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ASSESSMENT OF THE SELECTIVITY OF PROTEINURIA BY GEL FILTRATION

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ABSTRACT

The selectivity of proteinuria was assessed in 25 patients by a gel filtration technique utilising a G 200 Sephadex column. The results were compared with those obtained using gel diffusion and immunoprecipitation to assess selectivity. In five patients, where the protein excretion was less than 1.0 g. per day, the results obtained by gel filtration through Sephadex were found to be suspect. In the remaining 20 patients, where the protein excretion was over 1.0 g. per day, the relationship between the gel filtration selectivity and that determined by immunoprecipitation could be expressed as a straight line passing through the origin. The correlation coefficient for this line (0.84) was highly significant statistically.

The selectivities obtained on gel filtration were assessed in the light of the findings on renal biopsy. Two of the twenty patients with proteinuria of over 1.0 g. per day had minimal lesion glomerulonephritis. These patients also had the two highest selectivities in the series.

Three of the patients studied had chronic renal failure. These patients had the lowest, the second lowest, and the fifth lowest selectivities of the series.

Good renal function and minimal glomerular damage appeared to be associated with selective proteinuria, while poor renal function and severe glomerular damage appeared to be associated with unselective proteinuria.

Five patients with selective proteinuria were treated with steroids. In two cases the proteinuria disappeared. In a third it fell to trace amounts.

Ten patients with intermediate or unselective proteinuria were treated with steroids. In only one case was there a significant reduction of proteinuria.

INTRODUCTION.

Proteinuria may arise in a number of ways, but in the nephrotic syndrome it almost certainly results from increased permeability of the glomerular filter to substances of high molecular weight. (1 - 3).

Tubular reabsorption of filtered protein occurs, but this process is a non-specific one. The relative concentrations of the filtered proteins present in the urine are therefore the same as their relative concentrations in the glomerular filtrate. (1,2,4 - 7).

The pattern of proteinuria in various conditions may therefore be expected to yield information about the functional state of the glomerular filter in these conditions.

If the urine contains large amounts of low molecular weight protein and vanishingly small amounts of larger molecules, the proteinuria is said to be selective. Conversely, where relatively large amounts of high molecular weight protein are present in the urine, the proteinuria is said to be unselective. Since the amount of a given protein appearing in the urine depends on its plasma concentration as well as on the permeability of the glomerular basement membrane, selectivity must be assessed on the basis of the urine to plasma ratios of various proteins and not on the basis of urinary composition alone.

When the logarithms of the urine to plasma ratios are plotted against the logarithms of the molecular weights of the proteins concerned, a straight

line is obtained. (8,9,10,11). This lends support to the concept of graded pores being present in the glomerular basement membrane (1) - although no histological evidence of the existence of such pores has yet been obtained. In selective proteinuria, although the glomerular basement membrane is abnormally permeable to colloids, this increased permeability is restricted to the smaller colloid molecules. In unselective proteinuria, very large molecules pass with relative ease into the glomerular filtrate. Unselective proteinuria, therefore, implies a much more severe disruption of glomerular function and architecture than selective proteinuria. These considerations do not apply when a significant proportion of the protein present in the urine is derived from sources other than glomerular filtration. In the absence of haematuria, however, it appears that the measurement of the selectivity of proteinuria is a useful exercise in patients excreting over a gram of protein a day. Where the quantity of protein in the urine is less than this, the contribution of protein from the renal tubules, collecting ducts and bladder may be sufficient to mask the pattern of protein filtration and yield misleading information regarding the functional state of the basement membrane.

Bright and Bostock in 1827 recognised the association of proteinuria and renal disease. Qualitative and semi-quantitative studies on the nature of the proteins excreted have been carried out by many investigators using many techniques. Longsworth and McInnes in 1940 used free boundary electrophoresis (13). Electrophoresis on filter paper, cellulose acetate and in starch gel provided some information, as did the technique of immunoelectrophoresis (see review

by Lewis, L. A.)(14).

Accurate assessment of selectivity of proteinuria did not, however, become possible until the development of gel diffusion and immuno-precipitation (15) using specific antisera to determine urine to plasma ratios of a number of proteins.

Blainey, Brewer, Hardwicke and Soothill (1960) applied this technique to patients with the nephrotic syndrome. They determined the clearances of a number of proteins relative to transferrin and expressed their results by plotting the logarithm of the relative clearances of proteins against the logarithm of their respective molecular weights. The slope of the straight line obtained was steep in selective proteinuria and flat in unselective proteinuria (8).

Hardwicke and Soothill (1961) showed some correlation between the selectivity of proteinuria and the findings on renal biopsy, in that patients having minimal lesion glomerulonephritis had a proteinuria which was much more selective than that found in patients with established membranous or proliferative glomerulonephritis (1).

This work was repeated in America by Joachim, Cameron, Schwartz and Becker. This group concluded that the response to steroid therapy was significantly better in patients with selective proteinuria than in patients with an unselective pattern (9).

Cameron and White (1965) correlated the selectivity of proteinuria with the appearances on renal biopsy in children with the nephrotic syndrome.

While proteinuria in children appeared on the whole much more selective than in adults, severe histological abnormality was found to be associated with proteinuria of low selectivity (10).

Studies in Edinburgh point to the same conclusion (16).

Immunological techniques may be criticised on the grounds that degradation products, differing in molecular weight from their parent molecules, may give positive reactions with specific antisera. The antisera vary in potency, are difficult to prepare, and are not widely available.

Since the correlation of the selectivity of proteinuria with the appearances on renal biopsy can be expected to yield valuable information regarding the mechanism of proteinuria, and since the assessment of selectivity has diagnostic and prognostic significance, it is important to compare the results obtained by immunological clearance studies with these obtained by other methods.

This paper describes the use of a G 200 Sephadex gel in the fractination of the proteins of serum and urine and in the determination of the selectivity of proteinuria. G 200 Sephadex is a high molecular weight cross-linked dextran polymer. In aqueous suspension it forms a porous gel. If a protein-containing solution is passed through a column packed with this gel, high molecular weight substances will be eluted first, and low molecular weight substances last. This is because large colloid molecules (such as B lipoprotein) are excluded entirely from the interstices of the gel. Confined

to the fluid outside the Sephadex molecules, they are eluted in a small volume. Small molecules pass freely within the interstices of the gel and require a much larger volume of eluant to wash them from the column. With molecules of intermediate size, intermediate volumes of eluant are required (17).

