

Hepatitis B Virus Associated Nephropathy:

**A clinico-pathological study of patients presenting to the
Red Cross War Memorial Children's Hospital.**

By

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INTRODUCTION:

The nephrotic syndrome is probably the most common chronic renal illness in children. In Africa the nature of the renal lesions differs from the pattern seen in temperate areas¹. The main difference between Africa, and Europe and North America is the much higher incidence of glomerular pathology associated with infectious agents such as Lancefield group A streptococci, malaria and syphilis². Since the early 1980's there has been an increasing awareness of children with nephrotic syndrome and concurrent hepatitis B virus (HBV) infection. This study was undertaken to investigate this phenomenon as seen at the Red Cross War Memorial Children's Hospital.

Early descriptions:

In 1971 Combes et al³ described a 53 year old man who sustained severe injuries in a motor vehicle accident. He was resuscitated with 4 units of blood and 4 months later developed acute hepatitis. A year and a half after the accident he developed the nephrotic syndrome. His serum was found to be positive for hepatitis B surface antigen (HBsAg). Renal biopsy showed membranous glomerulonephritis. Immune deposits were positive for HBsAg using an indirect immunofluorescent technique.

This was the first description of the hepatitis B virus (HBV) as a causative agent of glomerulonephritis. Since then several reports have been published⁴⁻¹¹. Most authors have concentrated on the relationship of hepatitis B virus to membranous nephropathy (MGN), but there does seem to be a relationship with mesangiocapillary glomerulonephritis (MCGN) as well^{12,13}.

The first South African report of a possible association between hepatitis B virus infection and glomerular disease was published in 1973¹⁴. Ten years later Wiggelinkhuizen et al published their series of 25 children with MGN associated with HBV infection seen at the Red Cross War Memorial Children's Hospital.⁸ This paper was important for several reasons: Firstly it raised the level of awareness to the relatively high incidence of the condition in this country, and secondly it supported the relationship between resolution of the

nephrotic state and clearance of the hepatitis B e antigen (HBeAg) from the circulation.

Controversy regarding causality:

After the initial case report, papers began to appear reporting the incidence of hepatitis B carriage in patients with a variety of glomerulopathies and comparing this incidence to that of other populations, such as blood donors^{14,15}. The striking differences in incidence appeared to establish the pathogenic role of HBV on epidemiological grounds.

However, in a population with a large number of people infected with HBV there are inevitably going to be patients who are incidental carriers of the virus, with other, unrelated diseases. The problem thus arises how to determine whether or not the glomerular lesion in any particular patient is causally related to the hepatitis B virus. Initially, demonstration of HBV antigens within immune complexes situated within the glomerulus was considered proof of the causal role of HBV. However in 1981 Maggiore et al¹⁶ described artifactual demonstration of HBsAg in glomeruli of patients whose serum was negative for HBV antigens. This was presumed to be due to non-specific binding to the crystallisable fragment (F_c) of the labelled antibody by anti-immunoglobulin antibodies within the immune deposits as this false-positive staining was eliminated when labelled antigen-binding fragments F_{ab} directed against HBsAg were used. More recently, investigators in China tested a variety of renal biopsies for HBV antigens using monoclonal antibodies against HBsAg, HBcAg and HBeAg. They found no difference in antigen detection rates between

patients whose serum was positive for HBV antigens and those who were negative in a variety of glomerular pathologies.¹⁷ The deposition of HBV antigens followed the pattern of immune complex deposition, and massive immune complexes were usually positive for HBV antigens. In this study whole antibodies were used and not $F_{(ab')_2}$ fragments as used by Hirose *et al.*¹⁸ Non-specific binding to the F_c fraction in these massive deposits is likely to have occurred as in the study by Maggiore *et al.*¹⁶

A number of authors have reported being unable to demonstrate HBsAg in the glomeruli of patients who were suspected on clinical grounds of having HBV-related disease.⁶⁻⁸ Other authors, while demonstrating HBsAg inconsistently or not at all in these patients, obtained consistently positive results for HBeAg using highly specific antisera in those patients with active disease.¹⁸⁻²⁰

It would appear then that demonstration of HBeAg within glomerular immune complexes may be the most accurate method of making a prospective diagnosis of hepatitis B nephropathy provided that highly specific methods are used. However Venkateshan *et al.*²¹ were able to demonstrate HBeAg in only 6 of 12 patients. Moreover the antisera required are not readily available, and most clinicians make a diagnosis based on clinical, serological and histological grounds. In addition HBeAg deposits have not been sought in large numbers of patients. The best confirmation at present appears to be

retrospective, if resolution correlates with clearance of the HBV antigens from the serum.

AIMS:

The aims of this study were as follows:

- 1.) To determine the importance of hepatitis B virus infection as a cause of glomerulonephritis at the Red Cross War Memorial Children's Hospital.
- 2.) To describe the clinical, laboratory and histopathological features of the disease as seen by us.
- 3.) To describe the natural history of the disease.
- 4.) To attempt to define indicators of good or less favourable outcome.

TYPE OF STUDY:

This is primarily a retrospective study, based largely on the examination of hospital records. However 9 of the patients presented after the decision had been made to conduct this study and they were studied prospectively. The study is partly descriptive and partly analytic.

A small, uncontrolled interventional study was undertaken on the use of Interferon Alpha 2b in hepatitis B nephropathy.

PATIENTS AND METHODS:

All patients with nephrotic syndrome seen by the Renal Service from 1 August 1969 up to the end of October 1990 were reviewed. The identities of these patients were obtained from several sources, viz:

- 1.) Hospital computerised records of diagnoses
- 2.) Renal Clinic folders
- 3.) Renal biopsy reports
- 4.) A data base of patients with nephrotic syndrome used in previous studies by Professor Jan Wiggelinkhuizen
- 5.) A computerised data base of patients kept by Dr G. R. van Dugteren, a part-time consultant in the Renal Clinic.

The hospital records of all these patients were reviewed, and those with other conditions excluded. The ethnic group, sex, diagnosis, and hepatitis B status were recorded in those who had evidence of nephrotic syndrome. For these purposes the nephrotic syndrome was defined as persistent 4+ proteinuria with hypo-albuminaemia (less than or equal to 25 g/l) and hypercholesterolaemia or quantified protein excretion of more than 40 mg/m²/hour²² together with hypo-albuminaemia with or without hypercholesterolaemia.

Those with positive hepatitis B serology were then examined further and constitute the major part of this study.

All available records (hospital and Renal Clinic) of the 72 patients who were felt to have HBV related disease (defined below) were reviewed, and data collected on a computerised data base. These data included extensive clinical, demographic, laboratory and follow-up data (see appendix 1). The histopathology was reviewed and analysed according to a standardised protocol (appendix 2). The data obtained in this way were then subjected to analysis. Statistical analysis was done on an IBM compatible XT personal computer using STATGRAPHICS.

Routine haematological, microbiological and biochemical tests were done in the diagnostic laboratories of the Red Cross War Memorial Children's Hospital.

Renal function was estimated in 2 ways. Fifty (68%) patients had endogenous creatinine clearance studies performed shortly after presentation. In addition, corrected GFR was estimated from the serum creatinine at the time of first presentation and at the most recent Renal Clinic visit using the formula²³:

$$\text{GFR} = \frac{\text{Height in cm} \times 38}{\text{Plasma creatinine } \mu\text{mol/l.}}$$

Body surface area for calculation of corrected creatinine clearance and proteinuria was obtained from the formula²⁴:

$$\text{BSA (cm}^2\text{)} = \text{Wt}^{0,425} \times \text{Ht}^{0,725} \times 71,84$$

where BSA = body surface area, Wt = weight in kilograms and Ht = height in cm.

Proteinuria selectivity index was calculated using the formula:

$$\text{Selectivity index (\%)} = \frac{\text{Urine IgG} \times \text{plasma albumin} \times 100}{\text{Plasma IgG} \times \text{urine albumin.}}$$

Anaemia and microcytosis were defined as haemoglobin concentration and mean cell volume below the lower limit of normal for age as defined by Oski²⁵.

Before 1976 HBsAg was detected by passive haemagglutination, and no test was available for HBeAg. Since then, radioimmunoassays have been used to detect HBsAg (Ausria II and Ausab, Abbott Laboratories) and HBeAg and antibodies directed against HbeAg (Abbott-HBe).

Serum total haemolytic complement was assayed using sensitised sheep red blood cells as previously described²⁶, C3 and C4 were measured by single radial immunodiffusion using commercially prepared plates (Immuno-plate IV, Hyland diagnostics), and circulating immune complexes by the C1q binding method²⁷.

Rheumatoid factor was assayed by the latex particle agglutination method of Singer and Plotz²⁸, and the sheep erythrocyte agglutination test (SCAT)²⁹.

Anti-nuclear antibodies were assayed with fluorescein labelled sheep anti-human immunoglobulin (Wellcome Diagnostics) using mouse liver as substrate. Anti-DNA antibodies were measured as previously described³⁰ using a modification of the method of Ginsberg et al³¹.

All biopsies except 1 were performed percutaneously using a "Tru-cut" biopsy needle. One patient underwent open renal biopsy. Renal biopsy specimens were immediately divided into 3 parts. One part was fixed in Bouin's fluid, 1 part in 4% s-collidine buffered glutaraldehyde and the third part snap frozen in liquid nitrogen. Sections for light microscopy were stained with haematoxylin and eosin, periodic acid Schiff, Masson's trichrome and methenamine silver.

Material for ultrastructural examination was postfixed in osmium tetroxide and embedded in Spurr's resin. Sections were stained with uranyl acetate and lead citrate and examined with a Phillips 201 electron microscope.

Frozen sections cut at 4 μm were stained by a direct immunofluorescence technique using commercially available fluorescein isothiocyanate labelled anti-IgG, anti-IgA, anti-IgM, antifibrin, anti-C3, anti-C1q and anti-C4 antisera (Beringwerke AG Marburg, West Germany).

Hepatitis B antigens were detected as follows: Frozen sections were fixed in acetone. Endogenous peroxidase activity was

blocked using hydrogen peroxide. The sections were then incubated with goat anti-rabbit IgG or rabbit anti-mouse serum to prevent non-specific binding of labelled antibodies. After this they were incubated with either rabbit anti HBC or mouse anti HBs (Zymed laboratories Inc. San Francisco, U.S.A.) as the primary antibody, followed by biotinylated goat anti-rabbit or rabbit anti-mouse. The binding sites were then shown by incubating the specimens with streptavidin peroxidase conjugate and staining with buffered amino-ethyl carbazole. Haematoxylin was used as a counterstain.

The renal biopsies were reviewed and scored according to a standard protocol (Appendix II). The lesions of membranous nephropathy were staged on electron microscopy according to the classification of Churg³². Stage 1 has only a few subepithelial deposits overlaid by podocytes. In stage 2 these deposits are more numerous and completely encircle the capillary wall. Protrusions of basement membrane separate these deposits and are seen as "spikes" on silver stains. In stage 3 the spikes fuse over the deposits and some begin to resolve. In stage 4 the basement membrane is irregularly thickened and contains fading remnants of the deposits. Where more than 1 stage was present in a biopsy, it was graded according to the dominant stage.

Other glomerular changes were graded as absent, mild, moderate or severe. Mesangial hypercellularity was defined as 4 or more mesangial cells per mesangial area.

The term diffuse is used to describe a lesion or process that involves 80% or more of the glomeruli, and focal is used for lesions affecting less than 80% of glomeruli. Global lesions involve the whole glomerulus and in segmental lesions only a portion of the glomerulus is involved, leaving part of the glomerulus uninvolved by that particular lesion or process.³³

Terms describing race are taken from the hospital classification on the patients folder. White is used to describe patients of presumed European origin, Black to describe those of presumed indigenous African origin, and Coloured those of presumed mixed racial origin or Asian descent.

Hypertension was defined as a blood pressure above the 95th centile as defined by the second task force on blood pressure control in children³⁴.

The term asymptomatic proteinuria is used to describe patients with plasma albumin levels above 25 g/l but less than or equal to 35 g/l, 2+ or less proteinuria on dipstix or salicyl sulfonic acid testing and no oedema. Complete remission is defined as a plasma albumin level above 35 g/l, no more than trace amounts of proteinuria and no oedema.

During the trial of interferon therapy proteinuria was assessed by calculating the protein/creatinine ratio on a single voided specimen of urine³⁵. The normal range is below

20 mg protein per mmol creatinine and the nephrotic range above 200 mg/mmol.

RESULTS:**Patient identification:**

The records of 559 patients with nephrotic syndrome were traced. The clinical and/or histopathological diagnoses are shown in figure 1 and table 1.

TABLE 1: CAUSES/HISTOLOGICAL TYPES OF NEPHROTIC SYNDROME

TYPE	TOTAL(%)	Tested for HBV	HBV +VE	(%)
MCNS	242 (43)	175	9	(5,1)
Mes Prolif	97 (17)	71	13	(18,3)
MGN	92 (16)	81	71	(87,6)
Dif Prolif	33 (6)	22	4	(18,2)
Crescentic	20 (4)	10	0	(0)
APSGN*	11 (2)	9	1	(11)
MCGN	11 (2)	7	1	(14)
Chronic GN	11 (2)	9	0	(0)
FSGS	9 (2)	8	0	(0)
Cong Syphilis*	9 (2)	9	0	(0)
Miscellaneous	24 (4)	17	3	(17,6)
Totals:	559	418	102	(24,4)

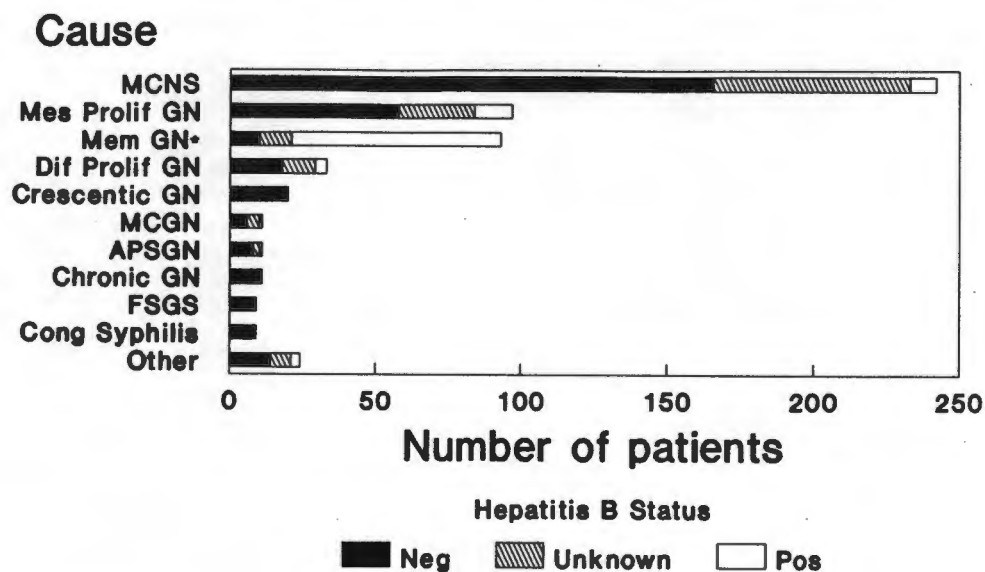
HBV = Hepatitis B virus antigens.

* Indicates clinical diagnosis with no biopsy.

MCNS = Minimal change nephrotic syndrome, Mes Prolif = mesangial proliferative glomerulonephritis, MGN = membranous glomerulonephritis, Dif Prolif = diffuse proliferative glomerulonephritis, Crescentic = crescentic glomerulonephritis, APSGN = acute post streptococcal glomerulonephritis, MCGN = mesangiocapillary glomerulonephritis,

Nephrotic syndrome

Frequency of causes and HB virus status



• $p < 0,000001$

Figure 1.

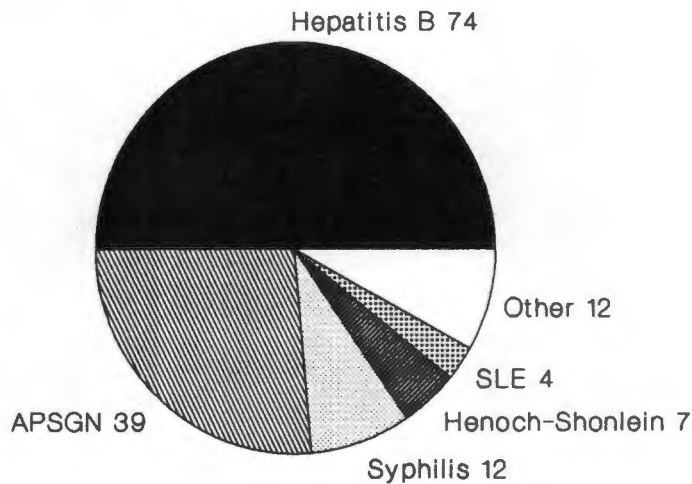
Of the 242 patients with a diagnosis of minimal change nephrotic syndrome (MCNS), 61 were biopsy proven. There were only 12 patients with congenital syphilis. This is the result of selection bias, as most patients with congenital syphilis are treated in the general paediatric wards or in the neonatal units and are not referred to the renal service. Of these, 3 underwent biopsy (2 membranous and 1 acute diffuse proliferative) and the rest were diagnosed on clinical and serological grounds.

Acute post streptococcal glomerulonephritis with nephrotic syndrome was diagnosed in 39 patients. Twenty-eight of these underwent renal biopsy which showed acute diffuse proliferative glomerulonephritis in all cases, and 11 were diagnosed on clinical and serological grounds.

There were 24 (4%) other patients with a variety of diagnoses. Included in this group are 2 patients who, on clinical grounds were suspected of having HBV associated nephrotic syndrome (NS), but in whom renal biopsy was not successful. One of these patients had a severe bradycardia at induction of general anaesthesia for biopsy and the procedure was abandoned, and the other biopsy specimen contained no glomeruli. These 2 patients have been included in the assessment of causes of NS in black patients and the causes of secondary NS, but have been excluded from further analysis.

There were 148 patients who had an identifiable cause of nephrotic syndrome (figure 2). In 74 (50%) of these nephrotic syndrome was felt to be secondary to HBV infection.

Secondary nephrotic syndrome Frequency of causes



n = 148

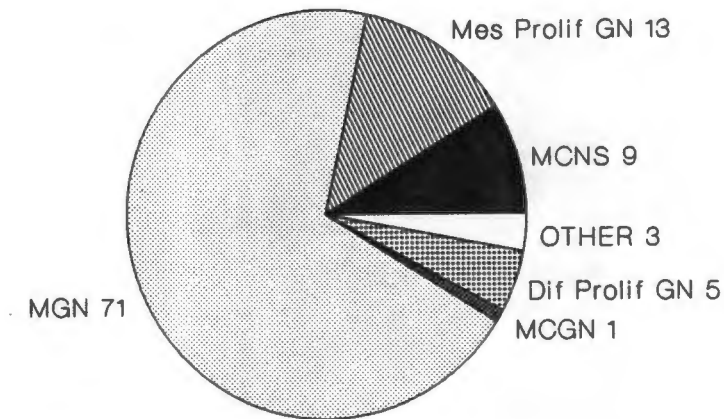
Figure 2

There were 92 patients with membranous nephropathy. Seventy-one (87,6%) of the 81 tested were HBV positive. Of those with membranous nephropathy and negative HBV tests, 2 had congenital syphilis, 2 were felt to have idiopathic membranous nephropathy and 1 systemic lupus erythematosus (SLE). Of the patients with unknown HBV results, 1 had SLE, 1 cystinuria with penicillamine-induced membranous nephropathy, and 1 was felt to have idiopathic membranous nephropathy. The records of the remaining 14 patients had been partly destroyed and

inadequate information was available to make any further diagnosis.

A total of 102 (24,4%) of the 418 patients tested for HBV infection were positive. The histopathological types seen on renal biopsy in these patients is shown in fig 3. Seventy-one (69,6%) of these patients had MGN. This distribution is highly significant ($p < 10^{-6}$) (Chi-squared). Thirteen patients had mesangial proliferative GN. HBV infection was significantly more frequent in these patients than in those with minimal change disease ($p < 0,005$).

Hepatitis B +ve patients with NS Distribution of histological types



n = 102

Figure 3.

