# Section of Pathology

*President* W M Davidson MD

*Meeting March 21 1967*

# Pyelonephritis [*Abridged*]

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## Serum and Urine Proteins in Pyelonephritis

The proteinuria of pyelonephritis has been examined by three methods: electrophoresis on cellulose acetate, immunochemical differential protein clearance, and thin-layer chromatography on Sephadex G75. To obtain a clear picture of the electrophoretic fractions, urine had to be concentrated to protein levels approximately equal to those in the serum and this had to be done quickly to avoid bacterial action on the proteins. Freshly passed midstream urine was filtered (or centrifuged) and 10 ml in a syringe was forced through graded Millipore filters (excluding 0·45 and 0·2μ) so that all the debris, the pus cells and the majority of bacteria were removed. The cleared urine was then concentrated to 20–200μl in a collodion shell in a glass holder (Sartorius) using ultrafiltration, achieved in 1–2 hours by a water vacuum pump. This represents a concentration of $\times 500 - \times 50$, depending on the initial protein level ($<10-100$ mg/100 ml).

*Electrophoresis on Cellulose Acetate*

The concentrated urine was then applied on the same piece of cellulose acetate side by side with a fresh sample of the same patient's serum from blood collected at the same time as the urine. Electrophoresis in veronal buffer (0·05M, pH 8·6) took about one hour, whereby identical fractions in serum and urine ran the same distance. After fixation and staining (Hobbs 1965) the serum strip was scanned with an empirical setting of the scanner. The urine strip was then scanned on to transparent paper, adjusted so that the area under the albumin peak equalled that of the donor serum scan which was left lying underneath. In the left-hand example in Fig 1 is shown the glomerular proteinuria from a patient with glomerulonephritis. The donor serum (the broken line) shows a well-marked $\beta_2$-globulin fraction, confirming that little denaturation had occurred

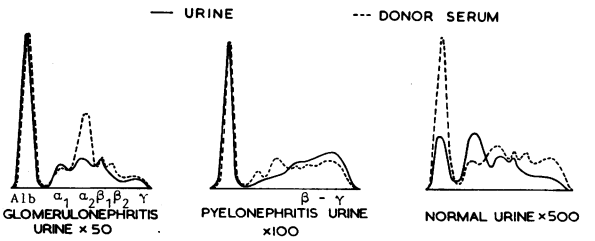


Fig 1 *Electrophoretic scans of concentrated urine superimposed over donor serum so that albumin areas are equal (except with normal urine). With V constant for the given urine sample, U/P for albumin becomes 1·0 and the globulin clearances can be visualized in relation to the albumin clearance. In glomerulonephritis the poorly selective glomerular leak shows that the clearances of the mainly small $\alpha_1$ molecules are slightly $>1$ and the mainly large $\alpha_2$ $<1$ of the albumin, with the β–γ region always $<1$. In pyelonephritis the β–γ clearance is apparently $>1$, and the pattern differs from the mainly $\alpha_1$, $\alpha_2$ pattern of normal urine*

during the 2–4 hours required for this whole procedure. After longer times than this the complement fraction of $\beta_2$-globulin denatures and migrates as $\beta_1$, invalidating comparisons of this latter fraction which would otherwise be mainly transferrin. The urinary pattern (the continuous line) is seen to be very similar to that of the serum though there appears to be some glomerular selection; there are more of the small $\alpha_1$-globulin molecules and less of the mainly large $\alpha_2$-globulin molecules present in the urine than in the serum.

For any substance the renal clearance of the plasma in ml/min can be derived using the formula UV/P where U and P are the urine and plasma concentrations of the substance and V the urinary output in ml/min.

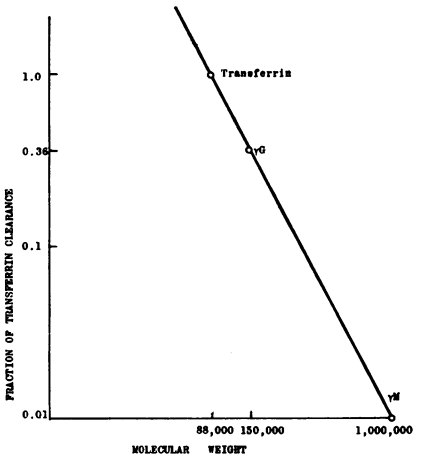


Fig 2 *The glomerulus as a molecular sieve. With γG clearance <0·36 of the transferrin clearance, γM clearance of glomerular origin can be expected to be <0·01 of the transferrin clearance*

For a given sample of urine, V is a constant so that the clearance of the different proteins in that sample may be expressed as U/P. By adjusting the albumin peak area to make U/P equal to 1·00 for albumin, the other protein clearances can be visualized as fractions of the albumin clearance. Thus in the above example $\alpha_1$ clearance is >1 and $\alpha_2$ clearance is <1. This example also shows that even in the most poorly selective glomerular proteinurias the β-γ clearance is always <1 of the albumin clearance, and this has been our experience in over 70 patients.

Tidstrom (1963) showed that in patients with pyelonephritis, paper electrophoresis of concentrated urine showed a definite predominance in the β–γ region. The central example in Fig 1 is representative of our results in 36 patients in whom a diagnosis of pyelonephritis was made from the presence of both pus cells and a pure growth of Gram-negative bacilli in the urine.

In 14 of these patients there was later histological confirmation of the diagnosis at operation or post-mortem. The pattern obtained is one of an excess of protein in the β–γ region where the apparent clearance related to albumin is >1. This pattern is only seen clearly when the total protein excretion is 0·1–1·0 g/day (*c.*10–100 mg/100 ml urine). At levels above this a nonselective pattern like the left hand example appears, presumably due to associated damage to the glomeruli.

We have seen β–γ excess in only 9 other patients without good evidence of pyelonephritis, though this group all had renal calculi. In 6 patients thought to have only cystitis, the pattern resembled the normal (right hand example in Fig 1) and no β–γ excess was seen.

*Differential Protein Clearance*

This approach was pioneered by Blainey *et al.* (1960) who measured protein levels by immunochemical methods and adjusted U/P to a transferrin clearance of 1·00. Fig 2 shows on double-log paper the plot of the clearance of a protein (as a fraction of the transferrin clearance) against the molecular weight of that protein. In a given patient, using a range of proteins, Blainey *et al.* found there was an approximately linear result, clearance decreasing with increasing molecular weight. After many others we have confirmed this and in 22 patients with the nephrotic syndrome where γG-globulin clearance has been 0·36 or less of the transferrin clearance the γM-globulin clearance has always been 0·01 or less of the transferrin clearance. In contrast our findings in 18 patients with pyelonephritis are shown in Fig 3. Despite a γG clearance 0·36 or less of the transferrin clearance, the γM clearance was much greater than would be expected from leaking glomeruli acting as a molecular sieve. Indeed in one patient with acute pyelonephritis, a baby aged 1 week in whom the serum level of γM was naturally very low, the clearance of γM was even greater than that of transferrin. Gel diffusion confirmed that this really was γM-globulin and not heavy chain fragments: our concentrated urines showed reactions of identity with pure γM-globulin, although in 2 patients some 20% of the reactivity was as faster diffusing fragments.

How does this γM excess arise? Selective reabsorption of the other proteins measured (trans-

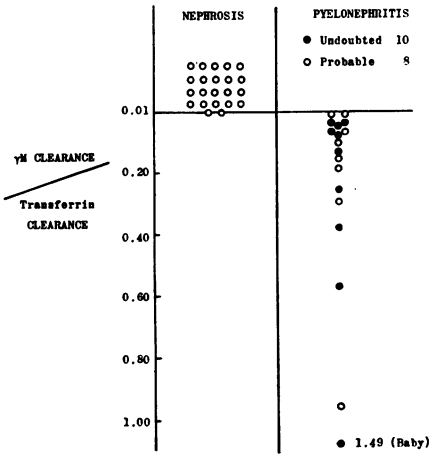


Fig 3 *γM/transferrin clearance contrasted in 22 patients with the nephrotic syndrome and 18 patients with pyelonephritis. Only those patients with γG/transferrin clearance <0·36 are included so that the γM/transferrin clearance through the glomerulus would be expected to be <0·01, showing that in pyelonephritis some other pathway seems probable*

ferrin, γG) may be possible but leakage of γM into the renal tract at a level below the glomerulus seems more likely in the presence of tubular inflammation. The presence of *Esch. coli* in the renal parenchyma is associated with increased antibody levels. These are presumably largely of γM-globulin since they are absent in newborn sera (γG only) (Schultze & Heremans 1966). It seems logical that in pyelonephritis γM antibodies might be synthesized by the interstitial plasma cells and might thereby gain the urine in greater amounts than if passed through the glomeruli. In absolute terms the quantity of γM is very small, 10–260 μg/100 ml of the original pyelonephritic urines, but γM antibody can be very effective at these levels (Robbins *et al.* 1965). It cannot account for the β–γ excess seen on electrophoresis which is reminiscent of some tubular proteinurias; thus concentrated urines have been subjected to the thin-layer analysis described by Davis, Flynn & Platt (personal communication).

*Thin-layer Chromatography on Sephadex G75*

The bottom line of Fig 4 shows the results obtained in 29 patients with the amount of tailing graded from 0 to +++ and illustrated above with four examples.

The two left-hand examples show tailing (++, +++) of the degrees seen in tubular proteinuria. These results were found in 17 of the patients with pyelonephritis, in 13 of whom the diagnosis had been confirmed histologically. Using antiserum kindly supplied by Dr Platt we have shown by immunoelectrophoresis that the tailing is due to low molecular weight proteins similar to those found in tubular proteinuria.

The right hand example of nephrotic urine (concentrated ×50 only) yielded a discrete spot with relatively no tailing (O) and urine from 8 patients with pyelonephritis (×100) showed a similar result. These 8 patients had the highest levels of urinary protein (80–180 mg/100 ml); glomerular leakage resulting from damage by ascending infection could have swamped any tubular pattern. The remaining example (of normal urine ×500) shows a slight degree of tailing (+) a result found in 4 of the patients with pyelonephritis and in the 6 patients thought to have mainly cystitis. These 10 showed very little proteinuria (5–15 mg/100 ml), a level at which any tailing is indistinguishable from that of normal urine. In urinary tract infections the finding of marked tailing (++, +++) may therefore be useful evidence of involvement of the renal tubules. With proteinuria <10 mg/100 ml and >100 mg/100 ml results are equivocal.

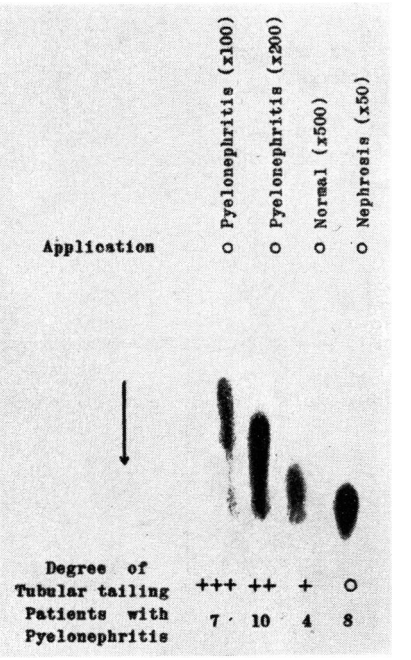


Fig 4 *Results of thin-layer chromatography of concentrated urines on Sephadex G75. Four examples above illustrate the degrees of tubular tailing and below are the results in 29 patients with pyelonephritis, 17 of whom showed marked tailing (++, +++) of the degree seen in tubular proteinuria. In urinary tract infections with proteinuria from 10–100 mg/100 ml this may be useful evidence of renal tubular involvement*

*Conclusions*

In summary, we have confirmed that in pyelonephritis, urine electrophoresis on cellulose acetate shows a distinctive β–γ excess which we have further shown to have an apparent clearance greater than that of albumin. The immunochemical clearance of γM-globulin is greater than would be expected through the glomeruli. The β–γ excess is due to the presence of proteinuria largely indistinguishable from the tubular type and thin-layer chromatography on Sephadex G75 offers a useful test.

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(1966) Molecular Biology of Human Proteins. London; **1**, 535
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The following papers were also read:

**Tests of Renal Tubular Function**
Dr O M Wrong
*(Royal Postgraduate Medical School, London)*

**Bacteriology and Serology of Urinary Tract Infection**
Dr W Brumfitt
*(St Mary's Hospital, London and Edgware General Hospital, Edgware, Middlesex)*

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(1965*a*) *Proc. roy. Soc. Med.* **58**, 783
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**Tubular Proteinuria**
Dr H S Platt
& Dr F V Flynn
*(University College Hospital, London)*

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**Davis J S, Flynn F V & Platt H S**
(1968) (in preparation)
**Flynn F V & Platt H S**
(1968) (in preparation)

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*Meeting June 24 1967*

A laboratory meeting was held at the Research Laboratories of the Imperial Chemical Industries Limited, Alderley Park, Cheshire. Demonstrations were given.

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*Meeting November 7 1967*

A laboratory meeting was held at St John's Hospital for Diseases of the Skin, London. Demonstrations were given.

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*Meeting December 5 1967*

A laboratory meeting was held at Guy's Hospital, London. Demonstrations were given.

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*Meeting February 13 1968*

A laboratory meeting was held at St Thomas's Hospital, London. Demonstrations were given.