Gel filtration thus separates molecules into fractions according to their molecular size. The limits of resolution for G 200 Sephadex are from 30,000 to 300,000 molecular weight. Within these limits, the elution volume for a substance is inversely proportional to the logarithm of the molecular weight of that substance (17, 20, 21).

MATERIAL AND METHODS.

Selectivity of proteinuria was assessed by gel filtration on G 200 Sephadex in 25 patients.

The diagnosis was established clinically and by renal biopsy in 24 patients and clinically in the remaining 1.

For a general account of the use of dextran gels in gel filtration the monograph by Flodin (1962) should be consulted (17).

The column used in these experiments was 100 cm. in height and 300 ml. in capacity. Constant volume aliquots of eluate were obtained using a Gallenkamp OA Ecenomia fraction collector and a 3 ml. syphon. The eluant was 0.1M tris buffer in 0.1M saline, pH 8.0. The column was operated at a pressure head of 10 to 30 cm. of buffer giving a flow rate of between 12 and 30 ml. per hour.

Aliquots of serum or urine containing 60 to 80 mg. of protein were applied. Serum was used neat. Urine was concentrated prior to application by dialysis through cellophane against polyethylene glycol till its protein content was between 30 and 70 mg./ml. (20). The urine concentrate was brought to 0.58M with hypertonic saline to precipitate Tamm Horsfall protein (18) and then centrifuged before application of the supernate to the column. As column characteristics tended to change slightly with time, serum and urine from the same patient were generally run successively.

The protein concentration of each fraction collected was determined by reading the optical density of 280 mp against a tris buffer blank. As a check on the specificity of the method, repeat determinations were done in some cases on an autoanalyser using a Folin and Ciocalteau method. The creatinine clearance, the total urine protein and the immunological selectivity of the proteinuria were assessed on each patient.

CALCULATION OF SELECTIVITY.

Optical density of 280 mp was plotted against tube number for serum and urine.

The urine to plasma ratio of protein concentration for each tube was calculated and (for arithmetical reasons) multiplied by 100. The logarithm of $\frac{100\ U}{P}$ was then plotted against tube number. Since the relationship between tube number and molecular weight is a log/log one (17), this was in effect a plot of the logarithm of the urine to plasma protein ratio against the logarithm of protein molecular weight. It approximated well to a straight

x axis - Tube Number (3ml fractions)

y axis - Optical Density at 280 mμ.

Nephrotic Serum run on G 200 S.
Showing predominance of high molecular weight protein.

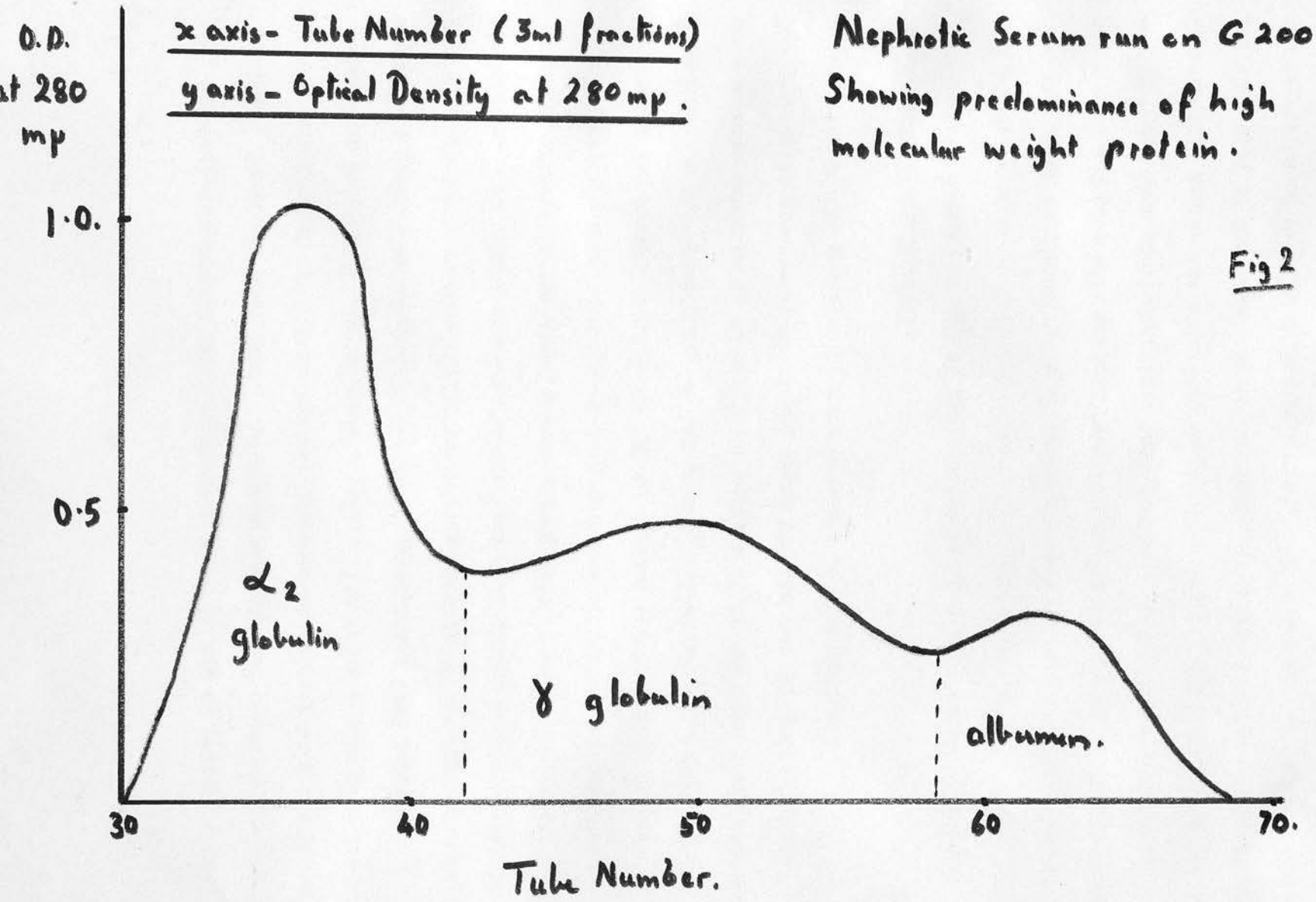


Fig 2

y axis - Optical Density at 280mμ.

x axis - Tube Number. (3ml fractions)

Normal Serum run on G 200 Sephadex
Showing protein separation into 3 peaks.