This distribution causes some difficulty. Firstly, although there is an apparently significant increase in HBV carriage in patients with mesangial proliferative glomerulonephritis when compared with other patients with NS, the HBV positive rate is only slightly higher than that found in certain sectors of the general population³⁶. Furthermore, the frequency of HBV infection in patients with membranous nephropathy is very much higher than that in mesangial proliferative disease ($p < 10^{-11}$). As there seemed to be no clear method of selecting those patients (if any) whose disease may have been caused by HBV infection, no patients with mesangial proliferative disease

have been included for further analysis. However, the relationship between HBV infection and mesangial proliferative GN merits further study.

The study group therefore consisted of the 71 patients with MGN and 1 patient with MCGN. The patient with MCGN was included for 2 reasons. Firstly MCGN has been described in association with HBV infection and this relationship seems to be generally accepted²¹, and secondly HBsAg and HbcAg were demonstrated within the immune complexes deposited in the glomeruli. In addition, many of our patients with MGN associated with HBV have at least some histopathological features of MCGN.

Clinical and demographic data:

RACE AND SEX:

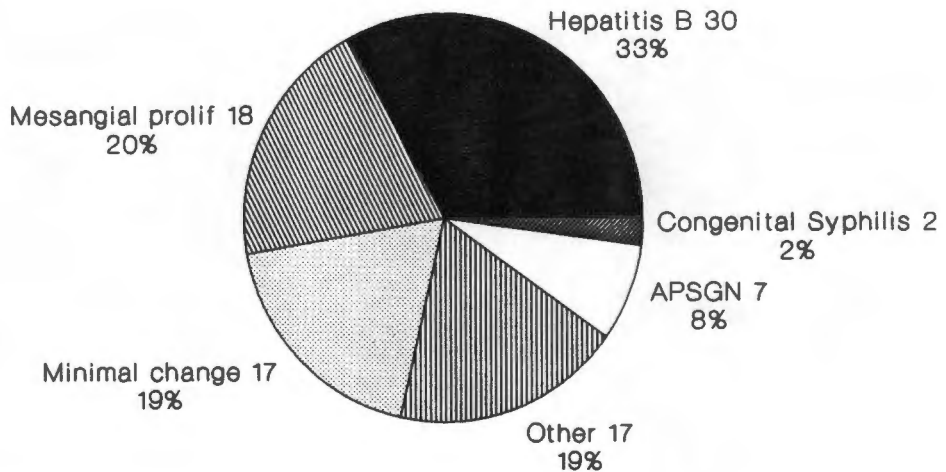
The group of 72 study patients was constituted as follows:

- a.) Sex: Boys: 60 (83%)
 Girls: 12 (17%)
- b.) Race: Coloured: 43 (60%) (37 boys, 6 girls)
 Black: 28 (38%) (23 boys, 5 girls)
 White: 1 (1%) (1 girl)

The frequency of minimal change nephrotic syndrome among Black children is known to be considerably lower than in Whites or

children of Asian origin¹. For this reason, the distribution of causes of the nephrotic syndrome in Black children was examined. The results are shown in figure 4.

Nephrotic syndrome in Blacks Histological types / Causes



n = 91

Figure 4.

Hepatitis B Nephropathy

Age at onset

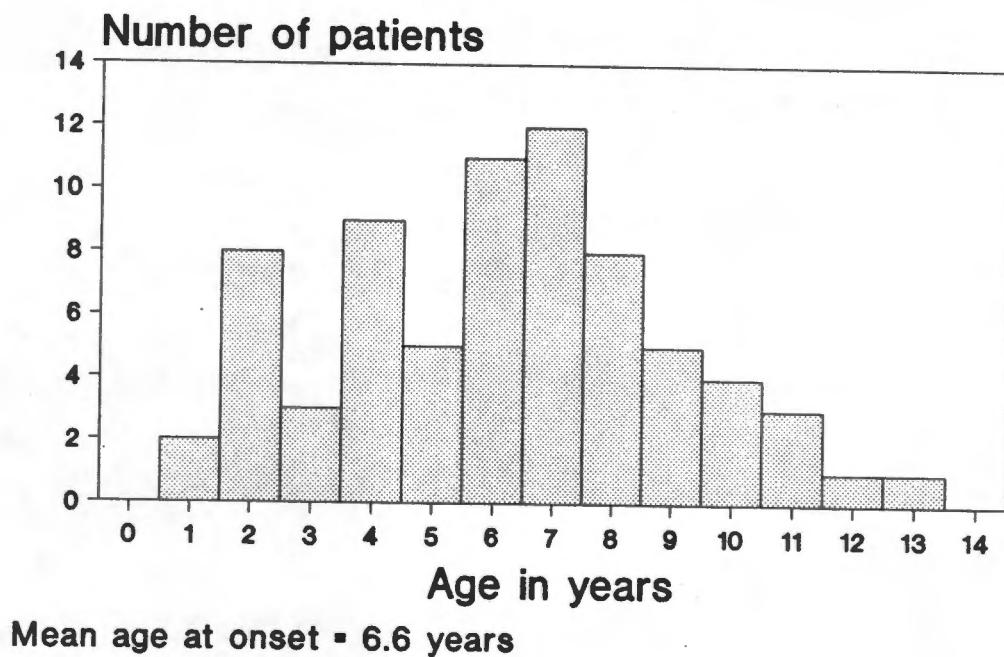


Figure 5.

C.) AGE AT ONSET:

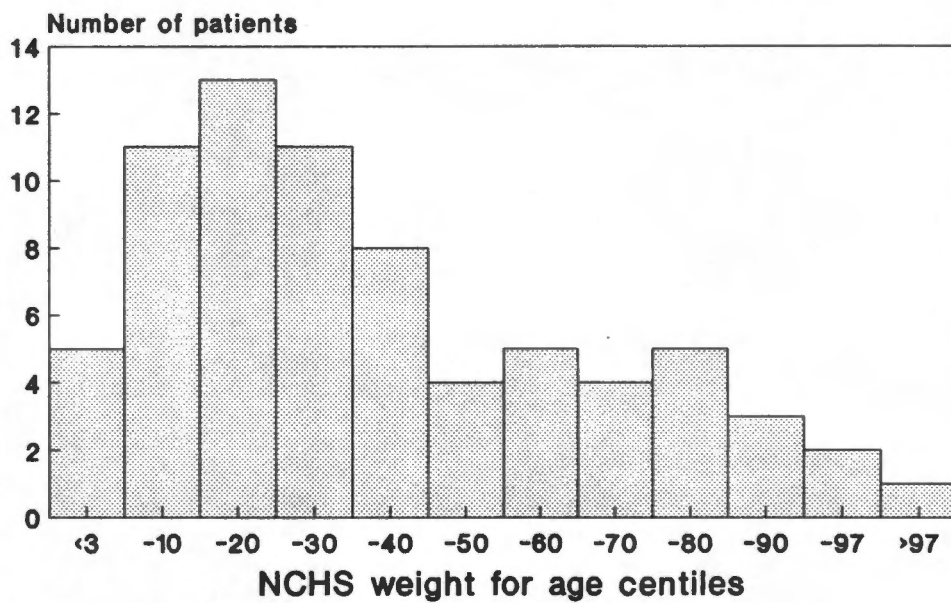
The distribution of ages at the time of onset is shown in figure 5. The ages ranged from 13 months to 164 months (13 years 8 months) The peak incidence is in the 6-7 year age group, and the mean age at presentation 79 months.

D.) GROWTH PARAMETERS

All patients were routinely weighed and had height measurements recorded. The weight used in this assessment was the lowest recorded within 6 weeks of presentation to Red Cross Children's Hospital. This was done to minimise over-estimation of weight due to oedema. Diuretic therapy with or without intravenous albumin infusions was given to severely symptomatic patients. Weight loss during this time averaged 12,9% (range 0 - 44%). Eight patients (11%) lost more than 25% of their weight at presentation with resolution of oedema.

The anthropometric data are summarised in the accompanying figures. There is a striking degree of stunting in these patients, as shown in figure 7. Weight for age and weight for height may be overestimated because these patients seldom, if ever, lose all their oedema within 6 weeks.

Weight for age Shown as NCHS centiles



Wt = lowest within 6 weeks

Figure 6.

Height for age at presentation NCHS centiles

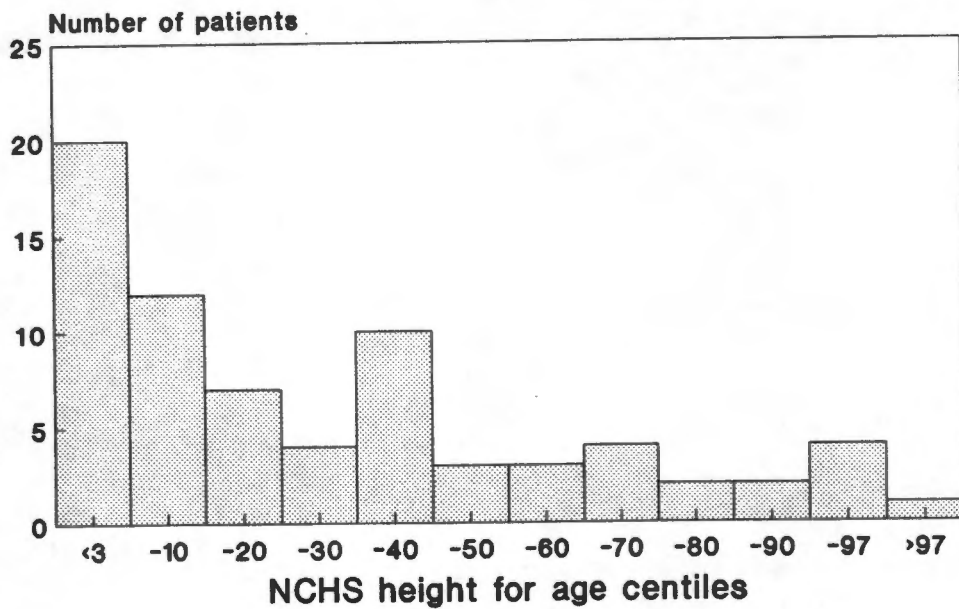
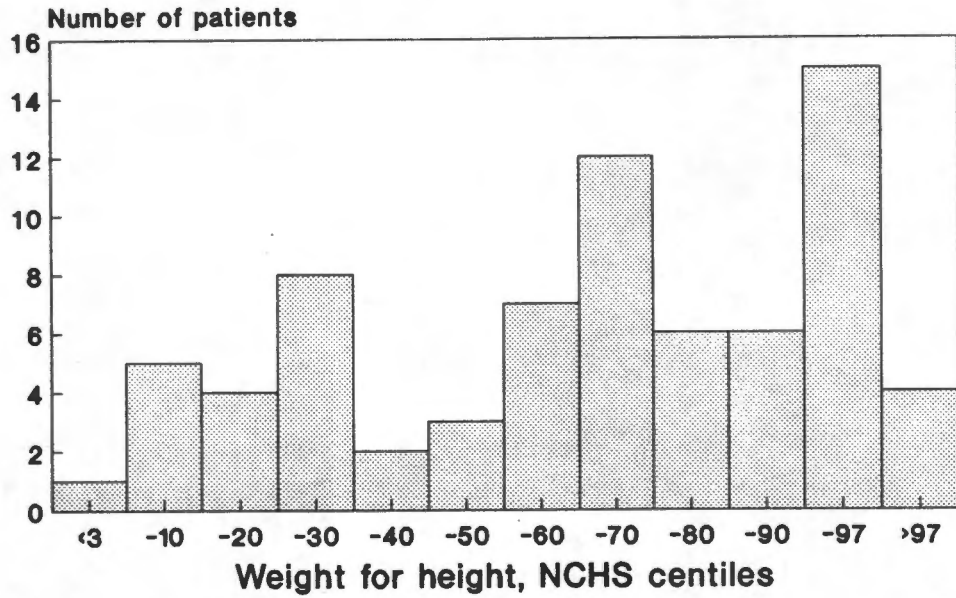


Figure 7.

Weight for height NCHS centiles



Wt = lowest within 6 weeks

Figure 8.

E.) CLINICAL SIGNS

All patients presented with heavy proteinuria and the clinical features of nephrotic syndrome. The clinical signs present are summarised in table 2.

TABLE 2: CLINICAL SIGNS AT PRESENTATION.

SIGN	NUMBER	(PERCENT)
Oedema	72	(100)
Proteinuria	72	(100)
Ascites	51	(71)
Micro-haematuria	51	(71)
Hepatomegaly*	29	(40)
Pleural effusion	24	(33)
Hypertension	19	(26)
Macro-haematuria	15	(21)
Splenomegaly**	8	(11)

* Hepatomegaly = liver palpable more than 2 cm below the costal margin in the mid-clavicular line.

** Splenomegaly = palpable spleen.

OTHER DISEASES:

Thirteen patients (18%) had tuberculosis (TB) at presentation. Eleven had pulmonary TB, 1 had abdominal TB, and 1 had tuberculous meningitis. Eight of the 13 have entered remission, an average of 32 months after onset of symptoms. Two defaulted after 13 and 38 months of follow-up, and 3 are still followed after 30, 49 and 114 months. The latter patient developed end stage renal failure.

Five patients had acute pneumonia at presentation, 3 had asthma, 2 had upper respiratory tract infections, 1 had Staphylococcal septicaemia and 1 had severe Herpes Simplex Virus stomatitis. One patient was from the Worcester School for the Deaf, and 1 had a congenital spinal cord defect associated with a lumbosacral lipoma.

LABORATORY DATA:Urine examination:

The results of urine microscopy are shown in table 3.

TABLE 3: URINE MICROSCOPY.

FEATURE	NUMBER POSITIVE	(PERCENT)
Red blood cells:	66	(92)
White blood cells:	22	(31)
Granular casts:	22	(31)
Hyaline casts:	17	(24)
RBC casts:	5	(7)

Microscopy for red and white blood cells was considered positive if there were more than 5 per high power (x 400) field of a centrifuged urine specimen.

Plasma chemistry profile:

The serum biochemistry results are summarised in table 4.

TABLE 4: SERUM AND URINE BIOCHEMICAL PROFILE

VARIABLE	(n)	AVERAGE	RANGE	S.D.
Albumin (g/l)	(72)	17,4	10 - 29	4,0
Total protein (g/l)	(72)	50,0	28 - 65	7,3
Cholesterol (mmol/l)	(72)	9,52	3,71 - 18,3	3,45
Urea (mmol/l)	(72)	4,5	1 - 10,4	1,97
Creatinine ($\mu\text{mol/l}$)	(71)	48,6	15 - 110	19,9
ALT	(58)	40,5	9 - 189	36,1
Cl_{creat} (ml/min/1,73m ²)	(57)	106,4	15 - 229	38,9
Calculated GFR (ml/min/1,73m ²)	(71)	101,0	32 - 288	36,5
Proteinuria (mg/m ² /hour)	(65)	313,6	26 - 1804	336,2
Selectivity	(34)	15,9	0,1 - 83,4	15,3

ALT = alanine aminotransferase, Cl_{creat} = corrected creatinine clearance, Calculated GFR = glomerular filtration rate calculated according to the formula $\text{GFR} = \text{height (cm)} \times 38 / \text{serum creatinine } (\mu\text{mol/l})$, Selectivity = proteinuria selectivity index.

All patients had decreased serum albumin levels. Two patients had initial plasma albumin levels above 25 g/l; in both the level subsequently fell below this. Total protein levels were similarly decreased in most patients.

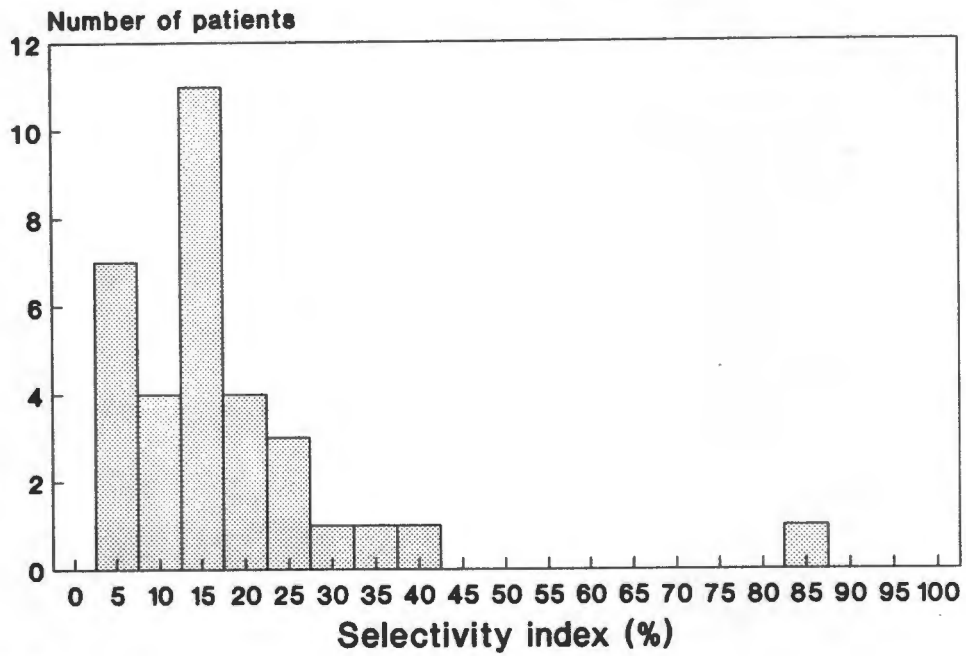
Using formal endogenous creatinine clearance studies, 15 of 57 (26%) patients had initial creatinine clearances of less than 80 ml/min/1,73m². Using the other method, 16 of 71 (23%) had values less than 80. Comparison of the two methods using linear regression analysis in the 50 patients in whom both were available showed only a fair correlation ($r = 0,59$).

Of the 16 patients with a calculated GFR of less than 80 ml/min/1,73m² at presentation, only 4 had results below this level at the last follow-up. In 1 patient the value remained unchanged and in 1 it rose from 32 to 47 ml/min/1,73m². In the other 2 cases the calculated GFR fell; in 1 case from 36 to 0, and in the other 79 to 54. Eight other patients had a calculated GFR of less than 80 at last follow-up.

Proteinuria is expressed as mg/m²/hour. The International Study of Kidney Disease in Children (ISKDC) definition of nephrotic syndrome is a protein excretion rate of more than 40 mg/m²/hour²². One patient had a measured protein excretion rate below this (26 mg/m²/hour). As she had a plasma albumin of 19 g/l and plasma cholesterol of 6,60 mmol/l it seems likely that this was an under-collection.

Proteinuria was highly selective (proteinuria selectivity index < 10%) in 10 patients (29%) and poorly selective (index > 20%) in 8 (24%). Most (47%) had moderately selective proteinuria.

Proteinuria selectivity index



n = 33

Figure 9.

Serum alanine aminotransferase (ALT) levels are summarised in table 4. While 43 of 59 (73%) had levels above 30 U/l (the upper limit of normal in our laboratory), in most patients the elevation remained modest and tended to fluctuate during the course of the disease. No patient was clinically jaundiced and only 1 had a history of jaundice, 4 months previously. None had symptomatic liver disease. Three patients underwent liver biopsy. This showed mild chronic persistent hepatitis in all 3.

Haematological data:

These are summarised in table 5. Sixteen patients had normocytic anaemias, usually mild (the lowest haemoglobin was 9,5 g/dl). Six patients had microcytic anaemias and a further 4 had microcytosis with haemoglobin levels within the normal range.

Most patients had normal leucocyte counts. The range was from $3,6 \times 10^9/l$ in the patient with hypersplenism to $31,8 \times 10^9/l$ in a patient with peritonitis at presentation. Differential counts were either normal or showed an increase in neutrophils in patients with infection.

Platelet counts tended to be elevated; 24 (36%) of 66 patients in whom counts were recorded had more than 450×10^9 platelets per litre. Only one patient had a low platelet count, secondary to hypersplenism.

TABLE 5: HAEMATOLOGICAL DATA

VARIABLE	(n)	AVERAGE	RANGE	S.D.
Haemoglobin (g/dl)	(71)	11,7	7,8 - 15,2	1,5
MCV (fl)	(64)	81,0	66 - 91	5,5
WCC ($\times 10^9/l$)	(70)	13,2	3,6 - 31,8	5,4
Platelets ($\times 10^9/l$)	(65)	412	105 - 971	178,0

MCV = Mean cell volume; WCC = White blood cell count.

