia, and oliguria.

TABLE I
Clinical Details of Patients Studied

Case No.	Age (yr)	Histology*	Steroid Response	Haematuria†	Plasma C3‡ % RNS	C _{creat} ml/min per 1.73 m ² SA	Calb/C _{creat} × 10 ³	C _{IgG} /Calb × 10 ²	C _{Iys} /C _{creat} × 10 ³	Comment§
Healthy (n=6)										
Range of observations:		25-44	—	0	54-230‡	117-171	0.00087-0.0036	—	0.58-2.1	
Nephrotic syndrome										
1	4	MC	S	0	92	47	11	3.1	0.78	
2	13	MC	S	0	112	74	1.7	19	0.71	
3	3	—	S	0	196	111	38	7.8	0.61	
4	4	—	S	0	116	101	1.0	3.7	0.84	
5	7	MC	S	0	96	126	0.69	5.9	0.49	
6	2	—	S	+	148	64	3.3	23	0.83	
7	10	FGS	R	0	68	136	4.7	12	0.34	
8	4	MC	R	0	88	51	13	28	6.8	
9	10	CGN	R	+	160	6.0	32	65	<100	
10	6	P	R	0	112	151	1.9	34	0.65	
11	6	P	R	0	112	148	1.3	24	1.0	
12	0.1	—	—	0	80	36	9.6	3.9	2.0	Congenital
Acute nephritic syndrome										
13	13	P+C	—	++	56	9.1	28	42	<8.8	Strep 0
14	5	—	—	++	54	33	0.67	43	12	Strep +
15	12	—	—	—	50	77	1.8	9.9	0.83	Strep +
Persistent glomerulonephritis										
16	8	P+C	—	+	144	143	0.20	14	1.4	ANS
17	11	CGN	—	+	60	95	0.84	20	0.83	ANS
18	7	P	—	0	92	159	0.086	—	0.34	ANS
19	5	P	—	0	164	178	0.84	11	—	
20	10	P	—	+	136	144	0.012	—	0.73	
21	14	P	—	+	172	91	0.19	16	0.40	
22	8	P	—	++	124	136	0.093	—	0.72	
23	10	P	—	+	104	170	0.72	8.4	0.51	
24	7	—	—	+	100	107	0.24	17	1.2	HSP
25	9	P	—	0	94	106	0.028	—	0.52	HSP
26	6	CGN	—	++	112	143	0.15	24	0.71	HSP
27	14	P	—	0	100	147	0.90	34	1.0	HSP
Recurrent haematuria										
28	15	MC	—	++	80	171	0.018	—	1.4	
29	6	MC	—	+	108	170	0.0057	—	<0.1	
Fanconi syndrome										
30	2	—	—	0	88	—	0.21	—	>150	Cystinosis
Haemolytic-uraemic syndrome										
31	4	HUS	—	++	116	4.6	14	61	75	

*MC, minimal change; FGS, focal glomerular sclerosis; CGN, chronic glomerulonephritis; P, proliferative glomerulonephritis; C, crescents; HUS, changes characteristic of haemolytic-uraemic syndrome.

†+, macroscopic; +, microscopic.

‡Plasma C3 expressed as percentage of reference serum. The range given for healthy subjects is that reported for children by Ngu and Soothill (1969).

§Strep, evidence of streptococcal infection (culture or ASO > 800 U/ml); ANS, acute nephritic syndrome at onset; HSP, Henoch-Schönlein purpura.

Results

The results of plasma fibrinogen levels and serum and urinary FDP levels are shown in Table II.

Fibrinogen. The plasma fibrinogen levels were either normal or raised in the children with renal disease. No clottable fibrinogen was detected in either fresh or concentrated urine.

Serum FDP. The child with the haemolytic-uraemic syndrome (Case 31) had the highest S_{FDP} titre. Two children with acute nephritis (Cases 13 and 14) and one with persistent glomerulonephritis (Case 17; acute nephritic onset) had S_{FDP} titres greater than the healthy adults; the remaining 27 children did not have raised S_{FDP} levels (Table II).

TABLE II
Plasma Fibrinogen and Fibrin Degradation Products in Sera and Urine

Case No.	Plasma Fibrinogen (mg/100 ml)	SFDP	UFDP (fresh)	U'FDP (concentrated)
<i>Healthy</i> (n = 6)				
Range of observations	155-187	1-4	<1	<1
<i>Nephrotic syndrome</i>				
1	186	2	<1	3
2	536	4	1	12
3	—	3	<1	4
4	459	3	<1	1
5	340	3	<1	4
6	588	4	<1	9
7	763	4	1	4
8	526	2	<1	16
9	839	4	12	20
10	526	3	2	17
11	479	4	2	13
12	454	4	<1	6
<i>Acute nephritic syndrome</i>				
13	541	5	1	11
14	—	5	1	8
15	278	4	<1	3
<i>Persistent glomerulonephritis</i>				
16	485	3	<1	4
17	273	5	<1	8
18	233	4	<1	6
19	665	4	<1	3
20	273	2	<1	6
21	349	3	<1	4
22	330	4	1	6
23	263	2	1	7
24	350	3	1	6
25	321	3	<1	5
26	342	3	2	13
27	289	2	2	16
<i>Recurrent haematuria</i>				
28	205	2	<1	5
29	324	4	<1	6
<i>Fanconi syndrome</i>				
30	209	3	<1	2
<i>Haemolytic-uraemic syndrome</i>				
31	356	6	9	25

Note: FDP are expressed as the logarithm (base 2) of reciprocal titres.