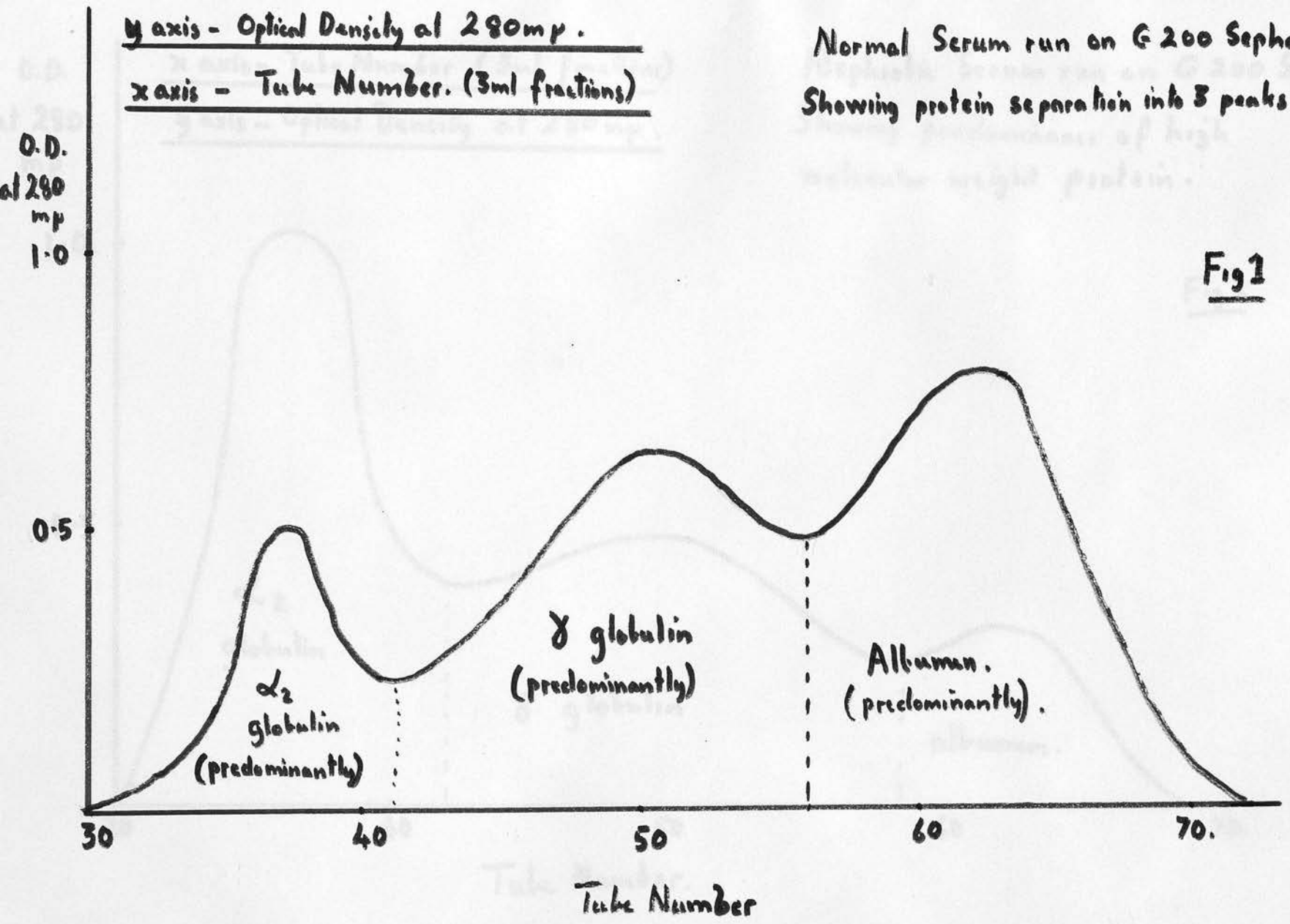


Fig 1

line in most cases.

The plot of optical density against tube number for a serum run yields three peaks with two intervening troughs (see Fig. 1). The first peak represents predominantly α_2 globulin, and the third albumin. Since G 200 Sephadex resolves poorly outside the range MW 300,000 to MW 30,000, only these tubes lying between the zenith of the first peak and the zenith of the third were taken into account in assessing selectivity (18).

The slope of the line $\log_{10} \frac{100\ U}{P}$ against tube number between these points was calculated by the method of least squares. This slope is proportional to the selectivity of the proteinuria, but is also affected by the number of tubes occurring between the apices of the first and third peaks. It is therefore an empirical index, and will vary greatly from column to column.

The following formula was therefore used to calculate selectivity:-

Let $y = \text{Log}_{10} \frac{100\ U}{P}$

Let x = Tube number

Then $\frac{dy}{dx}$ = slope of the plot of x against y.

(change in y per unit change in x)

Let Δ = total change in y between apex of first peak and apex of third peak.

If n = number of tubes between first peak and third peak

Then $\Delta = n \frac{dy}{dx}$.

Δ was taken as the index of selectivity in these experiments.

METHODS (Contd.)

RECOVERY OF ADDED PROTEIN.

Measured aliquots of pooled sera of known protein content were passed through the column. The optical density of each aliquot of eluate was measured. Serial dilutions of the same sera were made in tris buffer and used to construct a standard curve. The mean protein concentration of the samples of eluate collected multiplied by the total volume collected gave the amount of protein recovered from the column. The recoveries obtained in five such experiments ranged from 101 to 105%, the mean being 102.6%.

VARIABILITY OF ELUTION PATTERN.

With the column used, protein was eluted in tubes 31 to 77. If the layering of the sample on to the top of the column was carelessly done, the protein tended to spread over a larger number of tubes.

The position of the first peak varied from tube 37 to tube 41. The position of the second peak varied from tube 48 to tube 52. The position of the third peak varied from tube 61 to tube 65. For successive runs, however, the position of the protein elution peaks did not vary by more than one tube. Since the specimens of serum andurine used to calculate selectivity were run successively, variation in elution pattern did not produce any serious error in the determination of Δ .

Each time the column was packed, a standard serum was run to check its performance. Occasionally the separation obtained was found to be poor.

The column was then re-packed and re-checked before being used.

DUPLICATE DETERMINATIONS.

Five determinations of selectivity were done in duplicate. The results obtained agreed to within $\pm$ 7%. Since the error of the method is of this order, the results were expressed to two significant figures.