Microbiology:URINARY TRACT INFECTION:

All patients had urine cultures at presentation. Five (4 males) were positive, with a pure growth of more than 100 000 organisms per ml. Two grew Klebsiella species, 2 E. coli, and 1 a Proteus species. Further investigations for structural abnormalities predisposing to infection were negative in all 5 cases.

STREPTOCOCCAL INFECTION:

Fifty patients had skin and/or throat swabs taken for possible Lancefield group A beta-haemolytic streptococcal infection. Eight throat swabs were positive. None of the skin swabs were positive.

SEROLOGICAL TESTS FOR STREPTOCOCCAL INFECTION:

Anti-streptolysin O titre (ASOT): n = 69

>200 I.U.: 14 (20%)

μ200 I.U.: 55 (80%)

Anti-DNase B:

n = 23

>200 I.U.: 8 (35%)

μ200 I.U.: 14 (65%)

SEROLOGICAL TESTS FOR SYPHILIS:

Routine tests for syphilis (rapid plasma reagin (RPR) or the venereal disease research laboratory (VDRL) slide test) were done on 70 patients. All except one were negative. The exception was a child of 34 months who had previously been treated with a 10 day course of procaine penicillin for congenital syphilis diagnosed at 6 days of age. The *Treponema pallidum* haemagglutination assay remained positive despite a further course of penicillin therapy. His nephrotic syndrome continued for 42 months. He became asymptomatic within 5 months of clearing the HBeAg from his blood, and had complete resolution with no residual urinary abnormalities 17 months later.

TESTS FOR HEPATITIS B VIRUS INFECTION:

Prior to about 1980 the connection between HBV infection and renal disease was not generally appreciated, and HBV markers were not routinely sought in patients with nephrotic syndrome. HBeAg tests became available only some time after HBsAg tests had been in use (1976). In this series, 1 patient with well documented membranous nephropathy was tested only after his renal disease had undergone complete remission. His serum was positive for surface antibody and e antibody, but negative for all HBV antigens. A further 9 patients were first tested for HBeAg after resolution of the disease and were negative at that time with positive tests for anti HBe, but had

persistently positive tests for HBsAg, both earlier, during the active phase of disease and after remission.

No HBeAg results were available in 5 patients. All but 1 had presented before 1976. Fifty-seven patients had tests for HBeAg while the disease was active. Fifty-six were positive. The single patient who was persistently negative for HBeAg became asymptomatic with mild proteinuria only 24 months after presentation and initial testing.

At the time of the last clinic visit, 39 patients were HBeAg negative, and 33 remained HBeAg positive. Of the 39 patients who were HBeAg negative, 34 were in complete remission and 5 had asymptomatic proteinuria only. Only 7 of the 72 patients had cleared the surface antigen from the serum. The average interval between the last positive test for HBeAg and the first negative test was 8,3 months (range 1 - 23 months). This interval is influenced by the test interval rather than time taken for seroconversion.

Twenty patients had repeated HBV antigen tests during the course of their renal disease and after remission. The average interval from the first negative test for HBeAg to complete remission was 5 months (range: remission 24 months before seroconversion to 31 months after seroconversion).

Four patients were in remission but still had positive tests for HBeAg. Two of these were last tested for HBeAg on the same

day they were first found to be in remission. The other 2 were last tested 9 and 33 months after complete remission of proteinuria.

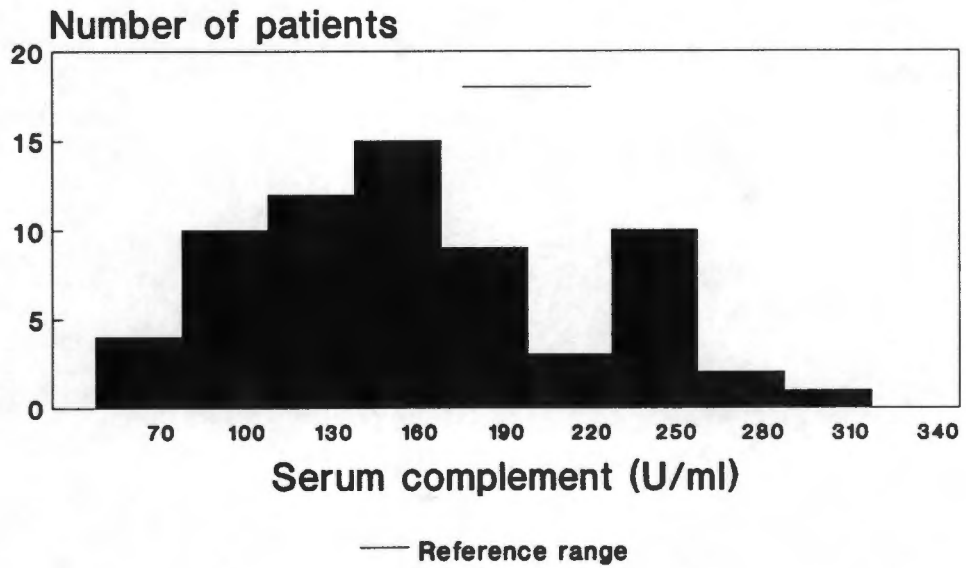
Hepatitis B serological studies were done on 39 mothers. Of these, 15 (38%) had evidence of present or past HBV infection.

Immunological activation and auto-antibodies:

The results of assays for serum markers of immunologic activation are shown in figures 10 to 12 and summarised in table 6.

Most patients had some depression of serum complement components, although some had elevated levels. Slightly more patients had low levels of the 3rd (C3) than 4th (C4) components (67% versus 47%), indicating probable alternate pathway activation in those patients.

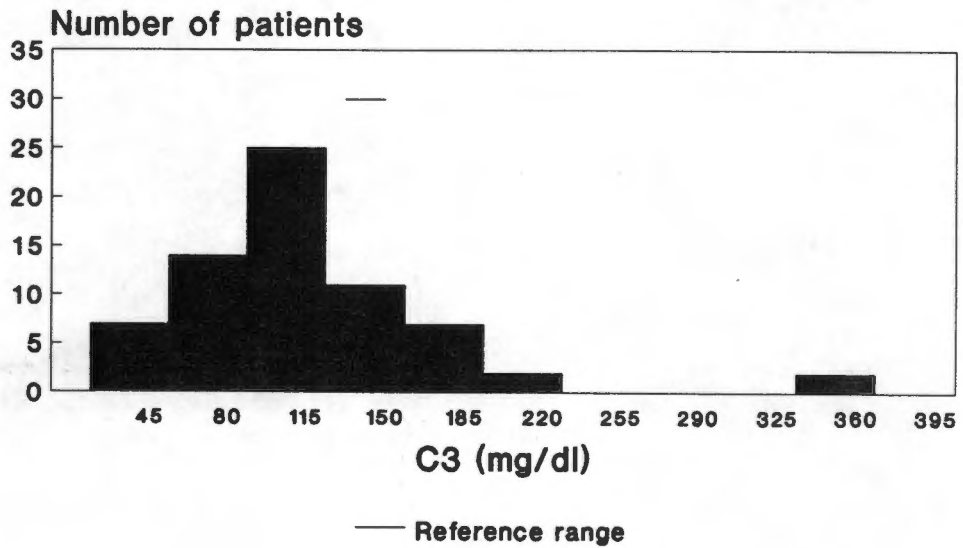
Serum total complement Units/ml



n = 66

Figure 10.

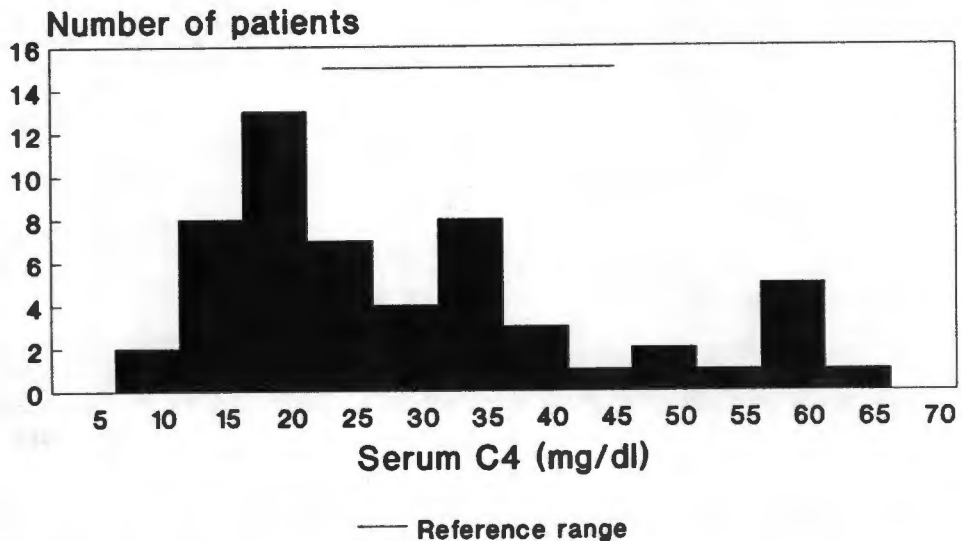
Serum levels of third component of complement



n = 67

Figure 11.

Serum levels of fourth component of complement



n = 55

Figure 12.

Of the 29 patients in whom circulating immune complexes were assayed, all but 6 were within the normal range (< 7% C1q binding), and only 3 had levels above 15% (68,3; 79 and 87,1%).

Rheumatoid factor was assayed in 32 patients. The SCAT was negative in 30 cases. The remaining 2 had positive tests to titres of 16 and 32. Latex tests were negative in 29 patients, and the remaining 3 had positive titres of 40, 80 and 320. Titres for both tests were positive in only one patient, who

had a SCAT of 32 and latex of 320. This patient had no evidence of rheumatoid arthritis, was asymptomatic within 13 months, and in complete remission within 25 months. Remission coincided with clearing the e antigen from her circulation.

Antinuclear factor (ANF) was measured in 58 patients, and was negative in 54. Of the positive results, 2 had titres of 10, 1 of 20 and 1 of 2500. None of these patients had any evidence of systemic lupus erythematosus (SLE).

Anti-DNA antibodies were measured in 44 patients, and were within the normal range of 0 - 10 μg DNA bound per ml of serum in 27. The distribution of elevated levels was as follows: 11 $\mu\text{g}/\text{ml}$: 6 patients; 13 $\mu\text{g}/\text{ml}$: 1 patient; 15 $\mu\text{g}/\text{ml}$: 2 patients; and 1 patient each had levels of 18, 20, 21, 23, 33, 34, 37 and 91 $\mu\text{g}/\text{ml}$. None had other features of SLE.

The patient with an ANF titre of 2500 had an anti-DNA antibody level of 5 $\mu\text{g}/\text{ml}$, and the patient with anti-DNA antibodies of 91 $\mu\text{g}/\text{ml}$ had a negative test for ANF.

TABLE 6: SERUM MARKERS OF IMMUNOLOGICAL ACTIVATION				
---	--	--	--	--

VARIABLE (Reference range)	(n)	AVERAGE	RANGE	S.D.
Total complement: (160 - 220 U/ml)	(66)	152	55 - 286	58,5
C3: (115 -150 mg/dl)	(67)	106	16 - 340	56,7
C4: (20 - 45 mg/dl)	(55)	27	6 - 61	15,1
C.I.C.*: (< 7%)	(29)	*	1,1 - 87,1	*

C3 = third component of complement, C4 = fourth component of complement, C.I.C. = circulating immune complexes.

*C.I.C median value = 2,9; geometric mean = 4,4.

Radiological features:

Prior to 1981 all patients underwent intra-venous pyelography (IVP). Since that time ultrasound has largely replaced IVP. As all X-ray films are destroyed after 4 years only 2 sets of films were available for examination. One patient had a duplex system on the left. Apart from large kidneys (+2 - +3 standard deviations) there were no other abnormalities. Those cases where the films were not available for examination were excluded from further analysis.

Renal ultrasound scans were available on 18 patients. In all except 2 cases the kidneys were of increased echogenicity. Renal length measured by ultrasound imaging was available for 14 patients (28 kidneys). The results are shown in figures 13 and 14. Fifteen (54%) of the kidneys were larger than the 95%

confidence level for the upper limit of normal³⁷, and only 1 was below the mean length for patient height.

Sonographically measured kidney length: Right kidney

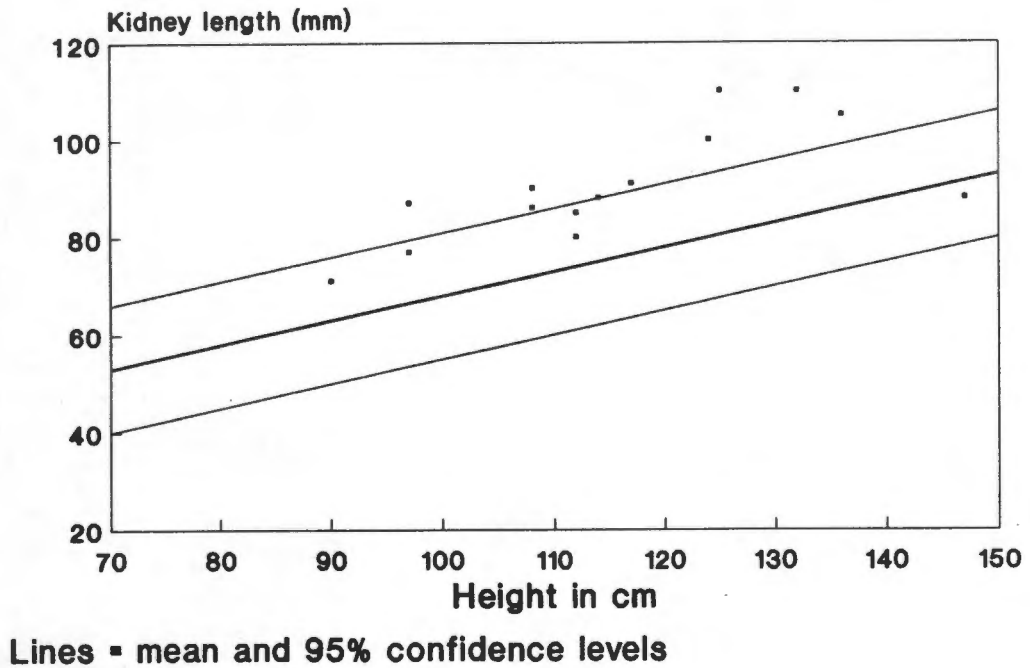
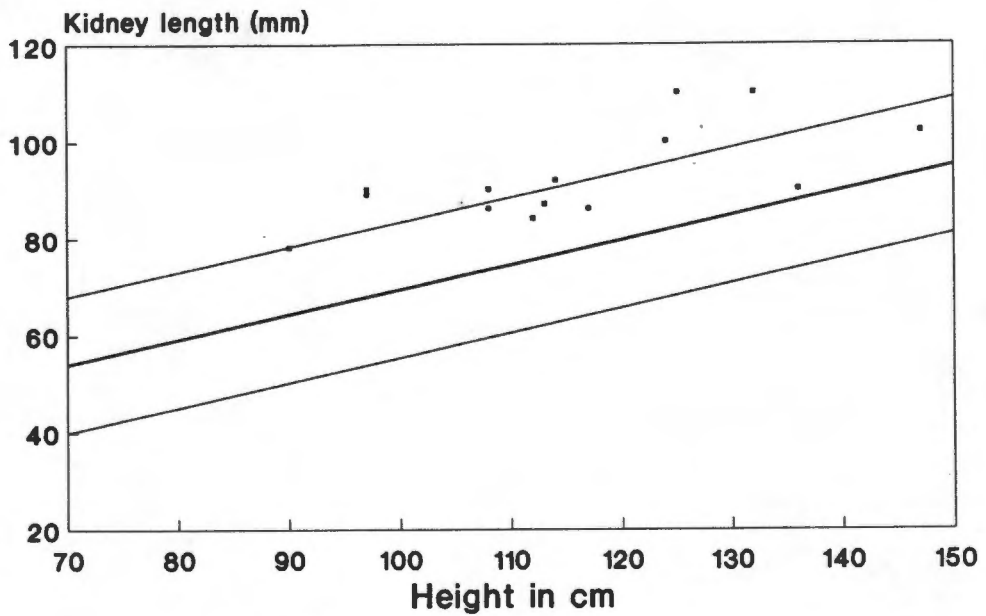


Figure 13.

Sonographically measured kidney length: Left kidney



Lines = mean and 95% confidence levels

Figure 14.

Clinical course of the disease:FOLLOW-UP

The 72 patients were followed for an average of 46,4 months, with a range of 2 to 139 months (median 41 months). Eleven patients were followed for less than 12 months. Twelve patients (16%) defaulted after a varying follow-up period and could not be traced. Of these 11 had active nephrotic syndrome when last seen and 1 had asymptomatic proteinuria. The duration of follow-up among the defaulters with nephrotic syndrome when last seen ranged from 2 to 38 months (mean 14,5 months, median 13 months). The patient who defaulted with asymptomatic proteinuria did so after 89 months. One patient died in a motor vehicle accident a few days after his last Renal Clinic visit, at which time he was asymptomatic but had mild persistent proteinuria. The follow-up status of all patients at their last clinic visit are shown in table 7.

TABLE 7: PROTEINURIA AT LAST VISIT

STATUS	NUMBER	(PERCENT)
Remission:	38	(53)
Nephrotic:	25	(35)
Asymptomatic proteinuria:	7	(10)
End-stage renal failure:	1	(1)
Post renal transplant:	1	(1)

The mean interval from onset of symptoms to becoming asymptomatic in the 45 patients was 22,9 months, with a range

from 2 to 80 months. The median interval from onset to the asymptomatic state was 18,0 months.

The average duration of proteinuria in the 38 patients in complete remission was 30,0 months, with a range of 5 to 95 months, and median of 27 months. Of those patients who went into remission, all but 5 did so in under 40 months. The average interval from becoming asymptomatic to complete remission was 10 months (range 0 to 32 months, median 8,5 months).

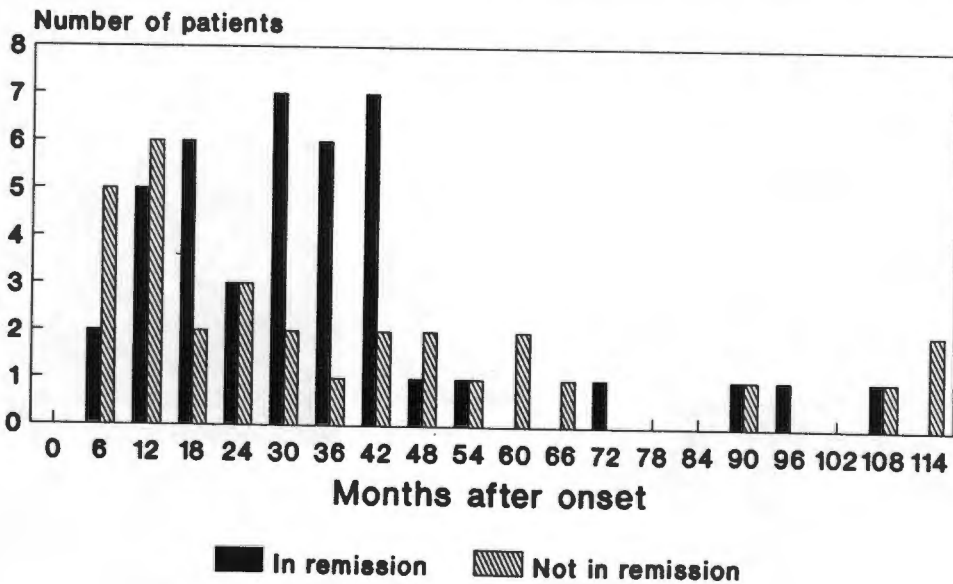
Twenty-six patients still had active nephrotic syndrome when last seen. The average duration of symptoms in these patients was 25,2 months, with a range of 2 to 82 months. Of these patients, 7 have had symptomatic disease for more than 40 months.

Figure 15 shows the follow-up status of all patients. The shaded bars represent the time of entering remission in those patients who did so. The hatched bars represent the most recent clinic visit by those patients not yet in complete remission.