Urine FDP. FDP were detected in the concentrates of urine samples of all the children studied but in none of the urine concentrates of the 6 healthy adults (Table II). The range of titres observed in the concentrated urines (U'FDP) was so wide that corrections for urine flow rate or creatinine clearance would have little effect upon the interpretation of the data and have therefore been omitted.

No correlation between FDP in serum and concentrated urine was observed ($P > 0.1$; Spearman). However, the child with the haemolytic uraemic syndrome (Case 31) had the highest FDP titre in both serum and urine.

In the 24 children with moderate or heavy

proteinuria ($C_{alb}/C_{creat} > 0.2 + 10^{-3}$) there was a significant correlation between the selectivity of proteinuria, C_{IgG}/C_{alb} , and the FDP titres in urine concentrates, U'FDP ($P < 0.01$; Spearman): those with the least selective proteinuria had the highest titre of FDP (Fig. 1). In the 11 children with poorly selective proteinuria ($C_{IgG}/C_{alb} > 20\%$), U'FDP was significantly correlated with C_{alb}/C_{creat} ($P < 0.05$; Spearman): thus increased glomerular permeability for albumin is accompanied by increased excretion of FDP. No correlation between C_{alb}/C_{creat} and U'FDP was observed in patients with more selective proteinuria (Fig. 2). The 2 children (Cases 31 and 9) with poorly selective proteinuria and very high levels of U'FDP also had

FIG. 1.—The relation between urinary FDP in concentrated urine (U'_{FDP}) and the selectivity of proteinuria (C_{IgG}/C_{alb}) in children with moderate or heavy proteinuria ($C_{alb}/C_{creat} \geq 0.2 \times 10^{-3}$).

a considerable increase of lysozyme excretion. However, they had low levels of creatinine clearance and therefore raised plasma lysozyme: under such circumstances increased lysozyme excretion does not necessarily imply impaired tubular reabsorption of protein (Harrison *et al.*, 1968). Tubular proteinuria itself is not necessarily associated with a high value for U'_{FDP} as in the patient with the Fanconi syndrome (Case 30).

Median U'_{FDP} levels did not differ in patients with and without haematuria ($P > 0.1$; Wilcoxon). Some subjects, e.g. Case 22, who had heavy haematuria, had low levels of FDP in the urine.

Discussion

Raised FDP levels in the sera of patients with glomerulonephritis have been previously reported (Wardle and Taylor, 1968; Stiehm and Trygstad, 1969): the highest titres we observed were in 2 children with acute glomerulonephritis (Cases 13 and 14), in 1 child with persistent glomerulonephritis following an acute nephritic onset (Case 17), and in the child with the haemolytic-uraemic syndrome (Case 31).

The presence of material antigenically related to

fibrinogen has been shown previously in the urine of patients with renal disease (Rayner *et al.*, 1969; Clarkson *et al.*, 1970a,b). However, it has not been certain whether the excretion of fibrinogen and FDP exceeds that which might be expected on the grounds of proteinuria alone. Analysis of FDP excretion by classical differential protein clearance theory (Hardwicke *et al.*, 1970) is restricted by ignorance of its physicochemical characteristics, by the quantitative uncertainty of the TRCHI technique (Ekert, 1970a,b), and by the number of theoretically possible origins of urinary FDP.

Thus FDP may be produced intravascularly and excreted through normal glomeruli or damaged glomeruli with varying permeability for proteins of different molecular size and might be subject to tubular reabsorption. Alternatively, FDP may be released following deposition of fibrinogen in glomeruli and passage into Bowman's capsule, or intact fibrinogen might be filtered through the glomeruli and degraded by fibrinolytic systems present in normal urine. The correlation of urinary FDP with selectivity of proteinuria, and, within the poorly selective group, with albumin excretion, is directly compatible with the latter

FIG. 2.—The relation between urinary FDP in concentrated urine (U'_{FDP}) and albumin excretion (C_{alb}/C_{creat}). ● = $C_{IgG}/C_{alb} \geq 20\%$; ○ = $C_{IgG}/C_{alb} < 20\%$; X = C_{IgG}/C_{alb} not measured.

hypotheses, but does not exclude the former, since localized IVC may be more active in subjects with heavy or unselective proteinuria.

The lack of correlation of FDP in urine and serum in our group of patients, and the low excretion of FDP in the patient (Case 30) with the Fanconi syndrome and tubular proteinuria suggest that urinary FDP do not usually arise from circulating FDP of low molecular weight; if this were so, FDP excretion would be increased with high serum levels or impaired low molecular weight protein reabsorption.

Two patients had exceptionally high U'_{FDP} : one (Case 31) had the haemolytic-uraemic syndrome with raised S_{FDP} , but the other (Case 9), who had the most rapidly progressive glomerulonephritis in the group, had S_{FDP} at the upper limit of normal. These 2 children also had high levels of FDP in unconcentrated urine (Case 31 = 9, Case 9 = 12). The highest level of FDP in random urines in the other patients was 5.

It is possible that the urinary excretion of FDP may be a more sensitive reflection of glomerular fibrin deposition than raised titres of FDP in the serum, and provide a more rational basis for the selection of subjects who might benefit from anticoagulant therapy (Kincaid-Smith *et al.*, 1968; Herdman *et al.*, 1970; Kincaid-Smith *et al.*, 1970), but other interpretations of the data have not been excluded; further clarification necessitates improvement of the present techniques of measurement and knowledge of the physicochemical characteristics of the degradation products of fibrin in the urine of patients with renal disease.

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