LIPAEMIC SERA.

When lipaemic sera were passed through the column, the first half dozen tubes containing protein were turbid and gave spuriously high results when protein content was assessed at 280 mp. Where possible, therefore, sera were obtained from fasting patients. With some outpatients fasting blood could not be obtained. Where turbidity was detected naked eye in the eluate, the calculated Δ was rejected, and the estimation repeated with a fresh pair of specimens.

CREATININE CLEARANCES.

These were determined in the Clinical Chemistry Department of Edinburgh Royal Infirmary by an autoanalyser method utilising the Jaffé reaction.

TOTAL URINE PROTEIN.

These were also determined in the Clinical Chemistry Department, using the Buiret reaction.

Pt No	Age	Sex	Diagnosis	△	Mean Tan θ	Protein in Urine in 24hr	Creat. Clearance
16	32	F	Lupus	1·5	1·8	10·0	98
17	51	F	Lupus	0·95	1·3	2·1	44
18	27	F	Lupus	0·89	1·6	6·6	163
19	59	M	Amyloid	1·92	2·9	24·0	88
20	29	F	Amyloid	0·90	2·4	0·8	143
21	54	F	Mixed	1·2	2·4	19·2	75
22	20	M	Hydronephrosis	1·0	1·8	5·0	3
23	50	F	Diabetic	0·78	2·2	<1	94
24	46	F	??Minimal	0·64	3·0	<1	155
25	41	F	Focal, ?aetiology.	0·54	1·6	<1	95.

Table 1 (Continued)

Pt. No	Age	Sex	Diagnosis	Δ	Mean Tan θ	Prot in 24hr	Cr Cl. (ml/min)
1	43	M	Minimal	2·5	3·2.	1·6	130
2	62	F	Minimal	2·3	2·7	3·5	86
3	50	F	Minimal	1·2	3·2.	<1	120
4	51	M	Membranous	0·64	1·0	3·4	7
5	52	F	Membranous	1·1	1·9	1·6	77
6	33	M	Membranous	1·64	1·7	5·0	170
7	62	F	Membranous	1·2	2·4	10·0	66
8	58	M	Membranous	1·3	1·7	11·0	95
9	21	M	Membranous	1·9	2·0	21·0	121
10	32	M	Proliferative	0·38	1·2	6·3	15
11	55	M	Proliferative	1·4	1·7	14·2	87
12	18	M	Proliferative	1·3	1·4	4·5	127
13	57	F	Proliferative	1·8	2·7	1·7	129
14	66	F	Proliferative	1·9	2·8	1·5	53
15	28	F	Proliferative	1·3	2·2.	5·5	128

Table 1.

continued over page.

IMMUNOPRECIPITATION SELECTIVITIES.

These were determined by Mrs. P. MacLean (*). Her method was a modification of that used by Soothill (8).

The clearances relative to albumin of transferrin, γ globulin, γ globulin, B lipoprotein and α_2 macroglobulin were plotted logarithmically along the y axis against the logarithms of the molecular weights of these proteins on the x axis. The angle made by this line with the x axis was denoted as θ, and tan θ (the slope of the line) was used as the index of selectivity.

Dr. J. S. Robson performed the renal biopsies and Dr. M. MacDonald interpreted the sections obtained in each case.

RESULTS.

Table 1 shows the age and sex of each patient and the diagnosis, based on renal biopsy in all but one case. It also shows -

1. The selectivity obtained by gel filtration (Δ).
2. The selectivity obtained by immunoprecipitation (tan θ).
3. The creatinine clearance.
4. The total protein excreted in 24 hours.

The selectivities determined by gel filtration ranged from 0.38 to 2.5.

Excluding the patients who excreted less than 1.0 g. per day of protein in the urine (see below), the mean selectivity for the remaining 20 on

(*) Graduate Research Assistant (S.H.E.R.T.) to Dr. J. S. Robson

gel filtration was 1.4, S.D. $\pm$ 0.5.

The following arbitrary classification was devised:-

Selective proteinuria - Δ = 1.8 and upwards.

Unselective proteinuria - Δ = 1.0 and downwards.

Intermediate proteinuria - Δ = 1.1 to 1.7.

COMPARISON of GEL FILTRATION SELECTIVITY WITH THAT OBTAINED BY IMMUNOPRECIPITATION.

Figure 1 shows the plot of Δ against tan θ for the 25 patients studied. The best straight line ($y = mx + c$) calculated by the method of best squares was

$$\Delta = 0.61 \tan \theta + 0.19.$$

The scatter about this line was fairly wide but there was nevertheless a strong correlation between the results obtained by the two methods.

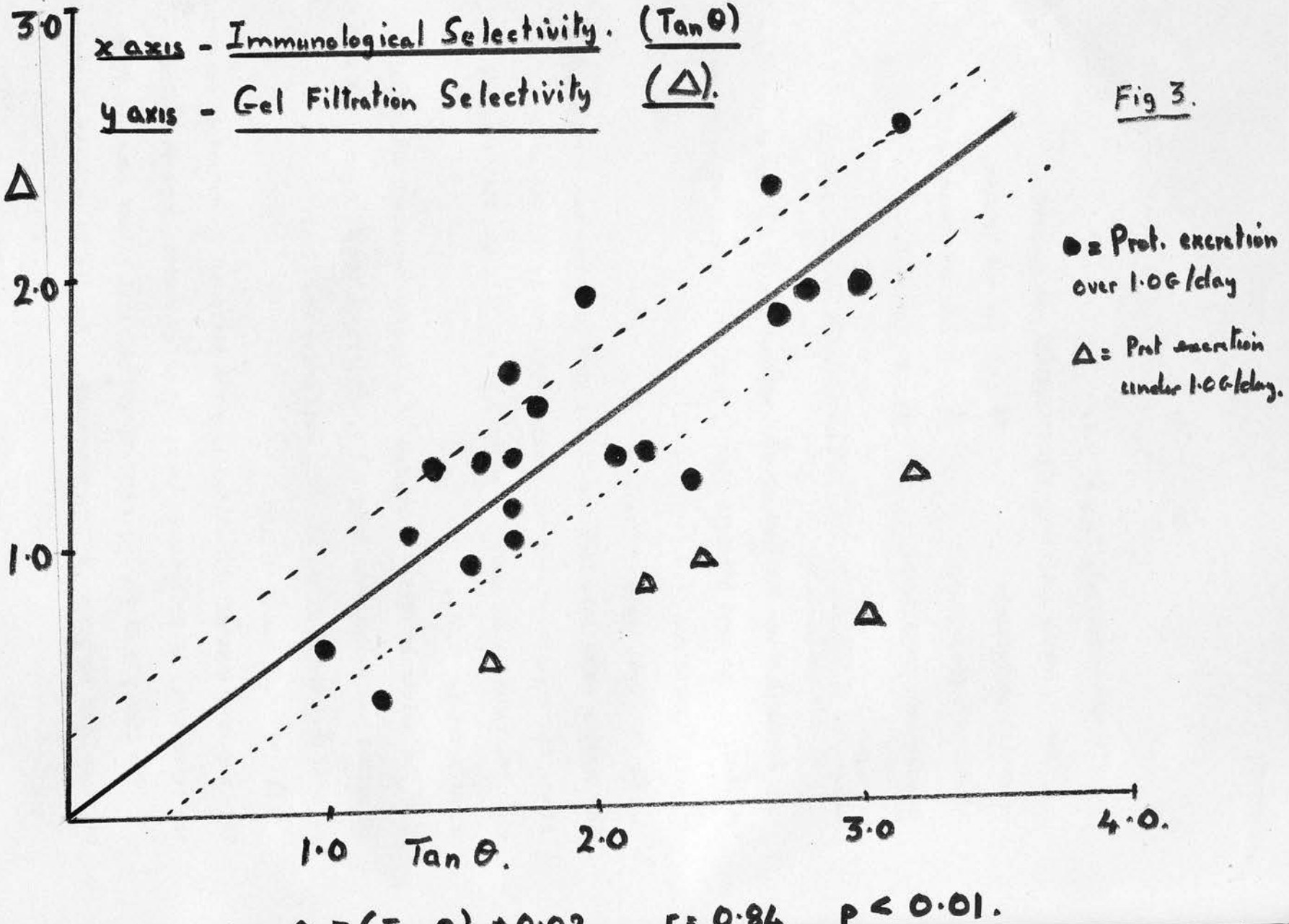
The correlation coefficient was 0.72 and this was statistically significant (p 0.01).

The values in Figure 1 denoted by triangles represent determinations on urines containing less than 1.0 g. of protein per 24 hours. It will be seen that these points all fall well below the line.

$$\Delta = 0.61 \tan \theta + 0.19.$$

This suggests that gel filtration is not a satisfactory method of determining the selectivity of proteinuria where the urine protein content is low.

The line $y = mx + c$ was recalculated omitting values obtained from patients with low urine protein excretion. For the 20 patients considered


x axis - Immunological Selectivity. (Tan θ)
y axis - Gel Filtration Selectivity (Δ).
Fig 3.
3·0
Δ
2·0
1·0
● = Prot. excretion over 1·0 G/day
Δ = Prot excretion under 1·0 G/day.
1·0
Tan θ.
2·0
3·0
4·0.
Δ = 0·7 (Tan θ) + 0·02. r = 0·84 p < 0·01.

Caption to Figure 3.

The Selectivity as determined on G200 Sephadex (Δ) is plotted against the immunological Selectivity, ($\tan \theta$).

The value taken for $\tan \theta$ represents the mean of between 3 and 6 determinations in each case.

The best straight line for the whole series ($\Delta = 0\ 61 \tan \theta + 0\ 19$) is not shown, the line plotted being the one obtained when the patients excreting less than 1 0 G /24 hr. are excluded from the calculation.

in this calculation, the best straight line was found to be

$$\Delta = 0.70 \tan\Theta + 0.01.$$

The correlation coefficient was 0.84 and this was again highly significant statistically.

CORRELATION OF SELECTIVITY (Δ) WITH RENAL PATHOLOGY, MINIMAL LESION.

In three patients renal biopsy showed the changes of minimal lesion glomerulonephritis (Patients 1-3, Table 1).

Patient 3 had only a trace of protein in the urine at the time of the determination. No conclusions can therefore be drawn from the of 1.2 obtained in this case.

In patient 1 Δ was 2.5.

In patient 2 Δ was 2.3 and 2.3 = 2.3.

These selectivities were the highest recorded in the entire series and lend support to the impression gained from immunological studies that in minimal lesion glomerulonephritis the proteinuria is highly selective.

In all three patients, proteinuria was abolished by steroid therapy.

In patients 1 and 2 proteinuria returned on the withdrawal of steroids to disappear again after the reinstitution of therapy.

MEMBRANOUS GLOMERULONEPHRITIS. (Patients 4-9, Table 1).

In six patients renal biopsy showed the changes of membranous glomerulonephritis.

Patient 4, whose selectivity was 0.64, had advanced renal damage and

a blood urea of about 200 mg. %.

In the remaining five patients Δ ranged from 1.1 to 1.9 with a mean of 1.42.

Steroid therapy had been tried at one time or another in all these patients but all continued to have proteinuria of 3.0 g. or more daily.

PROLIFERATIVE GLOMERULONEPHRITIS. (Patients 10-15).

In six patients the renal biopsy showed the changes of proliferative glomerulonephritis.

Patient 10 had a selectivity of 0.38. He had malignant hypertension and a blood urea of over 100 mg. %.

In the remaining five patients Δ ranged from 1.1 to 1.9 with a mean of 1.51.

Patients 11 and 12 (Δ 1.3 and 1.3 respectively) were treated with steroids but continued to have major proteinuria - 15.0 g. per day in patient 11, and 6.5 g. per day in patient 12.

Patient 13 (Δ = 1.8) was also treated with steroids. She improved slightly, her proteinuria falling from 4.0 g. per day to 1.5 g. per day.

Patient 14 (Δ = 1.9) improved markedly on steroids, a proteinuria of over 20.0 g. per day falling to 0.5 g. per day.

Although the numbers are small, these findings seem to suggest that within the group of patients with proliferative glomerulonephritis, those with selective proteinuria (Δ 1.8 or higher) tended to do relatively well on steroids.

(Patient 15 (Δ 1.3) was not treated with steroids).

RENAL LUPUS. (Patients 16-18, Table 1).

In three patients renal biopsy showed the changes of renal lupus. All were female. Patients 16 and 17 had rheumatoid arthritis.

Patient 16 (Δ 1.5) responded to steroids with a clinically useful diuresis and a reduction in proteinuria from 10.0 g. per day to about 2.0 g. per day.

Patients 17 and 18 (Δ 0.95 and Δ 0.89 respectively) showed no quantitative change in protein excretion with steroid therapy.

RENAL AMYLOIDOSIS. Patients 19 and 20).

In two patients renal biopsy showed the changes of amyloidosis.

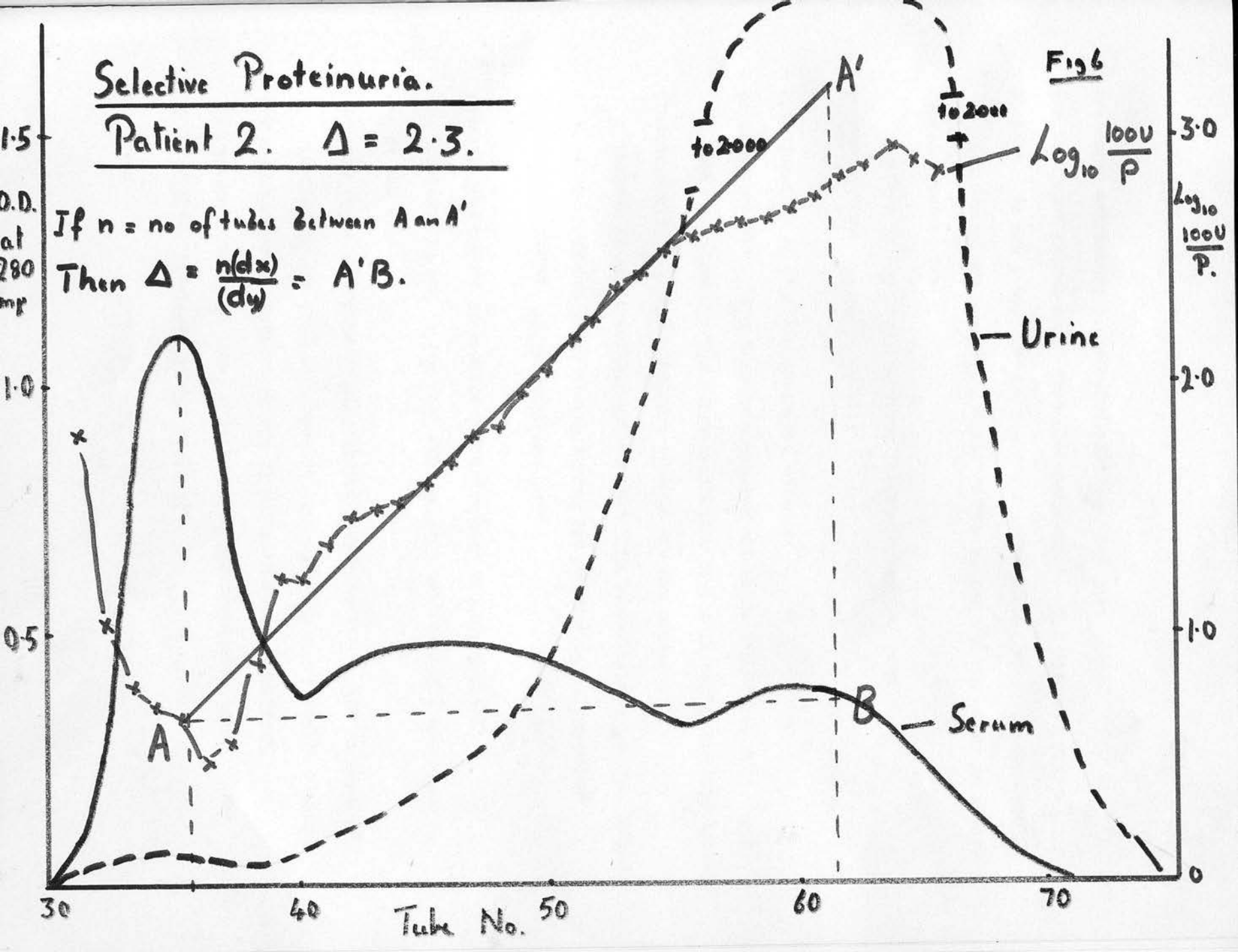
Patient 19 had a selectivity of 1.9. No primary cause for the amyloid disease could be found. Steroid therapy was not undertaken.

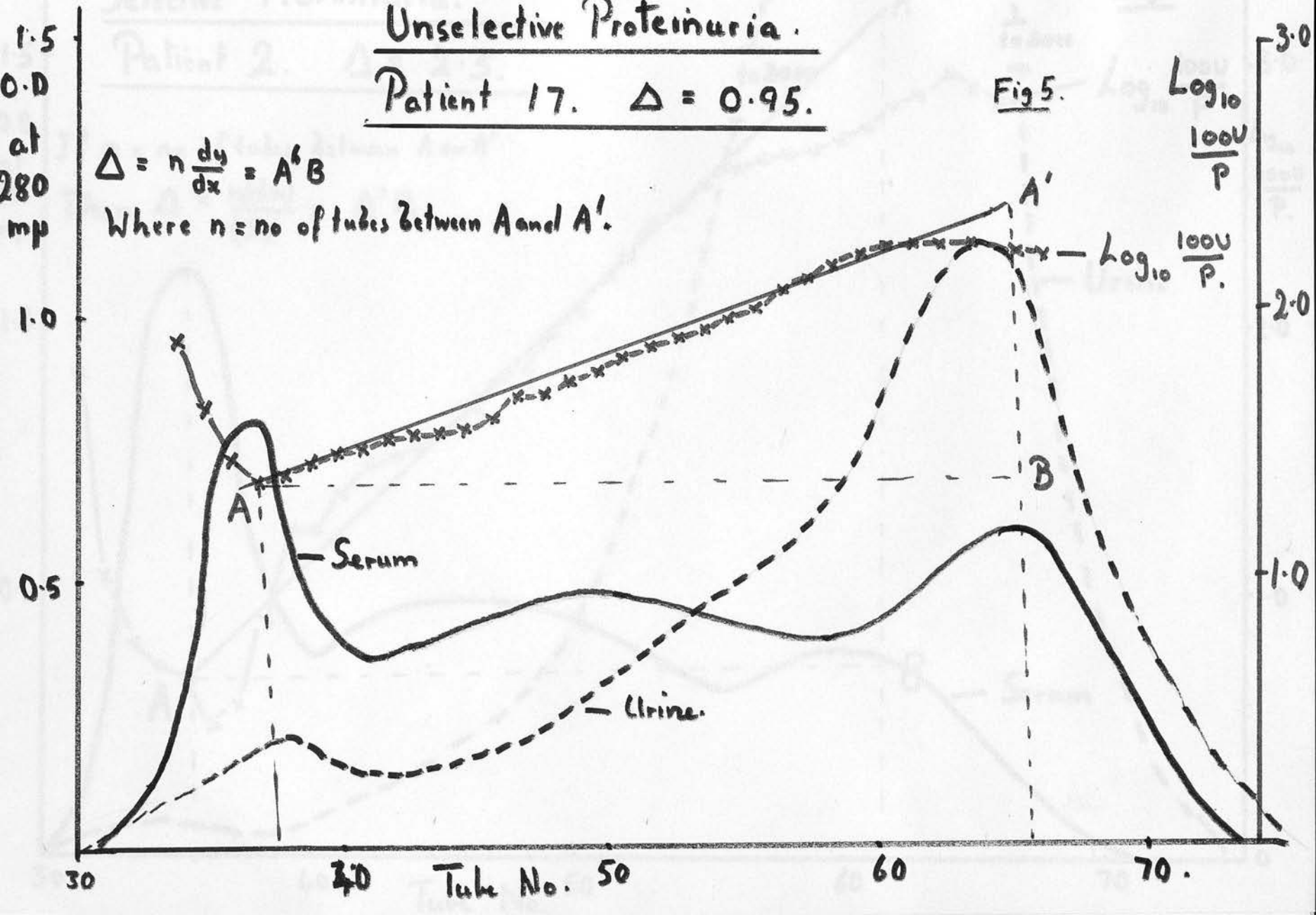
Patient 20 had chronic dental abscesses with very slight amyloid changes on renal biopsy. The protein excretion was under 1.0 g. per day. The calculated Δ of 0.90 was therefore suspect.

MISCELLANEOUS. Patients 21-25).

Patient 21 (Δ 1.2) had mixed proliferative and membranous glomerulonephritis.

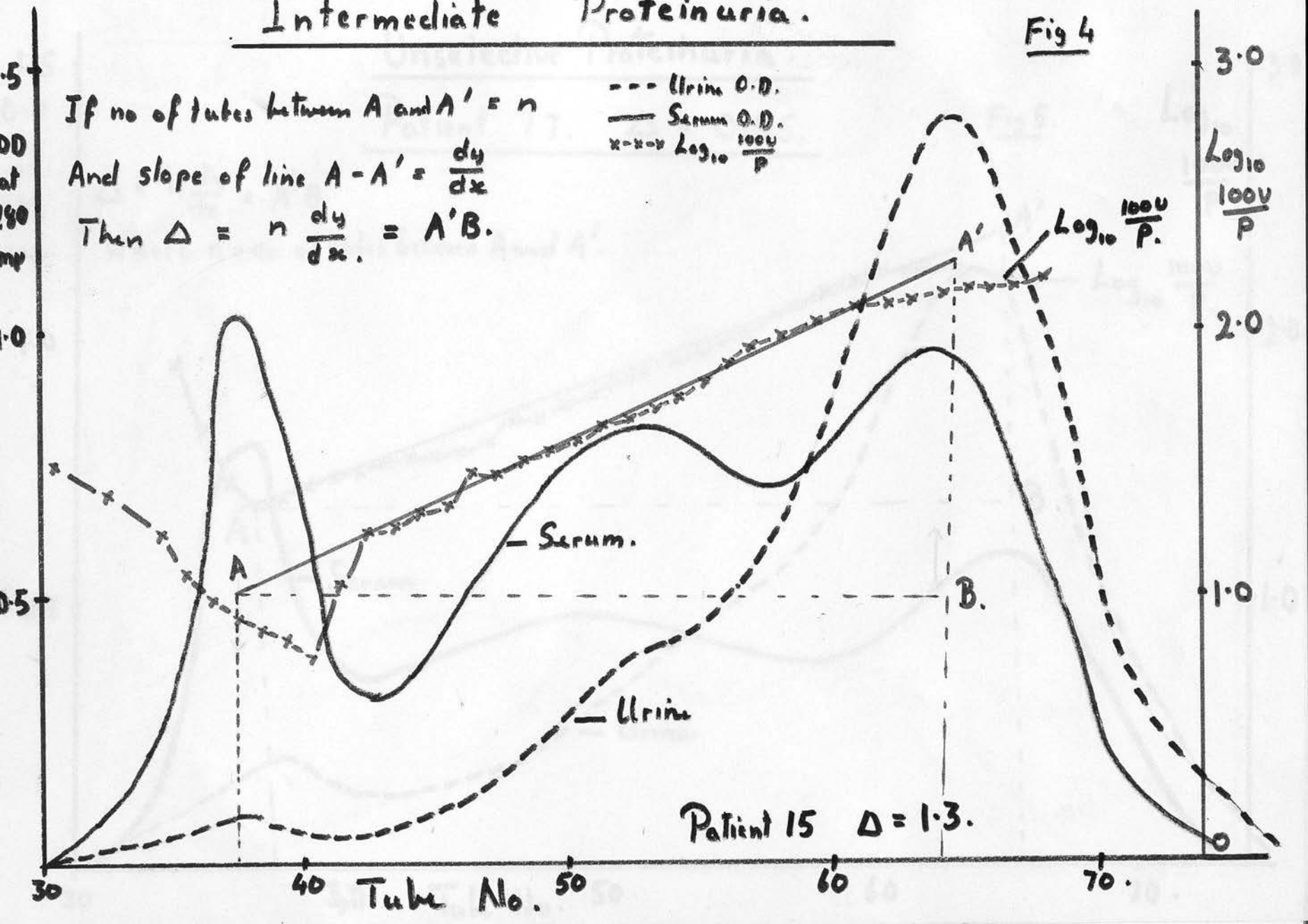
Patient 22 was the only patient in the series not subjected to renal biopsy. He was a patient who had had a nephrectomy for hydronephrosis, the remaining kidney being also hydronephrotic. His clinical history suggested superadded acute nephritis. Δ was 1.0.


Selective Proteinuria.
Patient 2. Δ = 2·3.
Fig 6
If n = no of tubes between A and A'
Then Δ = n(dx)/(dy) = A'B.
A'
A
B
1 to 2000
1 to 2000
Log10 100U/P
Urine
Serum
1·5
1·0
0·5
0
3·0
2·0
1·0
O.D. at 280 mμ
Log10 100U/P.
30
40
50
60
70
Tube No.


Unselective Proteinuria.
Patient 17. Δ = 0·95.
Fig 5.
Δ = n dy/dx = A'B
Where n = no of tubes between A and A'.
Log10 100V/P
Log10 100V/P.
A'
A
B
Serum
Urine.
1·5
O·D
at
280
mμ
1·0
0·5
3·0
2·0
1·0
30
40
Tube No. 50
60
70.

Intermediate Proteinuria.

Fig 4



Caption to Figures 4, 5 , and 6.

These figures show:-

1. The plot of optical density against tube number for serum.

2. The plot of optical density against tube number for urine.

3. The plot of $\log_{10}\frac{100U}{P}$ against tube number.

In the calculation of the best straight line for $\log_{10}\frac{100U}{P}$ against tube number, only those points between the first protein peak (A) and the third (A') are taken into account.

The slope of this line multiplied by the number of tubes between A and A' gives Δ.

AB is a line parallel to the X axis meeting the perpendicular from A' at B. A'B represents Δ.

Patients 23, 24 and 25 had trace amounts of protein in the urine only and the calculated Δ s (0.78, 0.64 and 0.54 respectively) were therefore suspect. Patient 23 had diabetic nephrosclerosis.

In patients 24 and 25 the renal biopsy findings were inconclusive.

PATIENTS WITH CHRONIC RENAL FAILURE.

Patients 4, 10 and 21 had renal failure, the blood urea being 100 mg. % or more and the creatinine clearance 15 ml./min. or less in each case.

The selectivities were 0.68, 0.38 and 1.00 respectively.

These selectivities were the lowest, the second lowest and the fifth lowest of the series (excluding the patients with less than 1.0 g. per day of proteinuria).

The two other patients with Δ s of under 1.0 were cases 17 and 18 (Δ 0.95 and 0.85 respectively). These patients had good renal function but biopsy showed severe glomerular damage.

These findings suggest that patients with severe glomerular damage tend to have unselective proteinuria.

DISCUSSION.

The method of immuno-precipitation differs markedly from the method of gel filtration.

Immunological clearance slopes are based on the urine and plasma concentrations of half-a-dozen antigenically pure proteins.

With the gel filtration technique all the proteins in the serum and

urine which are eluted between the first peak and the third peak contribute to the calculated selectivity. Each fraction collected from the column contains a mixture of proteins. The early tubes contain a preponderance of high molecular weight proteins; the later ones contain a preponderance of smaller molecules. At no point is the urine to plasma concentration ratio of a pure substance of known molecular size determined.

Both methods have errors inherent in them. Immunology is unable to distinguish between the parent protein molecule and degradation products of varying size which retain their antigenic specificity. Such degradation products occasionally occur in significant amounts. For example, substances in the urine with the immunological characteristics of β globulin (M.W. 170,000) have been shown by gel filtration to have molecular weights in the region of 40,000 (MacLean, personal communication).

Gel filtration can separate proteins according to molecular size, but cannot distinguish between proteins of urinary tract origin and those of serum origin. This fact is probably responsible for the unreliability of results where the total urine protein is less than 1.0 g./day. Where the total protein content of the urine is high, the contribution of renal tract protein appears to be quantitatively of little importance.

Despite the fact that the two methods used to determine selectivity differ so fundamentally, the relationship between their results could be expressed by a straight line passing through the origin and having a correlation coefficient which was statistically highly significant.

This indicates that selectivity is a real parameter of renal function and not simply a mathematical artefact.

Only two patients with minimal lesion glomerulonephritis were studied, but the Δ s obtained in these patients were higher than those found in any of the others in the series. Studies by other workers also indicate that selectivity is high in minimal lesion disease.

Since minimal lesion glomerulonephritis can be cured by steroid therapy in a majority of cases while its efficacy in other forms of the nephrotic syndrome is debatable, the detection of this condition is of obvious therapeutic importance. The diagnosis is best made by renal biopsy.

Selectivity determinations by either method may well prove useful as a screening procedure in the diagnosis of minimal lesion glomerulonephritis however. Blood and urine specimens could be sent to renal units from hospitals not possessing facilities for renal biopsy and, if the selectivity was found to be high, the patient could then be transferred for further investigation.

Joachim et al state that patients with selective proteinuria do relatively well on steroid therapy (9). The results obtained by Cameron and White (10) and by Robson, MacDonald and MacLean (16) are consistent with this viewpoint. All the above workers used immunological techniques.

The findings on gel filtration in this study point in the same direction.

Five patients with a selectivity of $\Delta = 1.8$ or more were treated with steroids. In three the proteinuria fell to trace levels with complete

clinical remission of the nephrotic syndrome.

Ten patients with a selectivity of Δ = 1.7 or less were treated with steroids. Significant proteinuria persisted in all ten but in one patient a good clinical response with loss of oedema and reduction in proteinuria from 10.0 g. per day to 2.0 g. per day occurred.

No attempt was made to conduct a controlled study and the numbers are small. Nevertheless, patients with selective proteinuria did appear to do better on steroid therapy than those with intermediate and unselective proteinuria.

It will be important to investigate this impression more fully as it may well be the case that a selective proteinuria is a relative indication for steroid therapy irrespective of the renal biopsy findings.

An interesting physiological point also remains to be resolved. In patients with proteinuria, good renal function and minimal glomerular damage correlate well with a high selectivity. One would therefore expect the proteinuria of normal people to be selective also. This point has not been studied with Sephadex, since the proteinuria of normal people is of the order of 30 mg./day or less. Immunological methods, however, give low selectivities in normal people, and patients with minimal lesion proteinuria on treatment with steroids change from a highly selective pattern of protein excretion to an unselective one as frank proteinuria disappears (16). This suggests that the "pores" of the normal glomerulus are relatively few in number (since proteinuria in normal people is minimal) and relatively large

in diameter (since the proteinuria seems unselective). It may, on the other hand, simply indicate that immunological selectivities (like Sephadex selectivities) are of limited validity where the urine protein content is low.

The use of a colloid other than protein to assess this situation is indicated. Dextran molecules of various sizes have already been used in this context. These substances have the advantage of not being reabsorbed in the tubule. Present techniques depend on pure dextrans of known molecular weight. (19)

Since gel filtration will fractionate dextran mixtures according to molecular size, G 200 Sephadex should be of use in the determination of selectivity using a mixture of dextrans of different molecular weights, and the precise composition of this mixture need not be known. Thus G 200 Sephadex may be applicable to the study of the selectivity of colloid filtration by the normal glomerulus.

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