The 38 patients who went into complete remission were followed up for an average of 27 months after remission (range 0 - 133 months, median 22 months). None had a relapse of nephrotic syndrome.

The cumulative probability of remission (from life table analysis) is shown in figure 16. A 70% 5 year remission rate was observed.

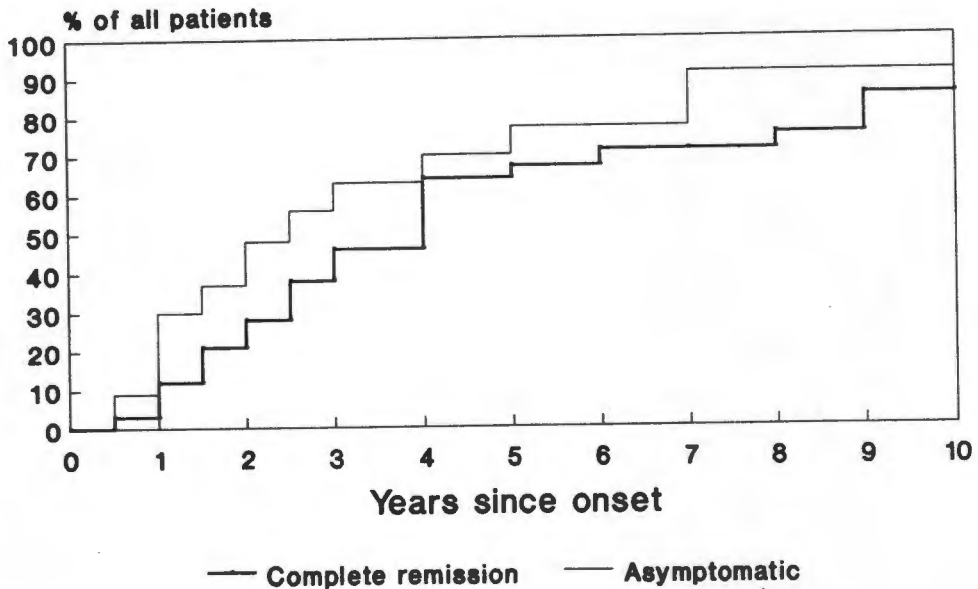
Duration of nephropathy Time to remission/duration of follow-up



n = 73

Figure 15.

Cumulative probability of remission or asymptomatic proteinuria



65% remission at 4 years

Figure 16.

RENAL FUNCTION:

In most cases renal function was well preserved. The average change in calculated GFR was +18,3%. Nine patients had a decline in GFR of more than 25% between presentation and last follow-up visit. Thirteen had a calculated GFR of less than 80 ml/min/1,73 m² at the last visit, of which 5 were under 50 ml/min/1,73 m². Two patients (2,8% of the total or 3,3% of those followed for more than 12 months) progressed to end-stage renal failure; 1 within 12 months and the other after 9,5 years. One has been successfully transplanted.

Complications:

1. Infections:

Peritonitis: There were 16 episodes of peritonitis in 8 children. Two patients had 2 episodes each and one had no fewer than 6 episodes. In 9 episodes *Streptococcus pneumoniae* was the responsible organism. In 2 cases there was no bacterial growth obtained although Gram stain showed numerous pus cells. In the remaining 6 cases peritoneal aspirate was either not cultured or the results could not be traced. Since it became available, all patients with nephrotic syndrome have been immunised against pneumococcal infection (Pneumovax, Logos).

Cellulitis: Two patients had episodes of cellulitis. One patient had a single episode and the other recurrent group A beta-haemolytic *Streptococcal* cellulitis.

Other infections: Four patients had 5 episodes of urinary tract infection during the course of their illness. Two patients had measles and 3 had chickenpox without major complications.

2. Thrombosis:

Three patients suffered 4 thrombotic complications. One patient had a left middle cerebral artery thrombosis with resultant right sided hemiplegia. Extensive investigations

found no vascular or other haematological abnormality. One patient had a thrombosis of his right popliteal artery which required embolectomy, and the third patient had 2 separate episodes of deep venous thrombosis involving each leg in turn.

3. Renal Failure:

There were no episodes of acute renal failure. Eight patients at last follow-up had mild renal impairment (GFR < 80 but > 50 ml/min/1,73m²) as assessed by the calculated GFR. A further 5 patients had a GFR of less than 50 ml/min/1,73m². Two had severe renal failure, with a GFR of 11 or less. One patient developed end stage renal failure within 1 year of onset of symptoms. He was initially maintained on chronic ambulatory peritoneal dialysis and given a course of Interferon alpha 2b (see below). He subsequently received a cadaver donor renal graft, and has done well. Seven months after the transplant he is normotensive, has no proteinuria, and maintains a serum creatinine concentration of approximately 70 µmol/l.

One further patient had slowly declining renal function. Ten years after onset of his disease he now has a GFR of 11 ml/min/1,73m².

4. Hypertension:

One child had convulsions due to hypertensive encephalopathy while receiving steroid therapy. Although 19 patients were

hypertensive at presentation, only 10 had hypertension at the last clinic visit.

5. Other:

One patient developed necrosis of part of the prepuce secondary to severe oedema of the genitalia.

THERAPY:

Fifty-eight of the 73 patients received only supportive treatment. Ten received steroids for 4-8 weeks, 2 received steroids and cyclophosphamide and 3 received interferon alpha 2b after steroid withdrawal.

1. Steroids:

Of the 10 patients given steroids, 6 had gone into remission by the time of their last visit. In none of these cases did remission occur during therapy, and only 1 went into remission within 6 months of steroid treatment. Two had defaulted while still having nephrotic syndrome at 22 and 15 months after onset. One had asymptomatic proteinuria after 45 months and a 4th still had nephrotic syndrome after 30 months. The remaining patients went into remission an average of 34,5 months after onset (range 5-90 months). This is similar to those patients who received no specific therapy (31 patients in full remission, mean duration of nephrotic syndrome 29,6 months, range 6-95 months), ($p > 0,5$). Steroid therapy was

complicated in 1 patient by severe hypertension and hypertensive encephalopathy.

2. Steroids and cyclophosphamide:

Two patients were given steroids and cyclophosphamide in combination for 8 and 12 weeks before the relationship between HBV infection and renal disease was recognised. They went into remission at 6 and 38 months after onset respectively. One of these patients had alopecia, leucopaenia and thrombocytopaenia as complications of therapy.

3. Interferon:

In 1989, after several promising reports on the use of interferon in patients with HBV related chronic liver disease³⁸⁻⁴⁰, it was decided to undertake a limited, uncontrolled trial of interferon therapy in 3 patients with particularly severe HBV related nephropathy. It was hoped that remission could be induced by eliminating the virus or at least the hepatitis B e antigen. Consent for this study was obtained from the Medicines Control Council and from the parents of the children concerned.

The proposed protocol was as follows: An initial 3 week period of steroid therapy (prednisolone 2 mg/kg/day to a maximum of 60 mg/day for the first week, half this dose during the second week and 1 quarter for the third week). Interferon alpha 2b

(Intron A, Scherag) therapy was started in a dose of 7,5 $\text{Mu}/\text{m}^2/\text{dose}$ (to a maximum of 10 Mu/dose) given by subcutaneous injection 3 times per week for 12 weeks. Blood was taken at the start of steroid therapy, at the start of interferon therapy, and at intervals of 4 weeks thereafter for full blood count (FBC) and full serum biochemical analysis. Random urine specimens were collected at the same times for urine protein: creatinine ratios and proteinuria selectivity index. In addition any further investigations were performed as dictated by clinical assessment from time to time.

The details of the 3 patients are as follows:

PATIENT 64:

Race/sex: Black male

Age: 10 years

Weight: 27 kg

Height: 130,5 cm

**Interferon
dose:** 7,5 Mu/dose

History: Presented in March 1988 with nephrotic syndrome and HBV infection. Renal biopsy showed grade III membranous nephropathy. Between March 1988 and October 1989 he had required 7 hospital admissions for treatment of complications of nephrotic syndrome (peritonitis twice, cellulitis once and anasarca 4 times.) Creatinine clearance fell from 119 ml/min/1,73 m² at diagnosis to 22 ml/min/1,73m² at the start of therapy.

PATIENT. 49:

Race/sex: White female

Age: 13 years

Weight: 43 kg

Height: 168,5 cm

**Interferon
dose:
Mu/dose.** Initially 10 Mu/dose, later reduced to 5

History: Developed umbilical sepsis at 3 weeks of age. This resulted in portal vein thrombosis. Between September 1977 and March 1983 she had numerous admissions for bleeding oesophageal varices, and received over 100 units of blood both in Cape Town and Zimbabwe. Her oesophageal varices were injected on numerous occasions. A mesocaval shunt and subsequently a Le Veen shunt both failed to relieve either the ascites or the portal vein pressure. Serum albumin at that time was normal. The Le Veen shunt was removed in May 1988 as the tip had migrated out of the vein.

In April 1986 she developed haematuria and nephrotic syndrome. HBV antigens were checked for the first time and HBsAg and HBeAg were both positive. Renal biopsy showed mesangiocapillary glomerulonephritis. Her course was complicated by bilateral deep vein thrombosis of the calves, 6 episodes of peritonitis and several episodes of cellulitis. Creatinine clearance was 88 ml/min/1,73m².

PATIENT 56:

Race/sex: Black male

Age: 7 years

Weight: 24,6 kg

Height: 114 cm

Interferon
dose: 6,5 Mu/dose

History: Presented in March 1988 with nephrotic syndrome, impaired renal function and positive tests for HBsAg and HBeAg. Renal biopsy showed membranous nephropathy with focal areas of sclerosis and some crescents.

His renal function deteriorated and in March 1989 he was started on Continuous Ambulatory Peritoneal Dialysis (CAPD). In his case interferon therapy was given in an attempt to reduce the risk of cross infection in the transplant unit and to prevent the possible recurrence of disease in a transplanted kidney.

Complications:

A) STEROIDS:

Patient 49 developed an acute exacerbation of her hypertension and of dyspepsia related to severe reflux oesophagitis. For these reasons her steroid course was shortened to 5 days for each dose.

B) INTERFERON:

All 3 patients experienced 'flu-like symptoms. These were most severe in the oldest patient and trivial in the youngest.

Patients 49 and 64 had fevers of up to 38,5°C after the first 3 injections. During the second week the highest temperature recorded was 38°C, and thereafter there was no elevation of temperature. Patient 56 had a fever of up to 37,5°C during the first week only. The 2 older patients complained of headaches following injections during the first 2 weeks.

Patient 49 developed crops of vesicles mainly on the extremities starting 10 days after the first interferon injection. Fluid from the vesicles was negative for viral and bacterial culture. Because of this rash the dose of interferon was reduced to 5 Mu/dose after 3 weeks and the course of treatment prolonged to 16 weeks.

There were no haematological or biochemical complications.

RESULTS:

A) HEPATITIS B MARKERS:

All 3 patients were HBsAg and HBeAg positive, and anti-HBsAg anti-HBeAg negative at the start of treatment. Patient 64 seroconverted to anti-HBe antibody positive after 4 weeks of interferon therapy and after 12 weeks he was negative for HBeAg but remained HBsAg positive. The other 2 patients remained both surface and e antigen positive.

B) PROTEINURIA:

A decline in proteinuria occurred in patients 49 and 64. Both patients had urinary protein/creatinine ratios of well over 800 mg/mmol at the start of therapy, and both fell to below 100 mg/mmol. There was no change in patient 56. The results are shown in figures 17 and 18. Patient 64 absconded 4 weeks after completing interferon therapy. Patient 49 had a temporary improvement in proteinuria which lasted 20 weeks before relapsing to former levels.

C) SERUM ALBUMIN:

Together with the decline in proteinuria there was a rise in serum albumin from 16 g/l to 27 g/l in patient 64 and from 22 g/l to 30 g/l in patient 49. There was no change in patient 56.

Interferon therapy

Serum albumin & proteinuria, patient 49

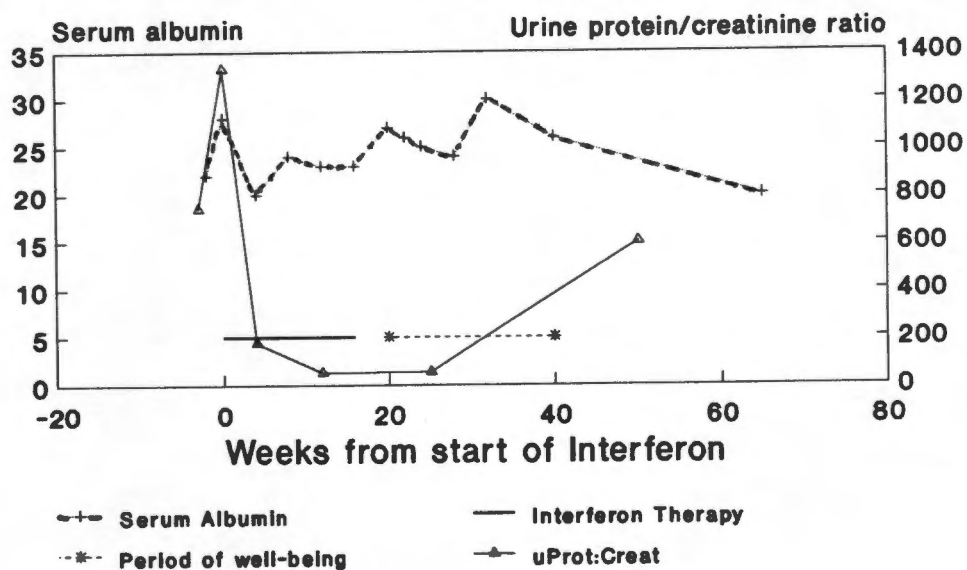


Figure 17.

Interferon therapy Serum albumin & proteinuria, patient 64

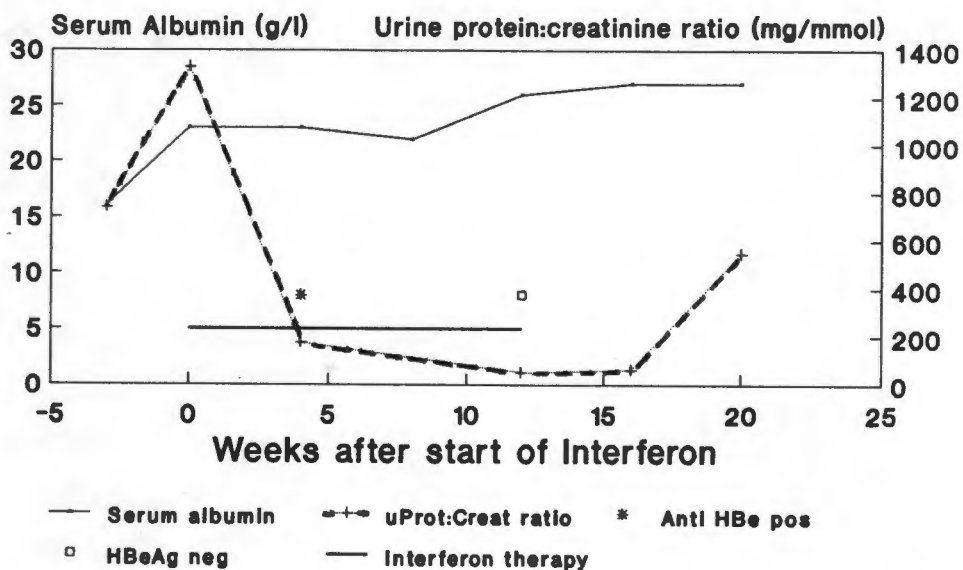


Figure 18.

D) RENAL FUNCTION:

Patient 64 absconded before formal renal function tests could be performed, but his serum urea fell from 11,7 mmol/l at the start of therapy to 5,1 mmol/l 4 weeks after completing treatment, and his creatinine fell from 125 μ mol/l to 93 μ mol/l (estimated GFR 53 ml/min/1,73 m²). There was no change in the glomerular filtration function of the other 2 patients.

HISTOPATHOLOGICAL DATA:

Seventy-five renal biopsies were performed on 71 patients. One patient was biopsied in Durban, and his biopsy was not available for review. One patient had 2 and 1 had 3 biopsies. The median interval from onset of symptoms to first renal biopsy was 2 months (range 0,5 to 50 months, average 3,8 months).

Seventy-one patients were classified as having membranous nephropathy and 1 had mesangiocapillary glomerulonephritis (MCGN) on the basis of the initial biopsy. Further details are given below.

Light microscopy:**GLOMERULI:****Glomerular basement membrane:**

Abnormalities of the glomerular basement membrane (GBM) were the most striking and consistent feature on light microscopy, seen in all 71 biopsies examined. The GBM was diffusely thickened in all cases. Thickening was global in 63 (89%) and segmental in 8 (11%). The degree of thickening was subjectively graded as mild in 4 cases (6%), moderate in 43 (60%), and severe in 24 (34%).

"Spikes" (protrusions of GBM material on the epithelial aspect seen on silver stained sections) were present in 69 (97%) of the 71 biopsies. (See figure 19.)

Splitting of the GBM seen with methenamine silver stains was present to some degree in 42 (59%) of biopsies. In 8 patients it was particularly pronounced, giving a light microscopic appearance of mesangiocapillary glomerulonephritis. (See figures 21 and 25.) One biopsy (patient 49) was classified as mesangiocapillary glomerulonephritis on the basis of absence of significant subepithelial immune complex deposits and massive subendothelial deposits seen on electron microscopy. (See figures 22 and 26.) The GBM features on light microscopy are summarised in table 8.

Of the 70 biopsies classified as membranous, 3 were stage 1, 34 were stage 2, 28 were stage 3 and 5 were stage 4. There was no correlation between the interval from onset of symptoms to time of biopsy and staging of membranous nephropathy, the degree of splitting of the GBM seen on light microscopy, or the degree of mesangial interpositioning between the GBM and endothelium seen on electron microscopy.

TABLE 8: GBM ABNORMALITIES ON LIGHT MICROSCOPY

DISTRIBUTION (%AGE of BIOPSIES)

FEATURE	F&S	F&G	D&S	D&G
Thickening:	0	0	8 (11)	63 (89)
Spikes:	11 (15)	1 (1)	28 (39)	29 (41)
Splitting:	16 (23)	0	18 (25)	8 (11)
Chaining:	18 (25)	0	24 (34)	14 (20)

F&S = focal and segmental; F&G = focal and global; D&S = diffuse and segmental; D&G = diffuse and global.

Capillaries:

Narrowing of the glomerular capillary lumen was present in 38 (54%) of biopsies. This was assessed as mild in 13 (18%), moderate in 18 (25%) and severe in 7 (10%). Endothelial cell proliferation was not observed.

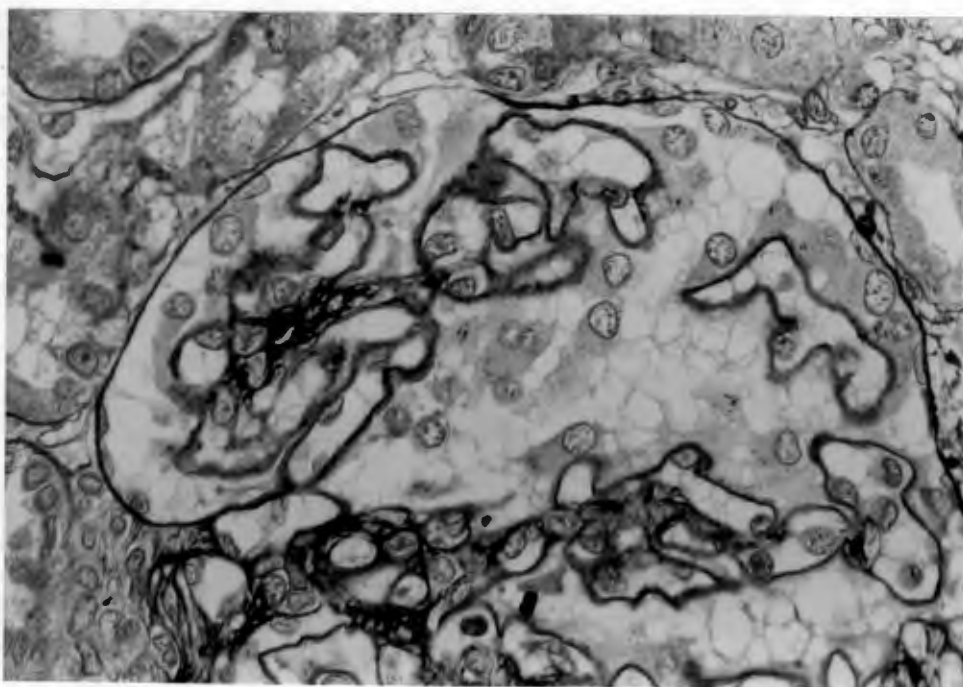


Figure 19. Methenamine silver stain showing pink subepithelial deposits and black "spikes". (Patient 67)

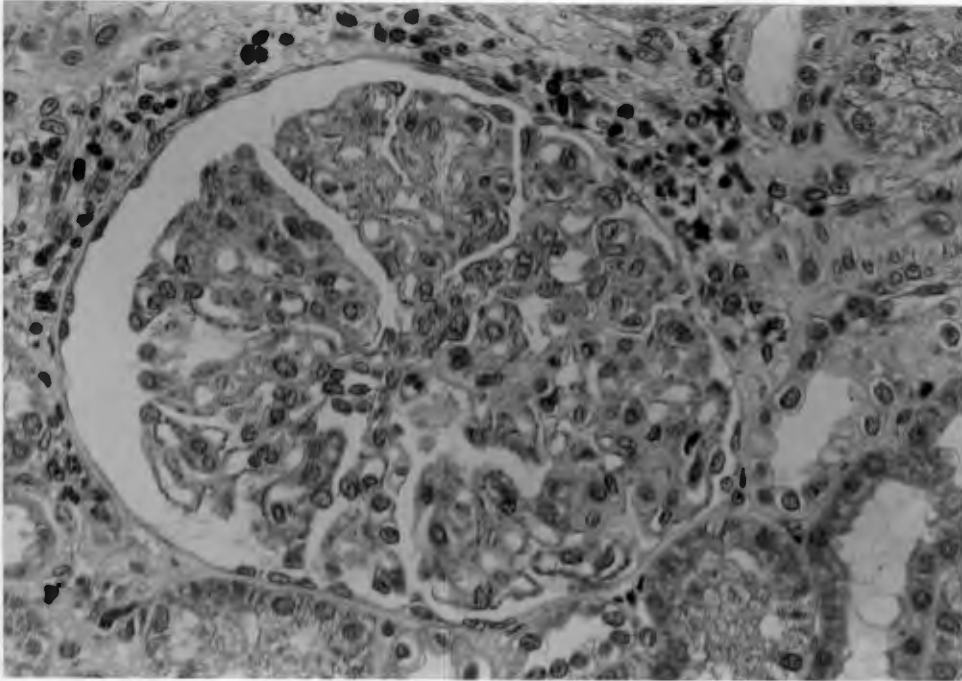


Figure 20. Masson's trichrome stain. The GBM is markedly thickened. Red immune complexes are visible in the subepithelial region and in the mesangium. (Patient 61)

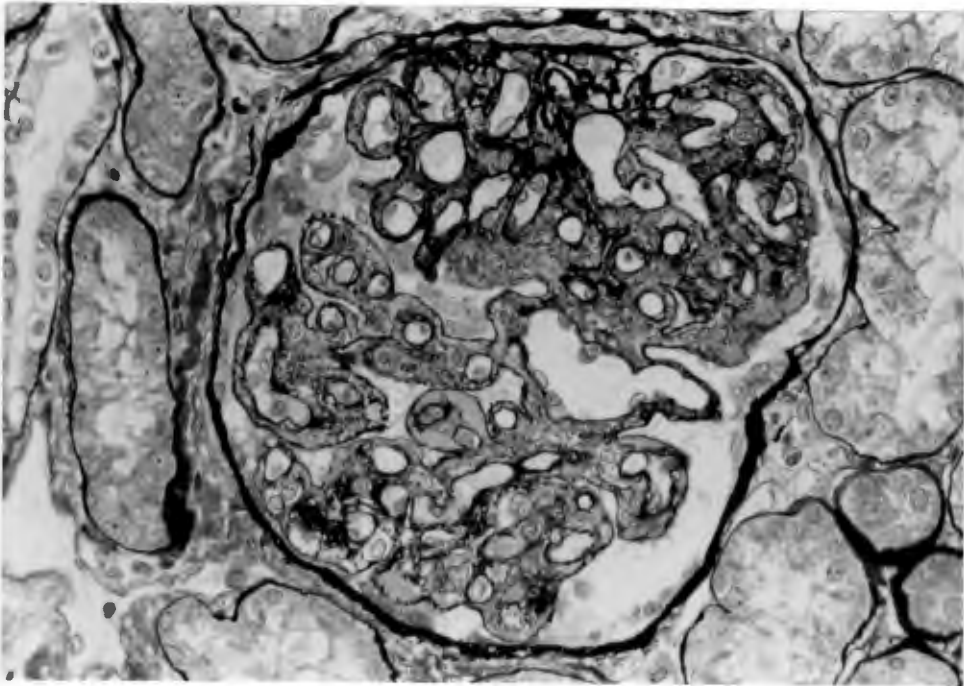


Figure 21. Methenamine silver stain showing extensive splitting of the basement membrane. "Spikes" are visible in a few areas. (Patient 66)

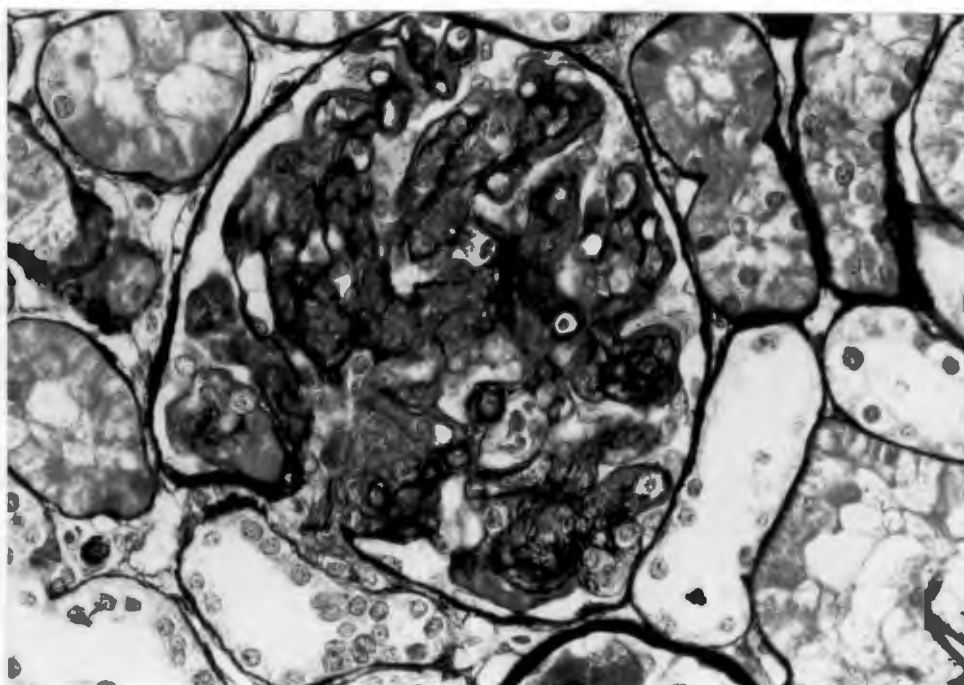


Figure 22. Methenamine silver stain of biopsy from patient 49 with mesangiocapillary glomerulonephritis showing mesangial proliferation and extensive splitting of the basement membrane. (Same biopsy as figure 25.)

Mesangium:

Mesangial abnormalities were present in all biopsies examined. All showed an increase in mesangial matrix, which was diffuse in 62 (87%) and segmental in 70 (99%). The increase in mesangium gave a lobulated appearance to the glomeruli in 7 biopsies.

Mesangial cellular proliferation (4 or more cells per mesangial area) was present in 66 cases (93%). In 47 (71%) it was diffuse and in 17 (25%) focal.

Other features:

A polymorphonuclear leucocyte infiltrate (5 or more cells per glomerulus) was present in 4 biopsies (6%).

Focal glomerular sclerosis was present in 10 cases (14%). This was segmental in 6 (8%) and global in 4 (6%). The percentage of glomeruli involved ranged from 5 - 55%, but 6 had less than 10% of glomeruli involved. Necrosis of glomeruli was not observed.

Cellular crescents were present in 5 cases. One patient had circumferential crescents in 35% of 20 glomeruli. The others all had small crescents affecting less than 30% of glomeruli.

Bowman's capsule was normal in 18 cases (25%). Adhesions were present in 53 cases (75%), 6 (8%) had mild or moderate fibrosis and 20 (28%) showed thickening of the basement membrane.

Sections treated with Masson's trichrome stain were available in 69 patients. These showed proteinaceous deposits in the subepithelial region in 55 (80%) and in the mesangium in 45 (65%). Only 1 patient (with mesangiocapillary glomerulonephritis) had deposits in the subendothelial position.

Tubules and interstitium:

The histology of the tubules is summarised in table 9.

TABLE 9: HISTOLOGY OF THE RENAL TUBULES

FEATURE:	NUMBER	(PERCENT)
Normal	55	(77)
Basement membrane thickening	12	(17)
Atrophy	11	(15)
Dilatation	4	(6)
Casts	3	(4)
Necrosis	0	(0)

INTERSTITIUM:

The interstitium was entirely normal in 40 cases (56%). In 24 there was an interstitial infiltrate of lymphocytes and plasma cells. This was subjectively characterised as scant in 12, moderate in 8 and heavy in 5. In 2 cases the lymphocytes formed lymphoid aggregates and in 1 the infiltrate was confined to the perivascular region.

Interstitial fibrosis was present in 9 biopsies. It was mild and focal in 7 cases and more diffuse in 2. Interstitial foam cells were present in 6 cases.

BLOOD VESSELS:

The blood vessels were normal in 66 biopsies (93%). In the remaining 5 there was mild medial proliferation. None of these patients was hypertensive at presentation.

Immunofluorescence:

The results of immunofluorescence studies were taken from the typed pathology reports as there was no way of repeating the studies without fresh, frozen biopsy material. Results were available in 65 patients. In all cases where immunoglobulins or complement components were demonstrated by immunofluorescence, they were deposited in a granular fashion. The results are summarised in tables 10-16.

One biopsy (patient 72) was tested for rheumatoid factor by direct immunofluorescence and found to be negative. This biopsy was positive for HBcAg and HBsAg.

TABLE 10: IMMUNOFLUORESCENCE: IgG DEPOSITS (n = 65)

GRADE	MESANGIUM	CAPILLARY
None	12 (18%)	2 (3%)
Light	1 (1%)	-
Moderate	3 (5%)	4 (6%)
Heavy	49 (75%)	59 (90%)

TABLE 11: IMMUNOFLUORESCENCE: IgM DEPOSITS (n = 65)

GRADE	MESANGIUM	CAPILLARY
None	19 (29%)	16 (25%)
Light	13 (20%)	16 (25%)
Moderate	22 (34%)	22 (34%)
Heavy	11 (17%)	11 (17%)

TABLE 12: IMMUNOFLUORESCENCE: IgA DEPOSITS (n = 65)

GRADE	MESANGIUM	CAPILLARY
None	35 (54%)	25 (38%)
Light	17 (26%)	24 (37%)
Moderate	11 (17%)	12 (18%)
Heavy	2 (3%)	4 (6%)

TABLE 13: IMMUNOFLUORESCENCE: C1 (n = 63)

GRADE	MESANGIUM	CAPILLARY
None	15 (24%)	6 (10%)
Light	12 (20%)	13 (21%)
Moderate	16 (25%)	25 (40%)
Heavy	20 (32%)	19 (30%)

TABLE 14: IMMUNOFLUORESCENCE: C3 DEPOSITS (n = 65)

GRADE	MESANGIUM	CAPILLARY
None	14 (22%)	3 (5%)
Light	6 (9%)	6 (9%)
Moderate	18 (28%)	26 (40%)
Heavy	26 (40%)	29 (45%)

TABLE 15: IMMUNOFLUORESCENCE: C4 DEPOSITS (n = 58)

GRADE	MESANGIUM	CAPILLARY
None	21 (36%)	11 (19%)
Light	11 (19%)	16 (28%)
Moderate	16 (28%)	22 (38%)
Heavy	12 (21%)	9 (16%)

TABLE 16: IMMUNOFLUORESCENCE: FIBRIN DEPOSITS (n = 63)

GRADE	MESANGIUM	CAPILLARY
NONE	11 (17%)	8 (13%)
LIGHT	18 (29%)	19 (30%)
MODERATE	25 (40%)	25 (40%)
HEAVY	9 (14%)	11 (17%)

Hepatitis B antigens:

Twenty-four patients were tested for deposits of HBsAg in the glomeruli and 22 were tested for HBcAg. The results are shown in table 17.

TABLE 17: HBV ANTIGENS DEPOSITED IN THE GLOMERULI

ANTIGEN	NUMBER TESTED	NUMBER POSITIVE (%)
HBsAg	24	15 (63)
HBcAg	22	16 (73)

Eleven biopsies were positive for both HBsAg and HBCAg and 3 were negative for both. Core antigen alone was positive in 6 and surface antigen alone in 2. In a further 2 cases only surface antigen was tested: both were positive.

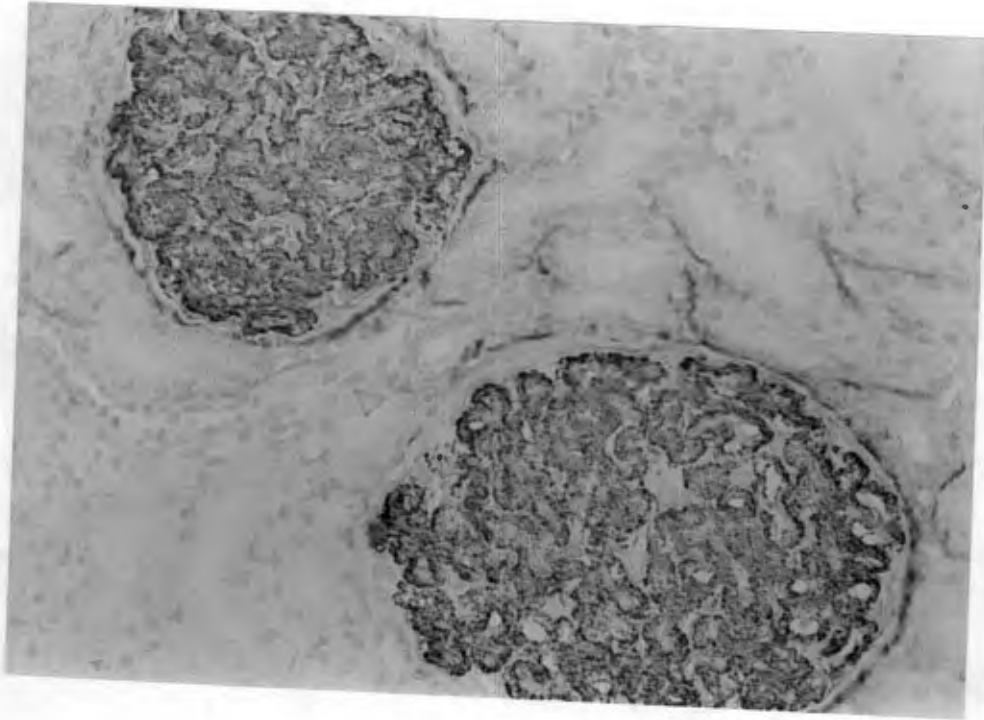


Figure 23. Positive staining for HBcAg in subepithelial immune complexes. (Patient 66: same biopsy as figure 21.)

Electron microscopy:

Electron micrographs were available from 68 of the 71 initial biopsies.

GLOMERULAR BASEMENT MEMBRANE:

The glomerular basement membrane was abnormal in all cases. The abnormalities were classified as thickening, reduplication (formation of new basement membrane between the epithelial cells and sub-epithelial immune complex deposits) and mesangial interpositioning between the GBM and endothelial cells. The frequency and severity of these abnormalities is shown in table 18.

TABLE 18: GBM ABNORMALITIES ON ELECTRON MICROSCOPY

ABNORMALITY	GRADE	NUMBER (%)
Thickening:	None	-
	Mild	3 (4)
	Moderate	23 (34)
	Severe	33 (49)
Reduplication:	None	23 (34)
	Mild	23 (34)
	Moderate	20 (29)
	Severe	2 (3)
Mesangial Interpositioning:	None	28 (41)
	Mild	15 (22)
	Moderate	11 (16)
	Severe	12 (18)

ELECTRON-DENSE DEPOSITS:

Electron-dense deposits were found in all biopsies examined. All had subepithelial deposits. In 10 cases some of these deposits resembled "humps" as seen in diffuse endocapillary proliferative glomerulonephritis⁴¹. Of the 4 patients with subendothelial deposits, 2 had only scanty deposits and 1 each had moderate and heavy deposits. The distribution of the deposits is shown in table 19.

TUBULO-RETICULAR BODIES:

These were seen within the endothelial cells in 29 of the 68 (43%) cases reviewed. In this study only existing electron micrographs were used and the sections themselves were not re-examined. In a previous study involving some of the patients included in this study, as well as biopsy specimens from Cecilia Makiwane Hospital in Ciskei, Mills and Emms were able to identify these structures in all cases of hepatitis B associated nephropathy examined⁴².

TABLE 19: ELECTRON-DENSE DEPOSITS
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SITE	NUMBER POSITIVE	PERCENT
Subepithelial	68	100
Intramembranous	54	79
Subendothelial	4	6
Mesangial	63	93

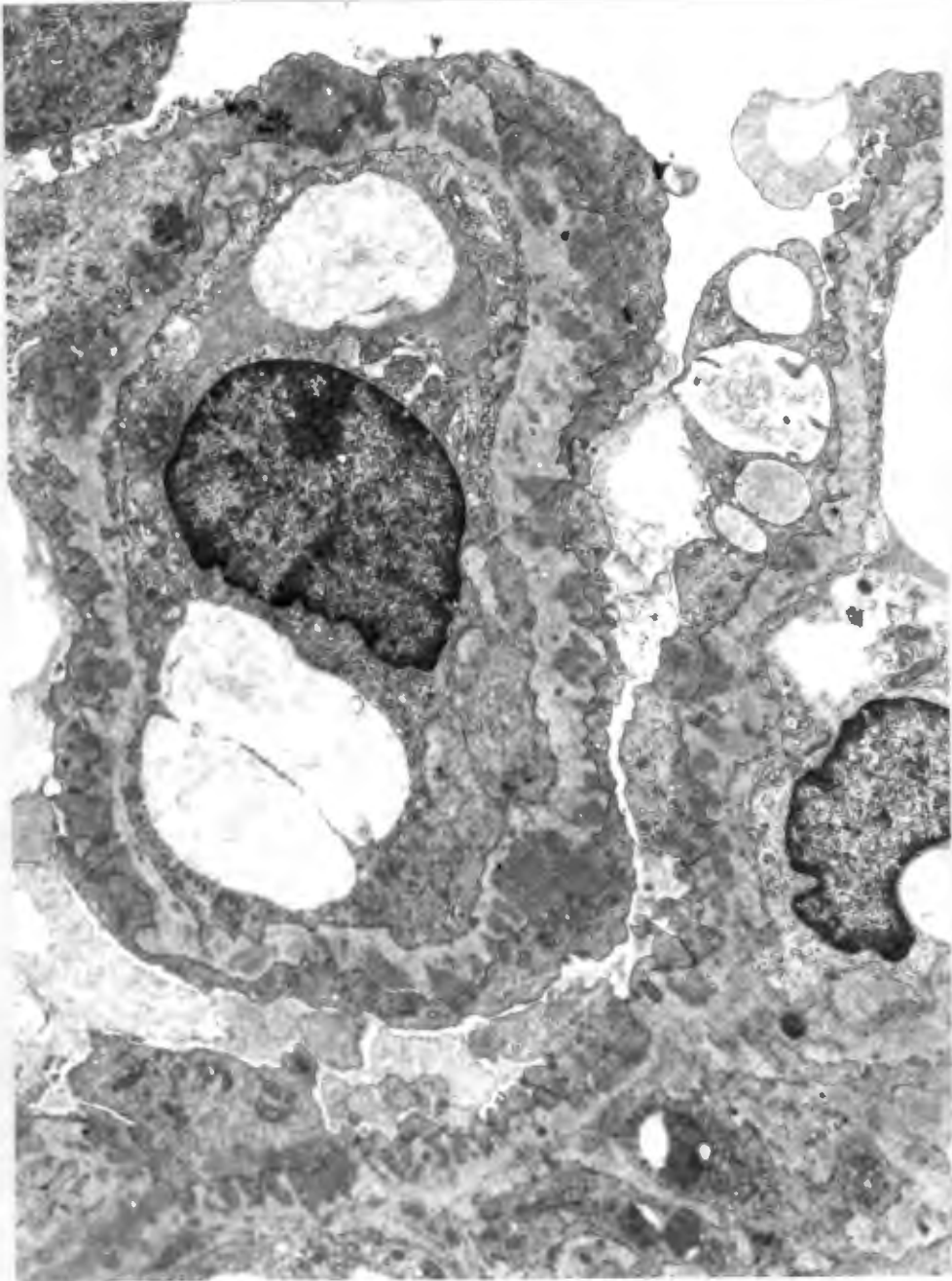


Figure 24. Electron micrograph showing subepithelial deposits separated by "spikes" of GBM. (Patient 67: same biopsy as figure 19.)

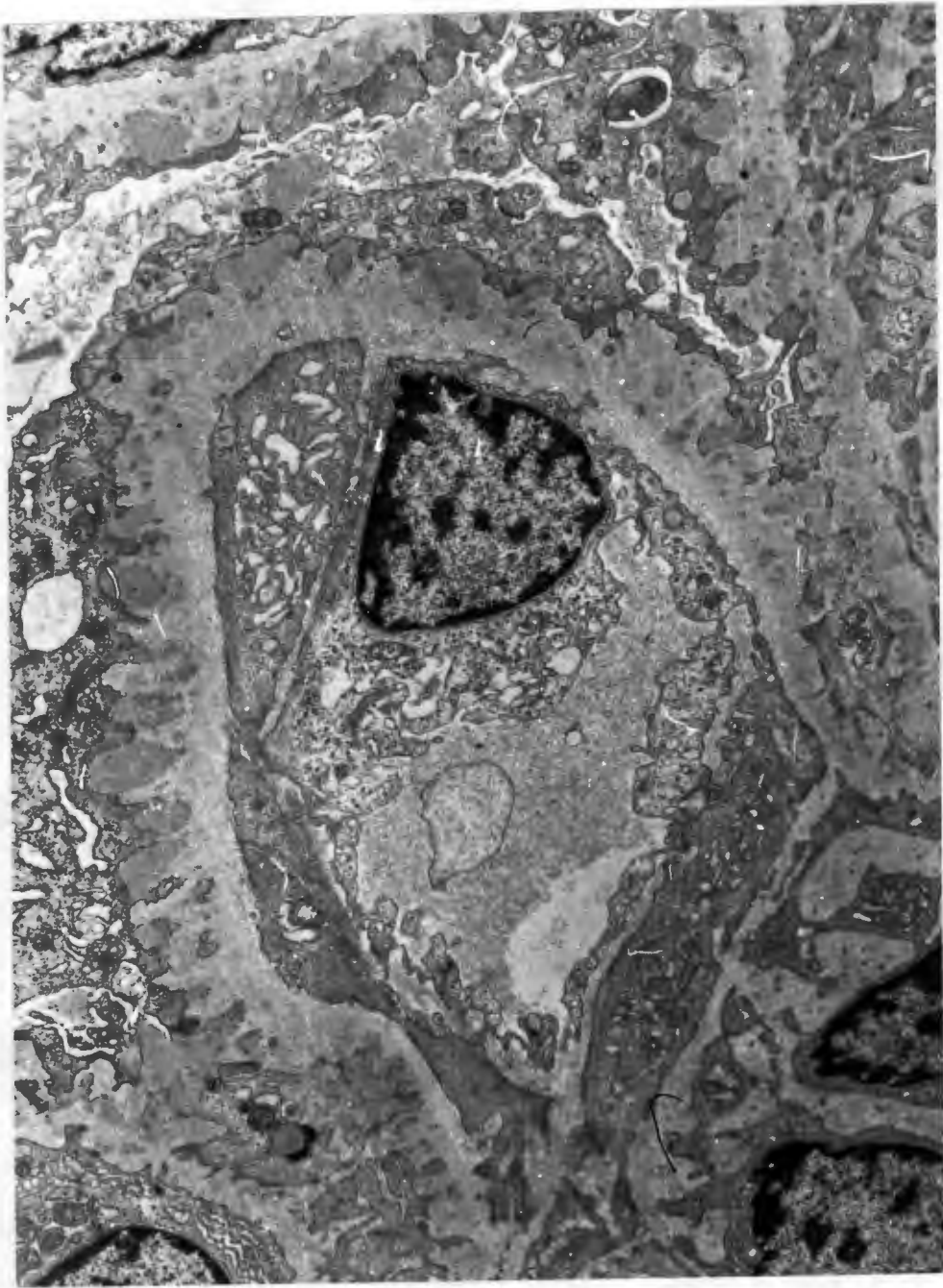


Figure 25. Electron micrograph showing subepithelial immune complexes and spikes. There are also significant areas of mesangial cytoplasmic interpositioning. (Patient 71.)

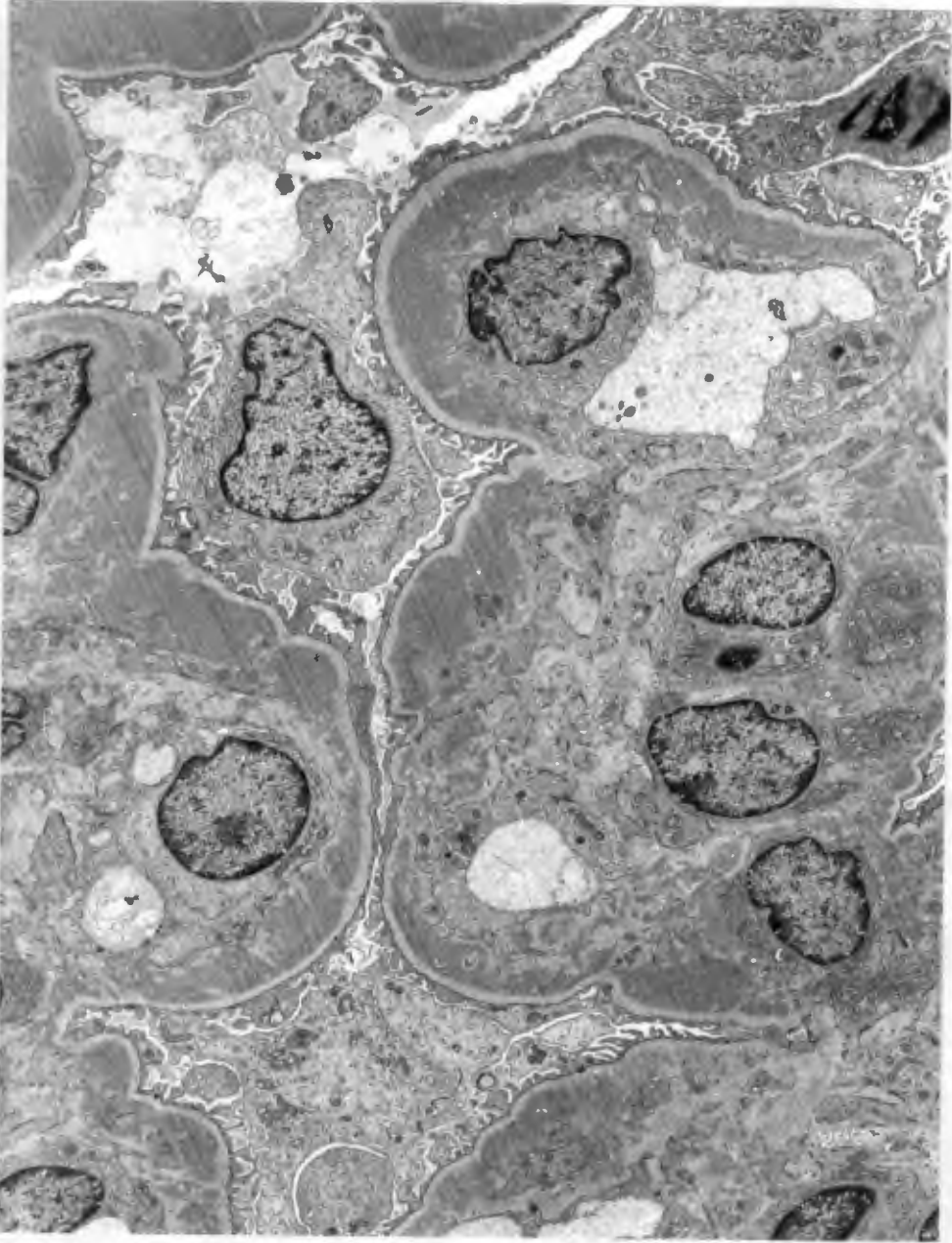


Figure 26. Electron micrograph showing massive supendothelial immune complex deposits. (Patient 49: same biopsy as figure 22.)

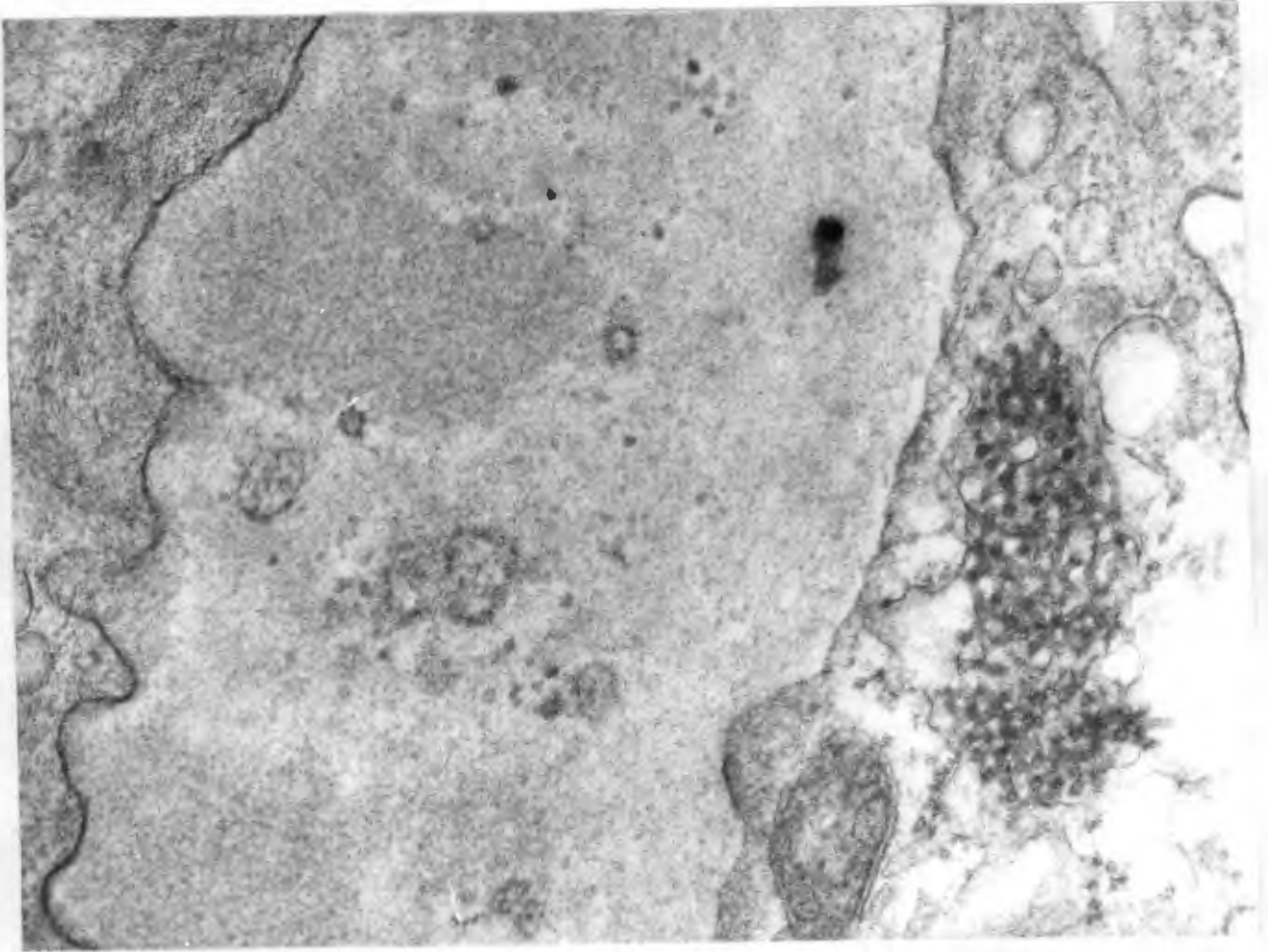


Figure 27. Electron micrograph showing tubuloreticular bodies within the cytoplasm of an endothelial cell to the right of centre. Fading subepithelial immune complex deposits are visible at the left of the picture.

Summary:

In summary, the typical biopsy features were:

1. Membranous nephropathy with an associated increase in mesangial cells and matrix
2. Heavy deposition of IgG and C3 in a granular pattern along the GBM and in the mesangium.
3. Extensive immune complex deposition in the subepithelial and mesangial areas.
4. Variable degrees of mesangial cytoplasmic interpositioning between the GBM and endothelial cells. This results in laying down of new GBM material giving an appearance similar to MCGN.
5. Variable demonstration of HBV antigens within the immune complexes.

Follow-up Biopsies:

Follow-up biopsies were performed in patients 22 (2 biopsies), 42 (3 biopsies) and 56 (3 biopsies).

Patient 22 had biopsies performed 4 and 18 months after onset of symptoms. The first biopsy showed stage 3 membranous nephropathy and the second stage 4. Both biopsies showed only mild mesangial changes and no other features of note. He became negative for HBeAg after 65 months, and asymptomatic after 78 months. He is still followed and remains asymptomatic and normotensive 114 months after onset of his illness. He remains HBsAg positive and has persistent mild proteinuria with normal renal function (calculated GFR 118 ml/min/1.73m²) and normal blood pressure.

Patient 42 had 3 biopsies 3, 35 and 63 months after onset of symptoms. The first biopsy showed stage 2 membranous nephropathy with a mild increase in mesangial matrix and cells. No evidence of basement membrane splitting or mesangial interpositioning was seen on either the silver stains or on electron microscopy. (Figure 28.) In the second biopsy the GBM showed significantly more thickening as well as several areas of splitting of the basement membrane. The electron micrographs showed several areas of mesangial interpositioning. The third biopsy, performed 5 years after the first showed only occasional membrane spiking, but very extensive basement membrane splitting or reduplication. On

light microscopy the appearances were of mesangiocapillary glomerulonephritis. The deposits seen on electron microscopy seemed to be divided into 2 populations. Some appeared older and were fading while others appeared to be of more recent origin (figure 32).

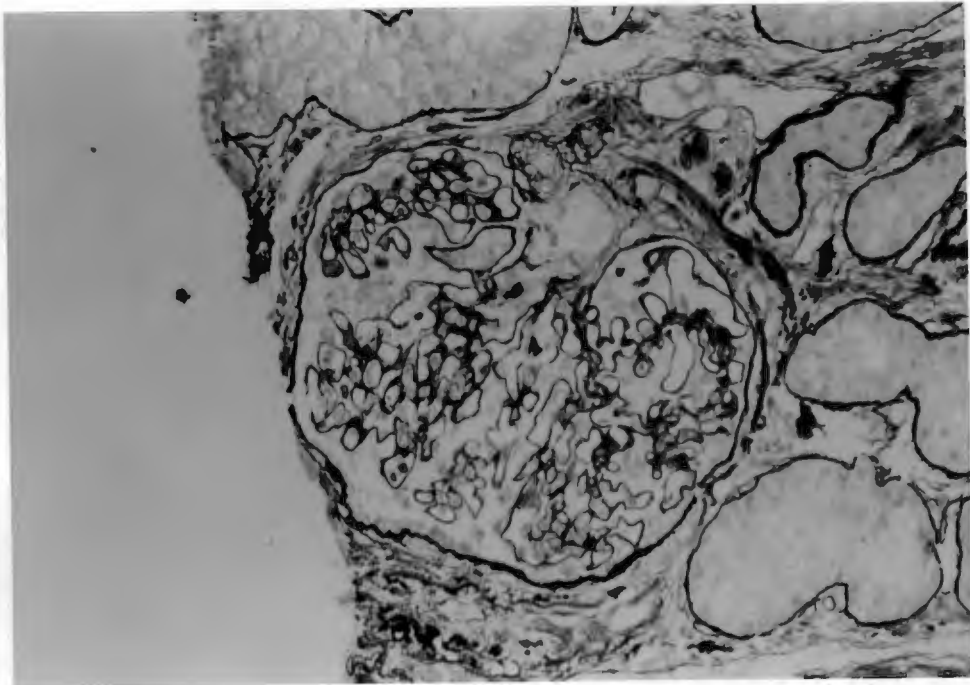


Figure 28. Methenamine silver stain of the first biopsy of patient 42

Patient 56 underwent his first biopsy within a month of onset of symptoms. This showed stage 2 to 3 membranous nephropathy with segmental sclerosis affecting 11 of 20 glomeruli. One year later he had progressed to chronic renal failure and repeat biopsy showed diffuse global sclerosis. The third biopsy was performed on the graft kidney 8 months after transplantation because of an acute rejection episode. There was no evidence of recurrent membranous nephropathy.

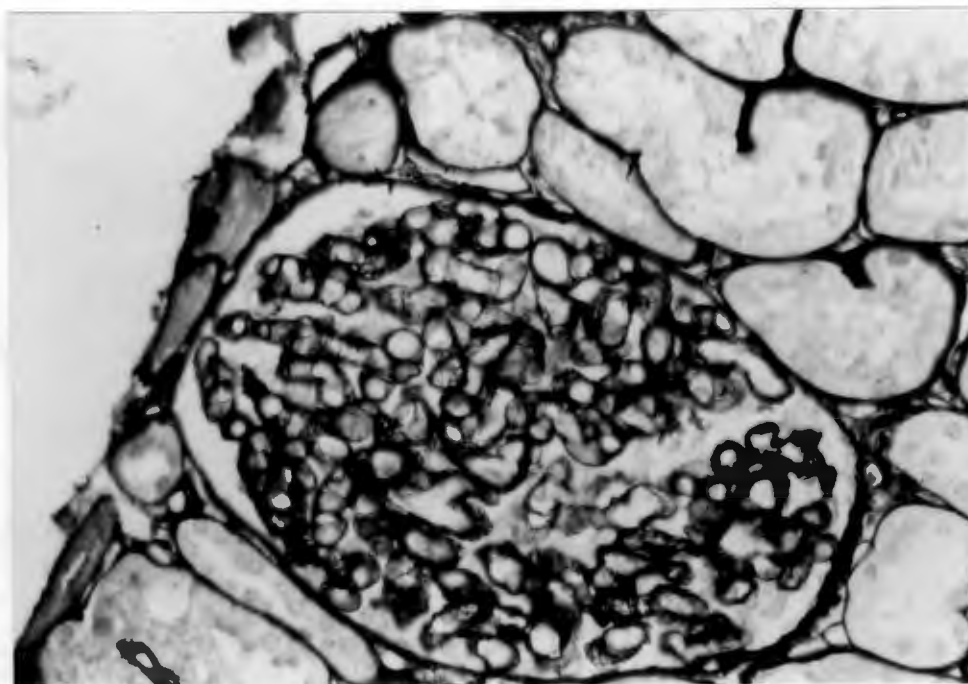


Figure 29. Methenamine silver stain of the second biopsy from patient 42, taken 32 months after the biopsy shown in figure 28. There are several areas where the GBM is split and in others a "chain-link" effect can be seen. Spikes can still be seen in some areas.

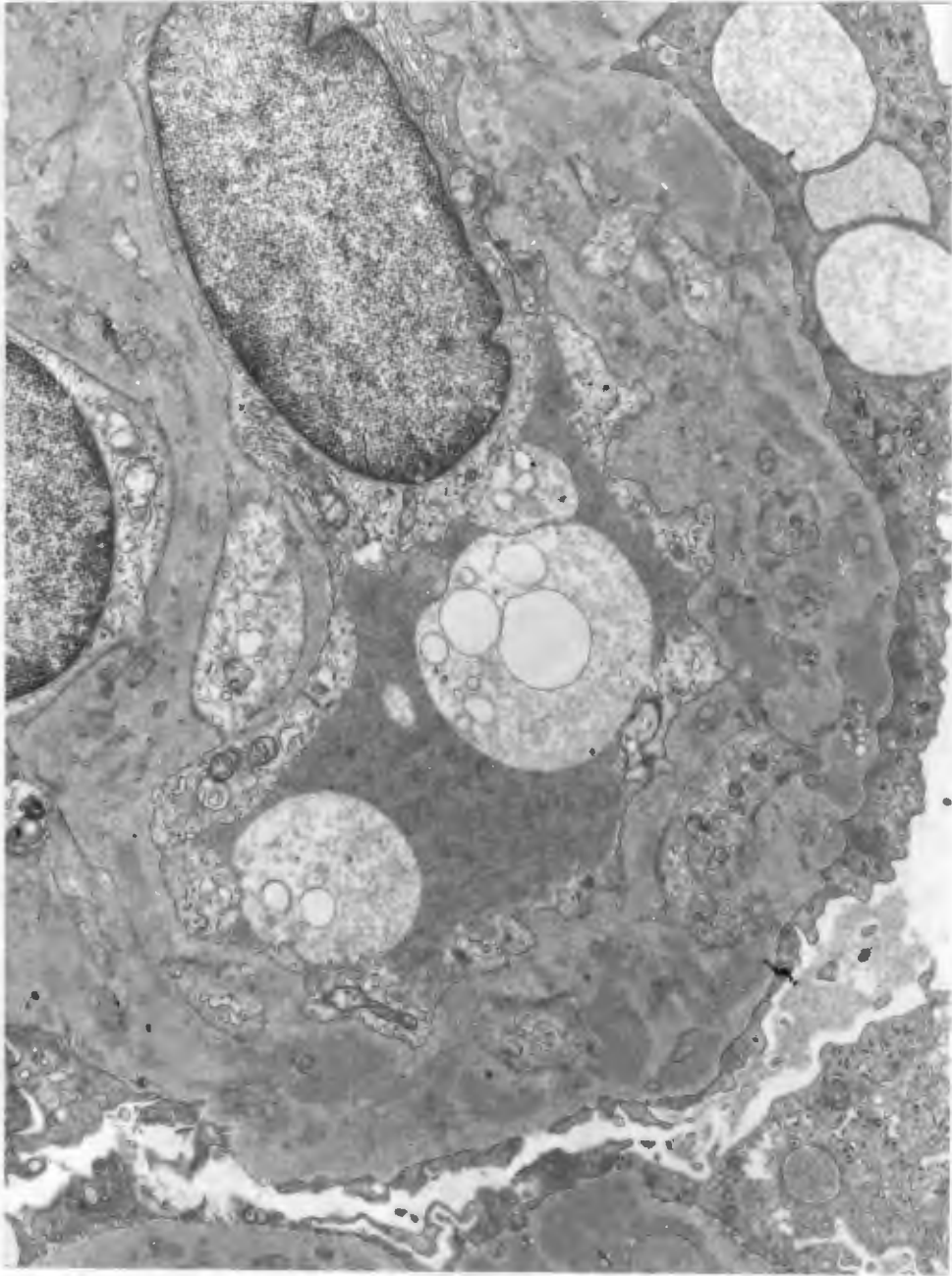


Figure 30. Electron micrograph from the same biopsy as figure 29. There are numerous intramembranous and occasional subepithelial immune complexes visible. In areas there is interpositioning of mesangial cell cytoplasm between the GBM and the endothelial cell with subsequent "splitting" of the GBM.

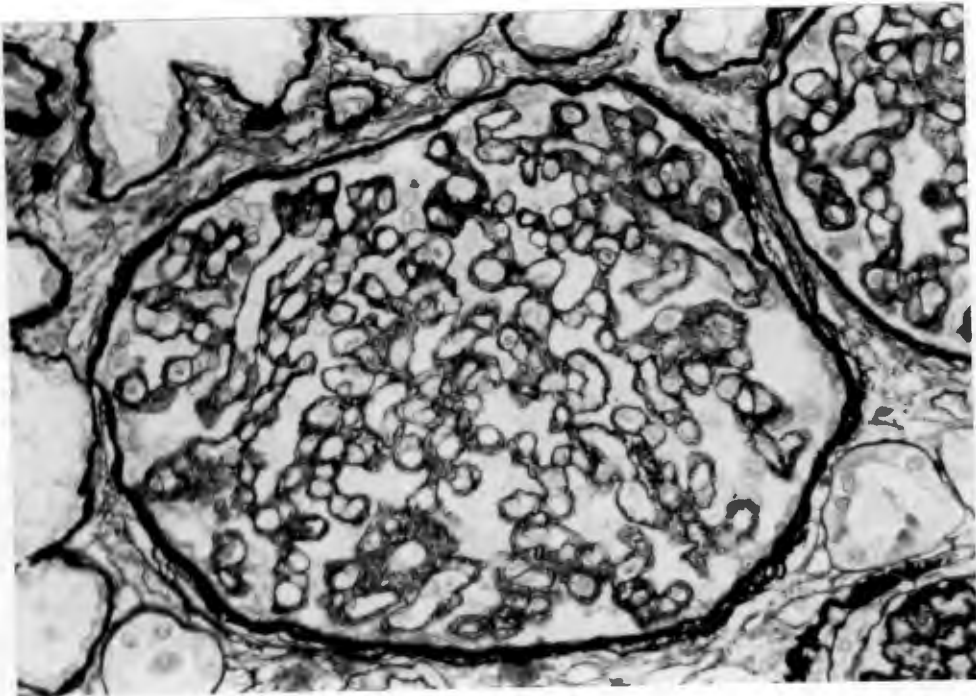


Figure 31. Methenamine silver stain of the third biopsy from patient 42. The GBM is massively thickened in areas with extensive splitting.

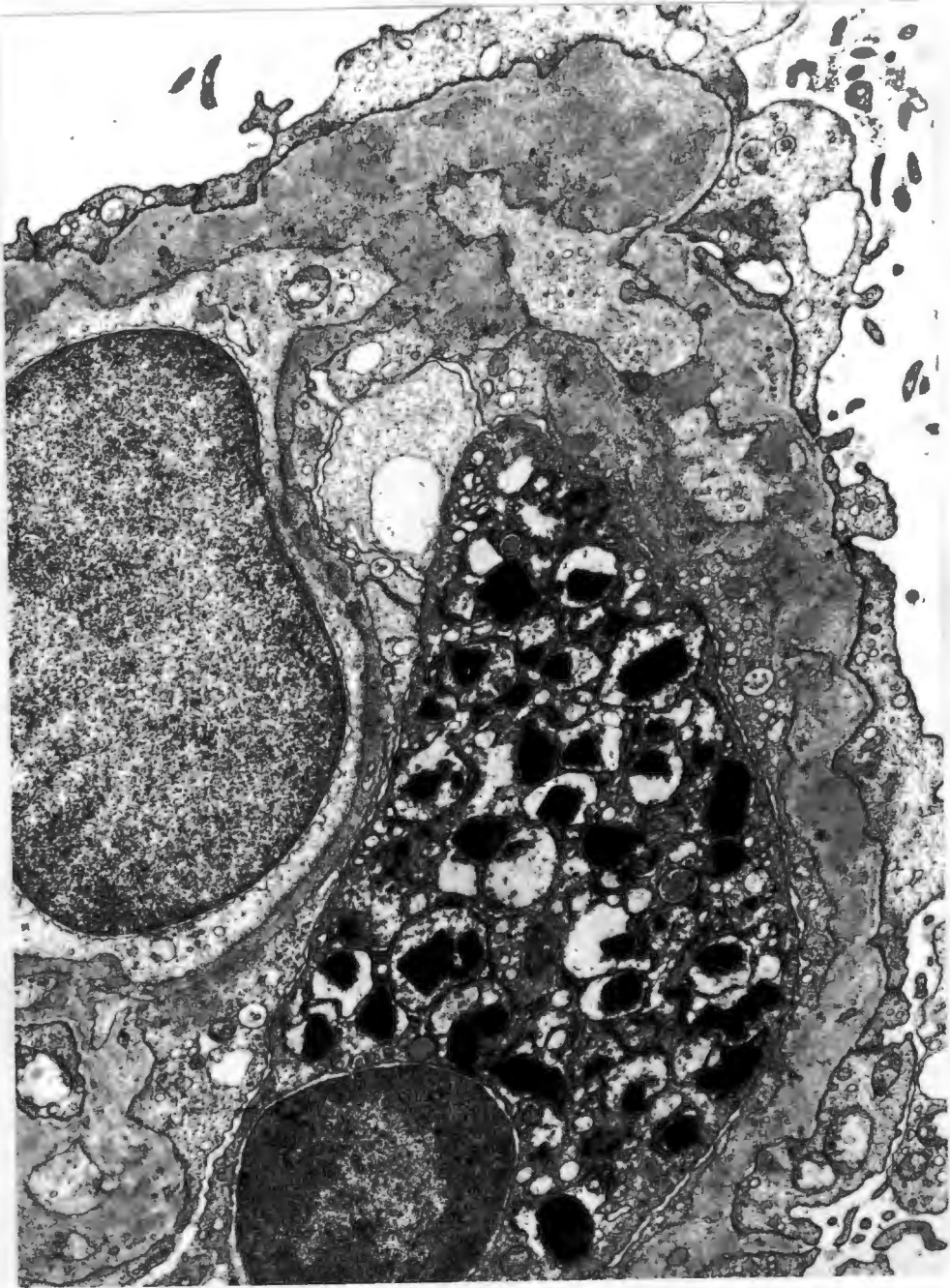


Figure 32. Same biopsy as figure 31. A polymorphonuclear leucocyte occupies the capillary lumen. Numerous subepithelial deposits and extensive mesangial interpositioning are shown.

ANALYSIS OF FACTORS AFFECTING OUTCOME

Using stepwise logistic regression analysis, an attempt was made to construct models to predict the duration of symptomatic disease and changes in calculated GFR. No meaningful models could be constructed using age at onset, sex, centile of height for age, calculated GFR at diagnosis, degree of proteinuria, stage of membranous nephropathy, or the degree of GBM splitting or mesangial interpositioning. The best correlation with duration of symptoms was with the interval from onset of symptoms to the first negative test for HBeAg. However, the correlation was rather weak (adjusted r squared = 0,21).

There were too many missing values to be able to calculate models using proteinuria selectivity index, complement levels, circulating immune complex levels, anti-DNA antibodies or anti-nuclear factor titres.

Using multiple regression analysis, no correlation could be found between any of the above factors and either duration of nephrotic syndrome or change in GFR. The best correlation was with seroconversion to the HBeAg negative state but even this failed to reach statistical significance ($p = 0,06$).

Hsu *et al* found that patients without focal glomerular sclerosis entered remission earlier than those with sclerosis⁴³. In this study there was no difference in average time to asymptomatic proteinuria only (25 vs 23 months) or

complete remission (38 vs 30 months). Nor was the presence of focal sclerosis useful in predicting declining GFR. As a test for estimated GFR of less than 80 ml/min/1.73m² at last follow-up, the presence of focal sclerosis had a sensitivity of 25% and a positive predictive value of 30%. The specificity was 88% and the negative predictive value 85%.

DISCUSSION

Clinical and demographic data

This confirms the 4:1 ratio described by Wiggelinkhuizen on the first 25 of these children⁸ and should be compared with the 1,4:1 ratio found in idiopathic membranous nephropathy in children⁴⁴, and a ratio of 1,5:1 for all patients with nephrotic syndrome in our series. Most other authors have described a similar large male predominance^{43,45}, although a few have described a more equal distribution^{4,21}. Venkateshan et al described only 12 patients, including 5 over the age of 21 years²¹. In the study by Brzosko et al there was no dominant histopathological type, and the question of coincidental HBV infection must arise⁴.

The reason for this sex distribution is probably a greater frequency of HBV infection in boys^{19,46-47,48}. Although there are no data regarding the prevalence of HBV infection in the Western Cape, this postulate is supported by data from the Gastroenterology Unit at Red Cross War Memorial Children's Hospital. Of 23 patients with HBV related chronic active hepatitis, 19 (83%) were boys (W.J. Frischman, personal communication.)

The average age of onset was 6,58 years. Again this is similar to the pattern described by other authors⁴⁵, although some have described a slightly earlier peak incidence, at about 4-5

years.^{11,43} It is probable that the age pattern simply reflects the age at which HBV infection is acquired.^{46,47,49} No data are available concerning the epidemiology of HBV infection in the Western Cape, and it is known that patterns of infection may differ widely in different areas.^{46,48} The average age of presentation of children with chronic active hepatitis was 6,38 years, again suggesting that this is the average age of acquisition of HBV infection in the population served by this hospital.

The transmission of HBV infection is mainly horizontal in Africa^{46,47,50}. The age of onset of disease in the current study is also compatible with horizontal spread of infection. In addition, 62% of mothers tested had no evidence of present or past HBV infection. Horizontal transmission was felt to be the principal method of infection in other studies as well, even in the Far East where vertical transmission is more common.^{43,51}

The stunting seen in the patients in this series is probably not related directly to their renal disease, but reflects the nutritional status of a large part of the community served by Red Cross War Memorial Children's Hospital^{52,53}, and are similar to the results of surveys of nutritional status carried out in the outpatient department of this hospital over several years⁵⁴. This study did not compare growth parameters of children with HBV related renal disease with other patients with nephrotic syndrome or glomerulonephritis, but it would be

usefull to do this as further confirmation of that stunting is not due mainly to the HBV related disease.

The majority of patients had oedema, ascites and microscopic haematuria. In addition, 26% had hypertension at presentation. These signs led to an initial clinical diagnosis of acute post streptococcal nephritis in several patients in this series. Even in an area with a high prevalence of HBV infection, the association of HBV with renal disease appears to be less widely appreciated than the association with other complications such as hepatocellular carcinoma and even polyarteritis nodosa.

Fourteen (19%) of the patients in this series had TB. Kala et al found TB in 37,5% of 40 patients with focal segmental glomerulosclerosis (FSGS) in Johannesburg, and these patients had a worse prognosis than those without TB⁵⁵. The incidence of TB is high in the Western Cape. In addition, the altered immune state in the nephrotic syndrome may promote activation of TB and impair host defense mechanisms. Alternatively the combination of TB and HBV infection may be particularly nephritogenic.

In this series the average change in calculated GFR from diagnosis to last follow-up was negligible (-0,5 ml/min/1.73m²). One of the 2 patients who developed end stage renal failure had pulmonary TB, but none of the other 3 patients with an estimated GFR of 50 or less had TB. There was

no difference in duration of symptoms between those patients with TB and those without. There is no evidence from these data that TB exerts a significant deleterious effect on the course of the disease, either in terms of duration of nephropathy or renal function.

Laboratory data

The results of urine microscopy were non-specific. Six patients (8%) had completely bland urinary sediments with no excess of cells or casts. Five had red cell casts in the urine. Of these, 2 subsequently received Interferon therapy (patients 56 and 64). Patient 56 has been successfully transplanted while patient 64 was asymptomatic when last seen, but with reduced renal function (calculated GFR 54 ml/min/1,73m²). Two of the other 3 patients entered remission after 5 and 11 months respectively, while the third had asymptomatic proteinuria only after 31 months. Although there were only 5 patients with urinary RBC casts, 2 had significant loss of renal function. Red cell casts may be an indicator of a more severe inflammatory process in the glomeruli.

Inaccurate urine collections probably account for much of the discrepancy between the 2 methods of estimating renal function. In addition, the calculation of GFR was done using the first creatinine value obtained, whereas formal clearance studies were often not done until days or even weeks later. Because calculated GFR was available on nearly all patients both at presentation and follow-up, it was used as the major method of estimating renal function

Approximately 25% of patients had impaired renal function (calculated GFR less than 80 ml/min/1,73m²) at presentation and 12 had reduced GFR at last follow-up. Of the 12, only 4

had reduced renal function at presentation. Of the 2 patients who progressed to end stage renal failure, 1 had a calculated GFR of 36 at presentation, and the other had an estimated GFR of 113 ml/min/1.73m².

Two patients had elevated estimated GFRs at presentation (GFR greater than 160 ml/min/1.73m²). Both had a normal GFR at last follow-up, when both were in remission.

Estimated GFR was not useful as a predictor of outcome.

All patients had nephrotic syndrome or heavy proteinuria. The proteinuria selectivity index varied widely. There were too many missing values to be able to use the selectivity index as a prognostic indicator. Seggie et al⁵⁶ found only 1 of 8 (13%) children with HBV associated membranous nephropathy had selective proteinuria (selectivity index < 10%). In this study proteinuria was selective in 29% of the 34 patients in whom it was measured.

Apart from hepatomegaly, there were no other clinical signs of liver disease in these patients. Most had mild elevations of ALT which fluctuated during the course of the disease. Three liver biopsies all showed mild chronic persistent hepatitis. In those reports that include descriptions of liver pathology, chronic persistent hepatitis in children and chronic active hepatitis or chronic persistent hepatitis in adults are the most frequent patterns^{3,9,12,45,51,57}. Isolated cases have been

reported of children with chronic active hepatitis, cirrhosis and fulminant hepatitis^{7,9,58,59}.

Patients with symptomatic nephrotic syndrome are almost always HBeAg positive. This has been confirmed by most other authors^{9,11,19-21}. Seggie et al did not demonstrate HBeAg in any of their patients. In this study 34 of 38 patients in remission were HBeAg negative and 34 of 39 who were HBeAg negative were in remission while the remaining 5 had asymptomatic proteinuria only.

Most patients become HBeAg negative shortly before or after entering remission. In this study it was not possible to show a very close correlation between remission and seroconversion. Possible reasons for this are the irregular nature of HBV antigen testing in this retrospective study, and the fact that a period of repair to the GBM is likely to be required before proteinuria stops.

The clinical correlation of seroconversion from HBeAg positivity to anti-HBe positivity with clearance of the HBeAg from the circulation with remission of disease has been noted by other authors^{8,21,43}. As in this study, Hsu et al found that patients who entered remission tended to lose the HBeAg while those whose disease remained active tended to remain positive⁴³. However a direct correlation could not be found. Remission occurred in 12 of 13 children before seroconversion,

but 12 of 16 cases who did not enter remission remained positive for HBeAg.

Milner et al found that HBeAg correlated better with renal disease than circulating HBV DNA. HBeAg was present in all cases whereas circulating HBV DNA was detected in only 6 of 11 (54%)¹¹.

The majority of patients had depressed serum complement levels, although some had elevated levels. Forty-seven (71% of 66 patients tested) had total serum complement levels below the normal range, while 13 (20%) had levels above the normal range. Similar results were obtained for C3 (67% below the normal range). The results for C4 were slightly different, with 47% below the normal range ($p = 0,04$ (Chi squared), odds ratio 2,3; 95 % confidence limits 1,0-5,1). These results suggest that alternate pathway activation of the complement cascade occurs more commonly than classical pathway activation.

Low levels of serum C3 and C4 in 15-64% of cases have been reported in most studies^{9,21,44,56}. A few studies have reported normal complement levels^{5,6,43}. Lin found low levels of both C3 and C4 initially, but normal levels of C3 in most after 6 months with C4 levels tending to remain low in almost 30%.⁵¹ Milner et al found low levels of C3 but not of C4, also suggesting alternate pathway activation¹¹.

In this series, 24 of 29 patients (83%) had normal levels of circulating immune complexes as assayed by the C1q binding assay. Other authors have found elevated levels in their patients^{19,45}. Furose et al found varying levels depending on the clinical organ involvement¹⁹. Twenty-five percent of those with renal disease alone had elevated CIC's, while 100% of those with only liver disease had elevated levels. Lin found that 67% of his patients had elevated IgM containing CIC's at presentation, but none still had elevated levels after 6 months⁵¹. IgG and IgA CIC's were not different from controls. Lin was also able to demonstrate HBsAg containing CIC's. The level did not correlate with the degree of proteinuria or haematuria⁵¹.

HBeAg is known to circulate both in the free form and in complexes with IgG^{60,61}, and Takekoshi et al have postulated that these circulating immune complexes may play a central role in the pathogenesis of HBV associated nephropathy⁶¹. It would appear that these HBeAg-IgG complexes are not detected by routine assays for CIC's.

The increase in size and echogenicity of the kidneys as seen on ultrasound scanning is non-specific and compatible with any inflammatory process.

Clinical course of the disease:

The majority of patients followed a fairly benign course. The median duration of symptomatic proteinuria was under 2 years, and about 2 thirds were in complete remission within 5 years. A few did have more prolonged symptoms. Thirty-seven patients were followed for more than 40 months; 7 still had active nephrotic syndrome, and 5 had asymptomatic proteinuria at the last visit. Of the 38 patients in complete remission, 5 took longer than 40 months to enter remission. Venkateshan *et al* analysed 8 previous publications and concluded that in children about 65% can be expected to be in spontaneous remission after 1 year, 85% within 2 years and by 5 to 7 years about 95%²¹. In the present study the early remission rates are somewhat lower.

Most patients had well preserved renal function, but 2 progressed to end stage renal failure and 3 others had an estimated GFR of under 50 ml/min/1,73m². Progression to chronic renal failure has been described by several authors. An analysis of 5 other studies with a minimum follow-up period of 1 year^{10,43,51,56,62} revealed 10 of 112 (8,9%) children with progressive loss of renal function. In this study, 5 of 72 (6,9%) children had an estimated GFR of less than 50 ml/min/1,73m² at last follow-up. Combining the 2 figures gives 8% of children developing chronic renal impairment.

The risk of chronic renal failure appears to be much higher in adults, with one third of patients progressing relentlessly to renal failure⁶³.

Steroid therapy was not effective in any of the patients receiving it, while it was responsible for severely aggravating the hypertension of at least 1 patient. In several other studies steroids have been found to be ineffective^{9,51,56,63}. Lin and Lo found that HBV DNA was present in patients with HBV associated membranous nephropathy for 12 months in untreated patients but persisted for up to 3 years in those who had received steroid therapy⁶⁴.

Specific anti-viral therapy has shown more promise^{57,63,64}. Of the 3 patients treated with alpha interferon in this study, 1 cleared the HBeAg from the serum, had a significant decline in proteinuria with a concomitant rise in plasma albumin and a small decline in serum creatinine levels by the time he defaulted; 1 showed temporary improvement and 1 showed no change. If these 3 patients are analysed with patients reported by other investigators^{57,63,65,66}, 9 of 16 patients (56%) showed sustained improvement in the degree of proteinuria and a further 5 had a temporary improvement. Seven of the 16 (44%) became negative for HBeAg and entered complete remission. This is not significantly different from the 36% seroconversion rate reported in a large study of patients receiving interferon for hepatitis B liver disease⁶⁷.

Lin and Lo described significant reductions in urine protein excretion rates in all patients treated with a combination of adenosine arabinoside and thymic extract, although only 1 of 24 became negative for HBeAg⁶⁴.

Histopathology:

Most authors have described a large majority of patients with membranous nephropathy in HBV associated renal disease. The current study supports this observation. As in previous studies,^{9,18,21,45,51} the patients seen at Red Cross War Memorial Children's Hospital had heavy deposition of IgG and C3 in the glomerular immune complexes.

There are several features which emerge from this study which have either not been mentioned or received only scant attention in previous reports.

In this study there were significant increases in mesangial cells and matrix in 93% and 100% of biopsies respectively. While some authors^{21,51,68} found mesangial proliferation in 50-100% of their patients with HBV associated membranous nephropathy, Yoshikawa *et al* described it in only 2 of 16 (12,5) of their patients⁴⁴ and several other authors make no reference to mesangial changes at all^{20,56,63}.

Mesangial cytoplasmic interpositioning between the GBM and endothelium was seen to some degree in 59% of the electron micrographs examined, and on light microscopy GBM splitting was visible in 58% of cases. In 8 cases there was diffuse, global splitting of the GBM, giving the appearance of mesangiocapillary glomerulonephritis. The decision to classify these lesions as membranous nephropathy might be

controversial, but was taken because of the following evidence:

1. The majority of biopsies examined (approximately 60%) showed some degree of mesangial interpositioning. There appears to be a continuum ranging from absence of interpositioning to circumferential interpositioning in virtually all capillary loops with a wide range of intermediate cases. It was therefore felt to be artificial and rather arbitrary to classify those with the most pronounced degree of interpositioning separately from the others.
2. Although the biopsies showed circumferential interpositioning as emphasised in the original description by Arakawa and Kimelstiel⁶⁹, immune complex deposition was heavy in the subepithelial area and mesangium with no subendothelial deposits.
3. There was no difference in outcome in those with extensive interpositioning compared with those without interpositioning. This is different from the situation described by Arakawa, where circumferential interpositioning was uniformly associated with progression to chronic renal failure⁶⁹.
4. Serial biopsies in patient 42 showed progression from stage 2 membranous nephropathy with no interpositioning

in the first biopsy to global interpositioning in the third, with an intermediate stage in the second biopsy.

Yoshikawa et al described minor degrees of mesangial interpositioning in 4 of their 16 patients with HBV associated membranous nephropathy and 1 of 12 with idiopathic membranous nephropathy⁴⁴.

HBsAg and HBCAg were demonstrated in the majority of biopsies tested. HBCAg does not appear in the circulation in detectable quantities, and its presence in large amounts in the glomeruli is enigmatic. HBeAg shares considerable amino-acid homology with HBCAg. HBeAg is produced by proteolysis of the translation product of the entire pre-core/core open reading frame of the HBV DNA. HBeAg consists of 10 amino acids coded by the pre-core region and most of the core protein, lacking only 34 amino-acids at the carboxy terminus^{70,71}. The polyclonal antibodies used have been shown to have anti-HBeAg activity and binding of these antibodies to HBeAg is the probable explanation of these results⁷².

Tubuloreticular bodies not been described in previous reports of HBV associated nephropathy, with the exception of the paper by Mills and Emms. One paper even suggested that they could be specific for the nephropathy associated with the acquired immune deficiency syndrome (AIDS)⁷³.

Prognostic indicators:

No factors which were useful in predicting outcome in hepatitis B associated nephropathy could be identified. This was a retrospective study and the possibility that a prospective study might be able to identify such factors cannot be ruled out.

Useful prognostic indicators would be of great benefit to those caring for patients with this disease, particularly as specific antiviral therapy becomes available. Those patients at highest risk for progressive renal failure or prolonged duration of nephrotic syndrome would be able to receive such treatment. At present the role of interferon in the treatment of HBV related renal disease is not firmly established. The disease has a favourable prognosis in most cases, and interferon is expensive and has numerous potential side-effects.

Brook et al analysed data from 114 patients given interferon for chronic HBV infection in an attempt to identify factors predictive of a good response to treatment. They concluded that negative human immunodeficiency virus (HIV) status, chronic active hepatitis on liver biopsy, high serum AST levels, a positive history of acute icteric hepatitis and low HBV DNA levels were useful predictors of a good response, defined as elimination of HBV DNA and HBeAg for at least 12 months after treatment⁷⁴. If a subset of patients with HBV nephropathy could be identified who are both at high risk from

their disease and are likely to respond to treatment, the expensive anti-viral agents could be used in a highly cost-effective manner.

Pathogenesis of Hepatitis B Associated Membranous Nephropathy:

The pathogenic mechanisms involved in the development of HBV associated nephropathy are unknown. There are 4 possible ways in which HBV infection and membranous nephropathy are related:⁴⁵

1. Preformed circulating immune complexes containing HBV antigens are deposited in the glomerulus.
2. Immune complexes containing HBV antigens are formed *in situ* in the subepithelial and mesangial areas.
3. Glomerular deposits of HBV antigen-containing immune complexes are not pathogenic and the deposits develop by another virus-induced mechanism.
4. HBV infection and membranous nephropathy are associated but the nephropathy is not caused by the viral infection.

The available evidence for and against these possibilities is discussed below.

Preformed circulating immune complexes containing HBV antigens are deposited in the glomerulus.

Circulating immune complexes containing HBV antigens have been demonstrated in some patients with HBV associated nephropathy^{8,19}, and HBeAg has been shown to circulate in

complexes with IgG⁶¹. For circulating immune complexes to be deposited in the subendothelial space they must be small and cationic⁷⁵. While HBsAg and HBcAg are large ($> 10^6$ Daltons) and anionic^{76,77}, HBeAg is small. In the free form it has a molecular weight of 19 000 Daltons, and forms complexes with IgG with a mass of 45 000 Daltons. Although HBeAg is also anionic the immune complexes are cationic⁷⁸. Further evidence for an important role for HBeAg is that circulating HBeAg tends to correlate with disease activity as shown in this study.

However experimental evidence suggests that membranous nephropathy is not mediated by circulating immune complexes but rather by 1 of a variety of possible mechanisms of *in situ* formation^{79,80}, but they could account for the mesangial deposits.

Immune complexes containing HBV antigens are formed *in situ* in the subepithelial and mesangial areas.

This would involve the initial trapping of either viral antigen or antibodies directed against such antigens in the free form in the subepithelial space. Rabbits chronically injected with bovine serum albumin develop predominantly membranous lesions⁸¹. This is due to initial charge interaction between the cationic antigen and the anionic GBM, and similar results have been obtained with other cationic

antigens⁸². All 3 major HBV antigens are anionic, and primary trapping of antigen would not occur.

Cationic IgG antibodies have been shown to become trapped in the subepithelial space, and when followed by an infusion of the appropriate anionic antigen give rise to subepithelial immune complexes⁸³. *In situ* formation of immune complexes may therefore play a role in the pathogenesis of HBV associated membranous nephropathy.

Glomerular deposits of HBV antigen-containing immune complexes are not pathogenic and the deposits develop by another virus-induced mechanism.

The classical experimental model of membranous nephropathy is Heyman nephritis⁸⁴. In this model, injection of homogenates of rat kidney ("active Heyman nephritis") or preformed antibodies against renal cortex ("passive Heyman nephritis") induce membranous nephritis in rats. The nephritogenic antigen was subsequently found to be a proximal tubule microvillar glycoprotein with a molecular weight of 330 kilo-Daltons, designated "gp330"⁸⁵. Subsequently gp330 was found to be present on the surface of glomerular epithelial cells in clathrin coated pits on the base of the foot processes where they make contact with the GBM⁸⁵. In a refinement of the passive Heyman nephritis model, membranous nephropathy has been induced by the injection of anti-gp330 antibodies⁸⁵.

A human equivalent of gp330 has been identified in proximal tubules but not in the glomerulus. This does not rule out the possibility of similar mechanisms being responsible for the formation of immune complexes in human membranous nephropathy. There is some evidence that certain kidney membrane antigens could play a role in membranous nephropathy⁸⁵, and Sekar et al have identified anti renal membrane antibodies in patients with HBV infection and concomitant nephritis⁸⁶. A variety of other auto-antibodies have also been demonstrated in chronic HBV infection⁴⁵.

Given the auto-immune nature of experimental and probably also of idiopathic human membranous nephropathy as well as the presence of auto-antibodies in chronic HBV infection, an auto-immune aetiology for HBV associated membranous nephropathy cannot be excluded.

HBV infection and membranous nephropathy are associated but the nephropathy is not caused by the viral infection.

Finally the frequent occurrence of HBV infection in patients with membranous nephropathy may not be due to the HBV infection or the immunological consequences thereof. An underlying immunological abnormality or genetic predisposition may increase the likelihood of these patients acquiring both diseases independent of each other. While this possibility has not been definitely excluded, it seems unlikely for several reasons. Firstly membranous nephropathy is far more common in

areas of high HBV prevalence such as Africa and the Far East than in areas of low prevalence⁴⁵. Secondly complete remission tends to correlate with clearance of the HBeAg from the serum. Finally there are serological and histopathological differences between idiopathic membranous nephropathy and the form associated with HBV infection⁴⁴.

CONCLUSIONS:

HBV infection is a common cause of nephrotic syndrome in children in Cape Town. It accounts for at least 13% (and possibly more) of all cases of nephrotic syndrome, and almost half of all cases in Black children.

Boys are affected 4 times more frequently than girls. The peak incidence is at about 6-7 years of age. These features probably reflect the epidemiology of HBV infection. HBV associated nephropathy is due mainly to horizontal spread of HBV.

The clinical features are those of nephrotic syndrome. Nearly all had some degree of haematuria, and hypertension is present in one quarter. Hepatomegaly occurred in 40% and splenomegaly in only 11%. These features provide important clinical clues to the aetiology when present. The diagnosis must be considered in all patients with a nephritic illness associated with heavy proteinuria in areas with a high prevalence of HBV infection.

Tuberculosis was commonly associated with HBV nephropathy in this series. TB must be carefully excluded in all patients.

Serum complement levels were depressed in the majority of patients, but some had elevated levels.

The histopathological features are those of membranous nephropathy with associated mesangial proliferation. Mesangial interpositioning between the endothelial cells and the GBM was seen to some extent in 60% of biopsies, and was extensive in 11%, giving the light microscopic appearances of mesangiocapillary glomerulonephritis. Serial biopsies in 1 patient showed progression from "pure" membranous features to extensive interpositioning with mesangiocapillary features.

Biopsy of these patients is essential for accurate diagnosis. Thirty per cent of patients with nephrotic syndrome and HBV infection had lesions other than membranous nephropathy. Nine per cent had minimal change nephrotic syndrome and responded to steroid therapy without complications related to the HBV infection.

The majority of patients will be asymptomatic after about 2 years, but some have a much more prolonged course. Renal function is well preserved in most patients, but progressive renal failure occurs in approximately 6-8% of cases. No useful indicators of prognosis could be identified.

While the ultimate prognosis is good, morbidity is moderately high. All the common complications of nephrotic syndrome were observed in this series.

A randomised, controlled trial is required to define the role of anti-viral therapy in the treatment of this condition.

Until this has been done, supportive therapy only is indicated for the majority of patients. This consists of a low salt diet with adequate protein intake, cautious use of diuretics, and in severely symptomatic patients infusions of salt-poor human serum albumin with diuretic cover.

The immunopathogenesis of HBV associated nephropathy is poorly understood and much research remains to be done to elucidate the underlying mechanisms.

Prevention of all the complications of HBV infection by universal immunisation remains the most desirable approach to this problem.

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APPENDIX 1: CLINICAL AND LABORATORY DATA.

1. Name:
2. Hospital Number:
3. Race and sex code:
4. Date of birth:
5. Date of onset:
6. Date referred:
7. Dry weight (kg):
8. Weight lost (%):
9. Centile weight/age:
10. Height (cm):
11. Centile height/age:
12. Centile weight/height:
13. Oedema at onset?:
14. Hypertension at onset?:
15. Ascites at onset?:
16. Pleural effusion at onset?:
17. Hepatomegaly at onset?:
18. Splenomegaly at onset?:
19. Haematuria at onset?:
macro
micro
none
20. History of jaundice? (months prior to onset)
21. Mother positive (any Ag/Ab)?
22. Kidney length (Right) (mm):
23. Kidney length (Left) (mm):
24. Kidneys measured IVP or US?:
25. Plasma albumin (g/l):
26. Plasma total protein (g/l):
27. Plasma cholesterol (mmol/l):

28. Plasma urea (mmol/l):
29. Plasma creatinine ($\mu\text{mol/l}$):
30. 24 Hr urine protein (g/day):
31. Proteinuria selectivity index:
32. Calculated GFR:
33. Creatinine clearance:
34. Plasma ALT (U/l):
35. Plasma LDH (U/l):
36. Plasma gamma GT (U/l):
37. Haemoglobin (g/dl):
38. Mean cell volume (fl):
39. White cell count ($\times 10^9/\text{l}$):
40. Neutrophils (%):
41. Lymphocytes (%):
42. Monocytes (%):
43. Eosinophils (%):
44. Basophils (%):
45. Platelets ($\times 10^9/\text{l}$):
46. Anti-DNase B (titre):
47. ASO titre:
48. Group A Strep culture: not done
 throat
 skin
49. Total complement (U/ml):
50. C3 (mg/dl):
51. C4 (mg/dl):
52. SCAT (titre):
53. Latex agglutination (titre):
54. Anti-nuclear factor (titre):
55. Anti-DNA antibodies ($\mu\text{g bound/ml}$):

56. Circulating immune complexes:
(% C1q binding)
57. Syphilis serology: neg
 pos: titre
58. Increased urinary RBC?
59. Increased urinary WBC?
60. Urinary granular casts?
61. Urinary RBC casts?
62. Urinary hyaline casts?
63. Urine culture: neg
 pos: (org)
64. Follow-up status: nephrotic
 asympt
 remission
 defaulted
 still follow
 other
65. Duration of follow-up (months):
66. Date asymptomatic:
67. Date of remission:
68. Interval onset-asympt:
69. Interval onset-remission:
70. Proteinuria at last visit: neg - 4+
71. Haematuria at last visit: neg - 4+
72. Calculated GFR last visit:
73. Hypertension at last visit?:
74. Relapses of proteinuria?
75. Relapses of haematuria?
76. Other diseases?
77. Complications of nephrotic syndrome?
78. Therapy:
79. Response to therapy:
80. Complications of therapy:

81. First HBsAg positive (date):
82. First HBeAg positive:
83. Last HBsAg positive:
84. Last HBeAg positive:
85. First HBsAg negative:
86. First HBeAg negative:
87. Last HBe antibody negative:
88. First HBe antibody positive:
89. Comment:

APPENDIX 2: HISTOPATHOLOGICAL DATA

Patient name:
Folder number:
Biopsy number:
Biopsy date:
Biopsy interval (onset to biopsy):

GLOMERULI:

Number in biopsy specimen:

1. Cellular proliferation

Endothelial:	none	segm/glob	focal/diff	grade
Mesangial:	none	segm/glob	focal/diff	grade
Epithelial:	none	segm/glob	focal/diff	grade
Crescents:	none	small %	circumferential %	

Polymorph exudate:

2. Glomerular basement membrane

Normal

Thickening:	none	segm/glob	focal/diff	grade
Splitting:	none	segm/glob	focal/diff	grade
Spikes:	none	segm/glob	focal/diff	grade
Chaining:	none	segm/glob	focal/diff	grade
Wire loops:	none	segm/glob	focal/diff	grade

Lumen narrowing grade 0-3

Deposits

(Masson)	none	segm/glob	focal/diff	grade
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3. Mesangial matrix

Proliferation	none	segm/glob	focal/diff	grade
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4. Glomerular lobulation

Absent: Present:

5. Glomerular sclerosis

None	segm/glob	focal/diff	% affected:
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6. Glomerular necrosis

None	segm/glob	focal/diff
------	-----------	------------

7. Haematoxyphil bodies

Absent/present

8. Bowman's capsule
Normal Thickening (0-3) Fibrosis (0-3) Adhesions (0-3)

TUBULES:

Normal Necrosis Atrophy Casts Dilatation
Tubular BM thickening

INTERSTITIUM:

Normal Cellular infiltrate Fibrosis Foam cells

BLOOD VESSELS:

Normal Intima Media Necrosis Infiltrate
Thrombi

ELECTRON MICROSCOPY:

1. GBM

Normal Normal Thickening (0-3) Reduplication
Interpositioning

2. Epithelial cells:

3. Foot processes:

4. Mesangial hypercellularity: (0-3)

5. Mesangial matrix:

6. Electron-dense deposits:

Position	solid	vacuolated	granular	striated
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Subendothelial:

Intramembranous:

Mesangial:

Subepithelial

Humps: Present/absent

Tubulovesicular bodies: Present/absent
Other:

IMMUNOFLUORESCENCE:

	Not specified	Mesangium	Capillaries	Tubules
IgG				
IgA				
IgM				
C3				
C1				
C4				
Fibrin				

HBV ANTIGENS:

1. HBsAg:
2. HBcAg:

FINAL DIAGNOSIS/STAGE: