QUANTITATIVE HISTOLOGICAL ASSESSMENT OF THE PATHOLOGICAL CHANGES FOUND IN RENAL BIOPSY SPECIMENS FROM CHILDREN. A CLINICO-PATHOLOGICAL STUDY.

by

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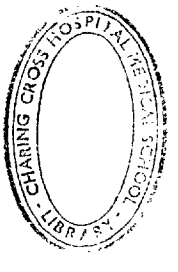

ABSTRACT

Evaluation of the histological changes found in kidney biopsies taken during the investigation of children with renal disease is complicated by the fact that in many instances the abnormalities seen by light microscopy are slight or absent, and may be prone to subjective errors of interpretation. In this thesis a number of renal biopsy specimens have been studied from 106 children presenting with a nephrotic syndrome, with idiopathic recurrent haematuria or with evidence of renal involvement in anaphylactoid purpura. In addition to a morphological classification, more objective histological techniques have been applied in analysing the changes found. Semi-quantitative methods of assessing the over-all degree of glomerular and tubular damage present have been used, and in addition, differential counts of the various types of cell composing the renal glomeruli have been made. These counts were found to be particularly valuable in the interpretation of slight degrees of 'proliferative' change in the renal glomeruli, the presence or absence of which is often difficult to establish subjectively. A number of preliminary studies were made to establish the reproducibility of the techniques of histological assessment used, and the renal biopsy findings were correlated with various clinical data.

In children presenting with a nephrotic syndrome the histological findings were correlated both with the response to treatment, and where appropriate, with the clinical outcome.

Amongst the children with nephritis following anaphylactoid purpura, a very good correlation was found between the degree of structural damage revealed on renal biopsy and the subsequent clinical course. In these biopsy specimens, and also in those taken from children presenting with idiopathic recurrent haematuria in which proliferative changes were found in the renal glomeruli, differential glomerular cell counts showed that in the majority of cases proliferative changes were diffuse rather than focal in distribution, affecting all the glomeruli present. In patients with idiopathic recurrent haematuria, renal biopsies were histologically normal in many instances. A very close correlation was found between the amount of protein excreted in the urine by these children and the presence of abnormalities on renal biopsy.

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INTRODUCTION

Percutaneous renal biopsy is now established as a valuable part of the investigation of patients with renal disease, whereby pathological changes in the kidney may be studied during life. The technique has been used at the Hospital for Sick Children, Great Ormond Street, for a number of years, particularly as part of the clinical assessment of children presenting with a nephrotic syndrome, symptomless recurrent haematuria or with evidence of renal involvement in anaphylactoid purpura. The application of newer techniques such as electron and immunofluorescent microscopy and enzyme histochemistry to the study of biopsy specimens obtained in this way has increased the understanding of the pathogenic mechanisms involved, and in some instances has revealed changes not discernable by more orthodox methods. Nevertheless, the routine histological examination of renal biopsies by light microscopy, both because it is a universally available technique, and because relatively large pieces of tissue can be examined, remains the principal method of evaluating the morphological changes in these biopsy specimens. In many instances the pathological changes seen may be very slight, and in some specimens, particularly those obtained from children with a nephrotic syndrome, abnormalities visible by light microscopy may be absent, so that their subjective histological interpretation can be open to error. This thesis sets out to investigate the application of more objective quantitative methods of assessment for the histological analysis of renal biopsies obtained from children with renal disease in order to provide a more rational basis for comparing the various

changes found between different biopsy specimens, and also in an attempt to improve diagnostic accuracy.

The literature concerning percutaneous renal biopsy is extensive. In presenting a review for this thesis emphasis has been placed on those aspects more directly related to the study performed. The development of the technique of percutaneous renal biopsy, particularly its use in children, has been outlined and special reference is made to the findings in the nephrotic syndrome, in recurrent haematuria and in the nephritis of anaphylactoid purpura. Previous methods of quantitative analysis of the histological changes in renal biopsies are also reviewed.

A section is devoted to the description and evaluation of the techniques of histological assessment, both subjective and quantitative, applied in this study and this is followed by sections on the application of these methods to renal biopsies from children with the nephrotic syndrome, with renal involvement in anaphylactoid purpura and with idiopathic recurrent haematuria. Full clinical descriptions of the patients studied are included and the results of the histological analyses are correlated with the relevant clinical findings.

The results are discussed in relation to the findings of other workers and some general conclusions are drawn showing how the techniques used may provide information of value in clinical management.

SECTION 1

REVIEW OF LITERATURE

Literature relating to the use of percutaneous renal biopsy in general.

Since it was first used by Pérez Ara (1950) and by Iversen and Brun (1951) percutaneous renal biopsy has become established as a valuable aid in the investigation and management of patients with renal disease. Previously, knowledge of the pathological changes present in diseases of the kidney such as glomerulonephritis depended largely on the examination of autopsy material (Volhard and Fahr, 1914; Longcope, 1929; Russell, 1929; Ellis, 1942). Occasionally renal tissue was obtained by open biopsy during abdominal operations on patients with renal disease (Gwyn, 1923; Jungman, 1924; Russell, 1934) and the changes in the kidneys in patients with hypertension was studied by a number of authors who performed open renal biopsies during lumbar sympathectomy (Castleman and Smithwick, 1934; Gelin, 1952; Heptinstall, 1954; Saltz, Sommers and Smithwick, 1957). However, it was only with the introduction of percutaneous renal biopsy that a technique became widely available which allowed the examination of renal tissue during life without a formal operation. Changes present at different stages in a variety of renal diseases could be studied, and, by the use of serial renal biopsies, their evolution over a period of time could be traced (Muehrcke et al, 1954; Pirani and Muehrcke, 1960).

Methods for performing percutaneous renal biopsies have varied little. Iversen and Brun (1951) performed the technique

by inserting a serrated needle into the loin with the patient in a sitting position. Tissue was removed from the kidney by aspiration with a special syringe in which the piston could be fixed in any desired position. In 1954, Kark and Muehrcke described a different method whereby the biopsy was performed with the patient in a prone position. A sand bag was placed under the costal margins so that the patient's weight pressed the kidneys against the posterior abdominal wall. An exploring needle was then introduced to locate the surface of the kidney, following which renal tissue was punched out and removed with a modified Vim-Silverman needle. With only minor modifications this technique remains the most widely used (Lancet, 1967). Improved methods for accurately locating the kidney have made the technique safer. These have included radioisotope positioning (Telfer, Ackroyd and Stock, 1964); television-monitored fluoroscopy with high-dose drip-infusion pyelography (Kark and Buenger, 1966) and retrograde pyelography with roentgen television used by Lindqvist and Nyström (1967) in severely uraemic patients. Such techniques also improve the chances of obtaining kidney tissue as a result of the biopsy manoeuvre. Although in experienced hands, a specimen of renal tissue is usually obtained, the failure rate in reported series has varied widely, ranging from 53% (Seltzer, 1956) to only 4% (Muehrcke, Kark and Pirani, 1955). Kark (1968) quoted the results of a questionaire on renal biopsies sent to a number of nephrology centres and noted that of a total of 8,081 biopsies performed 7,309 (91%) had provided renal tissue.

Muehrcke, Kark and Pirani (1955), Sala (1957) and Kellow et al. (1959) have examined the question of the adequacy of the small specimen of tissue obtained by needle biopsy of the kidney in making a pathological diagnosis. In each study autopsy kidneys were examined and comparisons were made between the findings in needle biopsies made from these kidneys and the appearances in conventional larger wedge sections. It was concluded that in diseases which involve the kidney diffusely needle biopsies are representative. The biopsies in Sala's study contained at least 10 glomeruli, and the same figure of 10 glomeruli was regarded as the minimum required for accurate diagnosis by Parrish and Howe (1955). Muehrcke, Kark and Pirani considered the biopsy specimen adequate if it contained 5 or more glomeruli. In certain circumstances, of course, even less tissue may be adequate. Brewer (1964) described a case in which a percutaneous renal biopsy was performed apparently unsuccessfully, only blood-clot being found in the biopsy needle. The clot was subjected to serial sectioning which showed it to contain a single isolated glomerulus, in which changes of renal amyloidosis were present.

A number of reports over the past twenty years, many based on large series of patients submitted to percutaneous biopsy of the kidney, have discussed the indications, contraindications and complications of the procedure and commented on the information it yields (Reubi, 1954; Kark et al., 1955; Parrish and Howe, 1955; Lich, 1957; Ross and Ross, 1957; Zollinger, 1957; Brun and Rasschou, 1958; Kark et al., 1958; Arnold and Spargo, 1959; Miatello et al., 1961;

Fairley and Kincaid-Smith, 1962; Turner, 1963; Ferris, 1965; Dittrich, 1966; Thaler, 1967; Scheurer, 1967; el Mahallawy, 1967; Kark, 1968; Takacs, 1969.). Arnold and Spargo (1959) stressed the clinical value of percutaneous renal biopsy in giving a precise pathological diagnosis for the management of patients with chronic renal disease. Though many such patients have remarkably few symptoms, and though the biochemical changes in their blood and urine may be non-specific, an accurate diagnosis can usually be made by renal biopsy. Parrish and Howe (1955), for example, in a series of 100 patients successfully submitted to renal biopsy, noted that a diagnosis was possible in all but 9 cases.

Because only a small piece of kidney is removed, percutaneous kidney biopsy is most useful in elucidating diseases which affect the organ diffusely, such as glomerulonephritis (Pollak et al., 1957; Kellow et al., 1959). The technique has proved most useful in managing patients with the nephrotic syndrome, in which a wide variety of pathological processes can result in a similar clinical presentation. (Bjørneboe et al., 1952; Ross and Ross, 1957; Joekes, Heptinstall and Porter, 1958; Berman and Schreiner, 1958; Kark et al., 1958; Hutt, 1963; Churg et al., 1965; Zollinger, 1968). It has also been widely used in connective tissue diseases with renal involvement, such as systemic lupus erythematosus (Muehrcke et al., 1957), progressive systemic sclerosis (Rodnan, Schriener and Black, 1957), in renal amyloidosis (Muehrcke et al., 1955) and in diabetic nephropathy (Brun et al., 1953; Sabour, el Mahallawy and Abou-el Naga, 1961).

Percutaneous renal biopsy is a less effective means of diagnosis in diseases such as pyelonephritis which affects the kidney focally. Depending upon the extent of renal involvement, there is a chance of the biopsy needle missing the affected tissue in such conditions. (Hutt and de Wardener 1961; Jackson, Poirier and Grieble, 1957).

Biopsy of the kidney can aid the management of patients by indicating the likely response to treatment and sometimes the ultimate prognosis. For example, the response to therapy with corticosteroid hormones in patients with the nephrotic syndrome can be predicted from the renal biopsy appearances (Blainey, Brewer, Hardwicke and Soothill, 1960; Jensen, 1967; Cameron, 1968; Black, Rose and Brewer, 1970). Brun (1954) and Fairley and Kincaid-Smith (1962) have described the use of needle biopsy of the kidney in selecting patients with reversible forms of acute renal failure for treatment by appropriate forms of haemodialysis.

Kark et al. (1958) listed the following as contra-indications to percutaneous renal biopsy; lack of cooperation by the patient, the presence of only one kidney, a haemorrhagic diathesis, large renal cysts, renal neoplasms, renal artery aneurysm, marked calcific ateriosclerosis, a peri-nephric abscess, hydronephrosis, an extremely ill patient in the "terminal" stages of his disease, or a blood urea nitrogen concentration that is either rising, or over 100mg/100ml. Most other authors have particularly stressed the first three factors as being absolute contraindications. Fairley and Kincaid-Smith (1962) and Brun and Raaschou (1958), however, have argued in favour of performing renal biopsies in uraemic

patients. These authors regard the value of making a diagnosis, particularly in anuric patients as worth the risks involved, such as an increased tendency for haemorrhage to occur. The questionaire on renal biopsies quoted by Kark (1968) provided the following list of contraindications; unilateral kidney, small kidney, congestive cardiac failure, severe hypertension, tuberculosis, "mobile" kidney and an unconscious or uncooperative patient.

A number of complications, including occasional deaths due to percutaneous renal biopsy, have been described. Alwall (1952) reported one death occurring a series of 13 patients submitted to percutaneous renal biopsy in 1944. He abandoned the procedure following this, but in a later publication (Alwall, Erlanson and Tornberg, 1955) he considered that the death of this patient could be attributed to retrograde pyelography which was carried out at the same time. Deaths due to renal biopsy alone are in fact very rare. Slotkin and Madsen (1962) reporting on the incidence of complications in 5000 renal biopsies note only four deaths (0.1%) amongst 7,309 patients submitted to percutaneous renal biopsy. A number of other complications have also been reported. The most common serious complication is haemorrhage, producing either a perirenal haematoma or gross haematuria (Karafin, Kendall and Fleisher, 1970; Immergut and Plotkin, 1970; Yatzidis et al., 1969; Ackerman, G.L., 1967). A renal anteriovenous fistula may also result from trauma by the biopsy needle (Blake, Hefferman, McCann, 1963; Ochsner and Busch, 1969; Sher, 1969 and River, 1970). Perforation of the calyceal system or the renal pelvis may result in extravasation of urine.

(River, 1970; Spong et al., 1970; and Stern, Langford and Grossman, 1970) and occasional perforation of a hollow viscus by the biopsy needle has been reported (Weiss, 1970; Mollard, Freycon and Marquet, 1967). The questionaires, both based on very large numbers of renal biopsies, reported by Slotkin and Madsen (1962) and by Kark (1968) inquired into the incidence of complications of the procedure. In both series the incidence of haematuria varied widely ranging between 2% and 50% in Slotkin and Madsen's series and 10% and 40% in the series referred to by Kark. This probably reflects different interpretations of significant haematuria. Brun and Raaschou (1958) comment that some degree of microscopic haematuria is almost universal after renal biopsy, but that frank haematuria particularly lasting longer than 36 hours following the biopsy, is much less common. Perirenal haematoma occurred in 27 (0.5%) of Slotkin and Madsen's series and in 16 (0.2%) in the series reported by Kark. A comment was also made that serious haemorrhage occurred most commonly in patients who were uraemic, hypertensive or who had diffuse connective tissue disorders. Other serious complications recorded were rupture of the spleen and liver, renal arteriovenous fistula, perforations of the renal pelvis and of other organs, septicaemia and renal abscess. The over-all incidence of all serious complications including deaths was, however, less than 1%, constituting 0.7% of Slotkin and Madsen's series of 5000 biopsies and 0.5% of the series of 8,081 cases recorded by Kark.

Literature relating to the use of percutaneous renal biopsy in children

Initially there was some reluctance to perform percutaneous renal biopsies in children, since it was considered that the much small renal mass in young subjects increased the risk of missing the kidney with the biopsy needle and either failing to obtain a biopsy specimen or of puncturing other vital structures (Rance, 1967). However, since the first series of percutaneous renal biopsies obtained exclusively from children was reported by Galán and Masó in 1957, a number of other publications (Vernier and Good, 1958; Bergstrand and Bucht, 1959; Dodge et al., 1962a; Fowler, Williams and Coldbeck, 1963; Urizar, Cassorla and Espinoza, 1963; White, 1963) have shown that in experienced hands the technique can be safely applied to children. The incidence of complications is very low and is comparable to that described in adults (White, 1963; Vernier, 1960; Karafin, Kendall and Fleisher, 1970). Failure to obtain an adequate biopsy specimen is rare in reported series in children; in the survey of the results of percutaneous renal biopsy referred to by Kark (1968) comparison between patients aged less than 14 years with those of 14 years or more showed very similar results, renal tissue being obtained in 93.5% of 1,382 cases in the former group compared with 89.8% of 6,699 cases in the latter group. Vernier and Good (1958), however, reported a poor yield of adequate biopsies obtained by percutaneous puncture in very young children (under 2 years), and recommended that open biopsies should be performed in this age group.

The indications for renal biopsy in children are broadly similar to those in adults (Lancet, 1965). The technique has been used most commonly in the investigation of children with the nephrotic syndrome (Galán and Masó, 1957; Farquhar, Vernier and Good, 1957; Dodge et al., 1962c) and those with suspected glomerulonephritis, including children presenting with anaphylactoid purpura, unexplained persistent proteinuria or with recurrent haematuria (Royer et al., 1962; Dodge et al., 1962b and d; Van Acker and Roels, 1964).

The Nephrotic Syndrome

In children, as in adults, the nephrotic syndrome, characterised clinically by heavy proteinuria, oedema and hypoalbuminaemia, is most commonly the result of a primary glomerular disease, although the incidence of the different pathological types of glomerular disease causing the condition varies quite considerably with age. Even before the introduction of percutaneous renal biopsy, on the basis of necropsy studies it was well recognised that some patients, particularly children, with proteinuria and generalised oedema, showed little or no structural abnormality in the renal glomeruli (Dunn, 1934). Volhard and Fahr (1913) in their clinical and pathological classification of Bright's disease used Müller's (1905) term "Nephrosis" to describe such non-inflammatory forms of renal disease. Munk (1913 and 1916) separated even more clearly a form of oedematous renal disease characterised pathologically by degenerative and fatty changes in the renal tubules which he called 'lipoid nephrosis'. He

regarded the condition as a generalised disease of fat metabolism and as such quite distinct from glomerulonephritis. Other authors were less ready to accept so sharp a separation between glomerulonephritis and nephrosis, for example, Aschoff (1917) argued that cases of "nephrosis" invariably showed at least some changes in the kidney which could be interpreted as indicating a preceding glomerulonephritis. The concept of lipoid nephrosis, however, proved attractive to clinicians (Leiter, 1931) and was widely adopted as a clinical description of patients, particularly children, presenting with a "pure" nephrotic syndrome lacking any complicating features such as gross haematuria.

Although some children with the clinical features of 'lipoid nephrosis' recovered spontaneously, others died, either as a result of intercurrent infection, or in chronic renal failure (Schwartz, Kohn and Weiner, 1943; Arneil, 1961). The introduction of sulphonamides and antibiotics stemmed the death rate from infection, and when corticotrophin and corticosteroids became available for the treatment of nephrotic children, the majority responded dramatically with loss of oedema and proteinuria, and an increase in the serum albumen concentration (Barnett, Forman and Lauson, 1952; Arneil, 1961; Arneil and Lam, 1966). Steroid therapy is not an ideal form of treatment since the majority of patients relapse at some stage, and the high dosage required to control proteinuria inevitably lead to side effects. However, more recently cytotoxic drugs, particularly cyclophosphamide, have been used successfully in combination with steroids, to obtain a more lasting control of the nephrotic state (West, Hong and Holland, 1966; Barratt and Soothill, 1970).

As experience grew with percutaneous renal biopsy in the investigation of patients with the nephrotic syndrome, it became increasingly apparent that a response to steroid therapy occurred in those subjects who lacked any marked histological abnormalities in the renal glomeruli, whilst those with morphological changes of glomerulonephritis tended to be steroid-resistant (Blainey et al., 1960; Vernier, Worthen and Good, 1961; Black and White, 1965; Drummond et al., 1966). In many instances the renal glomeruli observed in biopsies from steroid-sensitive nephrotics appeared completely normal to light microscopy. However, when such biopsies taken from nephrotic patients before commencement of steroid treatment, were examined with the aid of the electron microscope, it was found that despite the normal appearances by light microscopy, the epithelial cells of the glomerular tuft showed a coalescence of their foot processes where they were applied to the glomerular basement membrane (Farquhar, Vernier and Good, 1957). Though the exact significance of this change is still not clear, it seems more likely to occur as a result of heavy proteinuria rather than as a cause of the abnormality, since the same ultrastructural change occurs in experimental animals after induction of proteinuria by the parenteral administration of protein (Vernier et al., 1960; Anderson and Recant, 1962; Ashworth and James, 1961). It has also been shown that these electron microscope changes in nephrotic subjects revert to normal following diuresis (Folli et al., 1957). A variety of terms have been used to describe the presence of ultrastructural epithelial cell abnormalities in optically normal glomeruli from nephrotic subjects. These have included "minimal changes" (Hamberger, 1961), "glomerular

epithelial disease" (Earle and Jennings, 1960), "no change by light microscopy" and "foot process type" (Churg et al., 1956).

Despite the usually good prognosis in children with an uncomplicated nephrotic syndrome, occasional deaths from chronic renal failure occur. Heptinstall (1966) illustrates the case of a 12 year old boy with the nephrotic syndrome who died in renal failure; the kidneys at necropsy showed advanced tubular atrophy, interstitial fibrosis and hyalinisation of virtually every glomerulus, despite the fact that a renal biopsy performed less than a year previously had shown only minimal abnormalities. Studies which have included sequential renal biopsies from nephrotic children have demonstrated that some who initially show minimal or no changes in the glomeruli subsequently develop focal glomerular sclerosis (McGovern, 1964; Hayslett et al., 1969). The majority of cases showing focal glomerular sclerosis do not respond to treatment with corticosteroids, and the prognosis is bad since the lesion appears to be progressive (Churg, Habib and White, 1970; White, Glasgow and Mills, 1970). Apparently atypical cases who do not respond to steroid therapy yet exhibit minimal glomerular changes on renal biopsy may prove to be examples of focal glomerulosclerosis. In its early stages, because of the focal distribution of the lesions, a biopsy specimen may fail to include affected glomeruli, and thus the histological diagnosis may not be made. This likelihood is increased, since the biopsy usually samples tissue from the outer cortex, and there is evidence that focal glomerulosclerosis first affects the juxtamedullary glomeruli, and only later does the process spread to affect the rest of

the cortex (Rich, 1957).

Correlation of Glomerular morphology and proteinuria in the nephrotic syndrome

Investigation of the relative proportions of proteins of different molecular size in the plasma and urine of patients with the nephrotic syndrome have demonstrated that in some subjects the glomerular filter tends to selectively "dam back" the larger molecular weight plasma proteins, so that a much greater proportion of proteins of lower molecular weight such as albumen appear in the urine. In other cases the urinary protein excretion is quite unselective, the relative proportions of different plasma proteins in the urine approaching those seen in the plasma. In nephrotic subjects a good correlation exists between the presence of minimal glomerular changes in the renal biopsy and a highly selective pattern of urinary protein excretion (Blainey et al., 1960; Cameron and Blandford, 1966). Nephrotic children with minimal glomerular changes tend to be of pre-school age, and males are more commonly affected than females (White, 1970). Because of these clinical features, together with the fact that the histological changes can be predicted from the response to corticosteroid therapy and by the selectivity of the proteinuria present, the need for routine renal biopsy in nephrotic children has been questioned (Sonnenscheim, Minsky and Kramer, 1966; Cameron, 1968). There is little doubt, however, of the usefulness of this technique in the evaluation of nephrotic children with more extensive glomerular lesions.

Morphological Classification of the Histological Appearances found on Renal Biopsy in Children with the Nephrotic Syndrome not Responding to Steroids

Greater experience with the histological appearances in percutaneous renal biopsies from such patients had allowed a more satisfactory morphological grouping of the abnormalities found. Churg, Habib and White (1970) and White, Glasgow and Mills (1970) divide these cases into three main groups which they term membraneous (or epimembraneous) nephropathy, proliferative glomerulonephritis and advanced chronic glomerulonephritis.

Membraneous nephropathy is characterised by a diffuse thickening of the capillary walls in the glomerular tufts without any cellular proliferation. With the aid of the electron microscope this thickening can be seen to be due to the presence of dense deposits on the epithelial side of the basement membrane (Fiaschi et al., 1959). Projections of basement membrane material extend radially between these deposits and these projections or "spikes" can be demonstrated by light microscopy using silver impregnation techniques (Churg and Grishman, 1957). This condition, whilst one of the commoner glomerular lesions associated with the nephrotic syndrome in adults (Kark et al., 1958), is rare in children (White, 1967).

Cases of proliferative glomerulonephritis are of three main types. In mesangial proliferative glomerulonephritis there is a slight diffuse increase in mesangial matrix and the number of mesangial cells in the glomerular tuft, the appearances resembling those described by Jennings and Earle (1961) in resolving post-streptococcal nephritis. In

proliferative glomerulonephritis with crescents, in addition to proliferation of cells in the glomerular tufts, there is marked proliferation of the epithelial cells lining Bowman's capsules, to form "capsular crescents". In membranoproliferative glomerulonephritis there is a combination of mesangial proliferation and sclerosis, with diffuse thickening of the tuft capillary walls, as described previously by Habib et al., (1961).

Advanced chronic glomerulonephritis encompasses those cases in which glomerulosclerosis was so advanced that any distinctive morphological features were obscured. The condition is regarded as a non-specific end-stage of any progressive glomerulopathy.

The great majority of nephrotic patients with definite morphological changes in the glomeruli fail to respond to treatment either with corticosteroids or with cyclophosphamide. With the exception of cases of mesangial proliferative glomerulonephritis, in which there is evidence that spontaneous resolution may occur, (White, Glasgow and Mills, 1970) the presence of definite glomerular abnormalities in the renal biopsy is evidence of a progressive disease. Some patients with a steroid-resistant nephrotic syndrome do appear to have shown a favourable response to cyclophosphamide (White, Cameron and Trounce, 1966), but further evaluation of these patients has shown that they were cases of mesangial proliferative glomerulonephritis, in whom the apparent response to treatment with cytoxic agents may well be due merely to spontaneous resolution of their glomerular lesion (White, 1970).

The nephritis associated with anaphylactoid purpura (Schönlein-Henoch Syndrome)

Anaphylactoid purpura is a condition, usually occurring in children, which is characterised by a purpuric skin eruption, joint pains and various abdominal symptoms, including colicky pain, vomiting and intestinal bleeding (Gairdner, 1948). The skin rash may be accompanied by any of the other manifestations of the disease, and recurrences of any of these features can occur. Renal complications, judged by the presence of frank or microscopic haematuria, usually accompanied by proteinuria, are a frequent accompaniement even in the early stages of the disease.

Occasionally more serious renal involvement occurs, which results in renal failure and death. Although only a minority of patients whose kidneys are affected develop progressive renal disease, it has been appreciated since the earliest descriptions of the condition that the renal lesion could be fatal (Henoch, 1899) and indeed renal failure is the commonest cause of death in this disease. Interest has focused, therefore, both on the incidence of renal involvement in anaphylactoid purpura, and the proportion of such cases which might be expected to go on to renal failure. In addition, since the introduction of percutaneous renal biopsy, the pathological changes in the kidney have been studied during life.

The reported incidence of abnormal findings on urinalysis in children with anaphylactoid purpura has shown a surprising variation, ranging from as little as 12% (Roberts, Slater and Laski, 1962) to nearly 50% (Derham and

Rogerson, 1956; Philpott, 1952). The difference in the reported incidence of urinary abnormalities is difficult to explain, but the different series are all to a greater or lesser extent selected cases in as much as urinary abnormalities are more likely to be detected in hospitalised cases submitted to repeated urinalyses; these patients in turn are more likely to be those most seriously affected, since less-ill children will in most instances be treated at home. The extent to which such selection operates is difficult to gauge, but Allen, Diamond and Howell (1960) specifically state that in their series, in which the incidence of abnormal urinary findings was 40%, patients were not considered if they had been seen only in the out-patient department and not admitted to the hospital. In addition, the method used to examine the urine may affect the reported incidence of abnormal findings. Roberts, Slater and Laski (1962) comment that the low incidence of urinary abnormalities in their series may be due to the practice in their hospital of examining only the uncentrifuged urine for the presence of cells.

Although it appears that an appreciable number of children develop urinary abnormalities as a result of anaphylactoid purpura, constitutional disturbances, such as oedema, azotaemia and hypertension associated with acute post-streptococcal nephritis are seldom seen (Sterky and Thilén, 1960). Despite the fact that urinary abnormalities in anaphylactoid purpura may persist for some time, even for years, follow-up studies have emphasised the over-all good

prognosis. Sterky and Thilén (1960) in a study of 224 children with anaphylactoid purpura noted a 22% incidence of urinary abnormalities in the acute phase. 35 of these patients were seen again two years or more after their initial illness and only 4 (11.4%) of those who initially showed urinary abnormalities still had haematuria or proteinuria. However, one child who was initially free from urinary abnormalities was found to have haematuria at follow-up.

Allen, Diamond and Howell (1960) reported a 20% incidence of urinary abnormalities (15 out of 74) in children followed for periods ranging between 6 months and 10 years, whereas the incidence in children seen in the acute stages was 40% (53 out of 131). Allen, Diamond and Howell reported that three children in their series died from renal failure, but in the great majority of the remaining patients, as in the series described by Sterky and Thilén the only abnormality was in the urinary deposit. In every case the children were well and normotensive. There was no impairment of renal function apart from seven of the patients of Allen et al. in whom the creatinine clearance was measured as less than 50ml/min/sq.m. In both these large series the prognosis of the renal lesion in children is good, and this experience is similar to that described in other follow-up studies (Roberts, Slater and Laski, 1962; Oliver and Barnett, 1955; Derham and Rogerson, 1956). Although death in renal failure occurs in a small proportion of cases, in the majority a persistent abnormality in the urinary deposit is the only evidence of

renal disease. Although the abnormality may persist for up to a number of years, the fact that the incidence of haematuria and proteinuria diminishes with time in all the reported series, suggests that even this abnormality often eventually resolves.

The earlier studies of the pathological changes in the kidney in anaphylactoid purpura were confined to descriptions of the renal abnormalities found at necropsy in fatal cases; these were in general a chronic glomerulonephritis (Watson, 1903; Zothe, 1938).

Rathery and Derot (1934) described a 12 year old girl who died six months after developing anaphylactoid purpura. At autopsy the kidneys were described as showing a haemorrhagic nephritis with proliferation of cells lining Bowman's capsule. Levitt and Burbank (1953) described the autopsy changes in two of their five patients with nephritis following anaphylactoid purpura. The first, a 58 year old man, died from pulmonary tuberculosis one month after the onset of his symptoms. At necropsy both kidneys were slightly enlarged, but not scarred. Histological examination showed glomerular hypercellularity with adhesions and occasional "capsular crescents". The second case, a boy of 14 years, died in renal failure 51 days after admission to hospital. The kidneys at autopsy showed widespread arterial changes with areas of fibrosis, intimal thickening and focal fibrinoid arteritis. Similar acute arteritic changes were noted in vessels in the adrenal capsule.

Gairdner (1948) noted one death among 11 children with

anaphylactoid purpura. The patient was a four-and-a-half year old girl who died three months after the onset of her symptoms. At necropsy the changes in the kidney were described as advanced subacute glomerulonephritis. No detailed pathological description was made, but Gairdner regarded the changes as indistinguishable from the so-called Ellis type 1 nephritis (Ellis, 1942).

As percutaneous renal biopsy became more widely used in children a number of reports using this technique in anaphylactoid purpura have appeared. This has allowed the study of pathological changes at an earlier stage in the disease in non-fatal cases. Vernier et al. (1958) performed nine renal biopsies in eight children with anaphylactoid purpura. Six children had clinical evidence of renal involvement for periods ranging between two months and six-and-a-half years. Histological abnormalities were present in four of these six cases. The changes described were of two main varieties; firstly a segmental glomerulonephritis involving between 20% and 60% of the glomeruli present in the biopsy. The affected glomeruli showed segmental areas of cellular proliferation associated with amorphous deposits of material staining positively with periodic acid-Schiff reagent. The affected segments often showed adhesions to Bowman's capsule which in some instances showed localised proliferation of the lining epithelial cells. Other glomeruli (between 10% and 40% of those present) showed a more diffuse hypercellularity, and a number of glomeruli (between 20% and 50%) showed no recognisable abnormalities. Vernier and his colleagues regarded these changes, particularly the focal distribution

of the lesions which affected only some glomeruli and spared others, as quite distinct from the diffuse glomerulonephritis seen in acute post-streptococcal nephritis. They considered the changes to be similar to those seen in renal involvement by diffuse vascular diseases such as systemic lupus erythematosus. This view is supported by Norkin and Weiner (1965) in their description of the autopsy findings in two adult patients dying from anaphylactoid purpura. In addition to the changes in the kidneys, histological examination of the skin, intestines and lungs revealed widespread acute vasculitis with fibrin deposition in the walls of some affected vessels. Dodge et al. (1961) like Vernier et al. (1958) considered the renal lesions in anaphylactoid purpura to be distinctive. These authors studied biopsies from eleven children between the ages of 4 and 14 years with haematuria and proteinuria associated with anaphylactoid purpura. They described changes of a similar nature to those seen by Vernier and his colleagues. They remarked particularly on the variability in the severity of the lesions between parts of the same glomerular tuft. Berstrand, Bergstrand and Bucht (1960) and Kobayashi, Wada and Kifune (1961) also described the renal abnormalities in kidney biopsies from children with anaphylactoid purpura as a focal glomerulonephritis. Régnier and Bouissou (1960) on the other hand in a study of 11 renal biopsies from 6 children with anaphylactoid purpura regarded the renal lesion as a diffuse proliferative glomerulonephritis. Bernhardt, Chatelanet and Veyrat (1966) studied renal biopsies from both children and adults with anaphylactoid purpura, and they too

described the glomerular changes as diffuse rather than focal, but noted changes, such as the presence of focal fibrinous deposits in the glomerular tufts, analgous to those described by Vernier et al. (1958), Régnier and Bouissou (1960) were the first to use the electron microscope to study renal biopsies in cases of anaphylactoid purpura. They noted a proliferation of endothelial cells with segmental thickening of the basement membrane. In cases where a nephrotic syndrome developed, fusion of the foot processes of epithelial cells was also seen. Kobayashi, Wada and Kifune (1961) also studied 16 of their series of 21 renal biopsies with the aid of the electron microscope. In addition to the basement membrane thickening described by Régnier and Bouissou (1960) these authors noted focal interruptions in the basement membrane. This latter change was confined to cases exhibiting gross haematuria. Royer et al. (1963) studied the changes in renal biopsies from 15 children aged between 4 and 17 years at intervals ranging between 1 and 16 weeks after the onset of anaphylactoid purpura. All 15 presented with haematuria and proteinuria was found in 13. On the basis of the severity of the abnormalities present in the renal biopsy, these authors were able to distinguish four different groups. Group 1 (1 case) showed no definite histological abnormality. Group 11 (7 cases) was characterised by the presence of a focal and segmental glomerulonephritis with changes similar to those described by Vernier et al. (1958). Only some glomeruli were affected and the lesions often affected two or three segments of the

glomerular tuft. There were foci of fibrinoid necrosis, often with adhesions between the glomerular tuft and the capsule. In Group III (4 cases) the changes were similar to those described in Group II, being predominately segmental, they were, however, more severe and diffuse rather than focal in distribution. Group IV (3 cases) showed a severe diffuse proliferative glomerulonephritis with the formation of numerous conspicuous "capsular crescents" formed by proliferation of epithelial cells lining Bowman's capsule. Royer and his colleagues found an excellent correlation between the severity of the histological changes in the biopsy and the prognosis. The one patient without histological abnormalities in the renal biopsy (Group I) showed resolution of the urinary abnormalities two weeks after the biopsy. Of the seven cases in Group II, the urinary abnormalities resolved over periods between 4 and 18 months in three instances, and persisted in four instances. Of the four cases in Group III, one died after 23 months, and in the remaining three cases persistent haematuria and proteinuria were accompanied by the development of renal insufficiency. All three patients in Group IV died after intervals of between 5 and 7 months.

Idiopathic Recurrent Haematuria

Children, or young adults, with recurrent or persistent haematuria can present problems both of diagnosis and prognosis. This symptom may be the first evidence of a variety of conditions including glomerulonephritis, urinary tract infections and renal tuberculosis, calculi, urinary tract

neoplasms, traumatic damage, abnormalities of blood clotting, polycystic kidney, subacute infective endocarditis and systemic disease such as polyarteritis nodosa and systemic lupus erythematosus (New. Eng. J. Med., 1968). However, in a proportion of cases no such underlying cause can be demonstrated and haematuria of this type has been variously described as "essential" (Levy, 1922; Bumpus, 1928; Wyllie, 1955; Somerset and Harkness, 1960; Livaditis and Ericsson, 1962), "idiopathic" (Travis et al., 1962) or "benign" (Ayoub and Vernier, 1965; McConville, West and McAdams, 1966). Baehr (1926) described 14 young adults aged between 15 and 45 years with this abnormality which he referred to as a benign and curable form of haemorrhagic nephritis. These patients presented with recurrent or persistent painless haematuria; there were no constitutional symptoms and oedema and hypertension were absent. Although he did not report any long-term follow-up of his patients, Baehr stated that the prognosis was good and he noted that there was no impairment of renal function even when the haematuria continued for many months. Other studies in which such patients have been followed for a number of years are generally in agreement that the prognosis is favourable. Wyllie (1955) records 46 children with symptomless haematuria who were seen at Great Ormond Street between 1939 and 1955. Their ages ranged between 1 and 11 years, a third being under 3 years. Apart from occasional patients who complained of abdominal pain there were no other symptoms. None had more proteinuria than could be explained by the presence of haematuria and none had a raised blood pressure. Two patients showed a transient rise

in the blood urea concentration, but in none of the others was there any evidence of renal functional impairment. 30 patients had had retrograde pyelography and cystoscopy performed, and 5 others had had intravenous pyelograms. None of these investigations revealed any abnormalities. 34 patients were traced for follow-up. In 26 no recurrence of the haematuria occurred after intervals ranging from 5 to 15 years in 11 instances and for less than 5 years in 15. 8 patients continued to have recurrent haematuria for periods up to 8 years; in none of these 8, however, was there evidence of impairment in renal function. Ross (1960) described 9 patients aged between 6 and 25 years with episodes of recurrent haematuria. These appeared to follow an infection after brief intervals. There was no evidence of oedema, hypertension or renal impairment, and all 9 patients had remained well for intervals of between 9 months and 3 years. Livaditis and Ericsson (1962) followed up 50 children, aged between 1 and 15 years, with "essential" haematuria at intervals of between 4 months and 9 years since the onset of their symptoms. The average follow-up period was 4 years, and in 87% of cases was more than 2 years. 36 patients were well, without haematuria, 10 patients exhibited occasional macroscopic or microscopic haematuria, but were well in all other respects, and no information could be obtained in the remaining 4 cases. Ayoub and Vernier (1965) reported 17 children with "benign" recurrent haematuria aged between 5 and 18 years. Haematuria had been present for between 16 months and 15 years. The cases were followed for

between 2 and 10 years and in none were any adverse effects noted. McConville, West and McAdams (1966) described 17 children between 1 and 13 years old with "benign" haematuria. In 10 cases haematuria was also discovered on urine testing in a member of the patient's family. It was noted that haematuria tended to be persistent in the familial cases and episodic in the non-familial cases. These patients were followed for periods ranging between 6 months and 12 years. All were physically well and in none was there evidence of functional impairment. Johnson and Shuler (1969) reported a five year follow-up study on 36 of the 46 children with recurrent haematuria originally described by Bodian et al. (1965) 28 of these patients had no evidence of any underlying disease such as acute nephritis or Henoch-Schönlein disease (anaphylactoid purpura). Of these 28 patients only 14 (50%) continued to have haematuria; thus in 5 years haematuria had ceased in half the patients studied. All the patients were well and in none was there evidence of renal impairment.

Because of the good prognosis in children presenting with symptomless haematuria there was little opportunity to study the pathological changes present before the advent of percutaneous renal biopsy. Baehr (1926) noted the similarities in the clinical presentations of his patients with those of a group of cases described by Volhard and Fahr (1914). These patients developed haematuria at the height of an infection such as tonsilitis or scarlatina, and were described as examples of "focal" (herdformige) glomerulonephritis. Baehr assumed that similar pathological changes were present in the kidneys in his patients.

Studies of the histological changes seen in renal biopsies from patients with symptomless haematuria have shown a surprising variation. This, in part at least, is a result of differences in interpretation and in the use of nomenclature, but it does also strongly suggest that such patients do not form a homogeneous group. Travis et al. (1962) described two cases of "idiopathic" haematuria, one a ten year old boy and the other a girl aged thirty months. Symptoms had been present for 6 years and 7 months respectively, and in both renal biopsies showed no significant histological abnormalities. Ferris et al. (1967) performed renal biopsies in 11 adolescents and young adults (aged 15 - 35yr.) with recurrent haematuria. In two cases the biopsy changes were described as diffuse proliferative glomerulonephritis, but in the remaining 9 patients the only abnormalities were of a mild focal nephritis in which a minimal focal hypercellularity affecting parts of some glomerular tufts (focal glomerulitis) was seen. Ayouh and Vernier (1965) described 17 children with benign recurrent haematuria. Renal biopsies were performed in 14 of these patients. In the majority (9 of the 14) no histological abnormalities were seen and in the others (5 of the 14) only mild non-specific changes were present. McConville, West and McAdams performed renal biopsies in 11 of 17 children with benign haematuria. 10 biopsies were histologically normal, and in one biopsy there were changes of diffuse proliferative glomerulonephritis. Bodian et al. (1965) studied renal biopsies from 46 children presenting with recurrent haematuria. 7 were cases of Henoch-Schönlein purpura,

and 3 had initially had acute nephritis, but the remaining 36 were cases of idiopathic recurrent haematuria.

In 3 of these 36 children the renal biopsy appearances were those of chronic pyelonephritis, but in all the remaining 33 cases the biopsy showed a proliferative glomerulonephritis described by the authors as "focal segmental" or "focal diffuse" in type. Todd and Bouton (1966) and Joekes, Pugh and Pryor (1962) also reported abnormal changes in renal biopsy specimens from cases of recurrent haematuria. Todd and Bouton (1966) described 46 children with haematuria submitted to renal biopsy. They described the histological changes seen as a proliferation of endothelial and epithelial cells, either segmentally, or diffusely throughout the glomerular tufts. They mentioned that not all glomeruli were involved. The proportion of the 46 cases showing these changes was not stated. Joekes, Pugh and Pryor (1962) described the renal biopsy appearances in 43 patients of all ages with recurrent haematuria. Unequivocal glomerular changes were described in 38 cases, ranging from focal glomerulonephritis in 21 instances to a diffuse process in the remaining 17. Ross (1960) performed renal biopsies in 5 of his 9 patients with recurrent haematuria. A proliferative glomerulonephritis was observed in 4 of these 5 patients; in 3 this showed a focal distribution and in 1 it was diffuse. The remaining patient showed only interstitial fibrosis; proliferative changes in the glomeruli were lacking. Glasgow, Moncrieff and White (1970) reported the renal biopsy findings in 47 children with symptomless haematuria. Two children exhibited changes of membranoproliferative glomerulonephritis (Ogg, Cameron and

White, 1968) but in the remaining 45 the changes were much less severe. In 37 children the glomeruli were either entirely normal or exhibited minimal and equivocal abnormalities only. In the remaining 8 cases there was a diffuse mesangial thickening and hypercellularity similar in appearance to the changes described by Jennings and Earle (1961) in subsiding post-streptococcal glomerulonephritis. It was noted that a marginally increased incidence of β-haemolytic streptococcal infections and more markedly increased incidence of proteinuria was found in the cases with changes in the renal biopsy and it was suggested that these cases might represent an atypical form of post-streptococcal nephritis. Glasgow and his colleagues commented that none of their patients showed a focal glomerulonephritis. Arneil et al. (1969) reported the renal biopsy appearances in 17 children with recurrent haematuria. In 5 the histological appearances were normal. In the remainder there was some degree of proliferative glomerulonephritis. In the majority this was mild, but in one biopsy the changes were more pronounced and in this specimen an epithelial crescent was found in one glomerulus.

Singer et al. (1968) described the changes in renal biopsies from 31 children with recurrent haematuria studied both by light and electron microscopy. The cases were divided into two groups according to the presence or absence of small electron-dense deposits which the authors found in 11 of their patients. In both groups about half the cases showed no abnormalities on light microscopy and the remainder were described as having mild segmental glomerulitis. Electron-

dense subepithelial deposits have been described in post-streptococcal acute nephritis (Osawa, Beres and Kimmelstiel, 1966; Dodge et al. 1968). This change has been seen most commonly in the first six weeks of the illness (Herdson, Jennings and Earle, 1966) but occasionally later (Osawa, Beres and Kimmelstiel, 1966). However, other workers who have examined biopsies from cases of recurrent haematuria were unable to confirm the findings of Singer and his colleagues (Glasgow, Moncrieff and White, 1970). Lannigan and Insley (1965) commented on the striking variation in the thickness of the basement membrane which was in some places thinned and in others slightly thickened. These findings were confirmed by Glasgow, Moncrieff and White (1970) and by Chase (1968). Similar changes were also seen by Singer et al. (1968) but they regarded it as a normal variant.

Literature Relating to the Use of Quantitative and Semiquantitative Techniques for the Histological Assessment of the Changes Seen in Renal Biopsies

Evaluation of the histological abnormalities present in renal biopsy specimens, particularly the changes seen in the renal glomeruli, allows a precise pathological diagnosis in the majority of cases (Parrish and Howe, 1955). In addition, the severity of the various pathological changes seen can be measured by the application of quantitative or semi-quantitative assessment methods of histological assessment (Pirani and Salinas-Madrigal, 1968). The application of such techniques has the advantage of compelling a greater degree of precision and consistency from the observer (Pirani and

Salinas-Madrigal, 1968). In addition, where the reproducibility of such methods has been established (Pirani, Pollak and Schwartz, 1964), they allow an objective basis for quantifying the various changes present in order to correlate them with various clinical data. By this means it is possible to equate pathological changes in the kidney in various diseases with, for example, various parameters of renal functional impairment (Saltz, Summers and Smithwick, 1957; Suc et al., 1967; Howe and Parrish, 1955; Talbott et al., 1943; Kinoshita, Fryisaka and Eguchi, 1965; Hutt and Sommers, 1963; Risdon, Sloper and de Wardener, 1968).

Quantitative assessments have also been applied to evaluate more subtle histological changes the appreciation of which would normally depend on unreliable subjective criteria which are impossible to standardise from one observer to another. Brun et al. (1965) studied renal biopsy specimens from patients with rheumatoid arthritis to investigate previous reports based on subjective assessments of proliferation of glomerular tuft nuclei said to occur in this condition. These authors counted the number of nuclei seen in sections of four glomeruli in each biopsy. The image of each glomerulus was projected on to paper, and after drawing a line around Bowman's capsule, each individual tuft nucleus was drawn and counted. Sections cut at between 5 and 9μ thickness from individual glomeruli showed no significant change in the nuclear counts so obtained. By this method the authors were unable to demonstrate any significant increase in nuclear

counts, expressed as the number of glomerular tuft nuclei per unit area, in the biopsies from patients with rheumatoid arthritis when compared with controls. Suc et al. (1967) employed basically similar methods to count glomerular nuclei in cases of acute post-streptococcal glomerular nephritis and "lobular " glomerulonephritis. The counts in this study were performed on photomicrographs of individual glomeruli which were also used for planimetric measurement of the glomerular area. Both Brun et al. (1965) and Suc et al. (1967) also compared glomerular cell counts in standard areas of 625 μ^2 within the glomerular tufts in order to study the distribution of cells within the tuft. Both studies allowed the conclusion that in normal glomeruli the cells were distributed regularly. Brun et al. (1965) found that in cases of rheumatoid arthritis, although the total numbers of cells forming the glomeruli were not increased, their distribution was abnormal, focal aggregation of nuclei occurring in parts of the tuft. Suc et al. (1967) confirmed the presence of diffuse glomerular hypercellularity in acute nephritis. In "lobular" glomerular nephritis on the other hand, hypercellular was localised to the centrilobular and intercapillary zones of the tuft. Iidaka, McCoy and Kimmelstiel (1968) and Kawano et al. (1969) examined sections of kidney tissue from patients with so-called focal post-acute glomerulonephritis and with diabetic glomerulosclerosis. Quantitative determinations were made, not only of total number of cells in each glomerulus, but also differential counts were made in order to ascertain the proportion of endothelial, mesangial and endothelial cells present. In addition the proportion of the total area of the

glomerulus occupied by the mesangium was determined in each case. In order to make these calculations camera lucida drawings were made of each glomerulus in sections of 3 μ thickness. Using these techniques Kawano et al. (1969) were able to show that so-called focal post-acute glomerulonephritis was a diffuse process characterised by mesangial cell proliferation and deposition of mesangial matrix. The process varied in degree, but was found in nearly all glomeruli even those judged as normal but more subjective assessment.

SECTION 2

MATERIALS AND METHODS

Introduction

This section is presented in two parts. The first part deals with the histopathological apsects. The histological preparation of the percutaneous renal biopsy specimens, the morphological classification and the various methods of quantitative assessment of the morphological changes encountered are presented. In addition control studies are described which were designed to evaluate the glomerular cell counting techniques employed as part of the study.

The second part presents the clinical histories of the children from whom the renal biopsy specimens studied were obtained. The patients have been arranged in three groups, those presenting with anaphylactoid purpura, those with idiopathic recurrent haematuria and those with a nephrotic syndrome whose proteinuria was not controlled by treatment with corticosteroids. In these case histories emphasis has been placed on the relevant facts and investigations which directly influenced the diagnosis and management.

A summary of the clinical findings is also given of a group of children with steroid-sensitive nephrotic syndrome. The morphological findings in the renal biopsy specimens from this latter group of patients were used to compare and contrast with those from the children in other groups described.

Part I. Histopathological Methods

Histological preparation of renal biopsy specimens

Renal biopsy specimens were obtained by percutaneous puncture under fluoroscopic control, using a modified Vim-Silverman needle, and immediately fixed in buffered 10% formalin (pH 7.0) for 24 to 48 hrs. In some biopsies (those obtained after 1968) fixation in buffered formalin was followed by secondary fixation in Zenker-formal for 6 hr. They were then embedded in paraffin wax and serial sections were prepared between 2 and 4 μ in thickness. Sections were rountinely stained by Ehrlich's haematoxylin and eosin, periodic-acid/Schiff using a celestine blue-haemalum sequence (Lendrum and McFarlane, 1940) for nuclear counterstaining, Weiger's elastic tissue stain with van Gieson's mixture, Martius scarlet blue (Lendrum et al. 1962) and periodic acid/methenamine silver (Jones, 1957) using a weak Mayer's haemalum and eosin counterstain.

Secondary fixation with Helley's fluid (Zenker-formal) improved both fixation and staining properties with all the methods used, with the exception of period acid/methenamine silver. Satisfactory results were obtained, however, with this method if deparaffinised sections were first treated for 24 hr. with 2% ammonia in 96% alcohol (Meadows and Schoemaker, 1970).

Whenever possible, for a 14 month period between May, 1969 and July, 1970 part of each percutaneous renal biopsy

received in the laboratory was embedded in 'Araldite' so that sections of 0.5μ thickness could be obtained in addition to the conventional paraffin sections. During the period mentioned each biopsy was examined with the aid of a dissecting microscope. Usually it was possible to identify renal cortical tissue in the biopsy by the presence of glomeruli, and medullary tissue, if present, by the presence of the vasa rectae. Blood in the glomerular capillaries and in the vasa rectae differentiated these structures as small red spots, and parallel red streaks respectively. Provided sufficient glomeruli were present, a small piece of cortical tissue (up to 2 or 3mm. long) containing glomeruli was removed with a scalpel. In practice fewer glomeruli could be seen under the dissecting microscope than were ultimately seen in the sections from the biopsy, but tissue was only taken for 'Araldite'-embedding if an adequate number of glomeruli (5 or more) could be positively identified in the remaining tissue for embedding in paraffin wax. In all 58 renal biopsy specimens were received during this period and in 35 instances tissue was removed for embedding in 'Araldite'.

After fixing the small piece of cortical tissue removed from the biopsy specimen in 10% buffered formalin (pH 7.0) for 24 hr. it was thoroughly washed in phosphate-buffered saline (pH 7.1). The tissue was the post-fixed in 1% osmium tetroxide for 90 min. Following this the tissue was thoroughly washed again in phosphate-buffered saline and embedded in 'Araldite'. Sections of 0.5μ thickness were then prepared using an L.K.B. ultramicrotome. The sections were floated out on water and picked up on to glass slides. After

staining with toliudine blue they were examined by light microscopy (Eastham and Essex, 1969). Where necessary, after further trimming of the block of tissue, ultrathin sections were prepared for examination with the aid of the electron microscope.

Morphological Classification

I) The Nephrotic Syndrome

Each renal biopsy specimen was assigned according to the classification adopted by the International Study of Kidney Disease in Children (Churg, Habib and White, 1970) as follows:

"Minimal Changes"

The glomeruli for the most part show no unequivocal pathological changes, after allowing for any differences influenced by the section thickness or fixation effects. In all cases the glomerular capillaries are widely patent and have thin walls as assessed in P.A.S. or methenamine silver preparations (fig. 1). Tubules, interstitial tissues and blood vessels are normal.

Two variants are also included under the heading of "minimal changes". In one a small number of completely obsolescent glomeruli are seen, but segmentally sclerosed glomeruli are absent (fig. 2). In the other the glomeruli are optically normal, but minimal focal tubular changes, including basement membrane thickening are seen.

Fig. 1 Nephrotic syndrome - 'minimal changes'

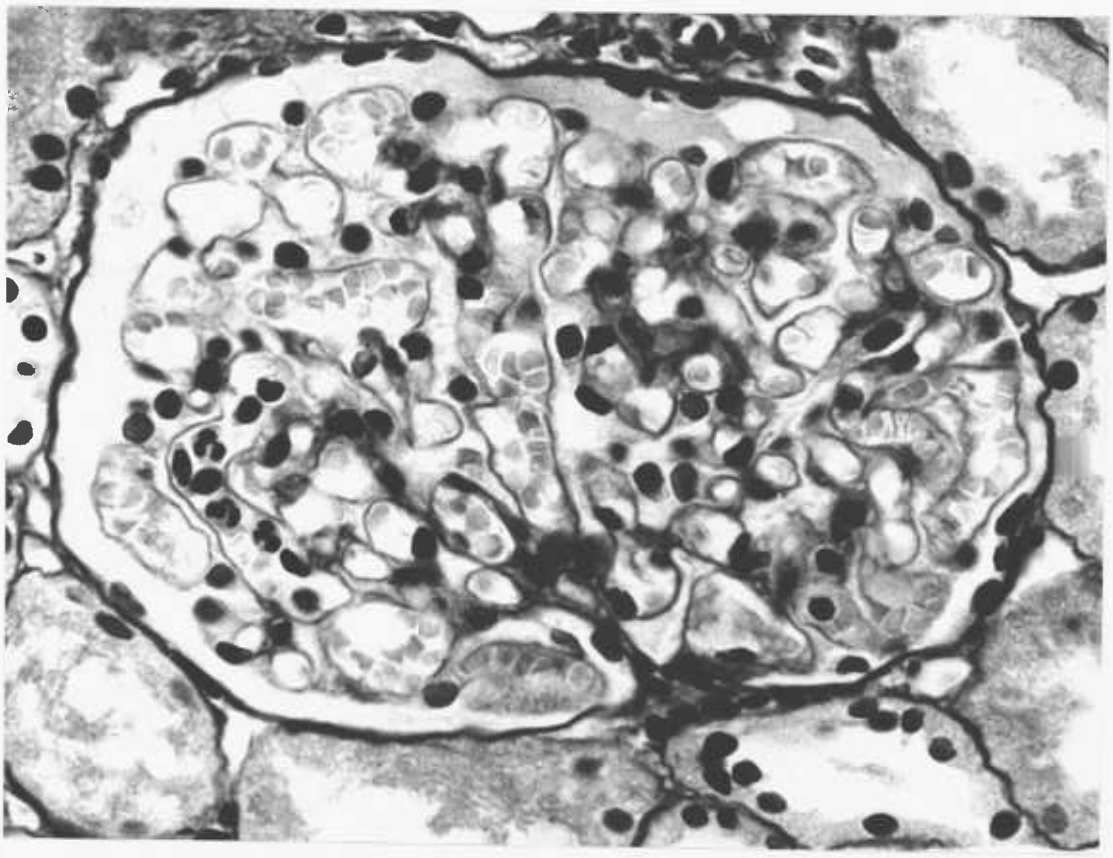
A glomerulus showing no definite morphological abnormalities. The capillaries are widely patent and have thin walls. There is no hypercellularity or excess of mesangial matrix M.S.B. x 150.

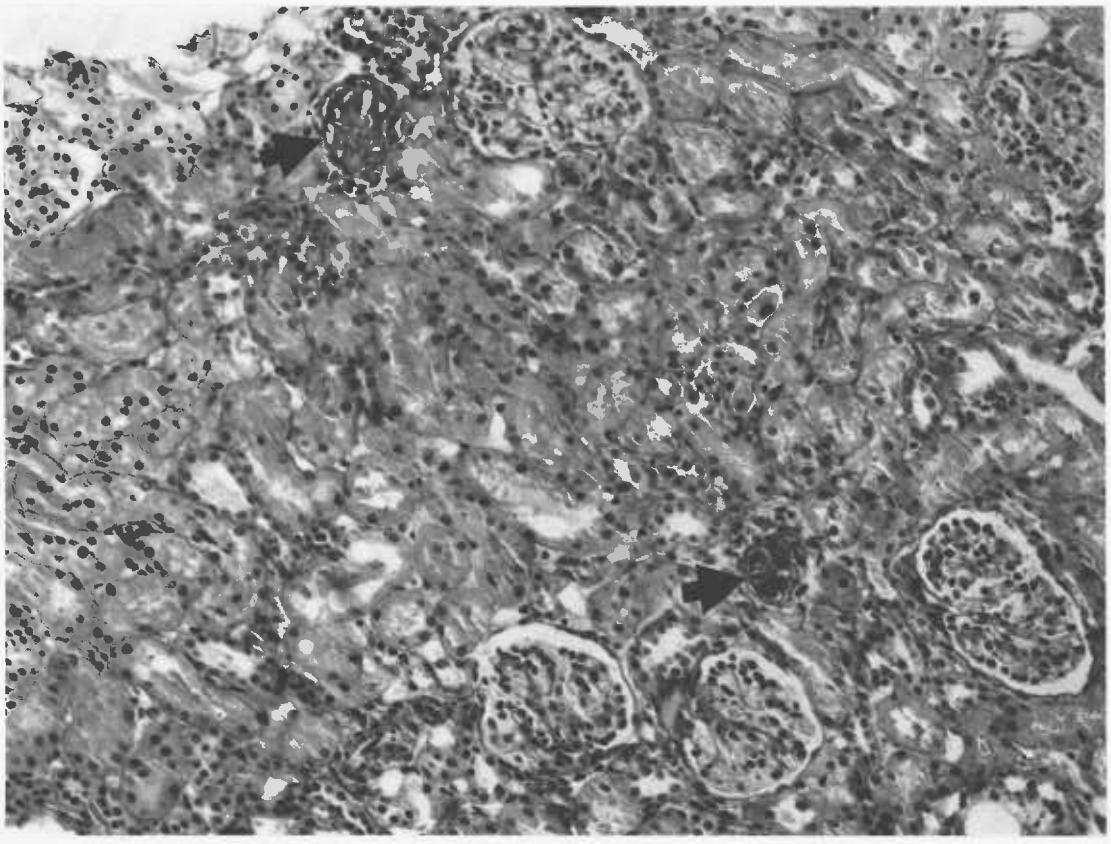
Fig. 2 Nephrotic syndrome - 'minimal changes'

In some biopsies, particularly those from young children, a small number of completely obsolescent glomeruli (arrowed) are present. However, segmentally sclerosed glomeruli are absent, and there is no tubular atrophy. M.S.B. x 16.

Fig. 1

Fig. 2





Focal Glomerulosclerosis

In its fully developed form, this glomerular lesion is both focal and segmental, in that some glomeruli are optically normal, while other glomeruli show areas of segmental sclerosis of the glomerular tuft (fig. 3). In its early stages this lesion may be difficult, or even impossible, in a biopsy specimen, to distinguish from the "minimal change" lesion, since only a very small proportion of glomeruli may be affected and then only to a slight degree. The segmental involvement is characterised by a localised increase of mesangial matrix extending into the capillary wall which becomes thickened and there may be narrowing or obliteration of the capillary lumen. Often there is a local adhesion to the adjacent Bowman's capsule (fig. 4). Sometimes a glomerular capillary is seen to contain a 'hyaline' thrombus (fig. 5). Cellular proliferation is usually absent but may be observed in some cases. The tubules associated with the affected glomeruli are almost always atrophic, except with very early lesions, and the tubular atrophy is associated with interstitial fibrosis (fig. 3). The presence of glomerular obliteration and significant tubular atrophy is very strong evidence of focal glomerulosclerosis, since glomerular obsolescence in the "minimal change" lesion is not associated with tubular atrophy (fig. 6).

Proliferative Glomerulonephritis

i) Diffuse exudative.

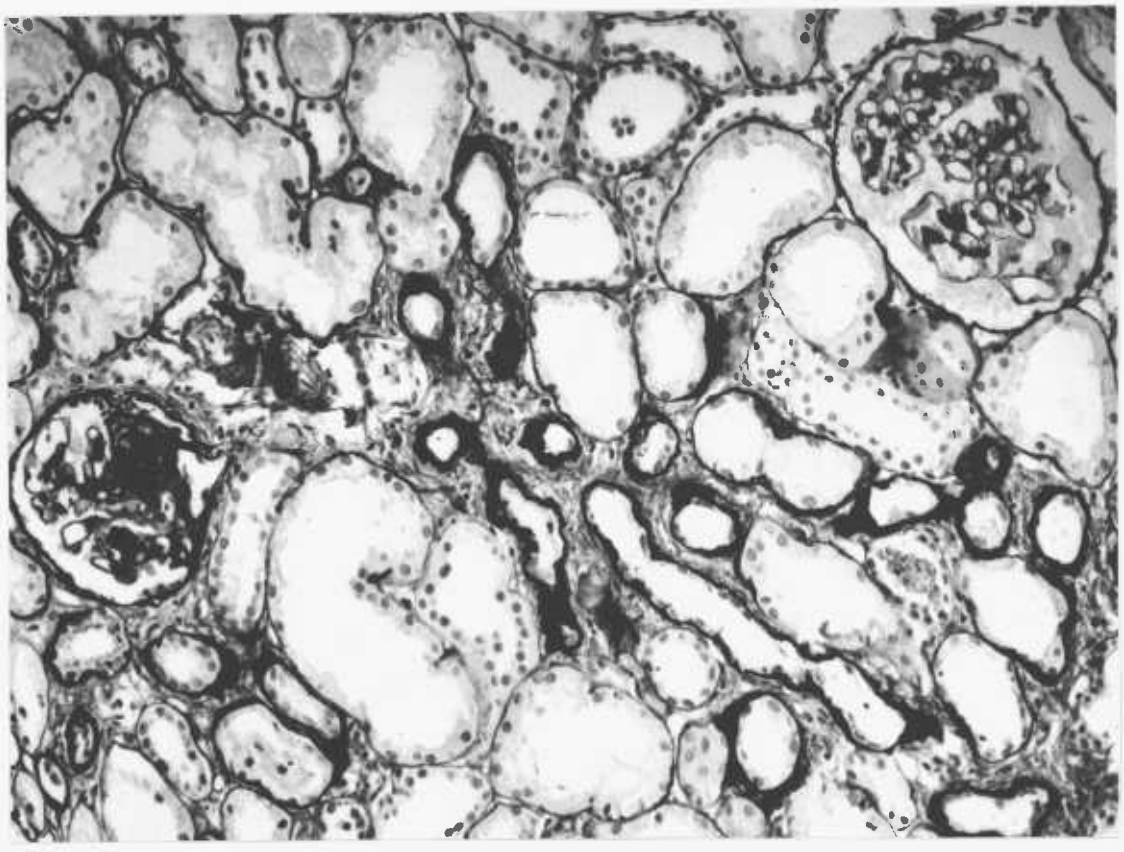
This type of glomerulonephritis, which is characterised by conspicuous proliferation of glomerular

Figs. 3a and 3b Nephrotic syndrome - focal glomerular sclerosis

Some glomeruli are normal, whilst others show areas of segmental sclerosis (3b). In the fully developed lesion completely sclerosed glomeruli are also present. Focal tubular atrophy is also seen (3a).
3a - P.A.S.M. x 16; 3b - P.A.S. x 16.

Fig. 3a

Fig. 3b



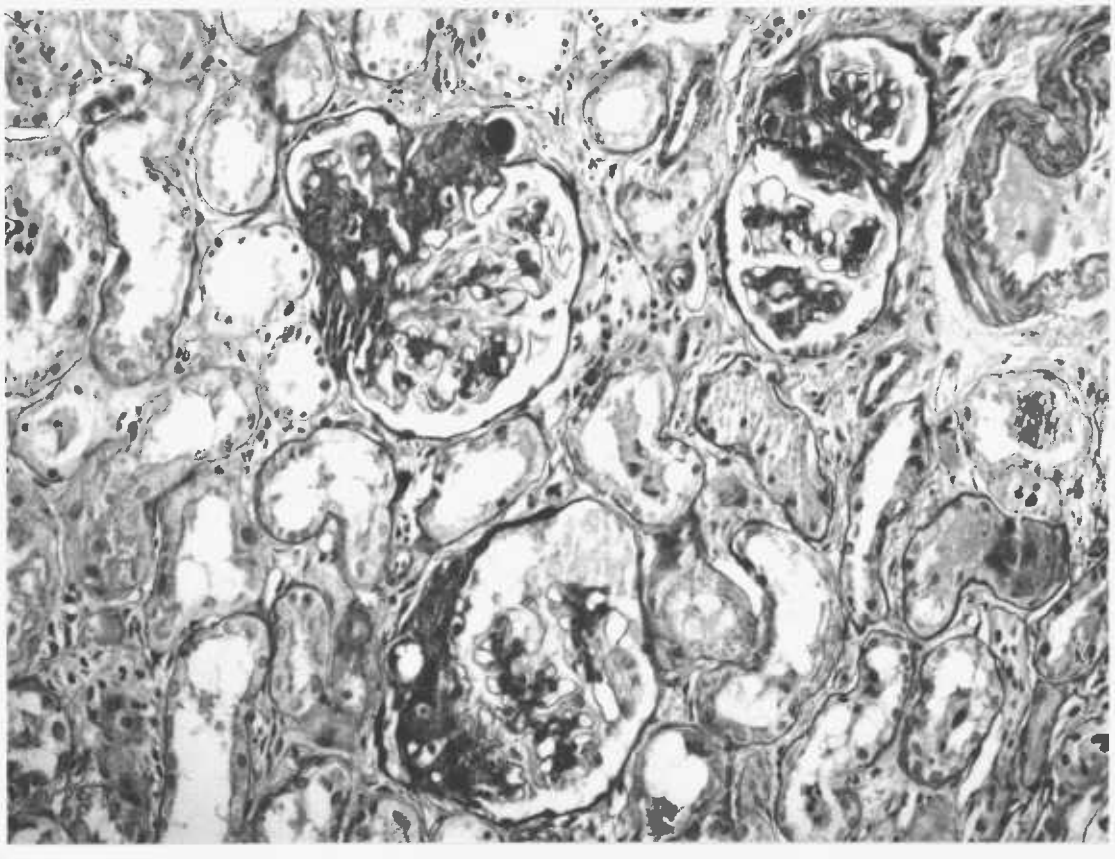


Fig. 4 Nephrotic syndrome - focal glomerulosclerosis

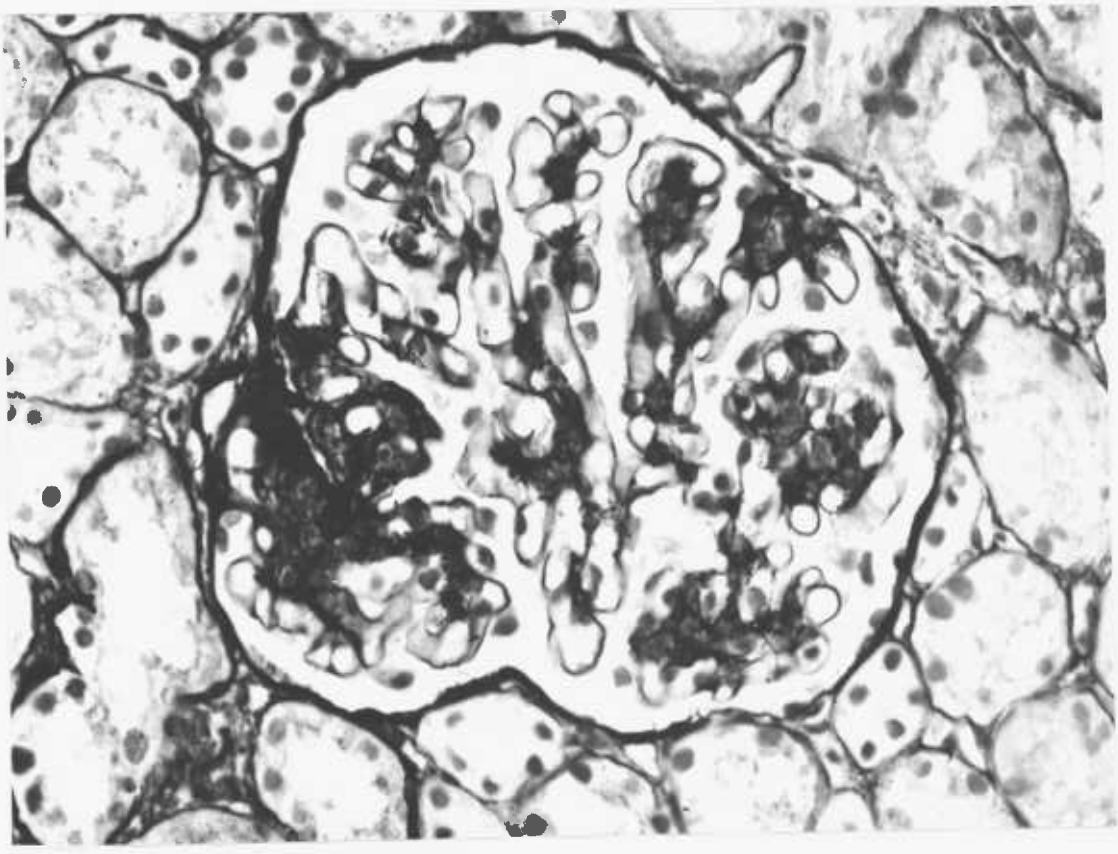
A glomerulus showing a localised area of sclerosis of the glomerular tuft associated with a capsular adhesion. The remainder of the tuft is normal. P.A.S.M. x 100.

Fig. 5 Nephrotic syndrome - focal glomerular sclerosis

A glomerulus showing partial sclerosis with a 'hyaline' thrombus blocking a capillary lumen. P.A.S.M. x 128.

Fig. 4

Fig. 5



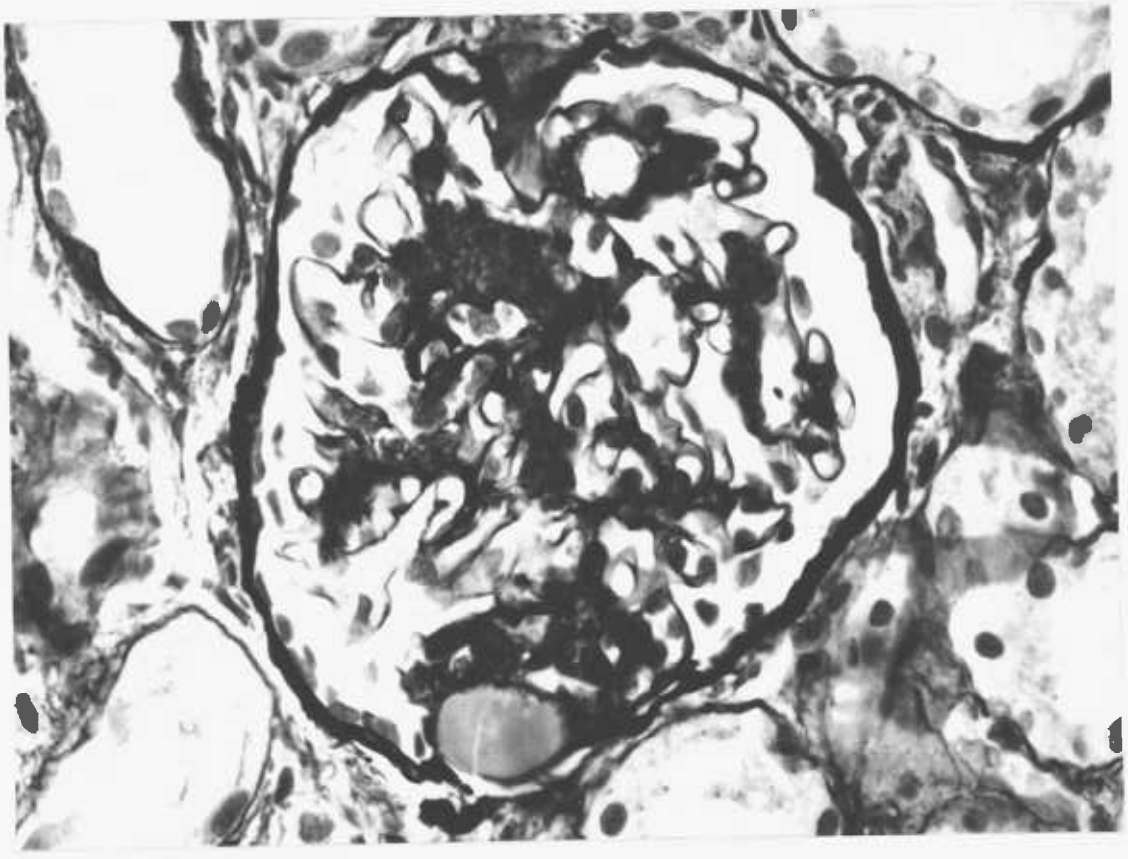
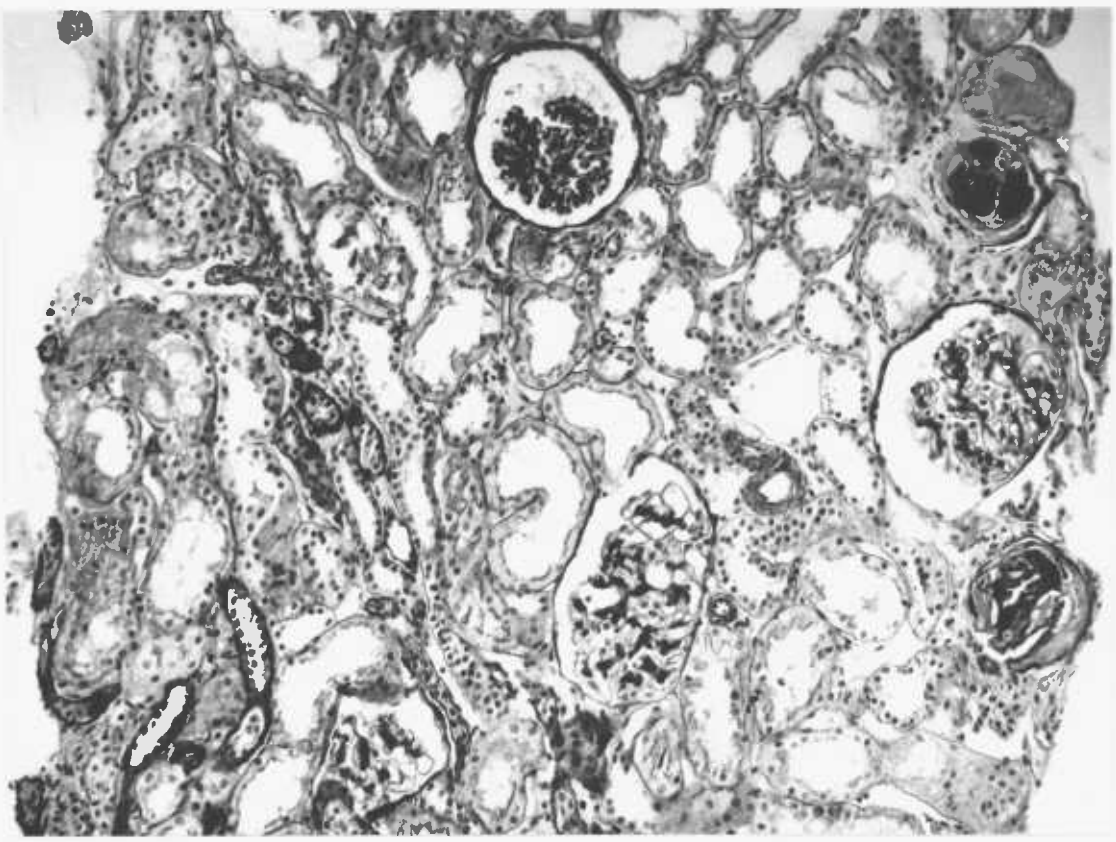


Fig. 6 Nephrotic syndrome - focal glomerulosclerosis

Some renal biopsy specimens from patients with focal glomerulosclerosis fail to sample glomeruli showing typical segmental areas of tuft sclerosis. However, in a child, the presence of completely sclerosed glomeruli associated with significant tubular atrophy in a biopsy is strong evidence of focal glomerulosclerosis. P.A.S. x 16.

Fig. 6



tuft nuclei, with narrowing or obliteration of the tuft capillary lumina and polymorphonuclear leucocytic infiltration, occurs classically in acute glomerulonephritis (fig. 7).

ii) Mesangial.

Proliferation of glomerular tuft nuclei is slight but diffuse and is most conspicuous in the lobular stalks (mesangial regions) which are slightly thickened due to an increase in fibrillar content best seen in a P.A.S. or silver/methanamine preparation (fig. 8). Polymorphonuclear leucocytic infiltration is not seen and the capillary loops are widely patent and have thin walls. Epithelial cell proliferation and capsular adhesions are not features of this type of lesion.

iii) Proliferative and sclerosing glomerulonephritis.

In addition to diffuse proliferative changes in glomerular tuft, occasional glomeruli are sclerosed, and foci of associated tubular atrophy and interstitial fibrosis may be seen.

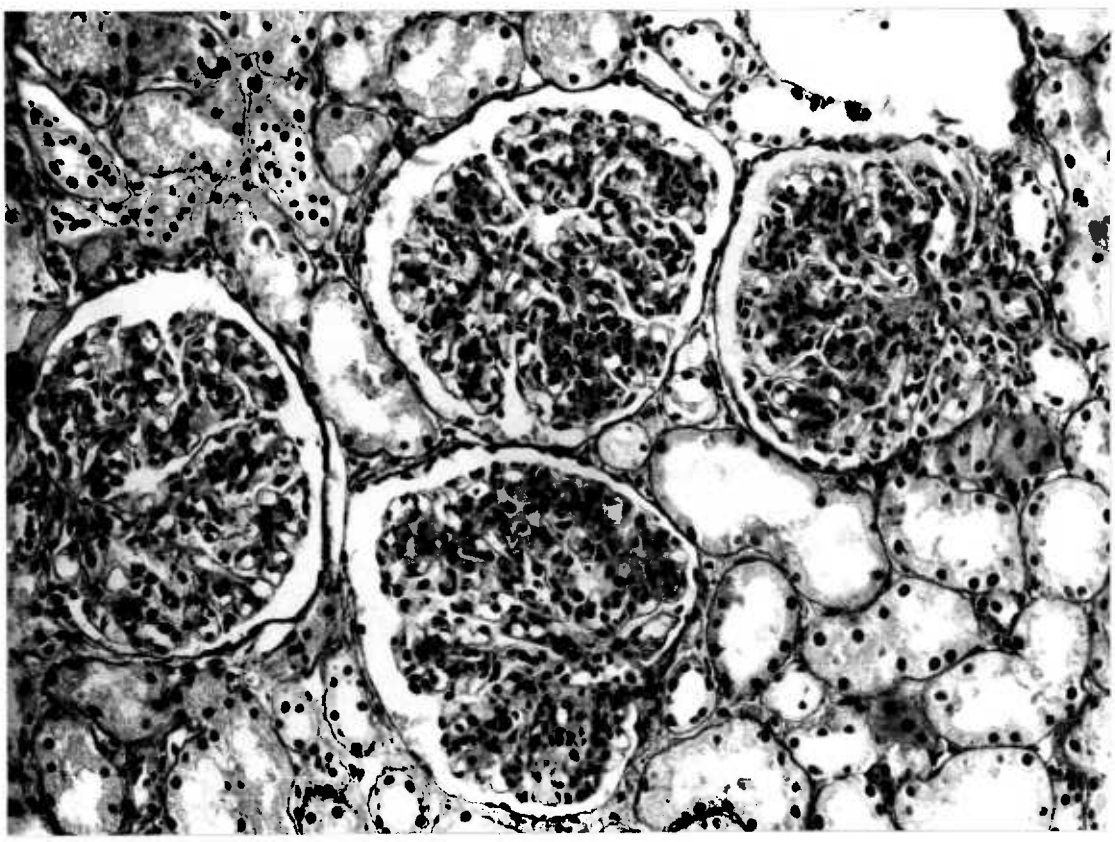
iv) With crescents.

Proliferation of epithelial cells, particularly those lining Bowman's capsule form multilayered structures termed 'capsular crescents' (fig. 9), and these are associated with proliferation of the glomerular tuft nuclei. Sometimes adhesions between the glomerular tuft and Bowman's capsule are associated with a slight localised proliferation of the adjacent epithelial cells (fig. 10). This reactive change

Fig. 7 Nephrotic syndrome - diffuse exudative glomerulonephritis

A glomerulonephritis characterised by conspicuous proliferation of the glomerular tuft nuclei and the presence of polymorphonuclear leucocytic infiltration ('exudation') in the glomeruli. This is an uncommon lesion to be associated with a nephrotic syndrome. M.S.B. x 40.

Fig. 7

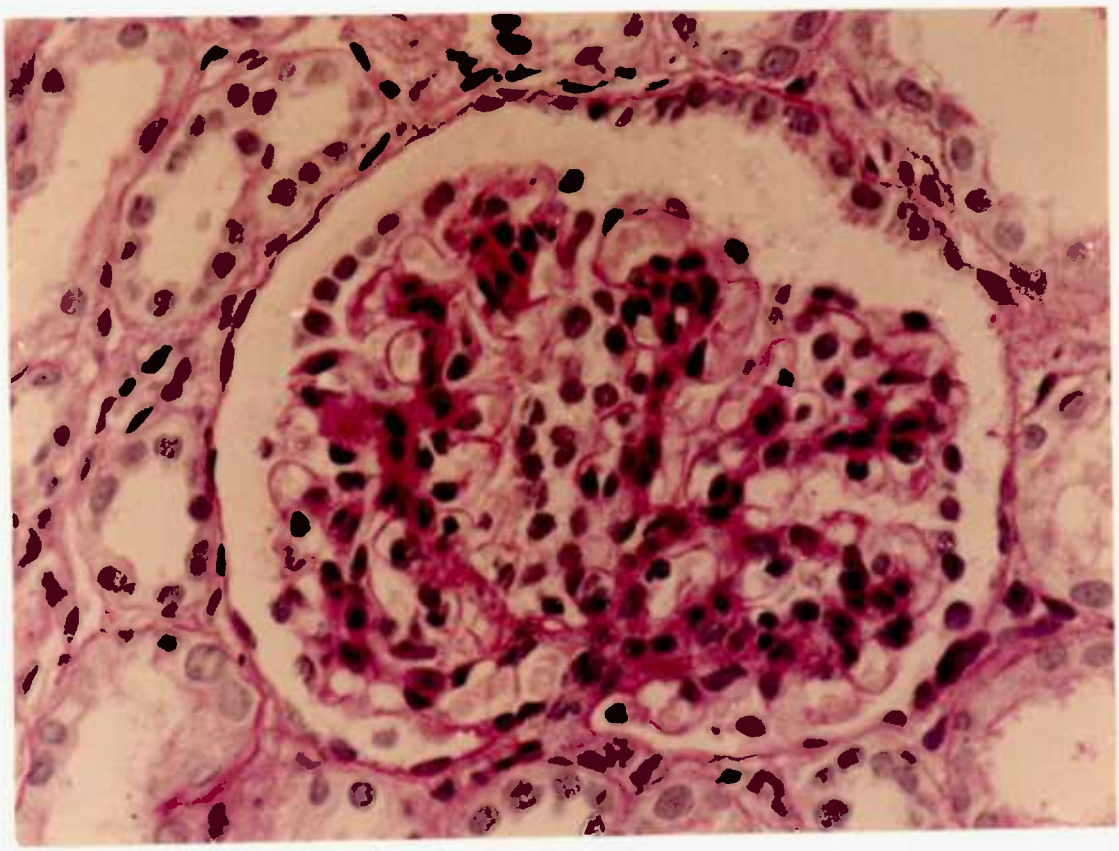


Figs. 8a and 8b Nephrotic syndrome - 'mesangial' proliferative glomerulonephritis

A glomerulonephritis in which cellular proliferation is most conspicuous in the lobular stalks (mesangial areas) of the glomerular tufts, and is associated with an increase in the amount of mesangial matrix. The capillary loops are widely patent and have thin walls.
8a - P.A.S. x 160; 8b - P.A.S. x 100.

Fig. 8a

Fig. 8b



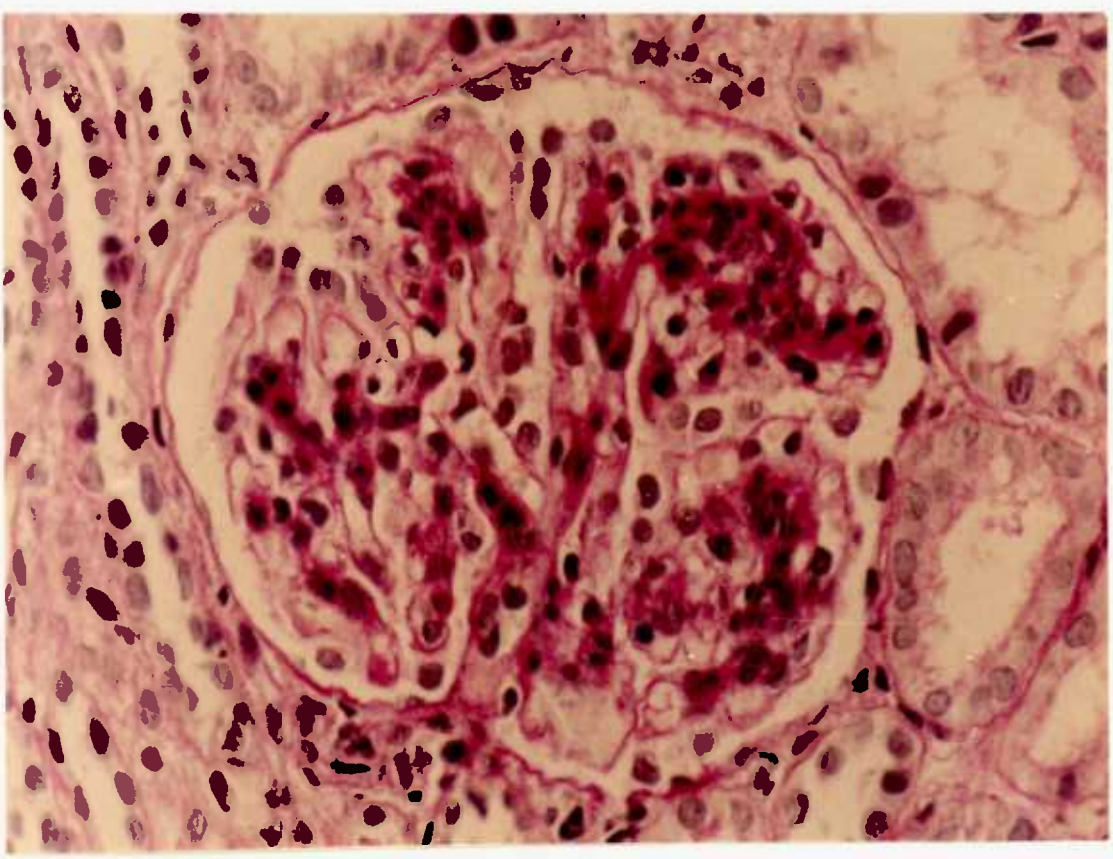


Fig. 9 Nephrotic syndrome - proliferative glomerlonephritis with 'crescents'

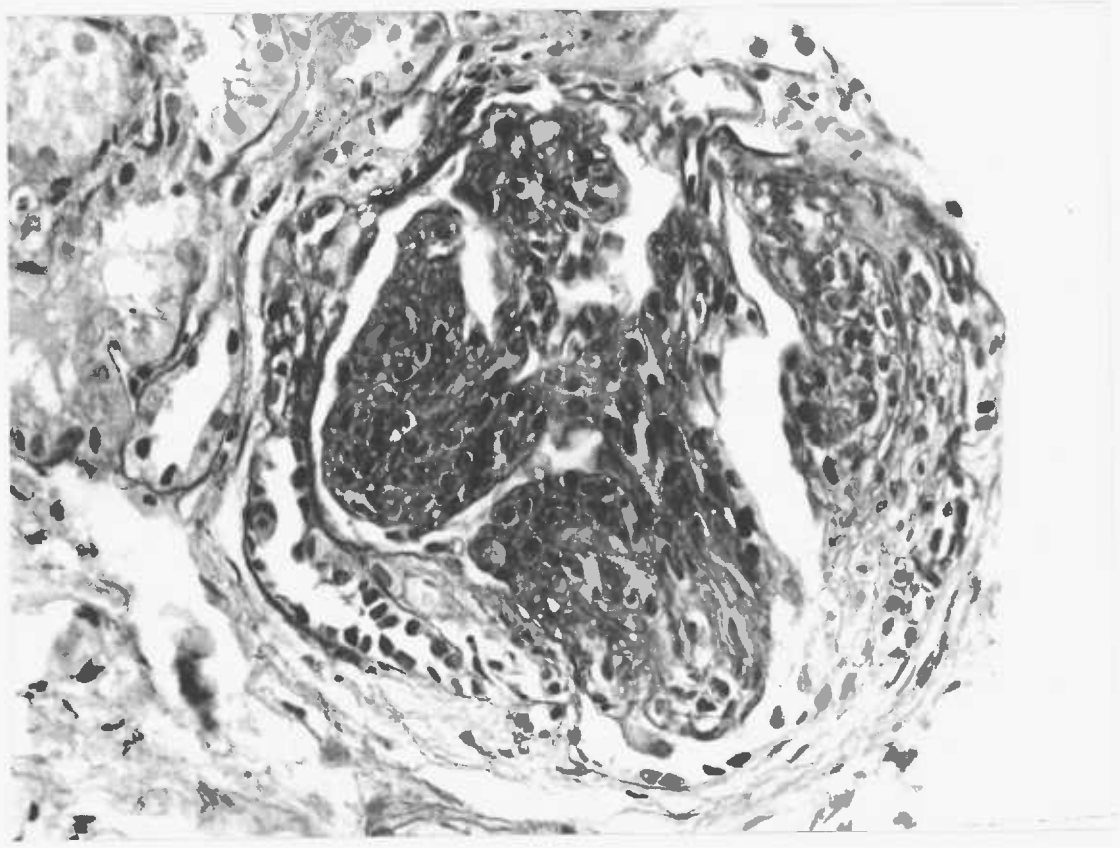
Conspicuous proliferation of the epithelial cells, particularly those lining Bowman's capsule, forms a multilayered 'capsular crescent' which compresses the glomerular tuft. P.A.S. x 128.

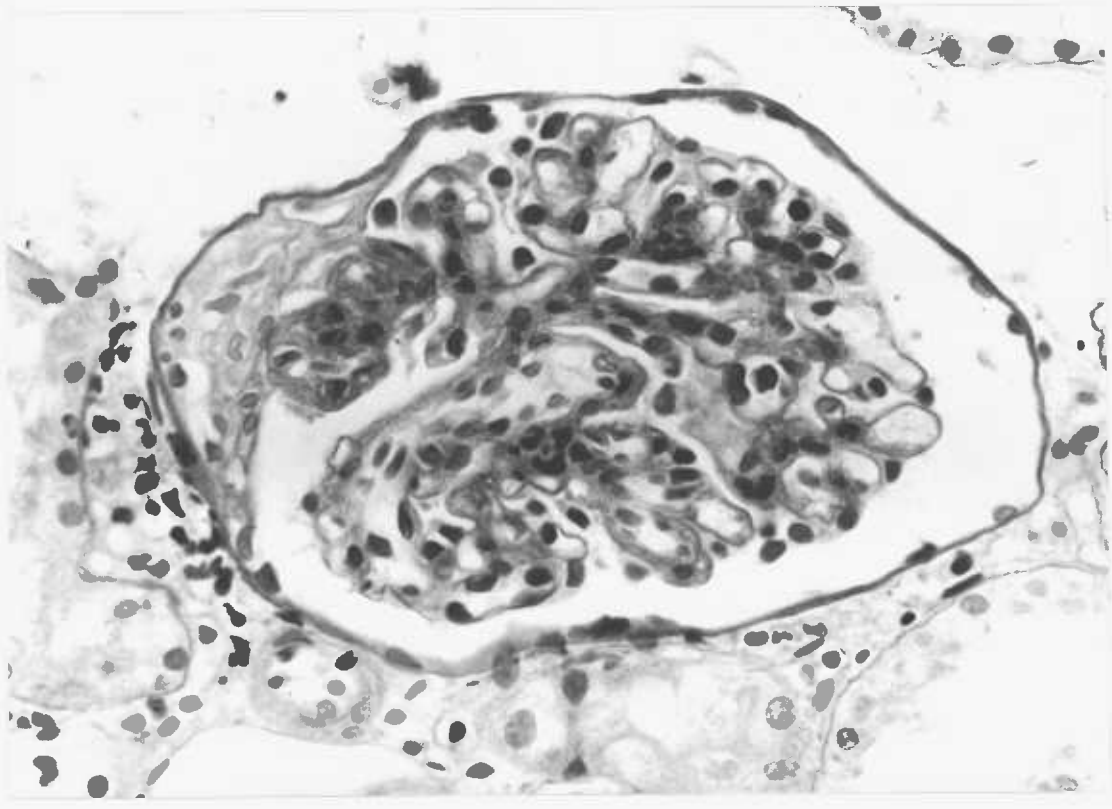
Fig.10 Proliferative glomerulonephritis with 'crescents'

In some cases of proliferative glomerulonephritis, adhesions forming between the glomerular tuft and Bowman's capsule are associated with a slight localised proliferation of the adjacent epithelial cells. This example is from the biopsy of a child suffering from anaphylactoid purpura.
P.A.S. x 160.

Fig. 9

Fig. 10





has also been referred to as "capsular crescent" formation, but in this thesis the term "glomerulonephritis with crescents" is used only to describe cases in which conspicuous epithelial cell proliferation is found in almost all the glomeruli present. In such cases tubular atrophy and tubular separation by interstitial oedema or fibrosis is usually conspicuous.

v) Membranoproliferative (mesangiocapillary).

The glomeruli show a combination of proliferation of glomerular tuft nuclei, increase in the mesangial matrix and thickening of the capillary wall (figs. 11 and 12). The proliferation and mesangial thickening give the tufts a markedly lobulated appearance. Examination with the electron microscope shows that the capillary wall thickening is due largely to an extension of mesangial and fibrillary material beneath the endothelial cell cytoplasm (fig. 13).

Membraneous (epimembraneous) nephropathy.

The glomerular capillary walls are diffusely thickened but there is no cellular proliferation. This thickening can be shown to be due to the deposition of immune deposits on the outer (subepithelial) aspect of the basement membrane (fig. 14). In the methenamine/silver preparations projections or "spikes" of argyrophilic basement membrane material can be demonstrated projecting from the epithelial aspect of the basement membrane between the deposits (fig. 15). In more advanced examples these spikes extend to encircle the deposits (Ehrenreich and Churg, 1968).

Fig.11 Nephrotic syndrome - membranoproliferative glomerulonephritis

A glomerulus showing a combination of proliferation of the glomerular tuft nuclei, increase in mesangial matrix and thickening of the walls of the capillary loops. Lobulation of the glomerular tuft is conspicuous. P.A.S. x 100.

Fig. 11

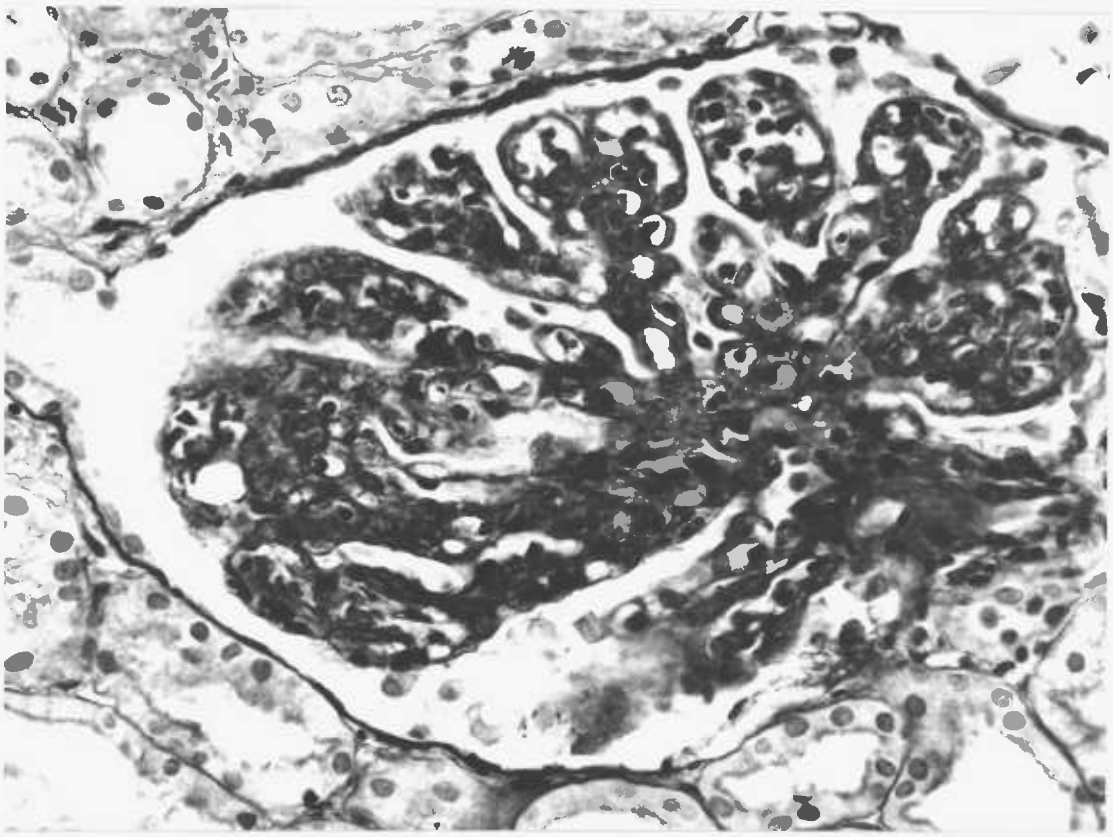


Fig.12 Nephrotic syndrome - membranoproliferative glomerulonephritis

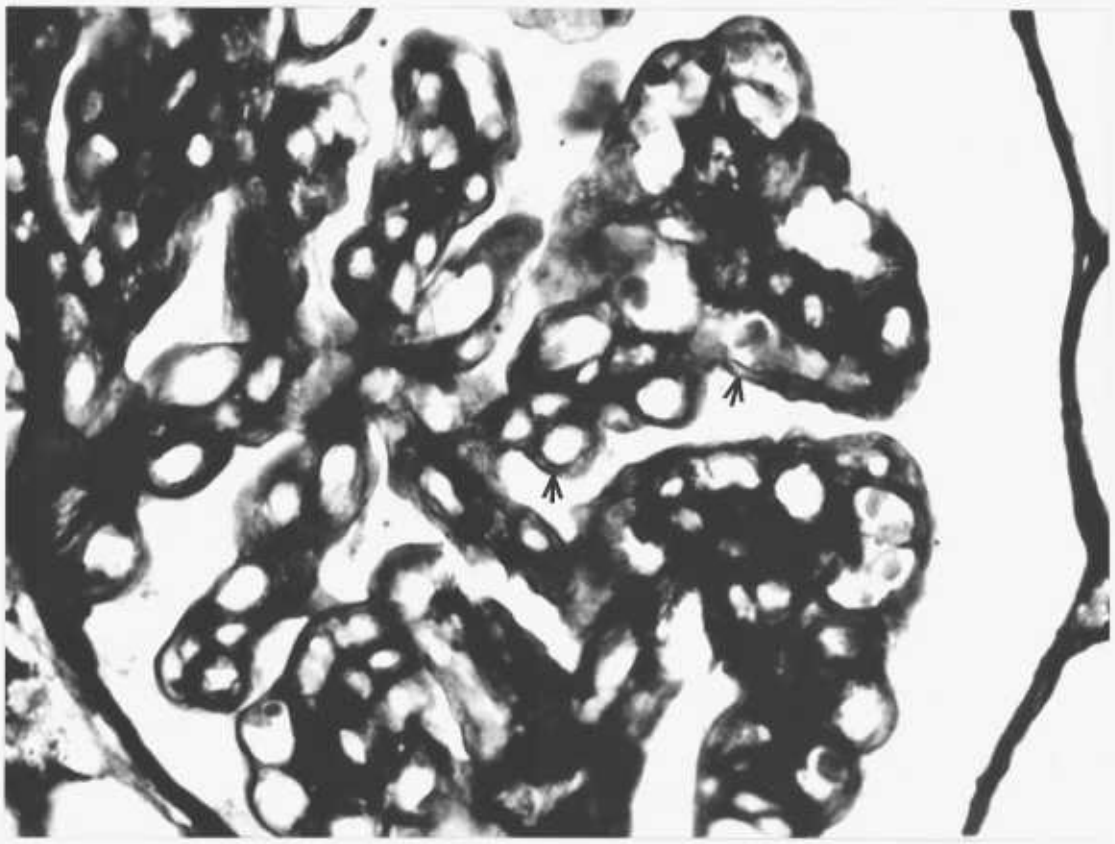
In this periodic acid - methenamine silver preparation, the basement membrane of the tuft capillary loops is seen predominantly as a thin, often irregular, black line. In some areas two parallel argyrophylic lines are seen, giving a 'tram-line' effect (see arrows). This is due to the infiltration of mesangial fibrils and cell cytoplasm beneath the endothelial cell cytoplasm lining the capillary loops.
P.A.S.M. x 256.

Fig.13 Nephrotic syndrome - membranoproliferative glomerulonephritis

Electron microscopic examination clearly demonstrates that the thickening of the capillary wall is only partly due to thickening of the true basement membrane. Between this structure and the endothelial lining of the capillary is a meshwork mesangial fibrillary material and intracapillary cell cytoplasm. B - basement membrane; E - fused foot-processes of an epithelial cell; M - mesangial fibrillary material; C - intracapillary cell cytoplasm; L - capillary lumen. (x 6,500).

Fig. 12

Fig. 13



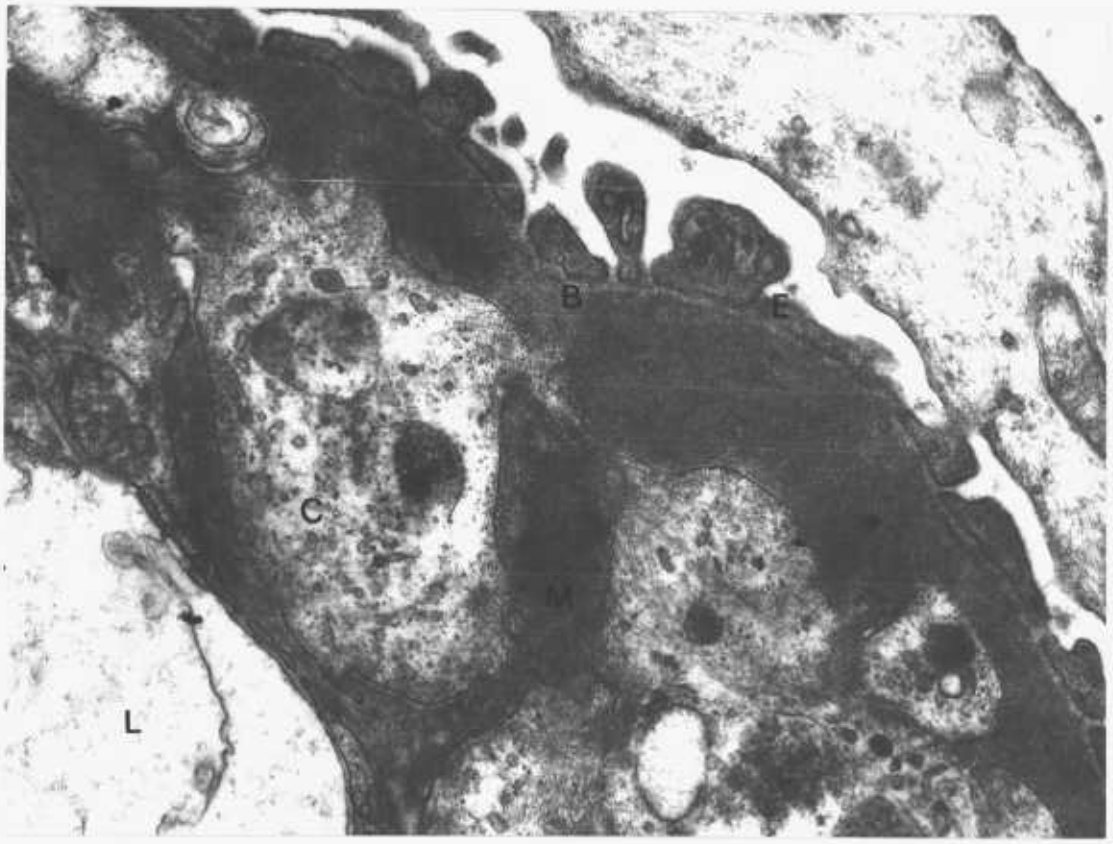

B
E
C
M
L

Fig.14 Nephrotic syndrome - membranous nephropathy

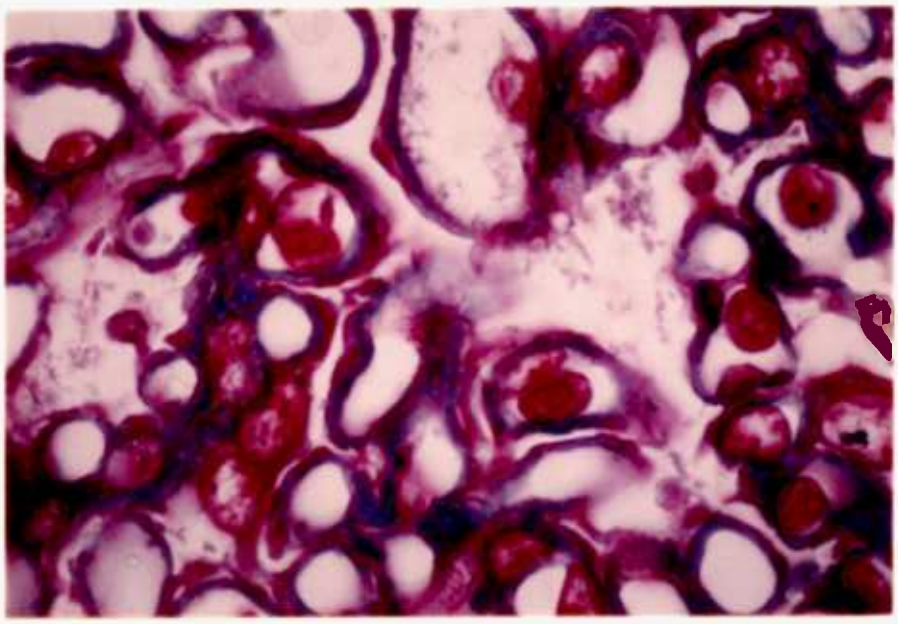
A diffuse thickening of the capillary wall in the glomerular tuft results from the presence of proteinaceous deposits (staining red) on the epithelial aspect of the basement membrane. This type of glomerular lesion is an extremely uncommon finding in biopsies from young children with a nephrotic syndrome. Mallory's trichromic stain x 400.

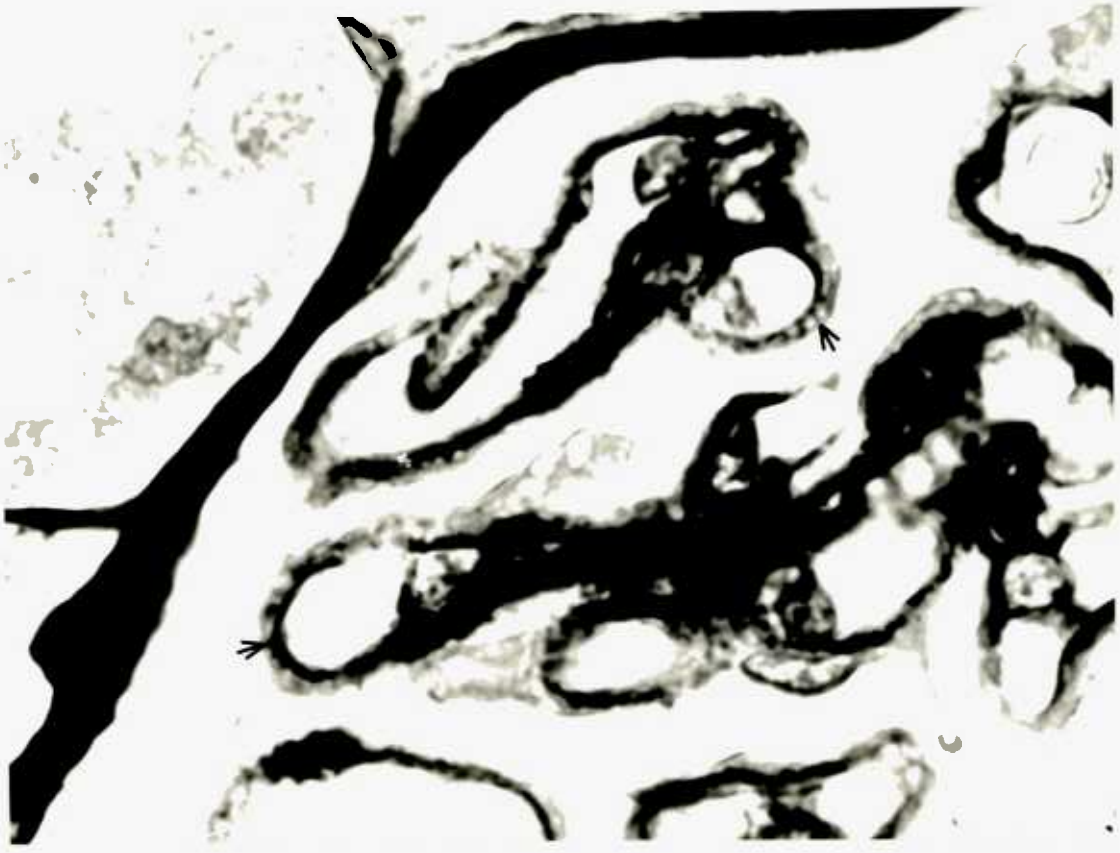
Fig.15 Nephrotic syndrome - membranous nephropathy

In this periodic acid - methenamine silver preparation projections, or 'spikes' (arrowed), of argyrophilic material can be seen extending from the epithelial side of the basement membrane. P.A.S.M. x 400.

Fig. 14

Fig. 15





Chronic glomerulonephritis.

In the advanced stage of any progressive glomerulopathy glomerular scarring and sclerosis, tubular atrophy and interstitial fibrosis may be so advanced that any distinctive morphological features may be masked. Such cases are included in the category.

II) Idiopathic Recurrent Haematuria.

Each renal biopsy specimen was classified either as morphologically normal or as proliferative glomerulonephritis.

Morphologically normal.

The glomeruli interstitial tissues and blood vessels show no definite pathological abnormalities, the appearances corresponding to those seen in the "minimal changes" group amongst the biopsies from children with a nephrotic syndrome (fig. 16). Occasionally red blood cells are visible in the tubules and occasionally haemosiderin deposition is seen in cortical or medullary tubules (fig. 17).

Proliferative glomerulonephritis.

Slight proliferation of glomerular tuft nuclei is seen. This change is diffuse, and affects the lobular stalks (mesangial region) which are slightly thickened (fig. 18.). The changes correspond to those described as "mesangial proliferative glomerulonephritis" amongst the children with the nephrotic syndrome. In some cases partially, or wholly, sclerosed glomeruli, and areas of tubular atrophy are seen (figs. 19 and 20).

Fig.16 Idiopathic recurrent haematuria - normal morphology

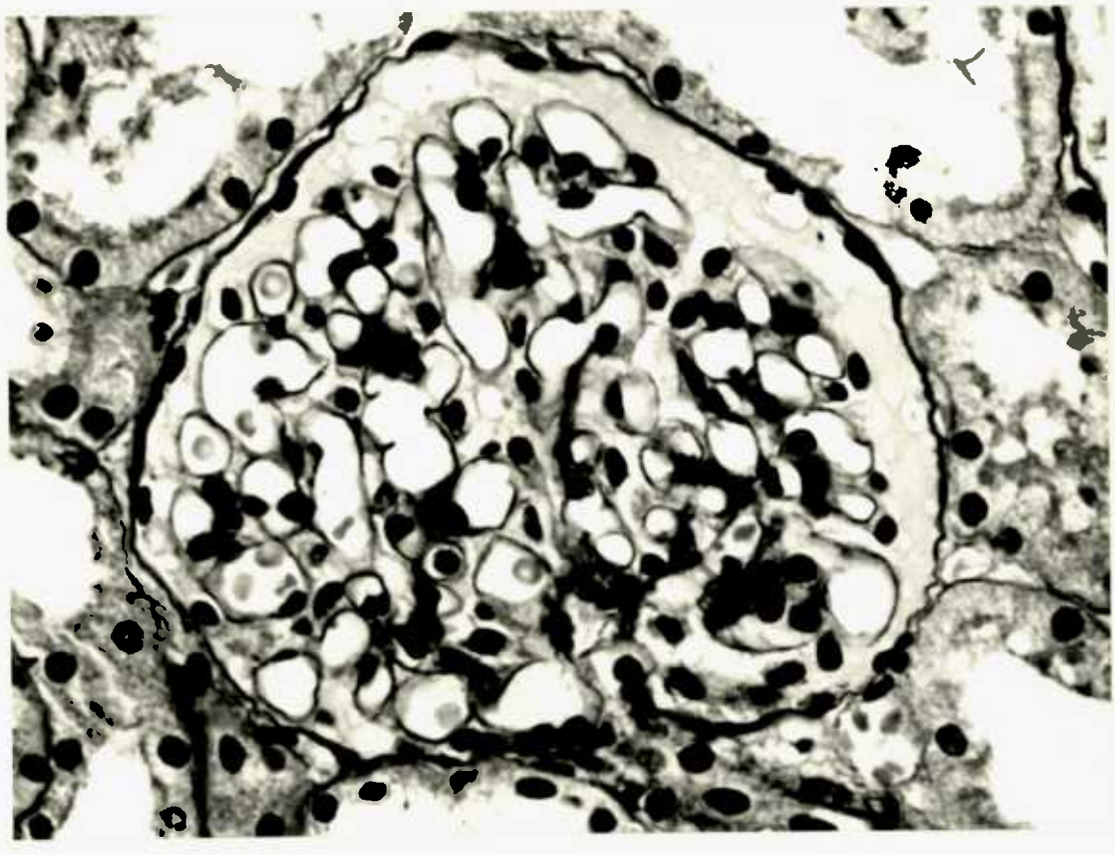
A glomerulus showing no definite pathological changes. P.A.S. x 150.

Fig.17 Idiopathic recurrent haematuria

Even where the glomeruli are normal, the renal origin of the urinary tract bleeding can sometimes be confirmed in a biopsy by the presence of haemosiderin deposits in the renal tubules. Perls' iron stain x 150.

Fig. 16

Fig. 17



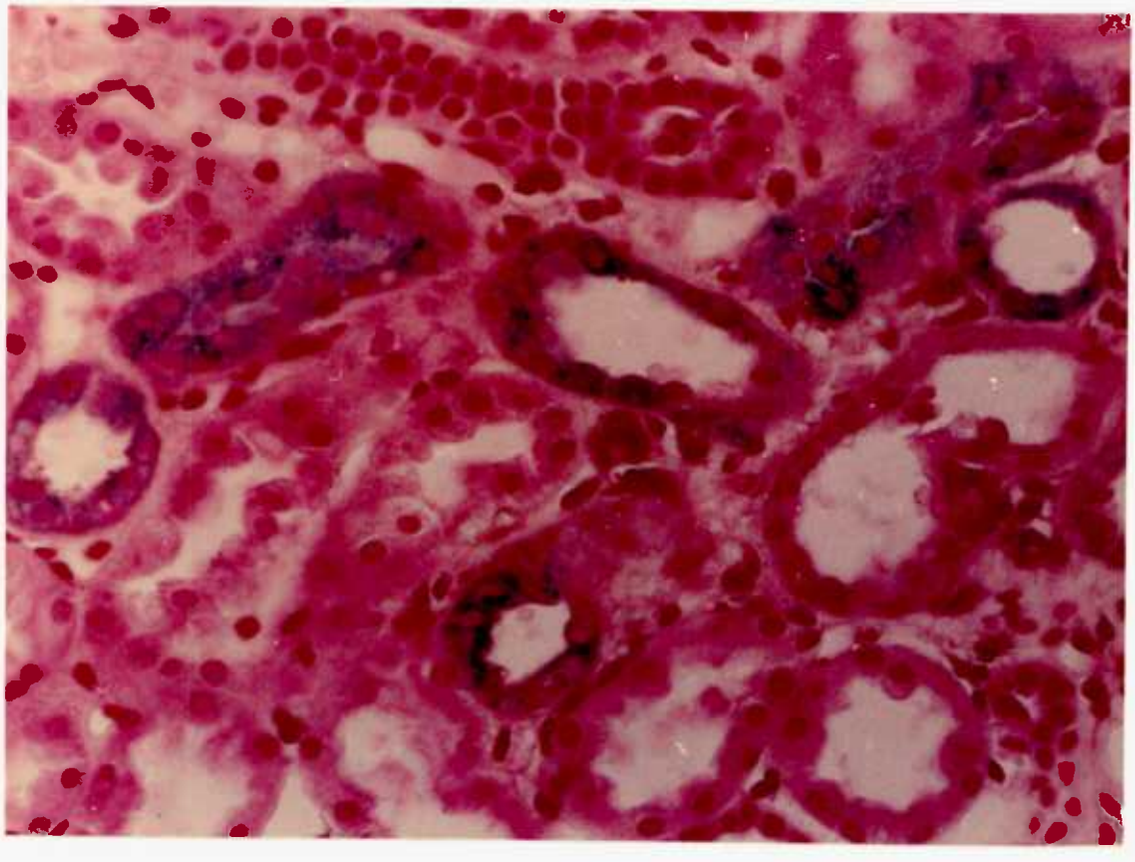
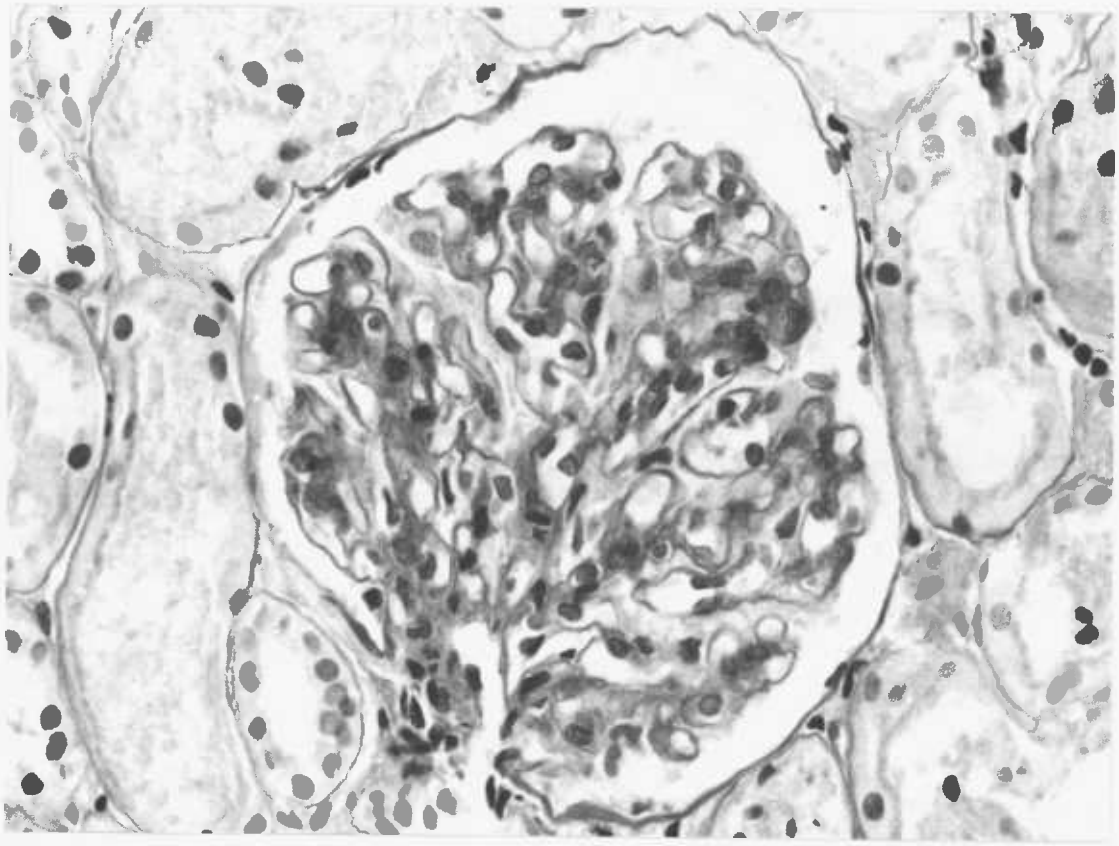


Fig.18 Idiopathic recurrent haematuria - proliferative glomerulonephritis

Glomerulus showing slight proliferation of tuft nuclei with some thickening of the lobular stalks. P.A.S. x 150.

Fig. 18

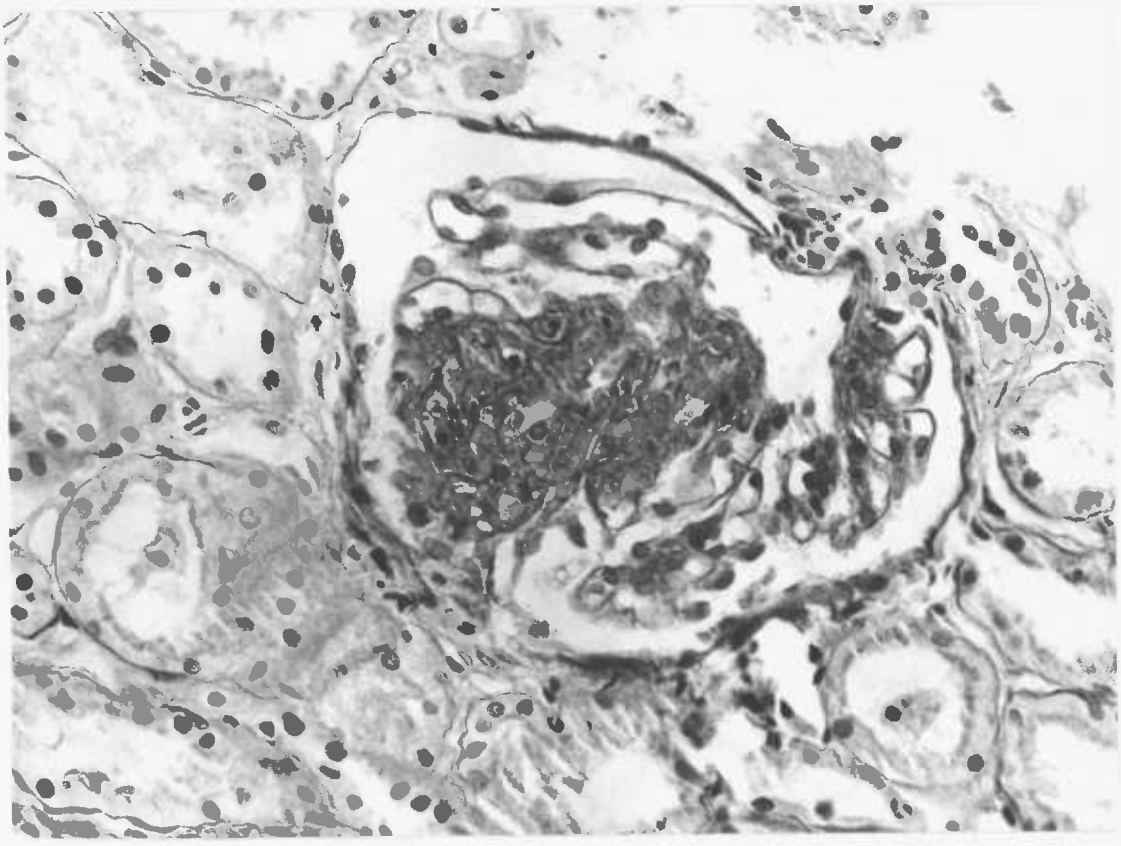


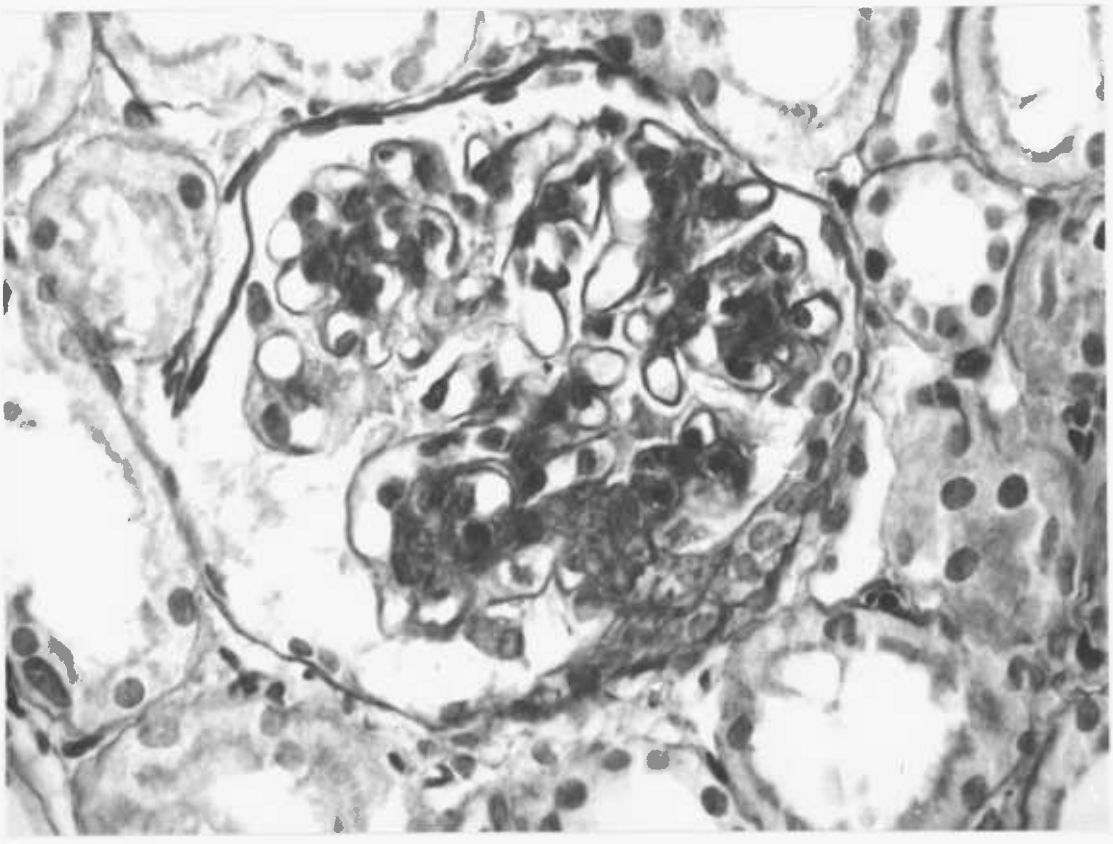
Figs. 19 and 20 Idiopathic recurrent haematuria - proliferative glomerulonephritis

In very occasional cases, in addition to slight proliferative changes segmentally sclerosed glomeruli (fig.19) sometimes associated with capsular adhesions (fig.20) are seen.
19 - P.A.S. x 100; 20 - P.A.S. x 150.

Fig. 19

Fig. 20





III) Anaphylactoid Purpura

All the renal biopsy specimens examined from children with anaphylactoid purpura showed changes of proliferative glomerulonephritis. In almost every instance proliferation of glomerular tuft cells was diffuse rather than focal, although the severity of the changes present often varied from glomerulus to glomerulus (fig. 21). The biopsies were divided into five groups according to the severity of the pathological changes encountered.

Group I

Proliferation of glomerular tuft nuclei is slight and is most conspicuous in the mesangial regions which are slightly thickened when examined in sections stained with P.A.S. or silver/methenamine, due to an increase in fibrillar content. Polymorphonuclear leucocytic infiltration is not a feature (fig. 22). The appearances in this group are very similar to those in the "mesangial proliferative glomerulonephritis" group of biopsies from children with a nephrotic syndrome. Abnormalities of the tubules, blood vessels and interstitial tissues are absent.

Group II

In addition to the changes described in Group I, some glomeruli show segmental areas of sclerosis of the glomerular tuft. This is characterised by a localised increase of mesangial matrix extending into the capillary wall and obliterating the capillary lumen. Characteristically there is an adhesion between the sclerosed segment of the

Fig.21 Anaphylactoid purpura

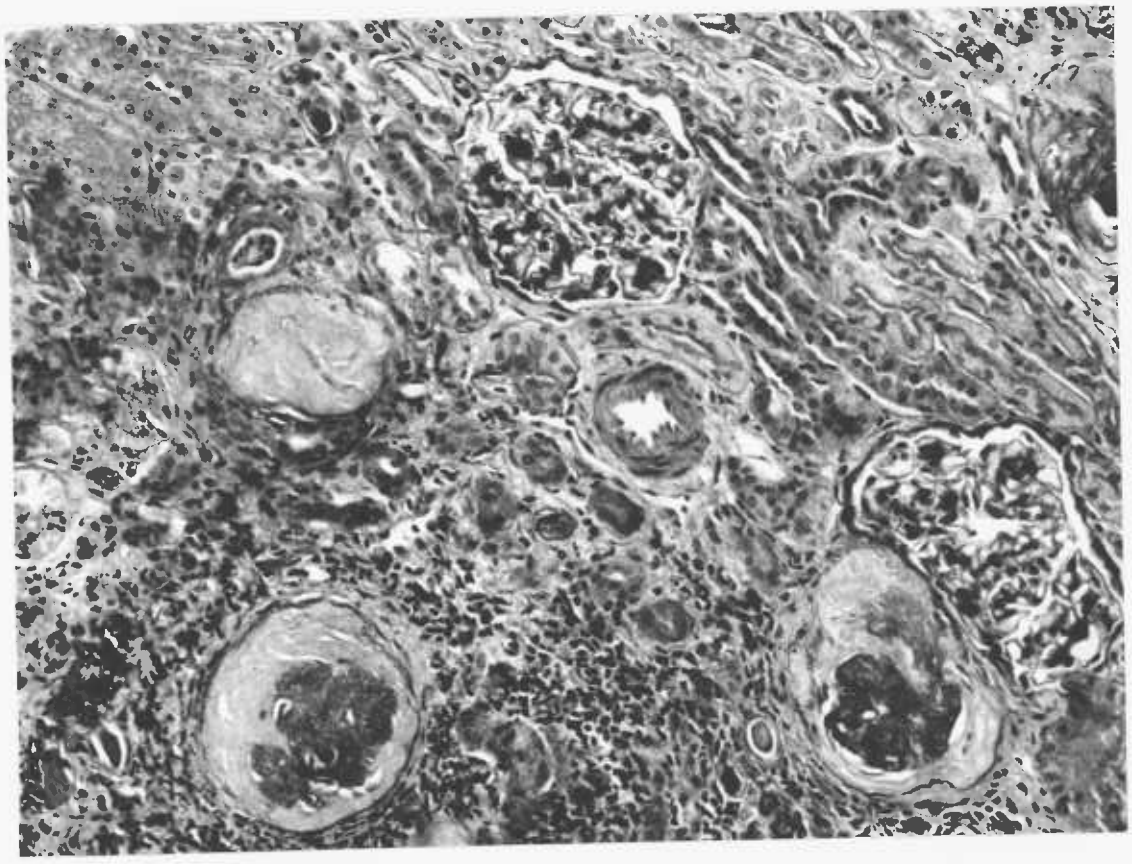
The severity of the glomerular changes may vary markedly from glomerulus to glomerulus. In this field very slightly affected and totally obliterated glomeruli are seen side by side. P.A.S. x 16.

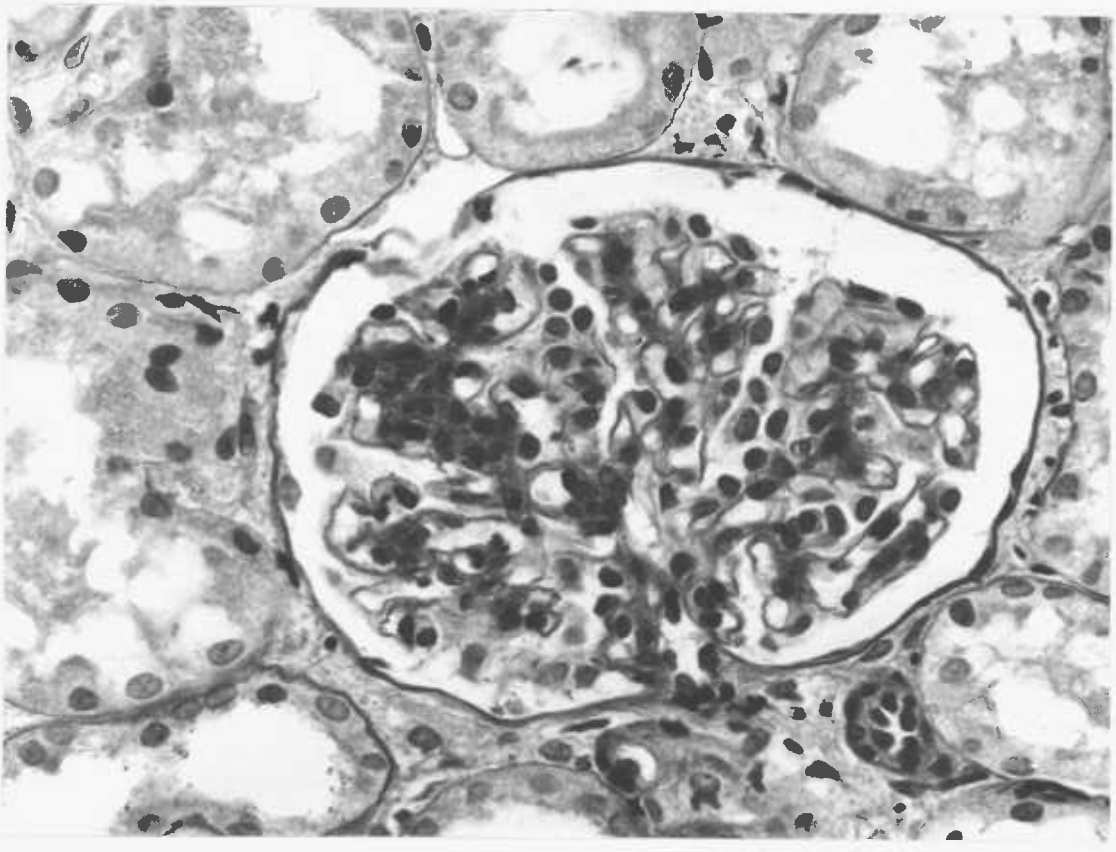
Fig.22 Anaphylactoid purpura - proliferative glomerulonephritis

Glomerulus showing slight proliferative changes most marked in the axial stalks and associated with an increase in the mesangial matrix. P.A.S. x 150.

Fig. 21

Fig. 22





glomerular tuft and Bowman's capsule, which is associated with a localised reactive proliferation of the adjacent epithelial cells (fig. 23). Some glomeruli may be entirely obliterated and foci of tubular atrophy and interstitial chronic inflammation and fibrosis may be seen. However, less than 10% of the glomeruli are scarred, and tubular atrophy is seen in less than 2 out of 10 consecutive high power fields examined with a 40x objective.

Group III

This differs only in degree from Group II in that more, but not all, glomeruli are scarred (more than 10% but less than 100%) and tubular atrophy is more extensive (more than 2 out of 10 high power fields examined).

Group IV

This again differs in degree. In this group are included biopsies in which all the glomeruli examined were scarred to a greater or lesser degree, and tubular atrophy is extensive (fig. 24). The changes correspond to those described as "advanced chronic glomerulonephritis" amongst the biopsies from patients with a nephrotic syndrome.

Group V

In all, or nearly all the glomeruli, in addition to conspicuous proliferation of the glomerular tuft cells, there is marked proliferation of the epithelial cells lining Bowman's capsule to form multilayered 'capsular crescents'. Proliferation of the epithelial cells is so marked that frequently the glomerular tuft is compressed and bloodless.

Fig.23 Anaphylactoid purpura - proliferative glomerulonephritis

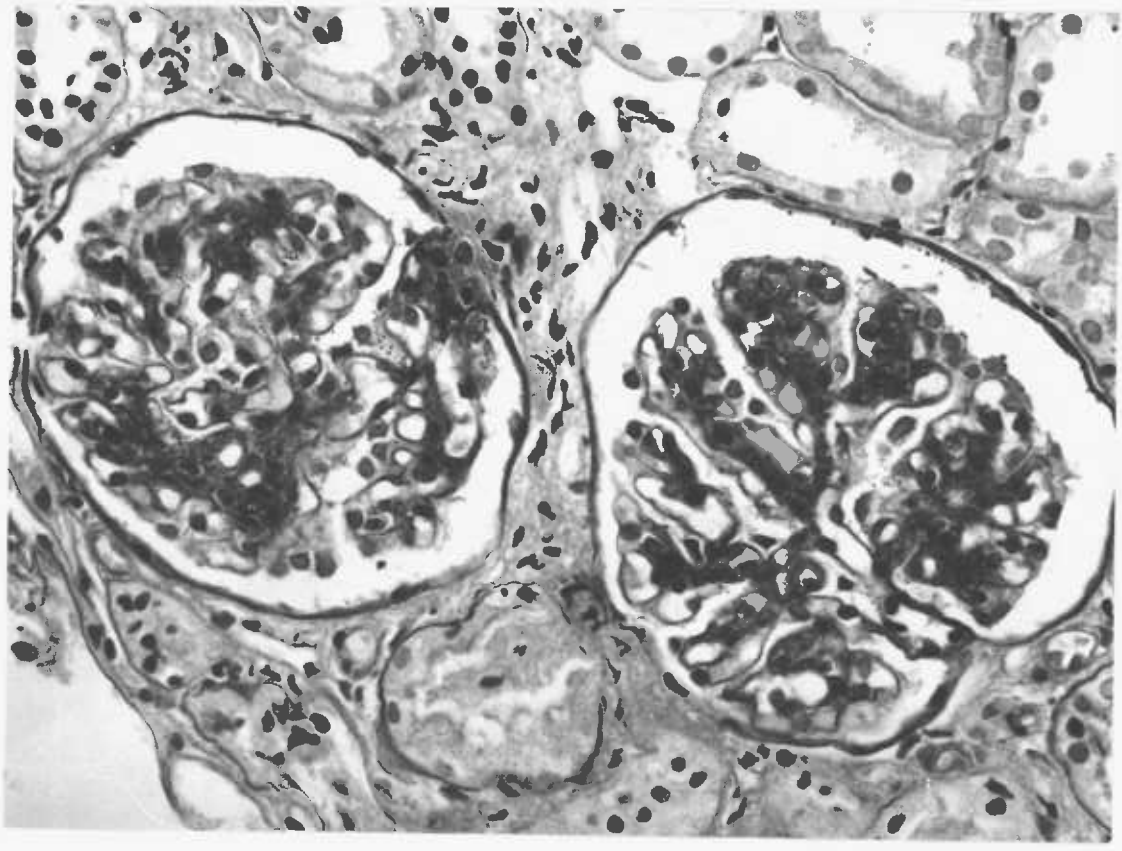
Two glomeruli showing slight proliferative changes. In one a small area of tuft sclerosis is associated with an adhesion to Bowman's capsule.
P.A.S. x 40.

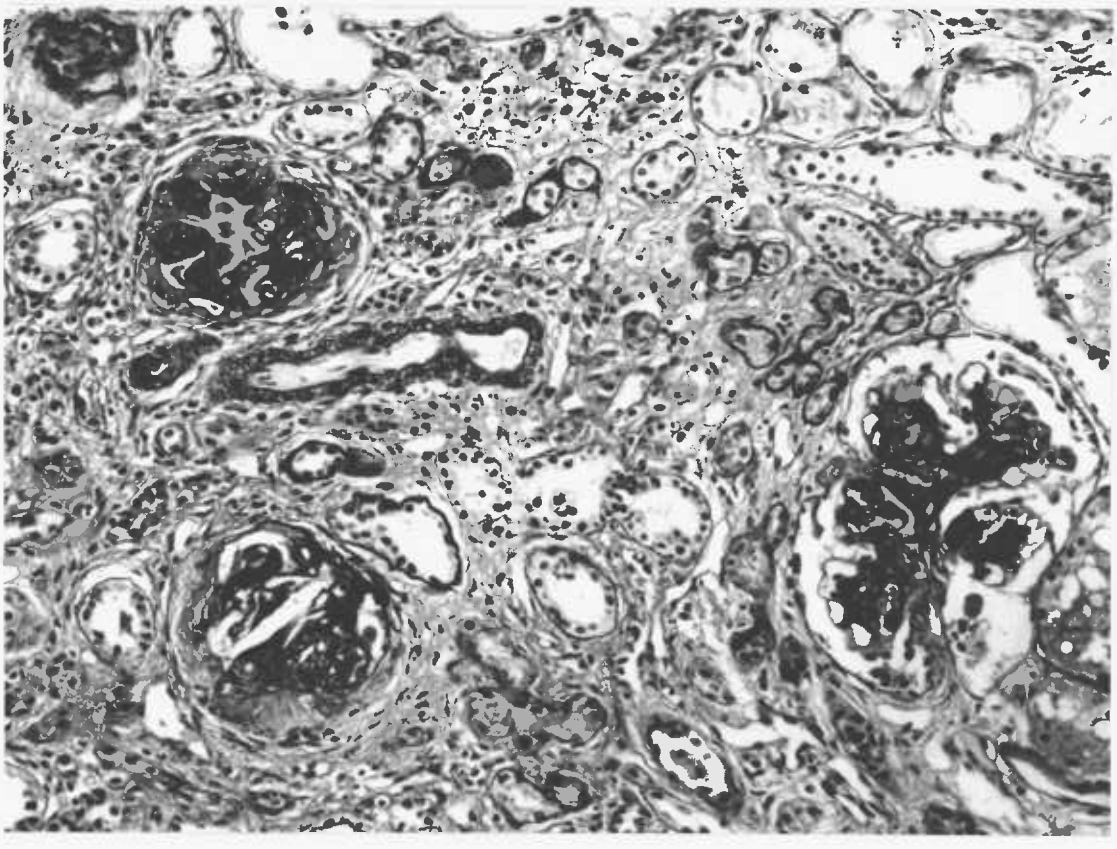
Fig.24 Anaphylactoid purpura - proliferative glomerulonephritis

Severe changes. All the glomeruli shown are scarred and areas of tubular atrophy are present.
P.A.S. x 16.

Fig. 23

Fig. 24





The appearances correspond to those in the group described as "glomerulonephritis with crescents" in the biopsies from patients with a nephrotic syndrome.

Quantitative and Semi-quantitative Assessments of the Histological Changes Found

I) Total and Differential Glomerular Cell Counts (Paraffin-embedded sections)

In each biopsy specimen total and differential counts were made of the cells composing each of five glomeruli. The biopsies were examined without knowledge of the clinical details of the patient from whom they were obtained. Only unscarred glomeruli, free from technical artifacts such as crushing, were considered, and the five glomeruli selected were chosen so that the plane of the section went through the equatorial region rather than the periphery of the glomerular tuft, i.e. near its maximum diameter. This was checked by examining the relevant serial sections, and counts were not made on glomeruli where the plane of the section cut through near the periphery. These were easily recognisable by their relatively small circumference compared with other glomeruli in the section.

The glomerular counts were made on sections stained with periodic acid/Schiff since the various cell types were most easily recognisable by this method. The sections were examined with an oil-immersion (x 100) objective. Each glomerulus chosen for counting was traversed systematically and the numbers of epithelial, endothelial and mesangial cells forming the glomerulus were recorded. Epithelial cells were

those cells on the outside of the basement membrane of the glomerular tuft and those lining Bowman's capsule (extracapillary cells). The cells inside the basement membrane (intracapillary cells) were endothelial and mesangial cells. The endothelial cells abutted directly on to, and lined, the capillary luminal spaces. The mesangial cells were situated within the stalks of the glomerular capillary loops. Although these cells were situated within the confines of the glomerular basement membrane, they were separated from the adjacent capillary lumens by endothelial cells (see fig. 25). In every glomerulus examined the number of epithelial, endothelial and mesangial cells were noted and expressed as a percentage of the total number of cells. Blood cells within the capillary lumina were ignored. In order to obtain a single figure to express the distribution of the three types of cell composing each glomerulus the percentage of extracapillary (epithelial) cells and the percentage of intracapillary (endothelial and mesangial) cells were subtracted one from the other. Where the proportion of extracapillary (epithelial) cells was in excess, a minus value was given to the percentage difference, and where the proportion of intracapillary (endothelial and mesangial) cells was in excess, a plus value was given to the percentage difference. For example, in section of a glomerulus containing 55% epithelial cells, 32% endothelial cells and 13% mesangial cells, that is 55% extracapillary cells and 45% intracapillary cells, the percentage difference is 10%. Since the extracapillary cells are in excess the difference would be expressed as -10%.

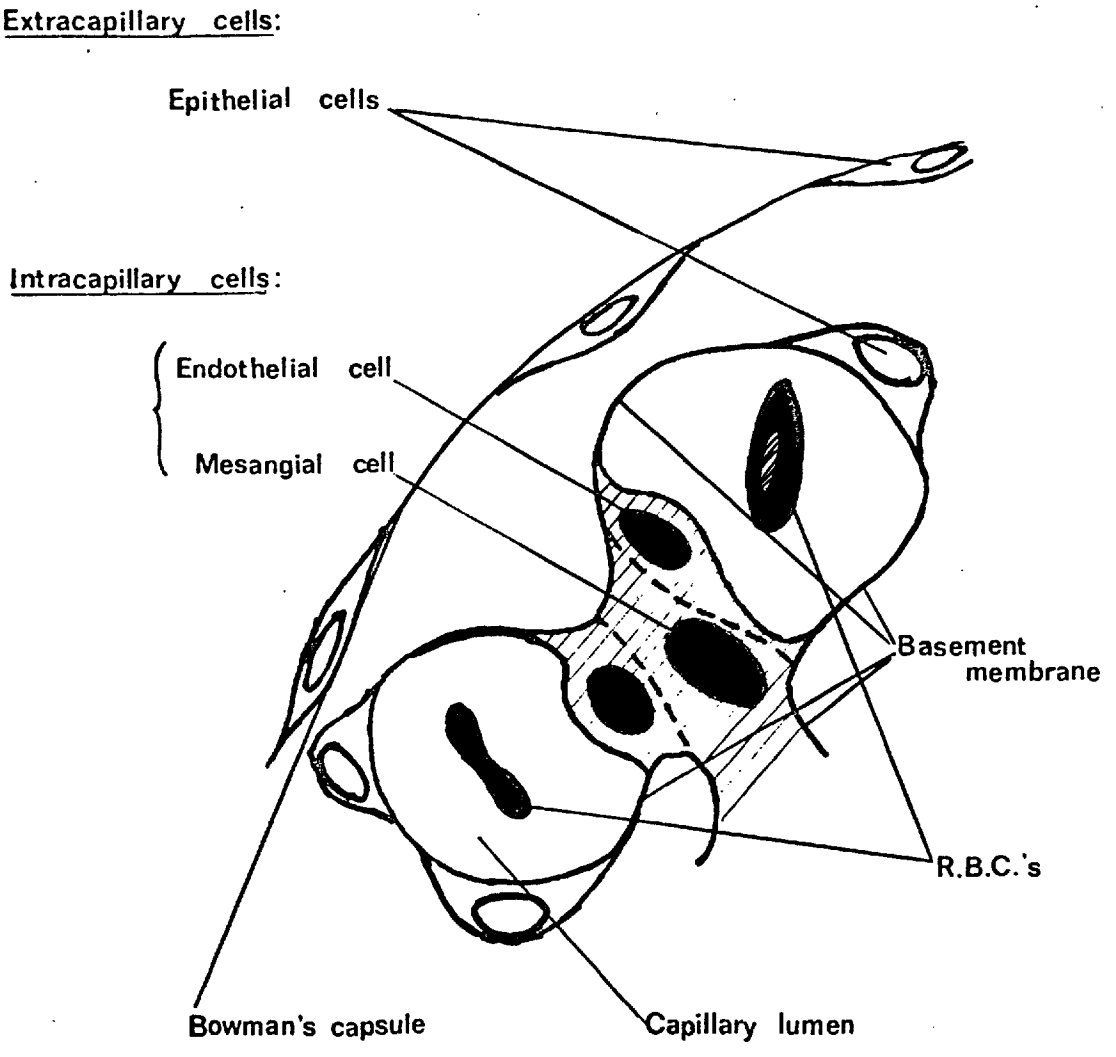


Fig. 25 A schematic representation of part of a glomerulus showing the various types of cell present. Epithelial (extracapillary) cells are situated on the outside of the basement membrane and also line Bowman's capsule. The cells inside the basement membrane (intracapillary cells) are endothelial and mesangial cells. The endothelial cells line the capillary lumina. Mesangial cells are situated within the stalks of the glomerular capillary loops, and although within the confines of the basement membrane, they are at all points separated from the capillary lumen by endothelial cells.

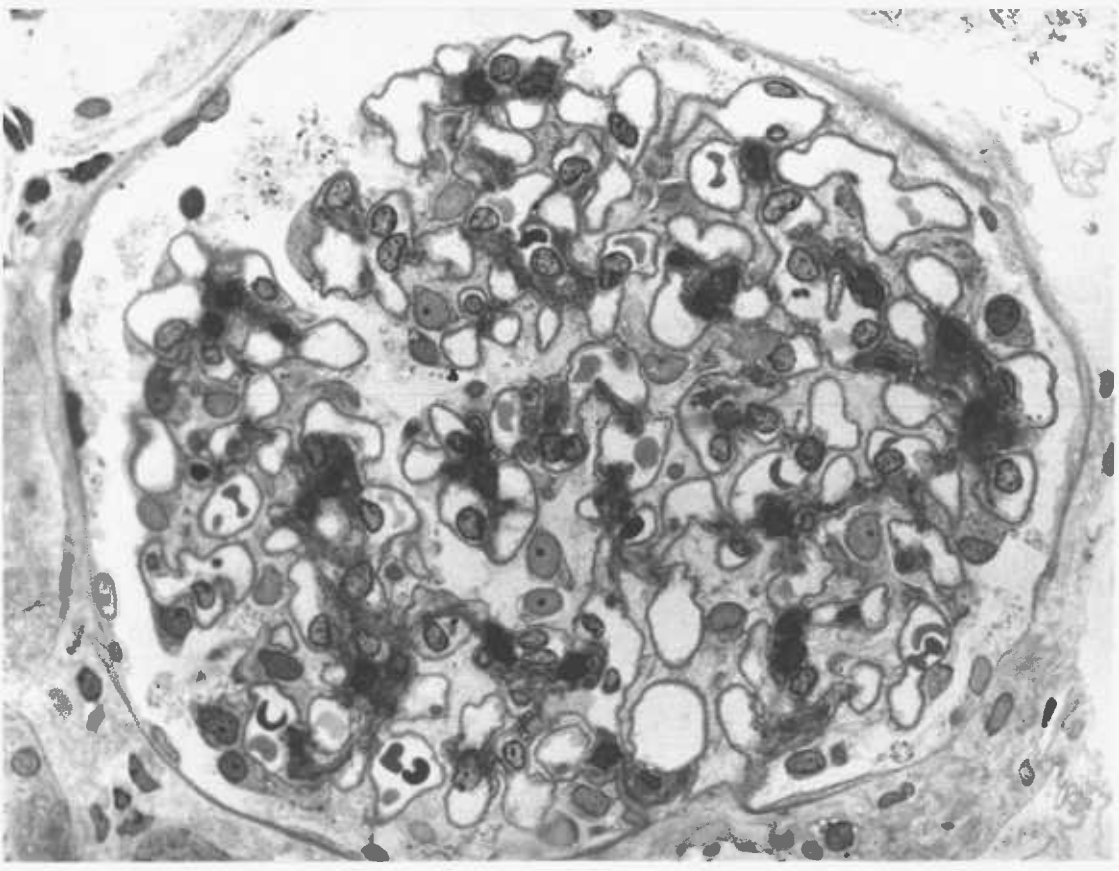
Since in each renal biopsy studied, counts were made from five glomeruli, the mean percentages of epithelial, endothelial and mesangial cells, the mean total number of cells, and the mean percentage differences between the intracapillary and extracapillary cells were calculated in each case.

II) The Use of Thin Sections (0.5 μ thickness) of 'Araldite'-Embedded Tissue for Glomerular Cell Counts

In those renal biopsy specimens in which tissue had been embedded in 'Araldite' as well as in paraffin wax, sections of the 'Araldite' embedded tissue cut at 0.5 μ thickness at various levels were examined by light microscopy. Photomicrographs of each unscarred and undistorted glomerulus present in each of these biopsies were made, the section of each glomerulus photographed being in the region of the equator of the glomerulus (see fig. 26). Differential counts of the epithelial, endothelial and mesangial cells were made from the photomicrographs. 35 biopsies were examined in this way, and in 24 instances three or more glomeruli suitable for counting were present in the 'Araldite'-embedded tissue. In these 24 cases the mean percentages of epithelial, endothelial and mesangial cells, the mean total numbers of cells and the mean percentage differences between the intracapillary and extracapillary cells were calculated for the number of glomeruli present in the 'Araldite'-embedded part of each biopsy. The results of the mean total and differential glomerular cell counts obtained by direct

Fig.26 A glomerulus in a section of 0.5 μ thickness from a biopsy embedded in 'Araldite' showing the high degree of histological clarity afforded by this method of preparation. Toluidine blue x 160.

Fig. 26



counting of glomeruli using an oil-immersion (100 x) objective in the paraffin sections, and by counts performed on photomicrographs of the glomeruli in the 'Araldite' sections were compared for each individual biopsy. The counts by these two methods were made on separate occasions, and no attempt to compare the results obtained was made until all the counts in every patient had been completed.

III) 'Glomerular Index' and Tubular Atrophy

Semi-quantitative assessments were made of the extent of any glomerular and tubular damage present in each of the renal biopsies studied using techniques which have been described previously (Risdon, Sloper and de Wardener, 1968).

The extent of any glomerular damage was assessed as follows. In each biopsy specimen, ten consecutive glomeruli were examined for the presence of excess material in the glomerular tufts staining positively with periodic acid/Schiff. Each glomerulus was graded from 0 to 5 in terms of the extent of the excess PAS - positive material. Where such material entirely obliterated the glomerulus, a grading of 5 was made. If excess PAS - positive material occupied more than three-quarters of the glomerulus, the grading was 4; if more than half, but less than three-quarters the grading was 3; if more than a quarter, but less than a half the grading was 2; if less than a quarter the grading was 1. If the glomerulus was considered entirely normal the grading was 0. In each instance the grading on ten consecutive glomeruli were added

together. Each biopsy was graded "blind" on two quite separate occasions several weeks apart, and a mean was calculated between the findings in each case on the two separate occasions, the figure being termed the 'glomerular index'. These indices ranged between 0 and 40. The largest difference between the sums observed in individual cases was 7. Table 1 gives details of the correlation between the two assessments.

Significant tubular damage sought was the presence of tubular atrophy, which was characterised by significant thinning of the tubular epithelial cells and by conspicuous thickening of the tubular basement membrane when stained by P.A.S. Such tubules were sometimes dilated by "colloid" material but more often they were narrowed, empty and separated from each other by interstitial connective tissue. The extent of any tubular atrophy present in each biopsy was assessed as follows. Ten consecutive microscopic fields were examined in the cortex of each specimen with the aid of a 40X objective. The extent of tubular damage was assessed by the number of microscopic fields in which there was unequivocal tubular atrophy. Assessments were made "blind" on two quite separate occasions several weeks apart, and where in a particular biopsy, the two assessments differed, a mean was taken. Agreement was close: the number of microscopic fields in which tubular atrophy was considered to be present differed by 3 out of 10 in two specimens, by 2 out of 10 in ten and by 1 out of 10 in 12 specimens. In the remainder the assessments were identical on both occasions (see Table 2).

TABLE I

Differences Observed in Calculating the 'Glomerular Indices' in 115 Renal Biopsies on Two Separate Occasions

Difference in "Glomerular Index" of:	0	1	2	3	4	5	6	7	Average Difference
No. of biopsies	71	12	9	7	8	3	3	2	1.24

TABLE 2

Differences Observed in Assessing Tubular Atrophy
in 115 Renal Biopsy Specimens on Two Separate Occasions

Difference in Tubular Atrophy (H.P.F./10) of:	0	1	2	3	Average Difference
No. of biopsies	91	12	10	2	0.33

IV) Estimation of the Variation in the Proportion of Epithelial, Endothelial and Mesangial Cells at Various Levels Through the Glomerulus

Two 'Araldite'-embedded biopsy specimens, chosen because they both contained an adequate number of glomeruli, were taken and serial sections (0.5μ thickness) were made. Every 20th section was mounted. Ten sections were mounted in this way so that by examining the sections in sequence a 100μ thickness of the biopsy could be examined at intervals of 10μ. All the sections were stained with toluidine blue. Two glomeruli in each biopsy, chosen because the step sections gave representative sections right through the glomerulus, from its periphery on one side, through the equitorial region to the periphery on the opposite side, were examined. The same two glomeruli in each biopsy were photographed at each of the ten levels examined. From the photographs the percentages of epithelial, endothelial and mesangial cells, and the total number of glomerular cells were calculated in the sections at each level through the glomerulus. In this way the effect of the plane of the sections through the glomerulus on the proportions of the various cell types was ascertained.

V) Variations in the Proportions of Epithelial, Endothelial and Mesangial Cells in the Glomeruli With Age

It is common experience that the glomerular epithelial cells are especially prominent in sections from the kidneys of very young children. In order to obtain some idea of the effect of age upon the portions of the various cell types in

the glomeruli, paraffin-embedded blocks of tissue from the kidneys of children aged between 1 and 12 yr. inclusive were taken from the autopsy files (see Table 3). Various criteria other than age for choosing these 12 cases were made. In none was there any clinical or pathological evidence of renal disease and none had suffered from any other condition, such as cyanotic heart disease, which might effect the glomerular morphology. In addition the blocks of kidney tissue in the files were technically satisfactory with regard to fixation and lack of post-mortem artefacts. Thin (3 to 4 μ thickness) sections were prepared and stained with periodic acid/Schiff. In each case counts were made under an oil-immersion (100x) objective of the cells constituting five glomeruli. The criteria for choosing the glomeruli counted in each case were the same as those applied on the biopsy specimens.

TABLE 3

Main autopsy findings in the cases of children aged between 1 and 12 yr. sections of whose kidneys were used as controls for glomerular cell counts

P.M. Case No.	Age (yr) last birthday	Principal Necropsy Findings	Kidneys	
			Combined Weight (gm)	Macroscopic Abnormalities
1	1	Cystic fibrosis of pancreas	61	None
2	2	Reticulum-cell sarcoma	93	None
3	3	Spinal degeneration (Biemond type)	107	None
4	4	Malignant thymoma	108	None
5	5	Leukaemia; Down's syndrome	125	None
6	6	Thoracic neuroblastoma	136	None
7	7	Craniopharyngioma	134	None
8	8	Arthrogryposis	130	None
9	9	Ulcerative colitis	192	None
10	10	Congenital hypotonia	180	None
11	11	Hydrocephalus.Down's syndrome	195	None
12	12	Aplastic anaemia	248	None

Part 2. Clinical Presentations of the Patients Studied

Introduction

Between October 1967 and December 1970 percutaneous renal biopsies were performed on 123 children. In 116 adequate specimens of renal tissue containing ten or more glomeruli were obtained. The clinical diagnoses made in these 116 children were as follows:

	No.
Nephrotic syndrome	59
Idiopathic recurrent haematuria	24
Anaphylactoid purpura	12
Acute glomerulonephritis	6
Pyelonephritis	5
Acute tubular necrosis	4
Renal dysplasia	3
Systemic lupus erythematosus	2
Fanconi's syndrome	1
Total	116

This study is based on the findings in the patients in the first three groups, that is those with a nephrotic syndrome, idiopathic recurrent haematuria and anaphylactoid purpura. Since the number of patients with anaphylactoid purpura was small, the records of renal biopsies performed between 1960 (when the technique was first used at Great Ormond Street) and 1967 were examined. A further 10 patients

biopsied because of evidence of renal disease following anaphylactoid purpura were discovered. These were included in the study so that the final number of patients in this group was 22.

The clinical histories of the 105 patients studied are now presented. The group of patients with idiopathic recurrent haematuria are presented first. These are followed by those with nephritis after anaphylactoid purpura and with a nephrotic syndrome. In this last group full clinical details are given only in those cases in which no response to steroid therapy was obtained. A summary of the relevant findings in the steroid-sensitive patients is given.

Children with Idiopathic Recurrent Haematuria

Patient No.1 (G.B.)

An eight-year-old boy who presented with frank haematuria on one occasion following an upper respiratory tract infection. There was no family history of renal disease. On examination the child appeared fit and well and no physical abnormality was found. The blood pressure was $^{120}/_{75}$mm. of mercury. Investigations showed a normal blood count, a creatinine clearance of 98ml/min/1.73m^2 and a plasma urea concentration of 30mg/100ml. A throat swab culture grew commensal organisms only and the serum antistreptolysin (A.S.O.) titre was less than 200 units/ml. The bleeding and clotting times, the prothrombin and kaolin-cephalin times were all normal. Examination of the urine showed no protein, and the urine culture was sterile. Microscopy revealed no cells. Radiological examination of the chest revealed no abnormalities, and the intravenous pyelogram showed both kidneys to be of normal size with normal excretion of contrast medium. Cystoscopy showed no abnormality of the bladder and urethra. A percutaneous renal biopsy was performed, and microscopy of the biopsy specimen revealed no histological abnormalities. The child was discharged and no follow-up of the case has been made.

Patient No.2 (S.B.)

A ten-year-old boy who complained of three episodes of frank haematuria during a seven month period. His urine had been examined between these attacks and microscopy had shown red blood cells to be present. The attacks of haematuria had

each been associated with upper respiratory tract infections, but apart from some aching in his legs during the third attack, there were no other symptoms. No family history of renal disease was found. On examination the child appeared well and no abnormalities were present. The blood pressure was $^{130}/_{70}$mm. of mercury. Investigations showed a normal blood count and a plasma urea concentration of 30mg/100ml. The creatinine clearance was recorded as 74ml/min/1.73m^3. The bleeding, clotting, prothrombin and kaolin-cephalin times were normal. Bacteriological cultures from a throat swab grew commensal organisms only and the serum A.S.O. titre was less than 200 units/ml. Radiological examination of the chest showed no abnormality. Intravenous pyelography showed both kidneys to be normal in size and excretion of contrast medium was normal. Urinalysis showed mild proteinuria (30mg protein/100ml) and bacteriological culture showed no growth. Microscopy of the urine showed 15 red blood cells (R.B.C.'s) and 4 white blood cells (W.B.C.'s) per cu.mm. The urinary albumin/creatinine ratio ($^{UA}/_{UC}$) ranged between 0.12 and 0.36. A percutaneous renal biopsy was performed and microscopy showed mild proliferative changes in the glomerular tufts.

The child was followed up as an out-patient and one year later, some 15 months after the first attack he was still getting episodes of frank haematuria. His general health was unimpaired, however, and he was normotensive.

Patient No. 3 (S.F.)

A six-year-old boy who presented with a history of frequent attacks of macroscopic haematuria. The first attack some six months previously had followed an acute attack of diarrhoea and vomiting, and some subsequent attacks appeared to be associated with exercise. There were no other symptoms and the family history was not contributary. No abnormalities were found on physical examination and the blood pressure was recorded as $^{95}/_{70}$mm. of mercury. Investigations showed a normal blood count and normal bleeding, clotting, prothrombin and kaolin-cephalin times. The plasma urea was 22mg/100ml. and the creatinine clearance 133ml/min/1.73m^3. Radiological examination of the chest was normal and intravenous pyelography showed both kidneys to be of normal size and shape with normal excretion of contrast medium. Cystoscopy revealed no abnormality of the bladder or urethra. Urinalysis showed no proteinuria and bacteriological culture of the urine was sterile. Microscopy revealed 20 R.B.C.'s/cu.mm. The 24 hr. urinary protein excretion was measured as less than 10mg. and the urinary albumin/creatinine ratio ranged between 0.046 and 0.064. Eight months after his discharge from hospital the child was fit and well and normotensive. There had been no further haematuria.

Patient No. 4 (.T.F.)

A seven-year-old boy with a three year history of painless haematuria occurring for two to three days every three to four weeks. There was no significant family history.

On examination there were no physical abnormalities. The blood pressure was $^{90}/_{50}$mm. of mercury. Investigations showed a normal blood count, a plasma urea concentration of 26mg/100ml. and a creatinine clearance of 164ml/min/1.73m^3. Urinalysis revealed slight proteinuria (up to 100mg/100ml) and microscopy showed up to 350 R.B.C.'s/cu.mm. The urinary $^{UA}/_{UC}$ ratio was 0.50. Radiological examination of the chest showed no abnormality and an intravenous pyelogram revealed both kidneys to be of normal size and to excrete contrast medium normally. Cystoscopy showed no abnormalities of the bladder and urethra. A percutaneous renal biopsy was performed, and microscopy showed mild diffuse proliferative changes in the glomeruli. The tubules and blood vessels were normal.

Patient No. 5 (S.H.)

A four-year-old girl who six months previously had had an episode of frank haematuria following an attack of tonsillitis. No family history of renal disease was elicited. On examination there were no physical abnormalities; the blood pressure was recorded as $^{110}/_{70}$mm. of mercury. Urine examination on a number of occasions revealed a trace of proteinuria and microscopic haematuria. The child was seen repeatedly at intervals over the next fifteen months. She remained physically well and normotensive, but urinalysis revealed persistent microscopic haematuria, and on occasions slight proteinuria (ranging up to 225mg/100ml). Blood urea estimations all revealed concentrations of 30mg/100ml. or less and an intravenous pyelogram showed both kidneys to be of normal

size and to excrete contrast medium normally.

Eighteen months after her initial illness she was admitted to hospital for investigation. No physical abnormalities were found and she was normotensive. Bacteriological culture of a throat swab isolated commensal organisms only. The serum A.S.O. titre was less than 200 units/ml. The serum β_{1C} globulin level was normal. The blood count, and bleeding, clotting, prothrombin and partial thromboplastin times were all normal. The creatinine clearance was 83ml/min/1.73m^2. Radiological examination of the chest was normal and a tuberculin skin test was negative. Urine microscopy revealed more than 1,000 red blood cells per cu.mm. The 24 hr. urinary protein excretion was 0.66g and the $^{UA}/_{UC}$ ratio ranged between 0.07 and 0.08. A renal biopsy was performed and this showed a mild proliferative glomerulonephritis.

Patient No. 6 (G.L.)

A nine-year-old boy with a four month history of continuous frank haematuria. He had no other symptoms and in other respects was well. There was no family history of renal disease. On examination no physical abnormalities were found. His blood pressure was $^{90}/_{50}$mm. of mercury. Investigations showed a normal blood count and normal bleeding, clotting, prothrombin and partial thromboplastin times. The plasma urea concentration was 33mg/100ml. and the endogenous creatinine clearance was 151ml/min/1.73m^3. A throat swab

culture grew commensal organisms only. A tuberculin skin test was negative. Urinalysis showed no proteinuria, but microscopy revealed large numbers of R.B.C.'s. An intravenous pyelogram showed no radiological abnormalities. A percutaneous renal biopsy was performed, and microscopy revealed no histological abnormalities. The child was seen again six months after discharge from hospital. He was quite well, and no haematuria had been noticed for two months.

Patient No. 7 (A.M.)

A child who first presented with haematuria and an upper respiratory tract infection at the age of 13 months. A throat swab grew commensal organisms only and urinalysis revealed a trace of proteinuria and numerous red blood cells. Haematuria recurred at the age of six years. At this time he was normotensive with a blood pressure of $^{110}/_{65}$mm. of mercury and investigation revealed a normal blood count and a plasma urea of 23mg/100ml. Culture of a throat swab grew no pathogenic organisms. A serum A.S.O. titre was less than 200 units/ml. Urinalysis showed slight proteinuria (40mg/100ml) and microscopy revealed numerous R.B.C.'s. A percutaneous renal biopsy was performed and this showed mild proliferative changes in the glomeruli. Subsequently it was found that a female sibling was found to be deaf and to have microscopic haematuria.

At the age of 12 years, when haematuria had been persistently present for nearly seven years, he was investigated again. He was normotensive, and investigation

revealed a normal blood count, a plasma urea of 29mg/100ml. and a creatinine clearance of 109ml/min/1.73m^3. Audiograms were performed in view of the family history but these were normal. An intravenous pyelogram showed no abnormalities. Urinalysis revealed proteinuria and the urinary $^{UA}/_{UC}$ ratio was 1.8. A second percutaneous renal biopsy again showed proliferative changes in the glomeruli with some glomerular sclerosis and focal tubular atrophy. It was decided to treat the child with cyclophosphamide for one year. At the end of this period microscopic haematuria (335 R.B.C.'s/cu.mm.) and slight proteinuria were still present; the urinary $^{UA}/_{UC}$ ratio was 2.0. The child was normotensive with a blood pressure of $^{120}/_{80}$mm.of mercury. A third percutaneous renal biopsy was performed and this again revealed proliferative glomerular changes with glomerulosclerosis and focal tubular atrophy. At follow up some six months later, proteinuria ($^{UA}/_{UC}$ ratio 1.8) was still present.

Patient No. 8 (C.P.)

A seven-year-old girl who presented with a three week history of frank haematuria. It was noticed that the first specimen of urine she passed on rising in the morning was normal in colour, but all subsequent specimens contained blood. On examination she was well and no physical abnormalities were found. The blood pressure was $^{110}/_{70}$mm. of mercury. Her blood count was normal and her bleeding, clotting, prothrombin and kaolin-cephalin times were normal. The plasma urea concentration was 34mg/100ml. and the endogenous creatinine

clearance was 86ml/min/1.73m^3. Bacteriological culture of a throat swab failed to grow any pathogenic organisms. Urinalysis showed no proteinuria; the urinary $^{UA}/_{UC}$ ratio was 0.06. Numerous red blood cells were present on microscopy of the urine. Radiological examination of the chest was normal and an intravenous pyelogram showed no abnormalities. On cystoscopy the bladder and urethra appeared normal. A percutaneous renal biopsy was performed and microscopy of the biopsy specimen showed no histological abnormality. She was discharged and seen again one year later. In the interval one episode of haematuria had occurred.

Patient No. 9 (J.S.)

An eight-year-old boy who presented with a two year history of painless haematuria. For the previous nine months, haematuria had been persistent. On examination no physical abnormalities were found. His blood pressure was $^{100}/_{70}$mm. of mercury. Investigations showed a normal blood count and normal bleeding, clotting, prothrombin and kaolin-cephalin times. His plasma urea concentration was 22mg/100ml. and his endogenous creatinine clearance was 112ml/min/1.73m^3. Radiological examination of the chest was normal and an intravenous pyelogram showed no abnormalities. On cystoscopy no abnormality of the bladder or urethra was demonstrated. Urinalysis showed slight proteinuria and on microscopy more than 200 R.B.C.'s/cu.mm. were seen. The urinary $^{UA}/_{UC}$ ratio was 0.16. A percutaneous renal biopsy was performed and microscopy showed no histological abnormality. The child was discharged and although haematuria occurred intermittently

during the next six months, in the subsequent six months following this period there was no further haematuria.

Patient No. 10 (E.W.)

A six-year-old boy with a three-year history of recurrent haematuria. There were no other symptoms. On examination no physical abnormalities were found. His blood pressure was $^{105}/_{70}$mm. of mercury. Investigation showed a normal blood count and normal bleeding, clotting, prothrombin and kaolin-cephalin times. Bacteriological culture of a throat swab grew commensal organisms only. The plasma urea concentration was 28mg/100ml. and the endogenous creatinine clearance was 116ml/min/1.73m^3. Radiological examination of the chest and an intravenous pyelogram were both normal. Urinalysis showed no proteinuria and microscopy revealed numerous R.B.C.'s present. The urinary $^{UA}/_{UC}$ ratio was less than 0.08. A percutaneous renal biopsy was performed and this showed no histological abnormalities. He was discharged from hospital. One year later he was well and normotensive. Occasional episodes of haematuria had occurred, apparently after colds. These attacks appeared, however, to be diminishing in frequency.

Patient No. 11 (T.G.)

A four-year-old boy who presented with a history of four episodes of frank haematuria occurring in the previous

six months. There were no other symptoms and no history of renal disease. On examination there were no physical abnormalities, and his blood pressure was $^{130}/_{75}$mm. of mercury. Investigations revealed a normal blood count, and normal bleeding, clotting, prothrombin and kaolin-cephalin times. Bacteriological culture of a throat swab grew no pathogenic organisms. Radiological examination of the chest and an intravenous pyelogram were both normal. The endogenous creatinine clearance was 165ml/min/1.73m^3. Urinalysis showed a trace of protein (25mg/100ml.) and more than 200 R.B.C.'s/cu.mm. present. The $^{UA}/_{UC}$ ratio was less than 0.06. A percutaneous renal biopsy was performed and this showed no histological abnormalities. The child was discharged from hospital, and when he was seen again six months later he was completely well and had had no haematuria since his discharge.

Patient No. 12 (A.D.)

A ten-year-old boy who presented with a five month history of intermittent painless haematuria. It was noticed that these episodes appeared to follow exercise. There were no other symptoms and no family history of renal disease. On examination there were no physical abnormalities; the blood pressure was $^{130}/_{80}$mm. of mercury. Investigation revealed a normal blood count and normal bleeding, clotting, prothrombin and kaolin-cephalin times. The endogenous creatinine clearance was 120ml/min/1.73m^3. Radiological examination of the chest and an intravenous pyelogram were both normal. Urinalysis revealed more than 100 R.B.C.'s/cu.mm. but no

proteinuria. The urinary $^{UA}/_{UC}$ ratio was less than 0.01. A percutaneous renal biopsy was performed and this showed no histological abnormalities. The child was discharged from hospital. He was seen again six months later, when he was completely well. Only one episode of haematuria had occurred in the intervening period.

Patient No. 13 (P.G.)

A twelve-year-old girl who presented with an eleven month history of persistent haematuria. There was no family history of renal disease. On examination there were no physical abnormalities. Her blood pressure was $^{100}/_{60}$mm. of mercury. Investigation showed a normal blood count and normal bleeding, clotting, prothrombin and kaolin-cephalin times. The plasma urea concentration was 22mg/100ml. and the endogenous creatinine clearancewwas 140ml/min/1.73m^3. Radiological examination of the chest was normal and an intravenous pyelogram showed no abnormalities. On cystoscopy, no abnormalities of the bladder or urethra were detected. Urinalysis showed numerous red blood cells present and a "trace" of proteinuria. The urinary $^{UA}/_{UC}$ ratio was 0.17. A percutaneous renal biopsy was performed and microscopy showed slight diffuse proliferative changes in the glomeruli. The tubules and blood vessels were normal. She was discharged from hospital, and remained well apart from further episodes of painless haematuria. Ten months later she was again admitted to hospital following a fainting attack in the street. Urinalysis showed moderate proteinuria (the $^{UA}/_{UC}$ ratio was 2.1) and numerous red blood

cells were seen on microscopy. Estimation of the serum β_{1C} globulin level showed a low value (34% of an M.R.C. standard serum) and this low level was confirmed on a number of occasions. She was again followed as an out-patient. She remained physically well, although she complained of occasional headaches. She was normotensive (her blood pressure ranged between $^{90}/_{50}$ and $^{110}/_{60}$mm. of mercury). Investigations showed a normal glomerular filtration rate (measured as 146ml/min/1.73m^2 using a 51 Cr. E.D.T.A. method) but she continued to have significant proteinuria and her serum β_{1C} globulin level remained low. Examination of her peripheral blood for L.E. cells was negative on a number of occasions.

Fifteen months after her original admission to hospital she was again admitted for a repeat renal biopsy. Bacteriological culture of a throat swab at this time isolated β haemolytic streptococci of Lancefield group A. Microscopy of the renal biopsy showed early changes of membranoproliferative glomerulonephritis. The proliferative changes present were significantly more advanced than those present in the previous biopsy and in some glomeruli they were associated with thickening of the walls of capillary loops.

Patient No. 14 (A.B.)

A nine-year-old boy who had had two episodes of painless haematuria, one three years previously and one three months previously. There were no other symptoms. On examination there were no physical abnormalities. His blood

pressure was $^{110}/_{70}$mm. of mercury. Investigations showed a normal blood count and normal bleeding, clotting, prothrombin and kaolin-cephalin times. The blood urea was 37mg/100ml. Urinalysis on several occasions showed no proteinuria and microscopy showed no R.B.C.'s or W.B.C.'s present. The urinary $^{UA}/_{UC}$ ratio was less than 0.03. Radiological examination of the chest was normal, but an intravenous pyelogram showed an abnormally-shaped calyx in the right kidney. A retrograde pyelogram confirmed this appearance, but there were no other abnormalities, and it was in any case considered to be of doubtful significance. A percutaneous renal biopsy was performed and this showed no histological abnormalities. The child was discharged from hospital. Six months later he was noted to be well, with a blood pressure of $^{110}/_{60}$mm. of mercury. No further episodes of haematuria had occurred.

Patient No. 15 (B.R.)

A six-year-old boy who had a five year history of recurrent haematuria. The first episode occurred at the age of 18 months and followed an upper respiratory tract infection. He was investigated at the age of 2 years at another hospital. An intravenous pyelogram was reported as showing no abnormalities, and the child was given a course of treatment with corticosteroids. Since that time he had had episodes of frank haematuria lasting about two to three days every three or four months. There were no other symptoms. No significant family history was obtained. On examination there were no physical abnormalities; the blood pressure was $^{80}/_{50}$mm.

of mercury. Investigations showed a normal blood count, and plasma urea concentration of 17mg/100ml. Normal bleeding, clotting, prothrombin and kaolin-cephalin times were found. The creatinine clearance was 75ml/min/1.73m^3. Urinalysis showed some proteinuria, and the urinary $^{UA}/_{UC}$ ratio was 2.2. Microscopy of the urine revealed 520 R.B.C.'s/cu.mm. An intravenous pyelogram and a cystogram were both normal. Cystoscopy revealed no abnormality of the bladder or urethra. A percutaneous renal biopsy was performed and this showed proliferative changes and focal tubular atrophy. No specific therapy was given at this time, but 15 months later the child was readmitted and treatment with cyclophosphamide was started. No effect on either the proteinuria or haematuria was noted, and this therapy was abandoned after four months because thrombocytopenia and neutropenia developed. Following curtailment of cyclophosphamide treatment the blood count quickly returned to normal. At follow-up four months later moderate proteinuria ($^{UA}/_{UC}$ ratio of 1.4) and haematuria were still present. The creatinine clearance ranged between 261 and 153ml/min/1.73m^3. The child remained physically well and his blood pressure was measured as $^{120}/_{75}$mm. of mercury.

Patient No. 16 (W.We)

A five-year-old girl who was admitted with persistent proteinuria and microscopic haematuria following an apparent acute nephritic episode six months previously. At this time she had developed facial oedema, haematuria and vomiting some ten days after a sore throat. She was admitted to hospital

where she was found to be hypertensive with a blood pressure of $^{170}/120$ and her blood urea was found to be 400mg/100ml. Her condition improved in hospital and her blood urea one month later had fallen to normal levels, but she was found to have microscopic haematuria and proteinuria, and was treated with prednisone. Steroid therapy was continued for two months, but was stopped because the child developed chicken-pox.

Proteinuria and haematuria persisted and she was admitted to hospital again for further assessment. On examination there were no physical abnormalities but her blood pressure was raised to $^{150}/105$mm. of mercury. A throat swab culture grew commensal organisms only. The plasma urea concentration was 27mg/100ml. and the creatinine clearance was 110ml/min/1.73m^3. 24 hr. urinary protein excretion was 1.24g. An Addis count of the cells present in the urine revealed 34×10^6 R.B.C.'s and 3×10^6 W.B.C.'s/24 hr. She was started on treatment with methyl dopa to control her hypertension. The drug made her sleepy, however, and her hypotensive therapy was changed to a combination of guanethidine and chlorthiazide. She was discharged from hospital but was again admitted for reassessment 15 months later. Her blood pressure was found to be normal ($^{110}/70$mm. of mercury) without therapy and accordingly no further hypotensive drugs were given. Investigations showed a normal blood count, a blood urea concentration of 54mg/100ml. and a creatinine clearance of 74ml/min/1.73m^3. 24 hr. urinary protein excretion ranged between 0.9 and 1.7g. The $^{UA}/UC$ ratio was 1.0. Microscopy of the urine revealed excess R.B.C.'s

and W.B.C.'s. It was decided to treat her with cyclophosphamide. A percutaneous renal biopsy was performed prior to therapy, and microscopy showed proliferative changes and a number of completely hyalinised glomeruli. Foci of tubular atrophy were also present. Six weeks after commencement of treatment with cyclophosphamide the urinary protein excretion had fallen to 0.1g/24 hr. Cyclophosphamide therapy was continued for 12 months following which a repeat percutaneous renal biopsy was performed. Mild proliferative changes were present and some glomeruli were sclerotic although their number was less in this biopsy specimen than in the previous one.

A year later, four years after her initial illness she was again admitted for assessment of her renal function. Her blood pressure was $^{110}/_{70}$mm. of mercury and there were no physical abnormalities present on examination. Her blood count was normal, the blood urea concentration was 38mg/100ml. and the creatinine clearance was 67ml/min/1.73m^3. There was no significant proteinuria (the urinary $^{UA}/_{UC}$ ratio was less than 0.04) and microscopy of the urine revealed no excess of red or white cells. She was followed up again as an out-patient one year later and remained in good health.

Patient No. 17 (K.D.)

A fourteen-year-old boy who presented with four month history of recurrent frank haematuria which was accompanied by abdominal pain. In infancy he had been diagnosed as suffering from adrenal insufficiency due to congenital adrenal hyperplasia, and ever since he had had

replacement therapy with cortisone. There was no family history of renal disease. On examination his blood pressure was $^{110}/_{70}$mm. of mercury. Investigation showed normal bleeding and clotting times, and a normal prothrombin and partial thromboplastin time. The blood count was normal, the plasma urea concentration was 26mg/100ml. and the creatinine clearance was 110ml/min/1.73^3. Examination of the urine showed numerous red blood cells. The urinary $^{UA}/_{UC}$ ratio was less than 0.04. A percutaneous renal biopsy was performed and this showed no definite histological abnormalities.

Patient No. 18 (R.E.)

An eight-year-old boy who presented with recurrent painless haematuria for four months. He had also suffered from sore throats for about a year. The first episode followed an influenza-like illness and subsequent attacks appeared to be related to exercise. On examination the child appeared well and there were no physical abnormalities. The blood pressure was $^{90}/_{60}$mm. of mercury. Investigations showed a normal blood count and a blood urea of 32mg/100ml. The 24 hr. creatinine clearance was 133ml/min/1.73m^3. Bleeding, clotting, prothrombin and kaolin-cephalin times were normal. A throat swab culture grew commensal organisms only. The serum A.S.O. titre was between 300 - 400 units/ml. A serum β_{1C} globulin level was reported as 116% of a normal control serum. Urinalysis showed no proteinuria and repeated urinary $^{UA}/_{UC}$ ratios ranged between 0.033 and 0.057. Microscopy of the urine showed more than 500 R.B.C.'s/cu.mm. A percutaneous renal biopsy was performed and microscopy on this revealed no histological abnormalities.

Patient No. 19 (W.Wa)

An eleven-year-old boy who presented after an episode of frank haematuria. There was no family history of renal disease. On examination there were no physical abnormalities. The blood pressure was $^{100}/_{80}$mm. of mercury. Investigations showed a normal blood count. Bacteriological culture of a throat swab failed to isolate any pathogenic organisms and a serum A.S.O. titre was less than 200 units/ml. Urinalysis revealed proteinuria and numerous red blood cells. He was treated with penicillin V. Two further episodes of haematuria and proteinuria occurred during the next year, the second followed an upper respiratory tract infection. He was admitted to hospital again. He was physically well, and his blood pressure was $^{110}/_{60}$mm. of mercury. The blood urea concentration was 32mg/100ml. and the creatinine clearance was 175ml/min/1.73m^2. The A.S.O. titre was 400 units/ml. Radiological examination of the chest was normal, and an intravenous urogram showed the kidneys to be of normal size and to excrete contrast medium normally. A blood count was normal, and bleeding, clotting, prothrombin and partial thromboplastin times were all normal. The serum β_{1C} globulin level was normal. Urinalysis revealed slight proteinuria and red blood cells present. The $^{UA}/_{UC}$ ratio was 0.24. A percutaneous renal biopsy was performed and microscopy revealed a mild proliferative glomerulonephritis. Occasional partially sclerosed glomeruli were seen. Treatment with cyclophosphamide (120mg/day) was commenced, but after two months therapy he developed a sterile cystitis, and immunosuppressive therapy was stopped.

Patient No. 20 (A.B.)

An eleven-year-old girl who presented with a two month history of intermittent frank haematuria. The attacks lasted for periods of two to ten days at a time, and haematuria was associated with the passage of blood-clots. There was mild abdominal pain during the attacks, but there were no other symptoms. No abnormalities were detected on physical examination and her blood pressure was $^{150}/_{50}$mm. of mercury. Investigations showed a normal blood count and normal bleeding, clotting, prothrombin and kaolin-cephalin times. The plasma urea concentration was 23mg/100ml. and the endogenous creatinine clearance was 131ml/min/1.73m^3. Bacteriological culture of a throat swab failed to grow any pathogenic organisms, and the serum A.S.O. titre was less than 200 units/ml. Urinalysis on several occasions showed no proteinuria and no cells were seen on microscopy. Radiological examination of the chest was normal, and no abnormalities were demonstrated in an intravenous pyelogram or in a cystourethragram. In view of the lack of positive findings nothing further was done. However, fifteen months later she was readmitted to hospital since three episodes of frank haematuria had occurred in the intervening period. On examination there were again no physical abnormalities, and her blood pressure was $^{110}/_{50}$mm. of mercury. Several urinalyses showed no proteinuria and the urinary $^{UA}/_{UC}$ ratio was 0.02. No cells were seen on microscopy of the urine. A tuberculin skin test was negative. During this admission a percutaneous renal biopsy was performed and this showed no histological abnormalities.

Patient No. 21 (K.McD)

A five-year-old boy with a seven month history of recurrent haematuria. No precipitating factors were evident, and the child was otherwise well. On examination there were no physical abnormalities. His blood pressure was $^{85}/_{55}$mm. of mercury. Investigations revealed a normal blood count and a normal bleeding, clotting, prothrombin and kaolin-cephalin times. Bacteriological culture of a throat swab grew commensal organisms only. The serum A.S.O. titre was less than 200 units/ml. The plasma urea concentration was 29mg/100ml. and the endogenous creatinine clearance was 265ml/min/1.73m^3. Radiological examination of the chest was normal and no abnormalities were demonstrated in an intravenous pyelogram. Cystoscopy revealed no abnormalities of the bladder or urethra. Routine urinalysis showed no proteinuria and 250 R.B.C.'s were seen on microscopy of the urine. The urinary $^{UA}/_{UC}$ ratio was 0.086. A percutaneous renal biopsy was performed and no histological abnormalities were seen on microscopic examination of the specimen. During a follow-up period of two years the child remained fit and well with no evidence of renal functional impairment, but he continued to have occasional episodes of painless haematuria.

Patient No. 22 (A.R.)

A six-year-old boy who presented with a two year history of repeated attacks of frank haematuria. At the age of two years he had developed a left-sided heiparesis; no cause for this could be found. The child had been previously

investigated for haematuria some eighteen months previously. At that time microscopy of the urine showed more than 1,000 R.B.C.'s per ml. and less than 1 W.B.C. per ml. A 24 hr. urinary protein excretion was 0.039. The plasma urea was 19mg/100ml. The blood count was normal, and bleeding, clotting, prothrombin and partial thromboplastin times were normal. Bacteriological culture of a throat swab grew commensal organisms only, and the serum A.S.O. titre was less than 50 units/ml. Radiological examination of the chest showed no evidence of pulmonary disease and an intravenous pyelogram was normal. Cystoscopy revealed no abnormalities of the bladder or urethra. Since that time he continued to have episodes of haematuria. This became particularly heavy and a few days before admission he developed colicky right-sided abdominal pain and renal tenderness. An intravenous pyelogram showed evidence of acute obstruction to the right kidney and a diagnosis of clot colic was made. The blood pressure was normal ($^{120}/_{70}$) and microscopy of the urine showed more than 1,000 R.B.C.'s/cu.mm. The urinary $^{UA}/_{UC}$ ratio was 0.17. A percutaneous renal biopsy was performed and this showed no definite histological abnormalities. The renal colic settled in hospital, and both repeat intravenous pyelogram and a right retrograde pyelogram were normal.

Patient No. 23 (A.B.)

A five-year-old boy who presented with a history of painless haematuria for two months. Haematuria was first noticed following an attack of tonsillitis and had been

intermittently present since; it appeared to be precipitated by exercise. There were no other symptoms and on examination the child appeared fit and well. His blood pressure was $^{110}/_{70}$mm. of mercury, and investigation revealed a normal blood count and a plasma urea of 27mg/100ml. Bleeding, clotting, prothrombin and kaolin-cephalin times were all normal. A serum A.S.O. titre was less than 200 units/ml. Urinalysis revealed no proteinuria on a number of occasions and microscopy showed numerous red blood cells to be present. An intravenous pyelogram was normal and cystoscopy showed no abnormalities of the bladder or urethra. A percutaneous renal biopsy revealed no histological abnormalities. He was discharged and followed in the out-patients department. One year later there had been no further episodes of haematuria.

Patient No. 24 (K.W.)

An eleven-year-old boy with a two-and-a-half year history of intermittent frank haematuria, occurring about every three weeks and lasting for one or two days. He was well in other respects and there did not appear to be any predisposing factors. There was family history of renal disease. On examination he appeared fit, and no physical abnormalities were found. His blood pressure was $^{120}/_{80}$mm. of mercury. Investigations showed a normal blood count and normal bleeding, clotting, prothrombin and kaolin-cephalin times. The plasma urea concentration was 27mg/100ml. A serum A.S.O. titre was less than 50 units/ml. Urinalysis showed no proteinuria but microscopy revealed 50 R.B.C.'s. Radiological examinatinn

showed no abnormalities in the chest. An intravenous pyelogram showed both kidneys to be of normal size and excretion of contrast medium was normal. Cystoscopy revealed no abnormalities of the bladder or urethra. A percutaneous renal biopsy was performed and no histological abnormalities were seen on microscopy.

Children with Nephritis following Anaphylactoid Purpura

Patient No. 25 (D.T.)

A six-year-old boy who presented with an attack of anaphylactoid purpura characterised by fever, pain and swelling of both hands, and a purpuric skin rash over the legs and buttocks. He was given a short course of treatment with prednisolone but on examination of his urine he was noticed to have proteinuria and microscopic haematuria. He was admitted to hospital three months after his initial illness. On examination his blood pressure was $^{145}/_{95}$mm. of mercury, but no other abnormal physical signs were noted. Investigation revealed a normal blood count and a creatinine clearance of 172ml/min/1.73m^3. Bacteriological culture of a throat swab grew no pathogenic organisms. The serum cholesterol concentration was 218mg/100ml. Examination of the urine showed microscopic haematuria (up to 300 red blood cells per 100ml) and proteinuria (150mg protein/100ml). The albumin clearance ranged between 0.1 and 0.04ml/min. A percutaneous renal biopsy was performed and microscopy of the specimen showed a diffuse proliferative glomerulonephritis. Treatment with cyclophosphamide was given for eighteen months. After about eight weeks treatment the albumin clearance had fallen to 0.004ml/min. Eighteen months later at the end of the course of therapy the child was again investigated. His blood pressure was $^{110}/_{70}$mm. of mercury, and the creatinine clearance was 114ml/min/1.73m^3. Routine urinalysis showed no protein and no cells present. The albumin clearance was measured as less than 0.0002ml/min. A second percutaneous renal biopsy was performed. Despite the absence of urinary abnormalities microscopy revealed slight diffuse proliferative changes in the glomeruli.

Patient No. 26 (K.D.)

A fourteen-year-old boy who presented with colicky abdominal pain, haematuria and a purpuric skin rash following a sore throat. The haematuria lasted for five days and then ceased spontaneously. Subsequently his urine was examined and found to contain protein but no cells. A serum A.S.O. titre was found to be 800 units/ml. Further urine testing showed the proteinuria to be persisting and he was admitted to hospital three months after the onset of his illness. On examination his blood pressure was $^{130}/_{60}$mm. of mercury. Bacteriological culture of a throat swab isolated β haemolytic streptococci of Lancefield group A. Examination of urine revealed proteinuria (250mg protein/100ml) and microscopy showed 125 red blood cells per cu.mm. A 24 hr. urinary albumin excretion was 610mg. Differential urinary protein clearances revealed an unselective pattern. Endogeneous creatinine clearances ranged between 78ml. and 89ml/min/1.73m^3. He was treated with penicillin. A percutaneous renal biopsy was performed, and microscopy of the specimen showed a diffuse proliferative glomerulonephritis. Some glomerular tufts showed capsular adhesions, and others exhibited "fibrinoid" necrosis. There were scattered foci of interstitial chronic inflammation and tubular atrophy.

Over the next fifteen months he remained normotensive with only a trace of proteinuria (the $^{UA}/_{UC}$ ratio ranged between 0.26 and 0.096). However, estimations of his glomerular filtration rate using 51 Cr. E.D.T.A. showed a progressive fall. Values taken at intervals were 119, falling

to 90, then 82 and finally 66ml/min/1.73m^2. Because of the deterioration in renal function he was admitted to hospital again for a repeat renal biopsy. The histological changes in this second specimen showed considerably greater damage in the glomeruli, the majority of which were partially or completely hyalinised. Tubular atrophy and interstitial fibrosis were extensive and many blood vessels showed medial thickening.

Patient No. 27 (M.H.)

A nine-year-old girl who presented with an attack of anaphylactoid purpura characterised by a purpuric rash on her legs and buttocks, swelling of her ankles and Hands, and joint pains. Over the next seven months frank haematuria occurred on three occasions and she was therefore admitted to hospital for investigation. No abnormalities were detected on physical examination. The blood urea was 28mg/100ml. and the creatinine clearance was 130ml/min/1.73m^3. Microscopic examination of the urine showed no cells present. A 24 hr. urinary albumin excretion was 0.44g.

A percutaneous renal biopsy was performed, and histological examination of the biopsy specimen showed very mild proliferative changes in some glomerular tufts. In one glomerulus there was a capsular adhesion with some proliferation of the adjacent capsular epithelial cells. She was treated with cyclophosphamide and penicillin. After one years therapy she was physically well and normotensive (her blood pressure was $^{100}/_{70}$mm. of mercury). The creatinine clearance was

144ml/min/1.73m^2. The urinary albumin excretion was 0.17g/24 hr. A second renal biopsy was performed and this showed essentially similar changes to those previously seen in the first renal biopsy. When last seen six months later she remained well and was normotensive. Examination of the urine revealed no proteinuria or haematuria.

Patient No. 28 (P.B.)

A boy who first presented with an attack of anaphylactoid purpura characterised by colicky abdominal pain, meleana, haematuria and a purpuric skin rash, at the age of five years. He was treated with prednisolone. One year later he was admitted to hospital with a second similar attack characterised by fever, abdominal pain, haematuria and a skin rash. Urinalysis revealed numerous red blood cells and some proteinuria. There was transient azotaemia, the blood urea rising from 39mg/100ml. to 104mg/100ml. before falling again to 30mg/100ml. A percutaneous renal biopsy was performed and microscopic examination of the biopsy specimen revealed slight proliferative changes in the glomeruli. No treatment apart from penicillin was given.

Eight months later the child again suffered an attack of abdominal pain and haematuria, unassociated on this occasion with a skin rash. He was admitted to hospital following the attack. On admission his blood pressure was $^{80}/_{50}$mm. of mercury. Examination of the urine revealed slight proteinuria (40mg/100ml) but microscopy failed to demonstrate any red blood cells. No treatment was given. When he was seen

again six months later he was physically well and normotensive. Urinalysis revealed no proteinuria or haematuria.

Patient No. 29 (S.Mo.)

A six-year-old girl who presented with a sore throat, fever and muscular weakness of her left leg. One week later she again became pyrexial; she developed abdominal pain and vomiting associated with a generalised purpuric rash over her legs, trunk and arms. A diagnosis of anaphylactoid purpura was made. Over the next few days her left elbow became swollen and painful. She vomited blood at times and was noted to have oedema of the ankles and vulva. She eventually became oliguric. Examination of urine revealed heavy proteinuria (71000mg/100ml) but no cells were seen on microscopy. The total serum protein concentration was 3.95g/100ml. with a serum albumin concentration of 1.8g/100ml. The blood urea concentration was 88mg/100ml. Examination of the blood for L.E. cells was negative. Treatment with prednisolone (10mg/day) was started and her symptoms gradually disappeared, so that it was decided to stop the steroid therapy. However, a fresh crop of purpura developed and steroids were recommenced. At this stage haematuria developed. She was admitted again to hospital five months after the onset of her illness. On examination she was noted to have "mooning" of the face as a result of steroid therapy and a macular rash was present on her legs. Her blood pressure was $^{130}/_{90}$mm. of mercury. The blood urea was 22mg/100ml. and examination of the urine showed heavy proteinuria with numerous red blood cells and occasional white blood cells present. An intravenous

pyelogram showed both kidneys to be enlarged; excretion of contrast medium was normal. The prednisolone therapy was stopped but following this the child became febrile and began to vomit. She became dehydrated and required treatment with intravenous fluids. Oliguria again developed and she became drowsy. The blood urea concentration rose to 141mg/100ml. Her condition subsequently improved and two weeks later her blood urea was normal. At this time, six months after the initial onset of her symptoms, a percutaneous renal biopsy was performed. Histological examination revealed severe changes. There was a diffuse proliferative glomerulonephritis. Many glomeruli were partially or wholly sclerotic and in others there were capsular adhesions, often with proliferation of the adjacent epithelial cells lining Bowman's capsule. There were areas of tubular atrophy and foci of interstitial chronic inflammation.

The child's condition continued to improve and she was discharged. One month later her blood urea was noted to be 30mg/100ml. and the creatinine clearance was 89ml/min/1.73m^3. Subsequently her urine was noted to be free from proteinuria and no cells were seen on microscopy. She has had regular urinalyses all of which were clear. When last seen at the age of sixteen years, ten years after her initial illness she was symptom free and her blood pressure was normal.

Patient No. 30 (T.T.)

A seven-year-old girl who presented with painful swelling of both ankles and knees two days after the onset

of a sore throat. Four days later she developed a purpuric rash which first appeared on her buttocks and then spread to her legs and hands. She then complained of abdominal pain and was noted to pass blood per rectum. About a week later she developed frank haematuria which persisted for three weeks. Treatment with prednisolone was started and she was discharged from hospital. However, she gradually developed facial oedema, and two months later she was again admitted to hospital. Investigations revealed heavy proteinuria (4g. protein excreted in 24 hr.) and microscopic haematuria was found on microscopy. The differential protein clearances showed a non-selective pattern. The blood urea was 58mg/100ml. and the creatinine clearance was 62ml/min/1.73m^3. The total serum protein concentration was 5.2g/100ml. and the serum cholesterol concentration was 400g/100ml. A serum A.S.O. titre was 400 units/ml. Her dose of prednisolone was increased to 60mg/day, but her proteinuria and microscopic haematuria persisted. She was also noted to be hypertensive, the blood pressure had been measured as ^{150}/110mm. of mercury. At this stage a renal biopsy was performed. Microscopy showed a diffuse proliferative glomerulonephritis. The majority of glomeruli were partially or wholly sclerotic, many showing capsular adhesions with proliferation of the adjacent capsular epithelial cells. Areas of tubular atrophy and interstitial fibrosis were also present. Her treatment was changed to a combination of azathioprine and a small dose of steroids, and this regime was continued for a total of fifteen months. After twelve months therapy blood pressure was ^{130}/80mm. of mercury. The blood urea concentration was 28mg/100ml. Proteinuria was still present, the 24 hr. protein excretion being measured as

0.57g. A second renal biopsy was performed and the changes present on microscopy were essentially similar to those in the previous specimen.

Patient No. 31 (S.G.)

A six-year-old boy who presented with an attack of anaphylactoid purpura characterised by pains in the back and abdomen, a generalised petechial rash and swelling of the right ankle. Two weeks previously the child had had a sore throat. On examination he was normotensive and there were no abnormal physical signs. Examination of the urine revealed no abnormality. He was treated with penicillin and discharged from hospital. Five weeks later he developed oedema of the hands, face and scrotum and was found to have ascites. The blood pressure was $^{130}/_{70}$mm. of mercury, and urinalysis revealed gross proteinuria and microscopic haematuria. He was again admitted to hospital. Investigations showed a blood urea concentration of 14mg/100ml. and a creatinine clearance of 154ml/min/1.73m^3. The serum cholesterol concentration was 250mg/100ml., and the total serum protein cnncentration was 5.5g/100ml. with a serum albumin concentration of 2.2g/100ml. An Addis count showed 69 x 10^6 red blood cells and 9 x 10^6 white blood cells excreted in the urine in 24 hr. There was heavy proteinuria with 24 hr. protein excretion of 5.9g. Differential urinary protein clearances revealed only a moderately selective pattern. A percutaneous renal was performed and histological examination of specimen obtained showed a diffuse proliferative glomerulonephritis. He was

treated with cyclophosphamide and small doses of steroids. This therapy was continued for a total of fifteen months although the treatment was temporarily discontinued at one point when the child developed measles. After twelve months treatment he was again admitted to hospital for assessment. His blood pressure was $^{110}/_{70}$mm. of mercury. Examination of the urine showed no significant proteinuria although microscopy revealed 240 red blood cells per cu.mm. A second renal biopsy was performed. Histological examination showed that there were scattered foci of tubular atrophy and a number of completely sclerosed glomeruli. Other glomeruli however were normal and proliferative changes were lacking.

He was seen again two years later, three years after his first illness. He was well and his urine was free of protein and cells. He was normotensive and again he showed no abnormality on urinalysis.

Patient No. 32 (S.Ma.)

A seven-year-old girl who presented with a purpuric skin rash, colicky abdominal pain and swelling of her left ankle. On examination her blood pressure was $^{120}/_{55}$mm. of mercury. Examination of the urine showed moderate proteinuria (400mg/100ml) and microscopic haematuria. Bacteriological culture of a throat swab isolated commensal organisms only and the serum A.S.O. titre was 50 units/ml. The blood urea concentration was 37mg/100ml. A percutaneous renal biopsy was performed and histological examination of the biopsy specimen revealed a diffuse glomerulonephritis. She was discharged

from hospital and was seen again three months later. She was physically well and normotensive. Examination of the urine revealed only a trace of proteinuria and on microscopy scanty red blood cells were seen.

Patient No. 33 (M.W.)

A five-year-old boy who presented with anaphylactoid purpura characterised by colicky abdominal pain, a purpuric skin rash and swelling of his right wrist and ankle.

On examination he looked ill and his face was puffy. The blood pressure was $^{120}/_{80}$mm. of mercury. Examination of the urine revealed heavy proteinuria and microscopic haematuria. A serum A.S.O. titre was 600 units/ml.

His blood urea concentration which was 55mg/100ml. on admission rose to 90mg/100ml. He was treated with azathioprine and then with cyclophosphamide. His condition improved slightly at first but then rapidly deteriorated. His heavy proteinuria continued, he became oedematous and his serum albumin concentration fell to 1.9g/100ml. Recrudescence of the purpuric skin rash and anthritis occurred and his blood urea concentration rose steadily to 180mg/100ml. The creatinine clearance fell from 78ml/min/1.73m^3 to 4ml/min/1.73m^3. A renal biopsy was performed and microscopy showed a severe proliferative glomerulonephritis. Many glomeruli were hyalinised and many others showed extensive proliferation of capsular epithelial cells forming 'capsular crescents'. Tubular atrophy and interstitial oedema were widespread. The child's condition continued to deteriorate and became hypertensive. He died six months after the onset of his illness. No necropsy was performed.

Patient No. 34 (M.C.)

A five-year-old boy who developed anaphylactoid purpura, characterised by colicky abdominal pain, a purpuric skin rash and frank haematuria following an upper respiratory tract infection. Subsequent urinalyses revealed persistent proteinuria and microscopic haematuria. Nine months after his original illness he was admitted to hospital for investigation. No abnormalities were detected on physical examination, and his blood pressure was measured as $^{100}/_{70}$mm. of mercury. The blood urea was 25mg/100ml. and the creatinine clearance 114ml/min/1.73m^3. A blood count was normal. Bacteriological culture of a throat swab isolated commensal organisms only. The serum A.S.O. titre was 260 units/ml. Examination of the urine showed microscopic haematuria (50 red blood cells/cu.mm.) and proteinuria. The urinary albumin excretion was 1.2g/24 hr. and $^{UA}/_{UC}$ ratio ranged between 1.8 and 2.5. A renal biopsy was performed and histological examination of biopsy specimen revealed a proliferative glomerulonephritis. Many of the glomeruli showed partial hyalinisation of the glomerular tufts, usually with capsular adhesions. In many instances there was some focal proliferation of the capsular epithelial cells adjacent to the adhesions. Foci of tubular atrophy and interstitial fibrosis and chronic inflammation were seen.

Ten months later the child was again admitted for reassessment and the findings were very similar. The blood urea concentration was 30mg/100ml. and the creatinine clearance was 174ml/min/1.73m^3. Estimation of the glomerular filtration

rate was made using radioactive chromium-labelled E.D.T.A. This method gave a value of 116ml/min/1.73m^3. Examination of the urine again revealed microscopic haematuria (more than 200 red blood cells/cu.mm. were seen on microscopy) and proteinuria (200mg protein/100ml).

Further investigations were made eight months later. His blood pressure at this time was $\frac{115}{60}$mm. of mercury. Proteinuria (100mg protein/100ml) and microscopic haematuria (more than 200 red blood cells/cu.mm.) were again noted. The blood urea concentration was 30mg/100ml. A repeat estimation of the glomerular filtration rate using 51 Cr. E.D.T.A. showed a significant drop from the former level of 116ml/min/1.73m^3 to 60ml/min/1.73m^3. A second percutaneous renal biopsy was performed. Microscopy revealed similar changes to those seen in the previous biopsy. The proportion of sclerotic glomeruli was increased in the second biopsy. In view of the severe changes in the renal biopsy and the deterioration in renal function it was decided to commence treatment with cyclophosphamide.

Patient No. 35 (S.H.)

A fourteen-year-old girl who suffered several attacks of anaphylactoid purpura over a two year period. Each attack was characterised by abdominal pain and the development of a purpuric rash, and was usually preceded by an upper respiratory tract infection. During one of these attacks she developed haematuria and was admitted to hospital. She was found to be normotensive, and investigation revealed a blood urea of

40mg/100ml. Examination of urine showed numerous red blood cells and proteinuria (850mg/100ml). A renal biopsy was attempted, but the specimen of kidney tissue obtained was inadequate for histological assessment. Two years after the initial attack she was admitted to hospital again. She was physically well and her blood pressure was $\frac{120}{80}$mm. of mercury. Her urine contained 300mg/100ml. of protein and numerous red blood cells were seen on microscopy. Her blood urea concentration was 26mg/100ml. A renal biopsy was performed and this showed proliferative glomerulonephritis with occasional scarred glomeruli and scattered foci of tubular atrophy. Following her discharge from hospital she was seen at three-monthly intervals over the next year. On the first three occasions only scanty red blood cells were present in the urine and proteinuria was recorded as either a "trace" or was absent. However, when last seen three years after the initial illness her urine contained numerous red blood cells and 300mg/100ml. of protein. She was physically well and her blood pressure was $\frac{110}{60}$mm. of mercury.

Patient No. 36 (S.W.)

A twelve-year-old girl who first presented with an attack of anaphylactoid purpura characterised by a purpuric rash over her legs, arms, shoulders and buttocks. She was admitted to hospital and on the day of her admission she was noted to have proteinuria. On examination her blood pressure was $\frac{120}{80}$mm. of mercury. The blood count was normal and the blood urea was 32mg/100ml. Bacteriological culture of a

throat swab isolated commensal organisms only. Examination of the urine showed moderate proteinuria (300mg protein/100ml), and on microscopy of the urine numerous red blood cells were seen. A percutaneous renal biopsy was performed. Histological examination revealed a mild diffuse glomerulonephritis. Occasional glomeruli showed partial hyalinisation of the glomerular tufts and in some of these capsular adhesions were present between the hyalinised areas and Bowman's capsule.

A short course of prednisolone was given. Five months later the child was well and normotensive. Urinalysis showed no proteinuria and no cells. She was seen again on a number of occasions over the next three years and on each occasion her urine showed no abnormality. On her last visit she was well, with a blood pressure of $^{100}/_{75}$mm. of mercury. Her urine was free from protein or cells.

Patient No. 37 (C.W.)

A five-and-a-half year old boy who presented with a history of persistent haematuria and proteinuria following an attack of anaphylactoid purpura nine months previously. This had been characterised by abdominal pain, a purpuric rash and frank haematuria. He was admitted to hospital for investigation of his persistent proteinuria and haematuria. On examination no physical abnormalities were found and he was normotensive, with a blood pressure of $^{110}/_{70}$mm. of mercury. The blood urea concentration was 20mg/100ml. An intravenous pyelogram showed no abnormality, but urinalysis showed numerous red blood cells and proteinuria (120mg/100ml) to be present. A percutaneous renal biopsy was performed and

histological examination showed a mild proliferative glomerulonephritis. The child was discharged from hospital but he continued to have episodes of frank haematuria at about three to four month intervals. He was admitted to hospital for reinvestigation five years later, six years after his original illness. On examination his blood pressure was normal ($^{115}/_{75}$mm. of mercury). Investigation showed a normal blood count, a blood urea concentration of 31mg/100ml. and an endogeneous creatinine clearance of 110ml/min/1.73m^3. Bacteriological culture of a throat swab failed to grow any pathogenic organisms, and a serum A.S.O. (antistreptolysin O) titre was less than 200 units/ml. The 24 hr. urinary protein excretion was 1.2g and an Addis count revealed 23 x 10^6 red blood cells and 0.7g x 10^6 white blood cells. He was again investigated in hospital five months later with essentially similar results. He was normotensive, his urine contained numerous red cells and a 24 hr. protein excretion was 2.1g. The creatinine clearance was 200ml/min/1.73m^3. On this occasion a second renal biopsy was performed. Histological examination again showed mild proliferative changes. In addition some glomeruli showed partial or complete hyalinisation of the glomerular tuft.

One year later, seven years after the onset of his illness, the child still had significant microscopic haematuria and proteinuria with a normal glomerular filtration rate and a normal blood pressure. It was decided at this stage to treat him with azathioprine. This therapy was continued for a fifteen month period. At the end of twelve

months treatment he was admitted to hospital for reinvestigation. His blood pressure was $^{110}/_{65}$mm. of mercury. The blood urea was 30mg/100ml. and the creatinine clearance 109ml/min/1.73m^3. Red blood cells were present in the urine. Radiological examination of the abdomen showed both kidneys to be normal in size and shape. Proteinuria persisted; the urinary albumin/creatinine ratio ($^{UA}/_{UC}$) ranged between 0.26 and 0.8.

A percutaneous renal biopsy performed at this time showed scattered areas of tubular atrophy and occasional partly or wholly sclerotic glomeruli. Other glomeruli appeared normal and proliferative changes were absent. Following therapy with azathioprine his clinical condition remained unchanged. When last seen, ten years after his initial illness, he was normotensive. He had continued to have occasional attacks of haematuria, and 24 hr. urinary albumin excretion was 0.68g.

Patient No. 38 (S.L.)

A child who first presented with an attack of anaphylactoid purpura at the age of eight years. This was characterised by a purpuric skin rash, abdominal pain and swelling of her joints. She was admitted to hospital. Her blood pressure was measured as $^{100}/_{70}$mm. of mercury. The blood urea was 30mg/100ml. and a serum A.S.O. titre was 684 units/ml. No abnormalities were detected in her urine which was examined on a number of occasions. She was discharged from hospital, but when her urine was again examined four months later she was noted to have proteinuria and microscopic haematuria. She

was admitted to hospital four months later, eight months after the original illness. On examination she was found to be hypertensive with a blood pressure of ^{190}/120mm. of mercury. The blood urea was 195mg/100ml. and she was anaemic with a haemoglobin of 7g/100ml. Examination of the urine showed proteinuria (100mg protein/100ml) and on microscopy more than 200 red blood cells were seen. The urinary ^{UA}/UC ratio was 2.7. The total serum protein concentration was 6.6g/100ml and the serum albumin was 3.4g/100ml. The serum cholesterol concentration was 240g/100ml. An intravenous pyelogram showed the kidneys to be of normal size and shape, but excretion of contrast medium was poor. Bacteriological culture of a throat swab isolated no pathogenic organisms. A serum A.S.O. titre was between 300 and 400 units/ml. Estimation of the glomerular filtration rate using radio-active chromium-labelled E.D.T.A. gave a value of 11.2ml/min/1.73m^2. A percutaneous renal biopsy was performed and microscopy revealed advanced chronic glomerulonephritis. All the glomeruli were wholly or partially sclerotic, some exhibiting capsular adhesions. Tubular atrophy and interstitial fibrosis were widespread. She was treated with a low protein diet, blood transfusion and cyclophosphamide (75mg/day) was commenced.

Patient No. 39 (J.C.)

A five-year-old girl who presented with a one year history of recurrent haematuria following an attack of anaphylactoid purpura. Her initial illness had been characterised by a purpuric rash most marked on her ankles and

legs which was associated with pain and swelling of both ankles. On examination there were no physical abnormalities. Her blood pressure was $^{110}/_{75}$mm. of mercury. Investigations revealed a normal blood count, and a blood urea concentration of 33mg/100ml. A serum A.S.O. titre was 60 units/ml. The urinary protein was 70mg/100ml., and microscopy of the urine revealed numerous red blood cells. A renal biopsy was performed, and histological examination showed a mild proliferative glomerulonephritis. One month later the child had an attack of tonsillitis. Bacteriological culture of a throat swab isolated β-haemolytic streptococci. Examination of urine revealed 160mg/100ml. of protein and microscopy showed numerous red blood cells. One month following the attack tonsillectomy was performed. A serum A.S.O. titre at that time was 300 units/ml. The child was seen again two years later, three years after her initial illness. She was normotensive with a blood pressure of $^{110}/_{68}$mm. of mercury. Examination of the urine showed 80mg/100ml. of protein and on microscopy a few red blood cells were seen. A skin sensitivity test to old tuberculin gave a negative result.

When the child was seen three years later, six years after the start of her illness both proteinuria and haematuria had disappeared. She was clinically well and normotensive. When last seen seven years after her first illness she had remained well. Her blood pressure was $^{120}/_{85}$mm. of mercury and the creatinine clearance was 241ml/min/1.73m^3, Her urine was free both of abnormal cells and of protein.

Patient No. 40 (A.S.)

A five-year-old boy who presented with severe abdominal pain. He was submitted to laparotomy and at operation the small intestine was found to be haemorrhagic and distended. Post-operatively he passed blood per rectum and developed a purpuric skin rash. His blood pressure was found to be ^{145}/100mm. of mercury. A diagnosis of anaphylactoid purpura was made. Over the next eleven months he had several more attacks of abdominal pain and on a number of occasions he had haematuria and blood-stained stools. He was treated with corticosteroids. His blood pressure remained elevated and on two occasions he became oedematous and developed convulsions. He was admitted to hospital where his blood pressure was recorded as ^{160}/105mm. of mercury. On investigation his blood count was normal and the blood urea concentration was 76mg/100ml. The total serum protein concentration was 5.7g/100ml. with a serum albumin of 3.6g 100ml. The serum cholesterol was 281mg/100ml. Examination of the urine revealed numerous red blood cells and 900mg/100ml. of protein. A percutaneous renal biopsy was performed, and this revealed a diffuse proliferative glomerulonephritis. Occasional capsular adhesions were present and some glomeruli were partly or wholly sclerotic. Areas of tubular atrophy and interstitial fibrosis were seen. Following this procedure treatment with corticosteroids, which had continued for three months, was stopped. Some seven months later the child died in renal failure. At autopsy there was evidence of pulmonary and cerebral oedema and concentric hypertrophy of the left ventricle. The left kidney weighed

64g. and the right kidney 67g. The renal cortices were narrowed and mottled. Histological examination of the kidneys showed a severe diffuse proliferative glomerulonephritis. Some glomerular tufts showed areas of fibrinoid necrosis (fig. 27), and in others there were capsular adhesions. Many glomeruli showed partial or complete hyalinisation and scattered areas of tubular atrophy and interstitial fibrosis, were present. Some interlobular arteries exhibited fibro-elastic intimal and medial hypertrophy and the wall of some arterioles were thickened.

Patient No. 41 (A.C.)

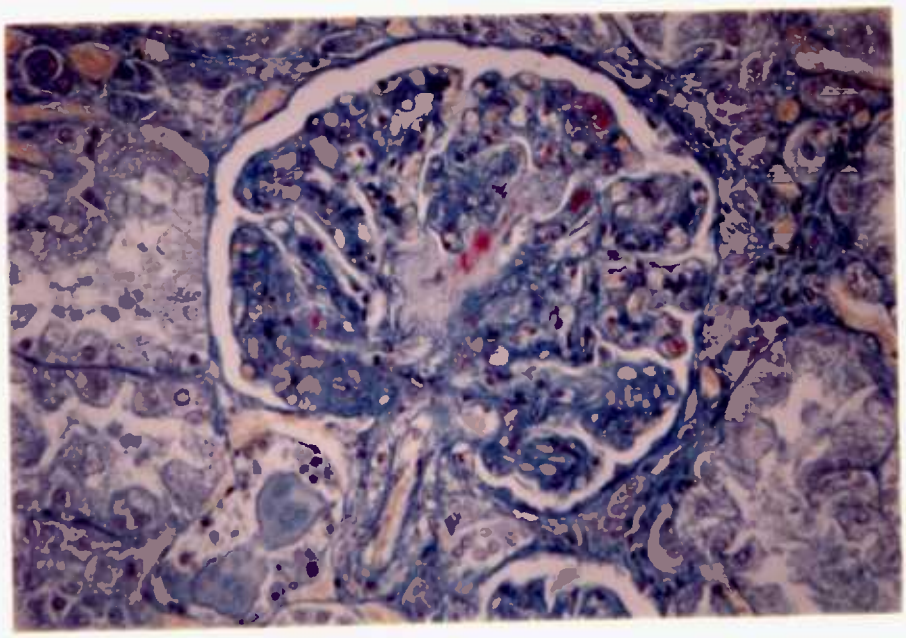
A boy who had an attack of anaphylactoid purpura at the age of nine years. This presented as severe colicky abdominal pain associated with vomiting and haematemesis. He also developed a purpuric rash on his legs. Over the next year frank haematuria occurred on three occasions each lasting for a few weeks. During the third episode he was admitted to hospital for investigation. On examination no physical abnormalities were found and his blood pressure was noted as $\frac{110}{60}$mm. of mercury. Investigations revealed a normal blood count and a blood urea concentration of 68mg/100ml. Examination of the urine showed 80mg/100ml. of protein and numerous red blood cells present. An intravenous pyelogram revealed both kidneys to be of normal size and shape and to excrete contrast medium normally.

Bacteriological culture of a throat swab failed to grow any pathogenic organisms and a serum A.S.O. titre was

Fig.27 Case No. 40 (anaphylactoid purpura)

A glomerulus in a section of the kidney taken at autopsy. There is a marked proliferative glomerulonephritis with an area of 'fibrinoid' necrosis. M.S.B. x 100.

Fig. 27



130 units/ml. A percutaneous renal biopsy was performed and histological examination of the biopsy specimen showed a diffuse proliferative glomerulonephritis with areas of chronic interstitial inflammation. Following his discharge from hospital he remained well although he continued to have attacks of haematuria lasting for a day or two every six months. He was admitted to hospital again some six years later, seven years after his initial attack, when he again presented with frank haematuria associated with a sore throat. On examination he was pyrexial, but there were no other abnormal physical signs. His blood pressure was $^{110}/_{60}$mm. of mercury.

The blood count was normal apart from an erythrocyte sedimentation rate (E.S.R.) of 33 min. in the first hour. The blood urea was 38mg/100ml., and the creatinine clearance 135ml/min/1.73m^3. Examination of the urine showed "+ + +" proteinuria, with numerous red blood cells and occasional white blood cells present. He was treated with penicillin and discharged. When last seen, eight years after his initial illness, his urine still showed microscopic haematuria and contained protein. He was physically well and normotensive.

Patient No 42 (R.le G.)

A seven-year-old boy who presented with colicky abdominal pain and a petechial rash most marked over the legs, ankles and buttocks. He was admitted to hospital where his colic continued intermittently, and on a number of occasions he passed blood per rectum. It was also noticed that both his

ankles became swollen. He was treated with penidllin. Examination of his urine revealed proteinuria (200mg/100ml) and a moderate number of red blood cells. Bacteriological culture of a throat swab grew no pathogenic organisms and his serum A.S.O. titre was 70 units/ml. The blood count was normal. Despite the fact that he still had proteinuria and microscopic haematuria, he was in other respects perfectly well and he was discharged from hospital after one week. Seven months later he was admitted to hospital again for assessment. On examination he was normotensive (his blood pressure was $^{100}/_{70}$mm. of mercury) and no abnormal physical signs were present. His blood urea concentration was 22mg/100ml. Examination of the urine showed slight proteinuria (40mg/100ml) and on microscopy occasional red blood cells were seen. A percutaneous renal biopsy was performed and histological examination showed a mild proliferative glomerulonephritis. It was decided to treat the child with prednisolone. He was seen again six months later. He was found to be free from proteinuria and no cells were seen on microscopy of the urine. Steroid therapy was gradually stopped. One year later he was physically well and normotensive. No abnormalities were found on examination of the urine. No further follow-up was made.

Patient No. 43 (P.A.)

A nine-year-old boy who first presented with haematuria and a purpuric skin rash which was associated with pain and swelling of both knees and ankles. A diagnosis of

anaphylactoid purpura was made. He remained well until twelve months later when he had a second attack of haematuria associated again with a purpuric skin rash. He was given a short course of prednisolone. A third episode of haematuria occurred eighteen months later, and one month after this he was admitted to hospital for investigation. On examination the blood pressure was $^{130}/_{90}$mm. of mercury. Investigation revealed a normal blood count, a blood urea of 27mg/100ml, and a creatinine clearance of 166ml/min/1.73m^3. Examination of the urine showed proteinuria (the 24 hr. albumin excretion ranging between 0.2 and 0.46g) and microscopy of the urine showed 790 red blood cells per cu.mm. The serum A.S.O. titre was 720 units/ml. A renal biopsy was performed and microscopic examination revealed a diffuse proliferative glomerulonephritis. Treatment with cyclophosphamide together with small doses of steroids was given and continued altogether for fifteen months. After twelve months of treatment he was admitted to hospital for reinvestigation. His blood pressure was $^{120}/_{70}$mm. of mercury. His blood urea concentration was 32mg/100ml. and the creatinine clearance was 185ml/min/1.73m^3. Urine examination showed some proteinuria (the albumin excretion was 0.6g/24 hr.) and microscopy revealed more than 1,000 red blood cells per cu.mm. A second renal biopsy was performed, and histological examination again showed a diffuse glomerulonephritis. When he was last seen one year later his clinical condition was unchanged, both microscopic haematuria and slight proteinuria persisting. He had also developed a transient rash which was thought to be a further episode of anaphylactoid purpura.

Patient No. 44 (D.C.)

An eight-year-old girl who presented with a purpuric rash on her legs and buttocks three days after an attack of acute tonsillitis. A week later she was admitted to hospital because her ankles had become swollen and painful. ,On examination her blood pressure was ^{100}/60mm. of mercury. Examination of her urine revealed microscopic haematuria and proteinuria; her blood urea concentration was 35mg/100ml. A diagnosis of anaphylactoid purpura was made. Two months later she developed ankle oedema and began to gain weight. On readmission to hospital she was found to have heavy proteinuria, with a urinary protein excretion of 6.8g/24 hr. The total serum protein concentration was 4.1g/100ml. with a serum albumin concentration of 1.9g/100ml. The serum cholesterol was 500mg/100ml. The blood urea was 28mg/100ml. and the creatinine clearance 65ml/min/1.73m^3. She was treated with prednisolone and penicillin but one month later her condition was unchanged. Her blood pressure was ^{110}/60mm. of mercury. The blood urea was 42mg/100ml. and the creatinine clearance 66ml/min/1.73m^3. Examination of the urine showed heavy proteinuria (the ^{UA}/UC ratio was 7.2) and microscopic haematuria. Bacteriological culture of a throat swab failed to grow pathogenic organisms, and a serum A.S.O. titre was less than 200 units/ml. Treatment with prednisolone was continued, but no diuresis occurred. One month later she was again admitted to hospital. Her blood pressure was ^{130}/90mm. of mercury. Investigations revealed a blood urea of 28mg/100ml. and a creatinine clearance of 57ml/min/1.73m^3. Heavy

proteinuria was still present, the $^{UA}/_{UC}$ ratio being measured as 8.1. A percutaneous renal biopsy was performed, and histological examination of biopsy specimen showed a diffuse proliferative glomerulonephritis. Treatment with cyclophosphamide and small doses of steroids was commenced. After three months treatment only a trace of proteinuria was present and no red blood cells were seen on microscopy of the urine. When the child was last seen after five months therapy with cyclophosphamide, again only slight proteinuria was found, the $^{UA}/_{UC}$ ratio being measured as 0.19. She was physically well and normotensive.

Patient No. 45 (C.S.)

An eight-year-old boy who presented with joint pains, swelling of the knees and a purpuric rash on his legs. Urinalysis revealed a trace of proteinuria and red blood cells. The rash faded, but two weeks later it recurred. Two months later he developed frank haematuria. He was admitted to hospital. Investigations revealed a blood urea concentration of 45mg/100ml. and a normal blood count with an E.S.R. of 42mm. in 1 hr. A serum A.S.O. titre was 250 units/ml. Examination of the urine again showed proteinuria and haematuria. One month later he had a further episode of abdominal pain and haematuria. On admission to hospital there were no abnormal physical signs and his blood pressure was $^{110}/_{70}$mm. of mercury. His blood count and bleeding, clotting, prothrombin and partial thromboplastin times were normal. Urinalysis revealed 50mg/100ml. of protein and 200 red blood cells/cu.mm. The

$^{UA}/_{UC}$ ratio was 0.96. The creatinine clearance was 126ml/min/1.73m^2. Bacteriological culture of a throat swab isolated commensal organisms only. An intravenous pyelogram showed both kidneys to be of normal size and to excrete contrast medium normally. Six months later a further episode of abdominal pain and haematuria occurred. He was normotensive, with a blood pressure of $^{110}/_{60}$mm. of mercury. Urinalysis again revealed traces of proteinuria and 20 red blood cells per cu.mm. A serum β_{1C} globulin level was normal. His blood urea was 21mg/100ml., and the creatinine clearance was 121ml/min/1.73m^2 and the 51 Cr. E.D.T.A. clearance was 184ml/min/1.73m^2. A renal biopsy was performed and this showed a mild proliferative glomerulonephritis.

Patient No. 46 (C.H.)

A six-year-old boy who presented with abdominal pain, a petechial skin rash, and swelling of the ankles, hands, knees and elbows. Examination of the urine showed no protein and microscopy of urine deposit showed no cells present. Bacteriological culture of a throat swab isolated commensal organisms only, and a serum A.S.O. titre was 50 units/ml. Two months later he developed a scrotal haemorrhage, the treatment of which required surgical intervention. Post-operatively he developed frank haematuria. He was normotensive with a blood pressure of $^{110}/_{70}$mm. of mercury. Examination of the urine at this time showed heavy proteinuria (100mg/protein/100ml) and on microscopy of the urine deposit showed 800 red blood cells/cu.mm. His blood urea concentration was

25mg/100ml. and the creatinine clearance was 84ml/min/1.73m^2. An intravenous pyelogram showed no abnormalities. A percutaneous renal biopsy was performed. Microscopical examination revealed a mild diffuse proliferative glomerulonephritis.

Patient No. 47 (K.P.)

A six-year-old boy who presented with abdominal pain, diarrhoea pain and swelling in the right ankle and a purpuric skin rash over the legs, arms and buttocks. Bacteriological culture of a throat swab isolated β haemolytic streptococci of Lancefield group A. He was treated with prednisone (30mg/day). One month later he developed oedema of the eyelids and frank haematuria. His blood pressure was $^{90}/_{50}$mm. of mercury. Radiological examination of the chest was normal. Urinalysis revealed proteinuria and on microscopy numerous red and white blood cells. The blood urea concentration was 20mg/100ml. and the creatinine clearance 47ml/min/1.73m^2. An intravenous pyelogram showed both kidneys to be normal in size and to excrete contrast medium normally. The serum A.S.O. titre was 200 units/ml. He was discharged from hospital but he continued to suffer attacks of abdominal pain associated with patches of petechial skin rash on his legs and arms. He was investigated again ten months after the onset of his illness. His blood pressure was $^{120}/_{70}$mm. of mercury. The total serum protein concentration was 4.7g/100ml. and the serum albumin concentration was 1.7g/100ml. The serum cholesterol concentration was 375mg/100ml. The creatinine clearance was 34ml/min/1.73m^2. Two months later a further episode of frank

haematuria occurred. On examination at this time the blood pressure was $^{100}/_{70}$mm. of mercury. The 24 hr. urinary protein excretion was 5.6g. Microscopy of the urine deposit revealed 100 white and more than 200 red blood cells/cu.mm. The haemoglobin was 10.1g/100ml. with a normal white count. Bleeding, clotting, prothrombin and partial thromboplastin times were normal. The blood urea concentration was 31mg/100ml. and the creatinine clearance 22ml/min/1.73m^2. A percutaneous renal biopsy was performed. Microscopy of the specimen showed chronic glomerulonephritis with extensive glomerular scarring affecting all the glomeruli present, tubular atrophy and interstitial fibrosis.

Children with a Steroid-sensitive Nephrotic Syndrome

Of the 59 renal biopsies studied from children presenting with a nephrotic syndrome, 42 were from children whose proteinuria disappeared within four weeks after commencement of treatment with prednisolone in a dosage of 2mg/kg. body weight/day (i.e. they were steroid-sensitive). These 42 children were not a random selection, since percutaneous renal biopsy was performed on steroid-sensitive nephrotic children only if relapse after steroid withdrawl had occurred on three or more occasions.

The clinical findings in these children were sufficiently uniform to make detailed consideration of their individual histories unnecessary. All presented with an insidious onset of oedema. 29 were boys and 13 were girls. Their ages at their last birthday ranged between 1 and 12 yr. (mode 2 yr.) at the onset of their symptoms. All were shown to have heavy proteinuria and hypoalbuminaemia. A few of these children were shown to have microscopic haematuria, but this was always transitory, and in none was macroscopic haematuria demonstrated. Many of these children were referred from other centres because of their frequent relapses after steroid withdrawl and were consequently in remission at the time of investigation prior to renal biopsy. For this reason, differential urinary protein clearances were performed in only 23 of the 42 patients in this group. Estimations were made in these 23 children of the ratios of the clearances of Ig G to albumin (C_{IgG}/C_A). Ratios of less than 10% were regarded as indicating highly selective proteinuria; values between 10 and 20% as indicating moderately selective proteinuria and values of more than 20% as indicating poorly selective proteinuria. In

the 23 patients investigated in this group, 12 showed highly selective proteinuria, 11 showed moderately selective proteinuria and none showed poorly selective proteinuria.

Children with a Steroid-resistant Nephrotic Syndrome

Patient No. 90 (A.A.)

A two-year-old girl who presented with generalised oedema and proteinuria following an upper respiratory tract infection. She had a sore throat for which antibiotics were given. Her proteinuria diminished in amount, but about three weeks later heavy proteinuria recurred. She was treated with auromycin and prednisolone but her proteinuria persisted and she became hypertensive. For this reason A.C.T.H. was substituted for oral steroids.

Three months later she was investigated again. On examination she was grossly oedematous, with ascites and bilateral pleural effusions. The urinary protein excretion was 2g/24 hr. and estimation of the serum albumin concentration gave a value of less than 1g/100ml. Differential protein clearance estimations showed a moderately selective pattern of urinary protein excretion. Microscopy of the urine revealed no cells present. The endogenous creatinine clearance was 81ml/min/1.73m^2 and the blood urea concentration was 60mg/100ml. The serum cholesterol concentration was 600mg/100ml. Bacteriological culture of a throat-swab isolated commensal organisms only. Treatment with prednisolone in a dosage of 40mg, later rising to 60mg/day, was given. However, the child's oedema increased and the blood urea rose to 90mg/100ml. The proteinuria continued unabated, and after four weeks therapy the steroid dosage was decreased, and treatment with cyclophosphamide (3mg/kg/day) was instituted. After three weeks on this regime, the proteinuria suddenly decreased and the child became much less oedematous. Treatment with cyclophosphamide was continued, but after three months it became necessary to stop the drug owing to the development of

neutropenia. The urinary albumin excretion fell from 3.0g/24 hr. to 0.7g/24 hr. during therapy. Since the amount of proteinuria did not increase when cyclophosphamide was stopped, no further therapy with this drug was given.

When seen again two years later, the child was well and had no proteinuria on routine testing. However, two months later, twenty-seven months after the onset of her first symptoms proteinuria recurred. She was admitted to hospital again one month later, by which time she was grossly oedematous. Investigation showed heavy proteinuria (1000mg/100ml) and a serum albumin concentration of 0.72g/100ml. The blood urea on admission was 143mg/100ml. Treatment with prednisolone (40mg/day) and cyclophosphamide (3mg/kg/day) was given. A diuresis occurred after 10 days therapy. A percutaneous renal biopsy was performed at this time. Microscopy revealed no definite histological abnormalities. After a diuresis had been established the dose of prednisolone was cut to 10mg/day. Treatment with cyclophosphamide was continued for eight weeks and then stopped. Subsequently the dose of prednisolone was further gradually reduced and finally stopped.

When seen again four months after her relapse, the child was physically well. Her blood pressure was $^{110}/_{70}$mm. of mercury, and there was no proteinuria.

Patient No. 91 (A.Be.)

A two-and-a-half year old girl who presented with oedema of her face and ankles following an upper respiratory tract infection. She was admitted to hospital where examination of her urine revealed heavy proteinuria. Estimation of her serum

protein concentration showed total serum proteins of 4.4g/100ml., and a serum albumin of 1.0g/100ml. Differential protein clearance estimations revealed a highly selective pattern of urinary protein excretion. Therapy with prednisone at a dosage of 40mg/day was given, but after one months treatment no diuresis had occurred. She was admitted to hospital again. Her blood pressure was measured as $^{100}/_{60}$mm. of mercury. Investigations showed a normal blood count and normal bleeding and clotting times. The total serum protein concentration was 6.1g/100ml., and serum electrophoresis showed diminution of serum albumin with an increase in the α_1 and α_2 globulin fractions. Examination of the urine showed proteinuria (more than 500mg/protein/100ml) and on microscopy five white blood cells/cu.mm. but no red blood cells were seen. The urinary $^{UA}/_{UC}$ ratio was noted to be 6.9. The blood urea concentration was 21mg/100ml and the serum cholesterol concentration 295mg/100ml.

A percutaneous renal biopsy was performed and histological examination of the specimen revealed a mild diffuse proliferative glomerulonephritis. Treatment with cyclophosphamide and small doses of steroids was given for eight weeks. There was an almost immediate diminution in proteinuria and a diuresis occurred. At the end of the eight week period of treatment the urine was free from protein. The child was physically well and normotensive.

Patient No. 92 (D.H.)

A three-and-a-half year old girl who first presented with facial oedema and oliguria. She was admitted to hospital,

where examination of her urine showed heavy proteinuria with an albumin excretion of 12.0g/24 hr. Estimation of her serum albumin concentration gave a value of 2.0g/100ml. The serum cholesterol concentration was 380mg/100ml. Treatment with prednisone was given and within three days diuresis occurred and the proteinuria ceased. One month later, however, whilst still being treated with steroids, her proteinuria recurred. On increasing the dose of prednisone the proteinuria again ceased. Two months later, again whilst still on the same dosage of prednisone a second recurrence of proteinuria occurred following a cold. The prednisone dosage was now increased to 60mg/day, and again the proteinuria disappeared. Subsequently the steroid dosage was gradually decreased until the child was receiving 15mg of prednisone on alternate days. A third relapse occurred, and the dose of prednisone was increased to 45mg/day. A fourth relapse then occurred and the child was readmitted to hospital ten months after the onset of her disease. On examination there were marked cushingoid features as a result of her treatment with steroids. Pretibial pitting oedema was present and her blood pressure was recorded as $^{120}/_{60}$mm. of mercury. Investigations showed a normal blood count and a blood urea concentration varying between 39 and 51mg/100ml. The serum cholesterol was 608mg/100ml. Radiological examination of the chest showed no abnormalities and an intravenous pyelogram showed both kidneys to be of normal size and to excrete contrast medium normally. Differential protein clearances showed a highly selective

pattern of urinary protein excretion. Microscopy of the urine showed no abnormal cells. A renal biopsy was performed and histological examination of the specimen obtained showed no morphological abnormalities. Treatment with cyclophosphamide and small doses of corticosteroids was given for eight weeks with dramatic improvement in her proteinuria. The $^{UA}/_{UC}$ ratio fell from 12.6 at the beginning of the treatment period to 0.04 after two weeks. Relapse again occurred four months later, with recurrence of proteinuria. The $^{UA}/_{UC}$ ratio was 1.5 and the creatinine clearance was 248ml/min/1.73m^3. Small doses of cyclophosphamide produced a diuresis within two days and three months later she remained physically well and normotensive and her urine was free from protein.

Patient No. 93 (A.M.)

A two-and-a-half year old girl who presented with periorbital oedema. One month later she was admitted to hospital where urine examination revealed heavy proteinuria. Differential protein clearances showed a highly selective pattern of urinary protein excretion. Treatment with prednisolone at a dose of 40mg/day was started. After seven days a diuresis occurred and the dosage of prednisolone was reduced to 10mg/day. A serum A.S.O. titre was 1,200 units/ml.

Over the next fifteen months proteinuria recurred on four occasions. On the first three occasions the proteinuria ceased on treatment with large doses of prednisolone. On the fourth occasion, however, heavy proteinuria persisted despite therapy for six weeks with prednisolone at a dosage of 40mg/day. She was admitted to hospital. On examination there

were marked cushingoid features due to her steroid therapy. There was facial "mooning" and a "buffalo hump" on her back. Dermal striae and telangectasia were present on her abdomen. There was marked dependant pitting oedema and ascites. The blood pressure was $^{130}/_{90}$mm. of mercury. Investigation revealed a normal blood count, a blood urea concentration of 20mg/100ml and a creatinine clearance of 202ml/min/1.73m^3. Radiological examination of the chest showed no abnormalities and an intravenous pyelogram showed both kidneys to be of normal size and shape and to excrete contrast medium normally. There was osteoporosis of the spine. Examination of the urine showed heavy proteinuria (the $^{UA}/_{UC}$ ratio was 10.6) and on microscopy five white blood cells/cu.mm. but no red blood cells were seen. Differential protein clearances showed a highly selective pattern of urinary protein excretion. The serum albumin concentration was 1.8g/100ml. A percutaneous renal biopsy was performed, and microscopic examination of the specimen showed occasional sclerosed glomeruli, but the majority of glomeruli showed no morphological abnormalities. Treatment with cyclophosphamide (3mg/kg ideal body weight/day) and reduced doses of prednisolone (10mg/day) was commenced. Initially the proteinuria decreased, the $^{UA}/_{UC}$ ratio falling from 10.6 to 1.1. After one months treatment the proteinuria had again increased, the $^{UA}/_{UC}$ ratio rising again to 11.2. The dosage of cyclophosphamide was therefore increased to 3mg/kg actual body weight/day. The proteinuria subsequently diminished slightly, the $^{UA}/_{UC}$ ratio falling to 4.8. However, after eight weeks of therapy with cyclophosphamide proteinuria was still present. Cyclophosphamide therapy was therefore

continued and the proteinuria diminished over the next month. The ratio of the albumin clearance to the creatinine clearance fell from a value of 0.87 to 0.004. After a further months therapy a haemorrhagic cystitis developed. As this was considered to be due to cyclophosphamide this drug was stopped. No recurrence of proteinuria occurred, and gradually over the following month the prednisolone dosage was gradually reduced, and this drug was also stopped. After two months without any drugs only a trace of proteinuria was present. The child was physically well and normotensive.

Patient No. 94 (J.R.)

A two-year-old boy who presented with a five day history of puffiness around the eyes and oliguria. Similar episodes had occurred on three previous occasions during the preceding year. On examination he was oedematous and had ascites. His blood pressure was $\frac{140}{90}$mm. of mercury. Investigations revealed heavy proteinuria (1,400mg protein/ 100ml) with a low serum albumin on serum electrophosis. Microscopy of the urine deposit revealed no cells. The serum cholesterol concentration was 645mg/100ml. The blood urea concentration was 16mg/100ml. and the blood count was normal. Treatment with prednisone (40mg/day) was given without effect, so therapy with A.C.T.H. (20 units/day) was given, again without effect. Over the next year the child's weight, and the degree of proteinuria varied, and various combinations of steroids and A.C.T.H. were given. His urinary protein excretion, when measured, was usually about 4g/24 hr. The total serum protein

concentration was about 5g/100ml., and electrophoresis invariably showed a marked diminution in the albumin fraction. The serum cholesterol level remained high at about 600mg/100ml. The child remained normotensive and the blood urea was not elevated. About a year after his first admission he again came into hospital in adrenal crisis. After initial treatment with intravenous hydrocortisone followed by prednisone (12.5mg/day) his condition improved, but his heavy proteinuria persisted. When he had recovered sufficiently, a percutaneous renal biopsy was performed. Microscopic examination showed the great majority of the glomeruli to be morphologically normal. In three glomeruli situated near the cortico-medullary junction, however, segmental sclerotic lesions in the glomerular tufts with adhesions between the scarred areas and Bowman's capsule were seen.

Steroid therapy was eventually stopped some eighteen months after his first admission. His proteinuria persisted, although he remained in reasonably good health and attended school.

He was investigated again eight years after his initial admission. On examination he was of short stature (133cm.) with ascites and bilateral varicocoeles. The blood pressure was recorded as $^{100}/_{60}$mm. of mercury. Investigations revealed a normal blood and normal bleeding, clotting, prothrombin and partial thromboplastin times. The E.S.R. was 60mm. in 1 hr. The blood urea concentration was 27mg/100ml., and the creatinine clearance 200ml/min/1.73m^2. The serum cholesterol concentration was 340mg/100ml. There was heavy proteinuria, the $^{UA}/_{UC}$ ratio being 11.8. Microscopy of urine

deposit revealed no abnormal cells. An intravenous pyelogram showed gross symmetrical enlargement of both kidneys. Excretion of contrast medium was prompt. A second renal biopsy was performed. Microscopy showed changes of focal glomerulosclerosis; the severity of the changes was not significantly different from those seen in the previous biopsy specimen. Treatment was again given with prednisone for four weeks. This had no effect and treatment was changed to cyclophosphamide (80mg/day) prednisolone (2.5mg/day) and bendrofluazide (5mg/day) with diuretics and potassium supplements. Cyclophosphamide was given for 12 months without effect on the degree of proteinuria; the $^{UA}/_{UC}$ ratio was 13.3 at the end of the course of therapy. Cyclphosphamide was therefore stopped and steroids tailed off. The creatinine clearance was noted to be 120ml/min/1.73m^2 and the child remained normotensive. When last seen ten years after his initial illness he remained physically reasonably well; he was normotensive and his creatinine clearance was 141ml/min/1.73m^2. In the interim period since he was investigated previously he was admitted to hospital with peritonitis. No localised cause for this was found at laparotomy but Streptococcus viridans was isolated from a blood culture. He quickly responded to treatment with antibiotics. His proteinuria has continued unabated.

Patient No. 95 (M.Sc.)

A boy who presented at the age of two years when a number of congenital abnormalities were noted. These included absent patellae, dystrophic nails, flexion

deformities of the elbows and, on radiological examination, the presence of iliac 'horns'. A diagnosis of 'nail-patella' syndrome was made. Two years later at the age of four years he presented with a nephrotic syndrome.

Examination of the urine revealed proteinuria (the urine protein excretion ranged between 0.3g and 1.32g/24 hr.) but microscopy revealed no red or white blood cells. The blood count was normal and the blood urea concentration was 19mg/100ml. The creatinine clearance was 89mg/min/1.73m^3. The total serum protein concentration was 5.0g/100ml. and serum electrophoresis revealed a diminution in the albumin fraction and an increase in the α_2 globulin fraction. A percutaneous renal biopsy was performed and this showed no definite histological abnormality. Treatment with prednisolone (2mg/kg/day) was given without effect on the proteinuria. One month later the ^{UA}/UC ratio ranged between 8.3 and 10.8. Microscopy of the urine showed no cells present. The total serum protein concentration was 4.3g/100ml. and the serum cholesterol was 390mg/100ml. Following this the dosage of corticosteroids was reduced and cyclophosphamide (3mg/kg/day) was given for eight weeks. At the end of this period the child was well and normotensive. The proteinuria continued unabated however (the ^{UA}/UC ratio was 10). When he was last seen six months later he remained well, but his proteinuria persisted, the ^{UA}/UC ratio being 5.8. A serum β_{1C} globulin level was normal.

Patient No. 96 (M.Sa.)

A seven-year-old boy who was admitted to hospital with a five week history of oedema of the face and ankles. On examination his blood pressure was $^{130}/_{90}$mm. of mercury. Urine examination revealed heavy proteinuria (more than 1,000mg protein/100ml). The total serum protein concentration was 4.3g/100ml. with a serum albumin concentration of 1.7g/100ml. The blood urea concentration was 110mg/100ml. Treatment with prednisone 60mg/day was commenced but no effect on the proteinuria was noted. The child's oedema became worse and his blood pressure ranged between $^{130}/_{90}$ and $^{150}/_{110}$mm. of mercury. Investigations at this stage showed a normal blood count and a blood urea concentration of 100mg/100ml. Radiological examination of the chest showed no abnormalities and an intravenous pyelogram showed slight enlargement of both kidneys with normal excretion of contrast medium. Bacteriological culture of a throat swab isolated no pathogenic organisms. A serum A.S.O. titre was more than 800 units/ml. Examination of the urine showed heavy proteinuria (more than 1,000mg/100ml of protein was present) and microscopy showed no abnormal cells present. Differential protein clearances revealed a moderately selective pattern of urinary protein excretion. A percutaneous renal biopsy was performed. Histological examination showed most glomeruli to be morphologically normal. One juxtamedullary glomerulus showed a focal sclerotic area occupying part of the glomerular tuft and a capsular adhesion was present between the scarred area and Bowman's capsule. Some lymphocytic and histiocytic

infiltration was present around the affected glomerulus. Elsewhere there was some interstitial oedema and focal areas of tubular atrophy and regeneration.

Because of the elevation and his blood pressure and the presence of azotaemia, treatment with steroids was tailed off and stopped. One month later treatment was restarted with cyclophosphamide (3mg/kg body weight/day) and small doses of steroids. Over the next eight weeks a marked diminution in proteinuria occurred. The urinary protein excretion fell from 2.7g/24 hr. to 0.19g/24 hr. and the albumin clearance fell from 0.52ml/min to 0.0046ml/min. The serum albumin concentration rose from 0.35g/100ml to 2.8g/100ml. Cyclophosphamide therapy was continued for a further 10 months. At the end of this period the child was well. His blood pressure was 140/80mm. of mercury. The creatinine clearance was 145ml/min/1.73m^3. A trace of protein only was present in the urine. The $^{UA}/_{UC}$ ratio was 0.11 and the 24 hr. urinary albumin excretion was 0.087g. When last seen three years later the child was physically well. The blood pressure was $^{125}/_{70}$mm. of mercury and his urine was almost free from protein.

Patient No. 97 (A.St.)

A two-year-old girl who presented with peripheral and facial oedema which had developed gradually over a number of days. She had been previously well, apart from pertussis some five months previously. Investigations revealed heavy proteinuria (the urinary protein excretion was 4.9g/24 hr.), hypoalbuminaemia (the serum albumin concentration was

1.2g/100ml) and hypercholestrolaemia (the serum cholesterol concentration was 615mg/100ml). The E.S.R. was 33mm. in 1 hr. and a serum A.S.O. titre was less than 100 units/ml. Treatment with prednisolone (40mg/day) was given a total of 19 weeks. After four weeks the oedema disappeared, but heavy proteinuria persisted.

She was admitted to hospital again eighteen months after the onset of her symptoms. Her blood pressure was $^{110}/_{60}$mm. of mercury. Heavy proteinuria (3.9g/24 hr.) was present. Differential protein clearance studies showed a poorly selective pattern of urinary protein excretion. The total serum protein concentration was 5.7g/100ml. with a serum albumen concentration of 1.5/100ml. The blood urea concentration was 15mg/100ml. and the creatinine clearance 120ml/min/1.73m^2. The serum cholesterol concentration was 645mg/100ml. Routine urinalysis showed occasional red and white blood cells present. Radiological examination of the chest and intravenous urography revealed no abnormalities. A percutaneous renal biopsy was performed and histological examination of the biopsy specimen showed a mild proliferative glomerulonephritis.

Treatment with cyclophosphamide (50mg/day) and small doses of prednisone (10mg/day) was instituted, and continued in all for 15 months. The proteinuria diminished in amount, but did not entirely disappear. After 12 months therapy the urinary protein excretion was noted as 0.6g/24 hr. and the urinary albumin excretion as 0.5g/24 hr. The serum albumin concentration was 2.8g/100ml. Urinalysis revealed no abnormal

cells. The blood urea concentration was 20mg/100ml. and the creatinine clearance 150ml/min/1.73m^2.

After 15 months therapy the urinary albumin excretion was 0.25g/24 hr. and the serum albumin concentration was 3.9g/100ml. The serum cholesterol concentration was 275mg/100ml. A second renal biopsy was performed and histological examination again revealed mild proliferative changes.

When the child was last investigated one year after cessation of the cyclophosphamide therapy (i.e. 4 years 4 months after the onset of symptoms) she was physically well and normotensive (her blood pressure was ^{90}/60mm. of mercury). Only slight proteinuria was present (the ^{UA}/UC ratio ranged between 0.34 and 0.51). The creatinine clearance was 110ml/min/1.73m^2.

Patient No. 98 (G.B.)

A four-year-old boy who presented with generalised oedema and heavy proteinuria preceded by a mild upper respiratory tract infection. It was noted that a sibling had recently suffered an attack of anaphylactoid purpura with evidence of renal involvement. Treatment with steroids, diuretics and a high protein diet was given, but no improvement occurred.

Three months after the onset of his symptoms he was admitted to hospital for investigation. On examination he was oedematous. His blood pressure was ^{120}/80mm. of mercury. His blood count was normal. Urinalysis revealed heavy proteinuria (more than 1,000mg/100ml) and urine microscopy showed 67 red

blood cells/cu.mm. present. The creatinine clearance was 112ml/min/1.73m^2. A serum A.S.O. titre was measured as 800 units/ml., but a repeat of this investigation ten days later gave a value of only 50 units/ml. Radiological examination of the chest and an intravenous urogram were both normal. Differential protein clearances revealed a moderately selective pattern of urinary protein excretion. A percutaneous renal biopsy was performed. Microscopical examination of this specimen showed changes of chronic glomerulonephritis with sclerosis of some glomerular tufts, and areas of tubular atrophy and interstitial fibrosis. Over the next six months various combinations of steroids with cyclophosphamide and with azathroprine were given without any demonstrable effect on the degree of proteinuria.

He was investigated again 12 months after the onset of his symptoms. His blood pressure was $^{140}/_{90}$mm. of mercury and urinalysis revealed heavy proteinuria (the $^{UA}/_{UC}$ ratio varied between 2:4 and 15:1). The creatinine clearance was 81ml/min/1.73m^2.

He was admitted to hospital again 18 months later because of increasing oedema. His blood pressure was $^{140}/_{110}$mm. of mercury. Urinalysis revealed heavy proteinuria (1,000mg/100ml) and microscopy showed 96 red blood cells and 10 white blood cells/cu.mm. The creatinine clearance was 95ml/min/1.73m^2. Control of his hypertension was achieved with diuretics and a low sodium diet. His proteinuria continued unabated, and he continued to become oedematous on occasions.

Patient No. 99 (G.C.)

A nine-year-old boy who presented with oedema of the face and ankles, haematuria and oliguria following a sore throat. He was admitted to hospital where his blood pressure was measured as $^{150}/_{90}$mm. of mercury. Examination of his urine showed it to be heavily blood-stained and heavy proteinuria to be present. Bacteriological culture of a throat swab failed to isolate any pathogenic organisms and a serum A.S.O. titre was less than 200 units/ml. After admission to hospital the oedema rapidly improved, his blood pressure fell to $^{140}/_{70}$mm. of mercury and his 24 hr. urine volume rose from 700ml. to 1500ml. However, repeated urinalysis showed persistence of his proteinuria and haematuria. Three months later his proteinuria was noted to be of nephrotic proportions, the $^{UA}/_{UC}$ ratio being measured as 8.8. The serum albumin concentration was 1.8g/100ml. Microscopy of the urine showed 14 white blood cells and 30 red blood cells/cu.mm. Prednisolone was given in a dose of 60mg/day for one month without effect on the proteinuria. Further investigations showed a creatinine clearance of 115ml/min/1.73m^2. An intravenous pyelogram showed no definite abnormalities. The total serum protein concentration was 3.7g/100ml. A percutaneous renal biopsy was performed. Microscopic examination of the specimen revealed an advanced chronic glomerulonephritis with widespread glomerular sclerosis, tubular atrophy and interstitial fibrosis. One month later therapy with prednisolone was tailed off to a dosage of 0.2mg/kg/day. Proteinuria persisted, the $^{UA}/_{UC}$ ratio varying between 2.6 and 4.3. Microscopy of the urine revealed 290 red blood cells

and 15 white blood cells/cu.mm. The glomerular filtration rate measured with radioactive chromium labelled E.D.T.A. was 76.4ml/min/1.73m^2. Treatment with azathioprine (2mg/kg/day) and prednisolone (0.2mg/kg/day) was started. Again the proteinuria persisted, the $^{UA}/_{UC}$ ratio measured after one months therapy was 6.4. After eight weeks treatment the $^{UA}/_{UC}$ ratio was 4.2. A serum β_{1C} globulin level was noted to be low. The creatinine clearance was 77ml/min/1.73m^2, and the glomerular filtration rate measured with Cr^{51} E.D.T.A. was 67.6ml/min/1.73m^2. Treatment was changed to cyclophosphamide (100mg/day) and prednisolone continued as previously. This treatment was continued for five months after which all treatment was stopped.

The child was reinvestigated eight months later, two years after the onset of his illness. On examination he was noted to be hypertensive, his blood pressure being measured as $^{160}/_{115}$mm. of mercury. The blood urea was 44mg/100ml, the creatinine clearance 61ml/min/1.73m^2. Urine examination showed proteinuria to be persisting although less in amount (the $^{UA}/_{UC}$ ratio ranged between 2.2 and 2.5). Microscopy of the urine revealed 5 red blood cells/cu.mm. Treatment was confined to hypotensive therapy with methyl dopa.

Patient No. 100 (B.C.)

An eight-year-old girl who presented with facial oedema. She was admitted to hospital for investigation. Her blood pressure was $^{130}/_{60}$mm. of mercury, and examination of the

urine revealed heavy proteinuria. Microscopy of the urine deposit showed no cells present. The erythrocyte sedimentation rate was 65mm. in the first hour. An intravenous pyelogram showed both kidneys to be enlarged and to excrete contrast medium normally. A serum A.S.O. titre was 60 units/ml. Treatment with prednisone at a dosage of 60mg/day was given without effect on her proteinuria. Because of this lack of response, steroids were tailed off and stopped. Four months after the onset of her disease she was admitted to hospital again following an episode of vomiting. Her blood pressure was noted to be $^{150}/_{95}$mm. of mercury. Examination of the urine showed heavy proteinuria, the 24 hr. protein excretion ranging between 2 and 3g. The total serum protein concentration was 4.3g/100ml with a serum albumin concentration of 2.5g/100ml. The serum cholesterol concentration was 480mg/100ml. The blood urea concentration was 40mg/100ml.

Steroid therapy was again given in conjunction with a high protein diet. The blood urea concentration rose to 105mg/100ml, but after reducing the protein content of her diet, the blood urea fell to 55mg/100ml. After five weeks steroid therapy was stopped. At this time her blood pressure was noted to be $^{135}/_{70}$mm. of mercury. A blood count was normal and the blood urea concentration was 46mg/100ml. The creatinine clearance was 72ml/min/1.73m^2. Radiological examination of the chest revealed no abnormalities. Bacteriological culture of a throat swab isolated no pathogenic organisms and a serum A.S.O. titre was less than 200 units/ml. The 24 hr. urinary protein excretion ranged between 1.5 and

2.9g. and the $^{UA}/_{UC}$ ratio was 5.1. Treatment with cyclophosphamide (3mg/kg/day) was started. After eight weeks the child's blood pressure was recorded as $^{130}/_{70}$mm. of mercury. The urinary albumin excretion was 3g/24 hr. and the $^{UA}/_{UC}$ ratio was 3.9. Cyclophosphamide therapy was continued altogether for fifteen months. After 12 months therapy the child was readmitted to hospital for assessment. The blood pressure was recorded as $^{134}/_{80}$mm. of mercury. The blood count was normal and the blood urea concentration was 74mg/100ml. The creatinine clearance was 52ml/min/1.73m^2. Examination of the urine revealed proteinuria (the $^{UA}/_{UC}$ ratio ranging between 2.8 and 7.8) and microscopy of the urine revealed numerous red blood cells (more than 1,000/cu.mm.) and scanty white blood cells (5/cu.mm.). A serum β_{1C} globulin level was low. After fifteen months therapy cyclophosphamide was stopped. At this time the child's blood pressure was $^{140}/_{90}$mm. of mercury. The total serum protein concentration was 5.4g/100ml and the serum albumin concentration was 2.9g/100ml. A 24 hr. urinary albumin excretion was 6g. and the $^{UA}/_{UC}$ ratio ranged between 3.2 and 6.1. Microscopic examination of the urine revealed 80 red blood cells/cu.mm. The blood urea was 77mg/100ml and the creatinine clearance was 52ml/min/1.73m^2. Bacteriological culture of a throat swab isolated haemolytic streptococci of Lancefield group A. A percutaneous renal biopsy was performed. Histological examination revealed severe changes suggestive of a membranoproliferative type of glomerulonephritis. Some glomeruli were sclerosed. Others were greatly enlarged. The

glomerular tufts were lobulated and both thickening of the walls of the tuft capillary loops and increased cellularity were present. Areas of tubular atrophy and interstitial firbosis were present. Following this a further eight weeks cyclophosphamide therapy was given but again no response with regard to her proteinuria or microscopic haematuria was noted.

Six months later she was readmitted to hospital. She had been vomiting and had complained of headaches. Her blood pressure was $^{210}/_{160}$mm. of mercury. The blood urea was 234mg/100ml and the creatinine clearance was 5ml/min/1.73m^2. The total serum protein concentration was 5.5g/100ml. Microscopy of the urine revealed 225 red blood cells and 5 white blood cells/cu.mm. Treatment with a low protein and low sodium and potassium diet and with methyl dopa was given. Three months later, two-and-a-half years after the onset of her symptoms, she was admitted again in left ventricular failure. Treatment with morphine and diuretics was given. Heavy proteinuria and microscopic haematuria were still present. Her blood urea was 159mg/100ml and the creatinine clearance was 50 ml/min/1.73m^2.

Patient No. 101 (C.F.)

A five-and-a-half year old boy who presented with oedema of the face and hands. Examination of his urine revealed heavy proteinuria. He was treated with prednisolone, but this had no effect on his proteinuria.

Two months after the onset of his symptoms he was again admitted to hospital. On examination he had "mooning"

of the face as a result of steroid therapy. His blood pressure was $^{110}/_{70}$mm. of mercury. Investigations revealed a normal blood count and an E.S.R. of 40mm. in the first hour. The blood urea concentration was 25mg/100ml and the creatinine clearance was 193ml/min/1.73m^2. The total serum protein concentration was 4.8g/100ml. Examination of the urine revealed proteinuria (the urinary albumin excretion ranging between 3.6 and 4.8g/24 hr.) and microscopic haematuria (275 red blood cells/cu.mm. were present). Differential protein clearances revealed a moderately selective urinary protein excretion. Treatment with azathioprine and cyclophosphamide were given for eight weeks. At the end of this period his heavy proteinuria continued (the $^{UA}/_{UC}$ ratio was 8). Treatment was continued with cyclophosphamide and prednisone. Three months later the child's oedema had disappeared. Treatment was continued for one year. At the end of this period the child's proteinuria was still present but had diminished in amount. The ratio of albumin clearance to the creatinine clearance fell from a value of 2.2 at the onset of treatment to a value of 0.075 at the end of treatment. The blood urea concentration was 20mg/100ml and the creatinine clearance 173ml/min/1.73m^2. A percutaneous renal biopsy was performed and this showed a mild proliferative glomerulonephritis. When the child was last seen, ten months later, two-and-a-half years after the onset of his disease, only slight proteinuria was present and no red cells were seen on microscopy of the urine. He was physically well and his blood pressure was $^{120}/_{80}$mm. of mercury.

Patient No. 102 (C.McG.)

An eight-year-old girl who presented with a three week history of periorbital oedema. On examination she was normotensive. Investigations revealed heavy proteinuria and a low serum albumin concentration. She was treated with cyclophosphamide and prednisone without effect on her proteinuria. She was admitted to hospital again after an interval of three months. On examination she was grossly oedematous. The blood pressure was $^{110}/_{70}$mm. of mercury. Examination of the urine showed heavy proteinuria. The blood urea concentration was 20mg/100ml, the serum albumin concentration was 1.1g/100ml. An intravenous pyelogram showed enlargement of both kidneys. Treatment with prednisolone (60mg/day) was given for four weeks without effect. Treatment was then changed to cyclophosphamide (150mg/day) with a reduced amount of steroids, but again without effect. She was reinvestigated at this time. On examination she was cushingoid and there was marked alopecia. Oedema of legs was present. Her blood pressure was $^{115}/_{85}$mm. of mercury. Her initial blood count showed a leucopenia of 2,600 W.B.C./cu.mm; however, this rose to 6,300 W.B.C./cu.mm. after one week, and subsequently remained normal. Bleeding, clotting, prothrombin and partial thromboplastin times were normal. The E.S.R. was 54mm. in 1 hr. Bacteriological culture of a throat swab isolated commensal organisms only and the serum A.S.O. titre was less than 50 units/ml. The blood urea concentration was 16mg/100ml and the creatinine clearance 135ml/min/1.73m^2. The 24 hr. urinary albumin excretion was 3.6g. and the $^{UA}/_{UC}$ ratio ranged between 10.2 and 20. Differential protein

clearances showed a poorly selective pattern of urinary protein. Microscopy of the urine deposit showed up to 95 red blood cells/cu.mm. and the red blood cell excretion rate was 9.71×10^6/12 hr. The total serum protein concentration was 3.8g/100ml and the serum albumin was 0.9g/100ml. The serum cholesterol concentration was 519mg/100ml. A percutaneous renal biopsy was performed, and microscopic examination revealed changes of advanced chronic glomerulonephritis. Treatment with cyclophosphamide (75mg/day), prednisone (12.5mg/day), chlorthiazide (250mg/day), spironolactone (50mg/day) and indomethacin (2mg/kg/day) was started and the child was discharged. No response in her proteinuria was noted, and after eight weeks therapy was changed to azathioprine (3mg/kg/day) and prednisolone (0.5mg/kg/day). Again no effect was noted. Various other combinations of drugs were tried, but the child's oedema could only be controlled by diuretics and sodium restriction. Two years after the onset of her illness she was again admitted to hospital in congestive cardiac failure. On examination her liver was enlarged, the jugular venous pressure was raised and there was gross ascites. Radiological examination of the chest revealed cardiac enlargement. Investigations revealed a haemoglobin of 9.6g/100ml and blood urea of 38mg/100ml. The creatinine clearance was 42ml/min/1.73m^2. There was heavy proteinuria, the $^{UA}/_{UC}$ ratio was 9.7. Microscopy of the urine deposit revealed 25 R.B.C.'s/cu.mm. She was treated with sodium and fluid restriction and a high protein diet. Her prednisolone therapy was tailed off and a diuresis occurred.

She was seen again two months later when for the first

time her diastolic blood pressure was noted to be raised to 90mm. of mercury. Increasing oedema was noted and an episode of frank haematuria occurred. The bleeding, clotting, prothrombin and partial thromboplastin times were all normal, but her haemoglobin was noticed to have fallen to 8.0g/100ml. The blood urea was 76mg/100ml and the creatinine clearance was 16ml/min/1.73m^2. Proteinuria was heavy (the $^{UA}/_{UC}$ ratio was 8.1) and differential protein clearances showed a very non-selective pattern. Five months later she was again admitted to hospital because of a rising blood urea concentration (27mg/100ml) and a falling haemoglobin level (4.7g/100ml). Her blood pressure was 150/110mm. of mercury. She was treated with blood transfusions and methyl dopa. Two months later, two and a half years after the onset of her illness, she died. No necropsy was performed.

Patient No. 103 (D.P.)

A six year old boy who presented with facial oedema and proteinuria following an upper respiratory tract infection. Bacteriological culture of a throat swab isolated β-haemolytic streptococci of Lancefield group A. A diagnosis of acute glomerulonephritis was made and treatment with penicillin was given. However, the child's proteinuria increased and generalised oedema developed. Treatment with prednisolone (initially 40mg/day, later 20mg/day) was given, but the proteinuria persisted. During the next eighteen months the child's oedema fluctuated in severity, and for most of the time he received steroid therapy. At the end of this time he was again admitted to hospital for assessment. On examination

he was cushingoid with gross oedema, ascites and bilateral pleural effusions. His blood pressure was $^{140}/_{100}$mm. of mercury. Investigations revealed a normal blood count. The blood urea concentration was 23mg/100ml and the creatinine clearance was 171ml/min/1.73m^2. Bacteriological culture of a throat swab isolated commensal organisms only, but the serum A.S.O. titre was more than 800 units/ml. Microscopy of the urine deposit showed no cells present on a number of occasions. The serum cholesterol concentration was 578g/100ml and the serum albumin concentration was 0.67g/100ml. Proteinuria was heavy, the 24 hr. urinary albumin excretion was 1.2g and the $^{UA}/_{UC}$ ratio ranged between 2.9 and 5. Treatment with prednisone (40mg/day) was given for four weeks but the proteinuria continued unabated. Treatment was changed to cyclophosphamide (60mg/day) and the dosage of steroids was reduced. A marked clinical improvement occurred. The proteinuria decreased (the $^{UA}/_{UC}$ ratio fell to 0.88) and the serum albumin level increased (to 5g/100ml). Cyclophosphamide was continued for the next 21 months. After one year's treatment only a trace of proteinuria was present (the urinary albumin excretion was 0.07g/24 hr) and the total serum protein concentration was 7.2g/100ml. The serum cholesterol concentration was 208mg/100ml. The blood urea was 37mg/100ml and the creatinine clearance was 130 ml/min/1.73m^2. A percutaneous renal biopsy was performed. Microscopic examination showed that the majority of the glomeruli were histologically normal. Four glomeruli in the region of the cortico-medullary junction were abnormal. Three were wholly sclerotic and the fourth showed areas of segmental sclerosis of the glomerular tuft.

Nine months later the child was again admitted to hospital for investigation of persistent frank haematuria which had first occurred six months previously following a fall. Investigations showed only slight proteinuria ($^{UA}/_{UC}$ ratio of 0.48) but microscopy of the urine deposit showed more than 200 red blood cells. Renal function was in other respects unimpaired. The creatinine clearance was 157ml /min/1.73m^2. Cyclophosphamide was withdrawn at this stage in case this drug was producing a cystitis. Cytoscopy revealed injection of the bladder mucosa.

Following withdrawal of cyclophosphamide haematuria persisted. At his last visit two months later, there was no change in renal function, and the child was normotensive, with a blood pressure of $^{140}/_{70}$mm. of mercury. The $^{UA}/_{UC}$ ratio was 0.54 and the ratio of the albumin clearance to the creatinine clearance was 0.006. A repeat cystoscopy showed haemorrhagic telangectatic patches in the bladder mucosa.

Patient No. 104 (D.S.)

A nineteen month old boy who presented with oedema and frank haematuria. Examination of the urine revealed heavy proteinuria with numerous red blood cells present. Other investigations revealed a blood urea of 38mg/100ml and a total serum protein concentration of 4.5g/100ml, with a serum albumin concentration of 1.6g/100ml. Treatment with prednisone (40mg/day) was given. After six weeks therapy no effect on the proteinuria was noted and the dosage of steroids was reduced. He was admitted to hospital again. On examination

"mooning" of his face was noted. His blood pressure was $^{90}/_{70}$mm. of mercury. Investigations showed a normal blood urea concentration of 36mg/100ml and a creatinine clearance of 234ml/min/1.73m^2. Bacteriological culture of a throat swab failed to isolate any pathogenic organisms, and the serum A.S.O. titre was 50 units/ml. The total serum protein concentration was 4.2g/100ml. Serum electrophoresis showed a decrease in the albumin fraction and a marked increase in the α_2 globulin fraction. The serum cholesterol concentration was 359mg/100ml. Bleeding and clotting times were normal. Proteinuria was heavy, with a urinary albumin excretion of 4.3g/24 hr., the $^{UA}/_{UC}$ ratio ranging between 13.1 and 28.9. Differential protein clearance studies showed a non-selective pattern of urinary protein excretion. Microscopy of the urine revealed more than 1,000 red blood cells and 5 white cells/cu.mm. An intravenous pyelogram showed both kidneys to be enlarged; excretion of contrast medium was normal. A percutaneous renal biopsy was performed. Histological examination showed a mild diffuse proliferative glomerulonephritis. Treatment with cyclophosphamide (3mg/kg/day) was given for eight weeks without effect on the proteinuria. Following this azathioprine (2mg/kg/day) was also given for an eight week period, again without noticable effect. However, over the next few weeks the proteinuria gradually diminished and disappeared spontaneously and when last seen the child was free from urinary abnormalities.

Patient No.105 (P.J.)

A twelve year old boy who presented with haematuria and

oedema of the ankles and eyelids, which developed one week after a sore throat. He was admitted to hospital where his blood pressure was recorded as $^{150}/_{85}$mm. of mercury. Examination of the urine revealed heavy proteinuria and numerous red blood cells present. The total serum protein concentration was 4.2g/100ml and the serum albumin concentratinn was 2.3g/100ml. The blood urea concentration was 90mg/100ml. The serum A.S.O. titre was less than 200 units/ml. Treatment with prednisolone at a dosage of 60mg/day later rising to 80mg/day was given for six weeks, without effect on the proteinuria. At the end of this time the child was readmitted to hospital. On examination he was oedematous and exhibited facial "mooning". Both ascites and bilateral pleural effusions were present. His blood pressure was recorded as $^{140}/_{90}$mm. of mercury. Investigations showed a blood urea concentration of 43mg/100ml and a creatinine clearance of 171ml/min/1.73m^2. The blood count was normal. Bleeding and clotting studies showed no abnormalities. An intravenous pyelogram showed both kidneys to be enlarged; excretion of contrast medium was normal. The total serum protein concentration was 4.2g/100ml and the serum albumin concentration was 1.9g/100ml. A serum β_{1C} complement level was low. Examination of the urine showed heavy proteinuria and microscopic haematuria. Estimation of the glomerular filtration rate using Cr^{51} E.D.T.A. gave a value of 146ml/min/1.73m^2. A percutaneous renal biopsy was performed. Histological examination of the specimen showed a membrano-proliferative glomerulonephritis. Treatment with cyclophos-phamide (3mg/kg/day) and prednisolone (10mg/day) was given for

eight weeks. At the end of this period no effect on the proteinuria was noted, the $^{UA}/_{UC}$ ratio being 4.4. It was decided to continue cyclophosphamide for one year. When last seen after two months therapy there was no change in his condition.

Patient No. 106 (A.Ba.)

A six-and-a-half year old boy who presented with oedema of the legs and ascites. On examination his blood pressure was recorded as $^{120}/_{50}$mm. of mercury. Investigations revealed a normal blood count, a blood urea concentration of 25mg/100ml, and a creatinine clearance of 149ml/min/1.73m^2. An intravenous pyelogram showed both kidneys to be of normal size and to excrete contrast medium normally. Bacteriological culture of a throat swab failed to isolate any pathogenic organisms, and an A.S.O. titre was 50 units/ml. A serum cholesterol concentration was 425mg/100ml. The serum β_{1C} globulin level was normal. Examination of the urine showed heavy proteinuria with a urinary protein excretion of 3.64g/24 hr. and a $^{UA}/_{UC}$ ratio of 5.9. Differential protein clearances showed a moderately selective pattern of urinary protein excretion. Microscopy of the urine revealed 200 red blood cells and 10 white blood cells/cu.mm. The total serum protein concentration was 4.2g/100ml with a serum albumin concentration of 1.2g/100ml. Treatment with prednisolone (40mg/day) was given for six weeks without effect on his proteinuria. Because of the lack of response, steroid therapy was tailed off at the end of this period. Investigations at

this time showed a creatinine clearance of 169ml/min/1.73m^2 and a normal serum β_{1C} globulin concentration. Examination of the urine revealed heavy proteinuria and microscopic haematuria. A percutaneous renal biopsy was performed. Histological examination revealed a moderate diffuse proliferative glomerulonephritis.

SECTION 3

RESULTS

Introduction

This section is presented in two main parts. The first part deals with the various studies made to evaluate the quantitative histological techniques used. With regard to the total and differential glomerular cell counts, these were firstly to correlate the counts made by direct microscopy using an oil-immersion objective on paraffin sections with those made on photomicrographs of glomeruli in epoxy-resin sections of the same renal biopsy specimens. Secondly, to ascertain the importance of the particular plane of section through a glomerulus chosen for making the counts. Thirdly, to consider the variations in the glomerular cell counts at different ages throughout childhood by examining paraffin sections of 'normal' kidneys obtained at necropsy.

In the second part the histological findings, including the quantitative methods of assessment described, are presented in each of the three groups of patients studied. In each group of children, those presenting with idiopathic recurrent haematuria, those with anaphylactoid purpura, and those with a nephrotic syndrome, the main clinical features and the pathological diagnoses are summarised and tabulated. The results of the quantitative histological analyses are given, and where relevant, these are correlated with the clinical and biochemical findings.

A short comment on each of the studies mentioned follows the presentation of the findings.

Part I. Studies to Evaluate the Quantitative Histological Techniques Used

I. The Correlation Between the Glomerular Cell Counts Made by Direct Microscopy on Paraffin Section with Those Made on Photomicrographs of Glomeruli in Epoxy-resin Sections of the Same Renal Biopsies

In figs. 28 and 29 the mean total numbers of cells, and the mean percentages of epithelial, endothelial and mesangial cells, found in glomeruli in paraffin sections examined with an oil-immersion objective are plotted against the mean total cells and the mean percentages of epithelial, endothelial and mesangial cells counted on photomicrographs of glomeruli in epoxy-resin sections from the same biopsy specimens.

In the paraffin sections, five glomeruli were counted in each case and the mean values were calculated from the results obtained in these five glomeruli. In the epoxy-resin sections the mean values were calculated from the percentages obtained in the number of glomeruli present in that particular biopsy. Only specimens with more than three glomeruli in the 'Araldite'-embedded tissue were used in this study, and the number of glomeruli present in the biopsies varied, therefore, between three and nine, with a mode of four. Full details of the glomerular cell counts are given in Table 4.

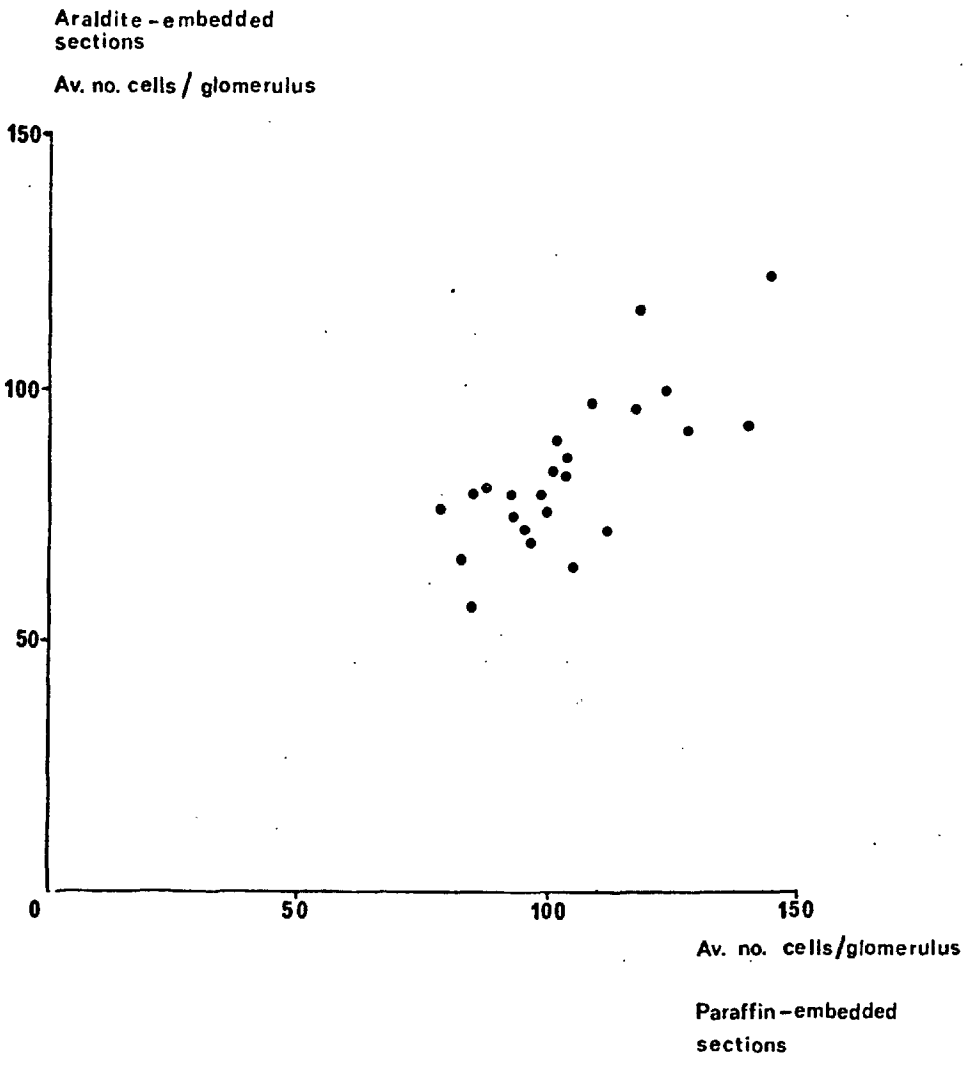


Fig. 28 Comparison of the mean total numbers of cells in 5 glomeruli made by direct microscopy in paraffin sections (2μ thickness) and on photomicrographs of all the glomeruli present in 'Araldite' sections (0.5μ thickness) from the same biopsies. The correlation coefficient (r) is 0.77.

Comparison between the average percentages of epithelial, endothelial & mesangial cells in paraffin-embedded & 'Araldite'-embedded sections from the same renal biopsy specimens

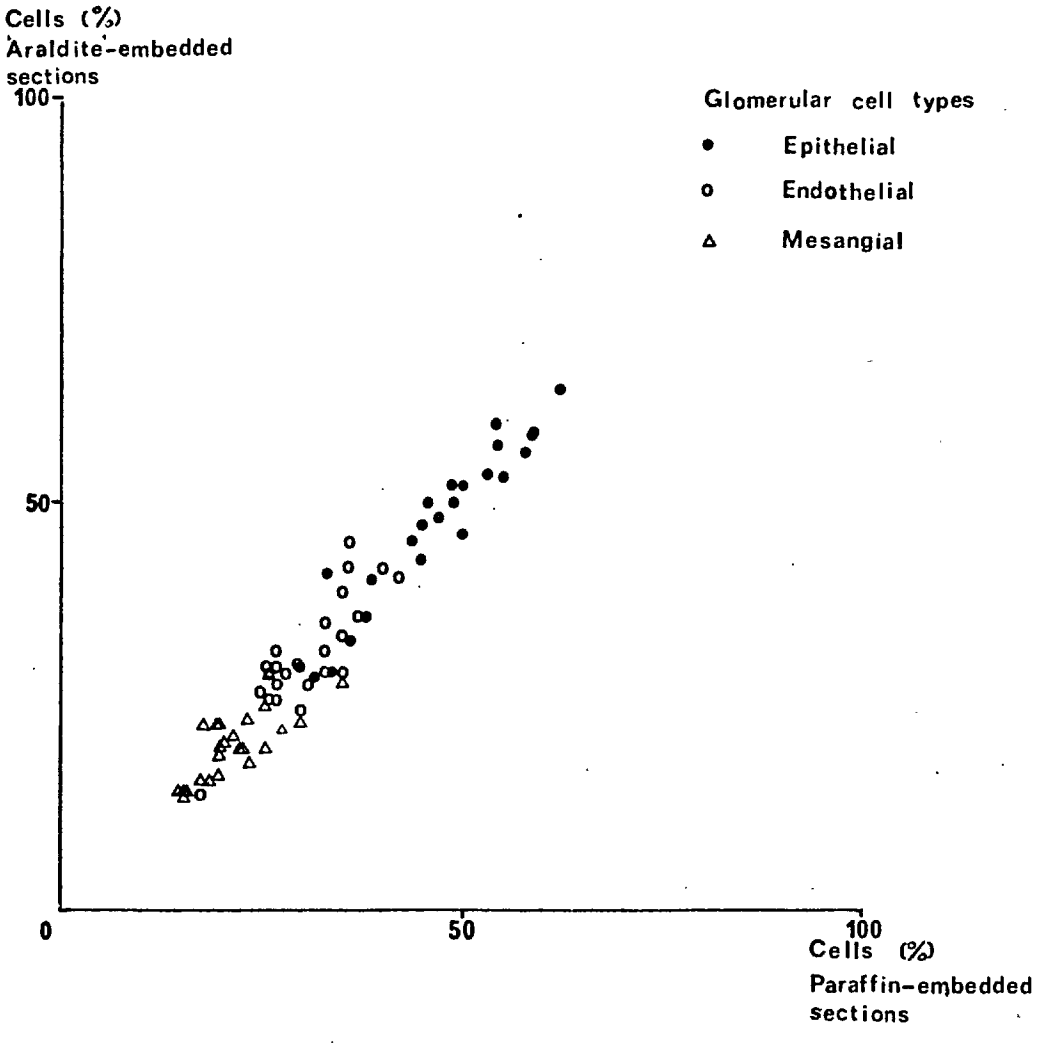


Fig. 29 Comparison of the mean percentages of epithelial, endothelial and mesangial cells in 5 glomeruli made by direct microscopy in paraffin sections (2μ thickness) and on photomicrographs of all the glomeruli present in 'Araldite' sections (0.5μ thickness) from the same biopsies. A very good correlation was found ($r = 0.97$).

Table 4. Total and Differential Glomerular Cell Counts Made by Direct Microscopy on Paraffin Sections and on Photomicrographs of Glomeruli in Epoxy-resin Sections From the Same Renal Biopsy Specimens

Patient		Glomerular cell counts made on paraffin sections				Glomerular cell counts made on epoxy-resin sections			
		Cells				Cells			
No.	Initial	Epithelial (%)	Endothelial (%)	Mesangial (%)	Total No.	Epithelial (%)	Endothelial (%)	Mesangial (%)	Total No.
44	D.C.	45	31	24	88	31	50	19	94
		26	36	38	135	41	44	15	120
		37	45	18	142	31	40	29	93
		39	33	28	131	30	44	26	76
		34	37	29	94				
		*36	36	28	118	33	45	22	96
26	K.D.	39	35	26	83	31	44	25	68
		30	41	29	75	35	36	29	45
		37	36	27	128	22	46	32	54
		36	42	22	74				
		26	45	28	64				
		34	40	26	85	29	42	29	56
27	M.H.	41	35	24	127	52	27	21	117
		38	32	30	116	38	35	28	100
		53	27	20	119	41	34	25	85
		46	30	24	137	48	23	28	94
		44	27	28	119				
		44	30	26	124	45	30	25	99

No.	Initial	Epithelial (%)	Endothelial (%)	Mesangial (%)	Total No.	Epithelial (%)	Endothelial (%)	Mesangial (%)	Total No.
1	G.B.	45	28	27	96	52	32	16	61
		53	29	18	70	45	32	24	76
		58	27	15	67	61	26	13	61
		50	30	20	96				
		58	20	22	87				
		53	27	20	83	53	30	17	66
2	S.B.	51	31	18	112	45	32	22	111
		45	33	22	82	35	44	21	91
		39	37	24	119	50	35	15	104
		39	29	32	90	42	28	30	50
		50	33	17	106				
		45	33	22	102	43	35	22	89
3	S.F.	48	38	14	100	64	28	8	67
		52	30	18	79	62	26	12	50
		60	24	16	86	50	23	27	80
		51	31	18	116	66	17	17	82
		60	29	11	104	57	29	14	65
		54	30	16	97	60	25	15	69
4	T.F.	40	32	28	124	38	37	25	115
		38	46	16	117	29	47	24	109
		36	46	18	142	40	38	22	120
		36	42	22	106				
		42	41	15	106				
		38	42	20	119	36	41	23	115

Table 4 cont/d

No.	Initial	Epithelial (%)	Endothelial (%)	Mesangial (%)	Total No.	Epithelial (%)	Endothelial (%)	Mesangial (%)	Total No.
5	S.H.	38	35	27	124	40	42	18	88
		40	36	24	107	47	37	16	74
		41	30	29	91	42	38	20	94
		50	28	22	78	37	35	29	93
		27	43	30	108	40	42	18	65
		39	35	26	101	41	39	20	83
6	G.L.	44	42	14	110	50	31	19	64
		46	31	23	96	48	33	19	76
		47	31	22	156	42	38	20	53
		43	30	27	87				
		45	39	16	75				
		45	35	20	105	47	34	19	64
7	A.M.	26	45	29	103	33	39	28	118
		30	26	44	174	32	36	32	92
		32	34	34	149	30	43	27	74
		32	37	31	148	26	50	24	84
		32	38	30	125				
		30	36	34	140	30	42	28	92
8	C.P.	45	36	19	101	45	32	23	87
		49	32	19	74	47	28	24	68
		49	31	20	110	43	29	28	83
		46	43	11	74	44	38	18	70
		47	34	19	106	55	22	23	82
						56	22	22	77
		47	35	18	93	48	29	23	78

Table 4 cont/d.

No.	Initial	Epithelial (%)	Endothelial (%)	Mesangial (%)	Total No.	Epithelial (%)	Endothelial (%)	Mesangial (%)	Total No.
9	J S.	56	29	15	106	60	26	14	65
		52	31	17	75	57	28	15	100
		59	21	20	61	59	22	19	67
		58	23	19	81	48	39	10	78
		64	24	12	104				
		58	26	16	85	56	29	15	78
10	E.W.	45	32	23	123	50	31	19	80
		54	29	17	136	55	33	12	40
		45	27	28	126	47	31	21	82
		50	27	23	90	48	27	25	81
		52	26	22	87	50	26	24	79
						51	28	21	72
		49	28	23	112	50	29	20	72
49	J.B.	60	23	17	70	44	38	18	74
		54	31	15	107	54	36	10	69
		58	25	17	80	45	33	22	92
		43	35	22	108	64	27	9	89
		60	20	20	74	55	26	19	77
		55	27	18	88	53	32	16	80
60	F.D.	64	23	13	82	54	25	21	72
		56	28	17	89	56	27	17	84
		65	27	8	87	59	29	12	63
		56	26	18	68	67	23	10	87
		56	27	17	66				
		59	26	15	79	59	26	15	76

Table 4 cont/d.

No.	Initial	Epithelial (%)	Endothelial (%)	Mesangial (%)	Total No.	Epithelial (%)	Endothelial (%)	Mesangial (%)	Total No.
86	S.N.	48	34	26	142	47	33	20	91
		54	24	25	111	53	21	25	92
		46	28	24	93	53	30	17	119
		50	32	25	115	54	26	20	87
		51	18	22	82				
		50	27	23	109	52	28	20	97
101	C.F.	34	32	34	152	29	44	27	115
		39	35	26	119	40	39	21	108
		40	32	28	133	48	28	24	78
		27	43	30	111	38	43	19	69
		27	43	30	126	45	33	22	63
						53	24	23	79
						43	36	21	90
						47	37	16	104
						29	37	34	118
		33	37	30	128	41	36	23	92
84	M.M.	41	34	25	133	45	37	17	67
		51	29	20	101	54	22	24	90
		45	35	20	110	57	24	19	99
		48	31	21	91	43	34	23	83
		45	38	17	87				
		46	33	21	104	50	29	21	85
73	G.R.	52	29	19	78	43	34	23	95
		60	14	26	77	48	30	22	87
		43	30	27	90	48	26	26	52
		45	30	25	160				
		48	28	24	92				
		50	26	24	99	46	30	24	78

Table 4 cont/d.

No.	Initial	Epithelial (%)	Endothelial (%)	Mesangial (%)	Total No.	Epithelial (%)	Endothelial (%)	Mesangial (%)	Total No.
76	W.S.	56	19	25	106	51	21	28	73
		70	15	15	82	68	68	24	81
		54	20	26	82	68	12	20	61
		59	21	20	113	68	12	20	72
		72	14	14	81	65	8	27	90
						62	21	16	68
		62	18	20	93	64	14	23	74
	D.T.	32	28	40	173	33	30	37	115
		31	32	37	171	21	38	41	117
		29	32	39	158	31	27	42	141
		35	36	29	109	32	32	36	113
		33	39	28	116				
		32	33	35	145	29	32	38	122
80	D.W.	55	30	15	82	55	28	17	72
		51	25	24	96	65	19	16	71
		52	30	18	76	52	32	16	73
		58	22	23	104				
		55	27	18	122				
		54	27	19	96	57	26	16	72
	A.G.	58	30	12	110	61	29	10	59
		63	24	13	104	62	22	16	91
		54	25	21	107	59	26	15	80
		58	22	20	103	56	34	10	99
		63	24	13	98				
		59	25	16	104	59	27	14	82

Table 4 cont/d.

No.	Initial	Epithelial (%)	Endothelial (%)	Mesangial (%)	Total No.	Epithelial (%)	Endothelial (%)	Mesangial (%)	Total No.
93	A.M.	48	32	20	100	50	30	20	664
		45	32	23	110	44	34	22	83
		52	32	16	101	55	25	20	79
		53	30	17	83	58	24	18	74
		46	28	26	104				
		49	31	20	100	52	28	20	75

Table 4 cont/d.

* Mean Values

Comment

Since the percentages rather than the absolute numbers of epithelial, endothelial and mesangial cells were compared, the difference between the epoxy-resin sections (0.5 μ thickness) and the paraffin sections (3 to 4 μ thickness) should not affect the result provided the distribution of cells is reasonably homogenous (as has already been shown). In fact the correlation was very close (see fig. 29) and the correlation coefficient (r) was 0.97. Since very accurate counts were possible on the photomicrographs of thin epoxy-resin sections, this very good correlation was regarded as evidence of the accuracy of the counts made by direct microscopy on the paraffin sections. The counts were purposely made on different occasions, and correlation of the results was deferred until all the counts had been made, thus avoiding any bias. The correlation between the mean total counts was less good (fig. 28) the correlation coefficient (r) being 0.77. It is not possible to produce sections of uniform thickness with standard microtomes and the "scatter" was understandably great since the total number of glomerular cells varies as a function of the section thickness. Also the mean total numbers were higher in the thicker paraffin sections than in the epoxy-resin sections. The correlation between the total mean numbers of glomerular cells was significantly less good than that between the percentages of epithelial, endothelial and mesangial cells; using Fischer's Z transformation, the difference in the two values for the two correlation coefficients was some six times the standard error.

II. The Variation in the Proportions of Epithelial, Endothelial and Mesangial Cells, and in the Total Number of Cells at Different Planes Through the Glomerulus

Table 5 shows the percentages of epithelial, endothelial and mesangial cells, and the total number of glomerular cells in step sections of 0.5 μ thickness cut at 10 μ intervals through two glomeruli in each of two renal biopsy specimens. One biopsy was from a child suffering from steroid-resistant nephrotic syndrome, and the other was from a child with idiopathic recurrent haematuria. The appearances in the former biopsy are of a slight proliferative glomerulonephritis, whilst in the latter the glomeruli appeared morphologically normal. Fig. 30 shows three of the photomicrographs taken from the first biopsy at various levels to compare the appearances at the equator and at the periphery of the glomerulus.

Comment

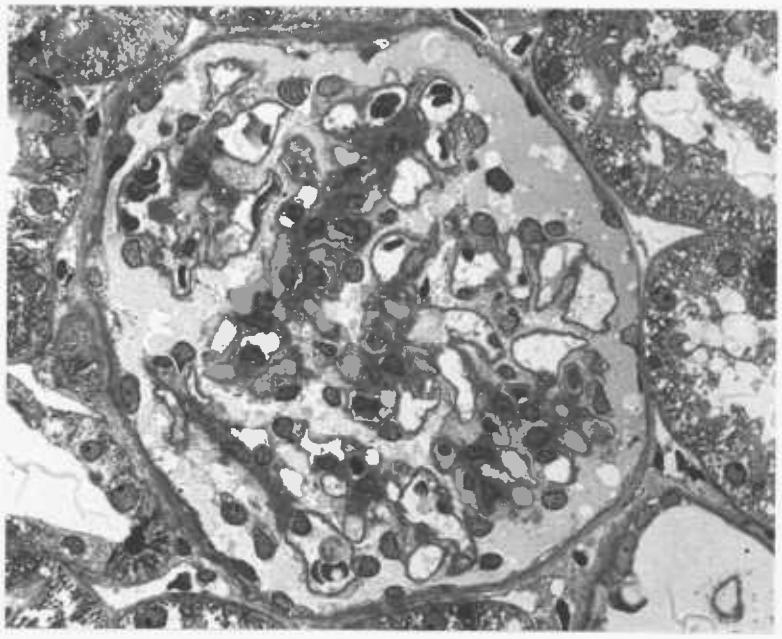
This study shows that if the primary purpose is to determine the relative numbers of epithelial, endothelial and mesangial cells, the plane of the section through the glomerulus makes surprisingly little difference. The ideal section is one made through the equator of the glomerulus, but little difference in the relative proportions of the various cell types is encountered until the periphery of the glomerulus is approached. In practive this is readily recognised by its relatively smaller circumference (see fig.30).

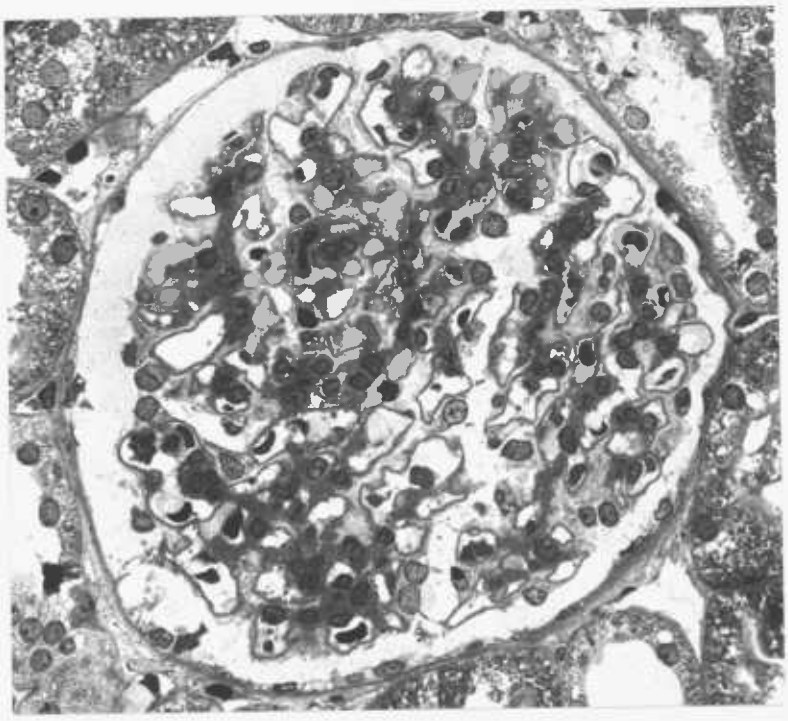
Table 5. Total and Differential Glomerular Cell Counts Made on Step Sections Through Two Glomeruli in Each of Two Renal Biopsy Specimens

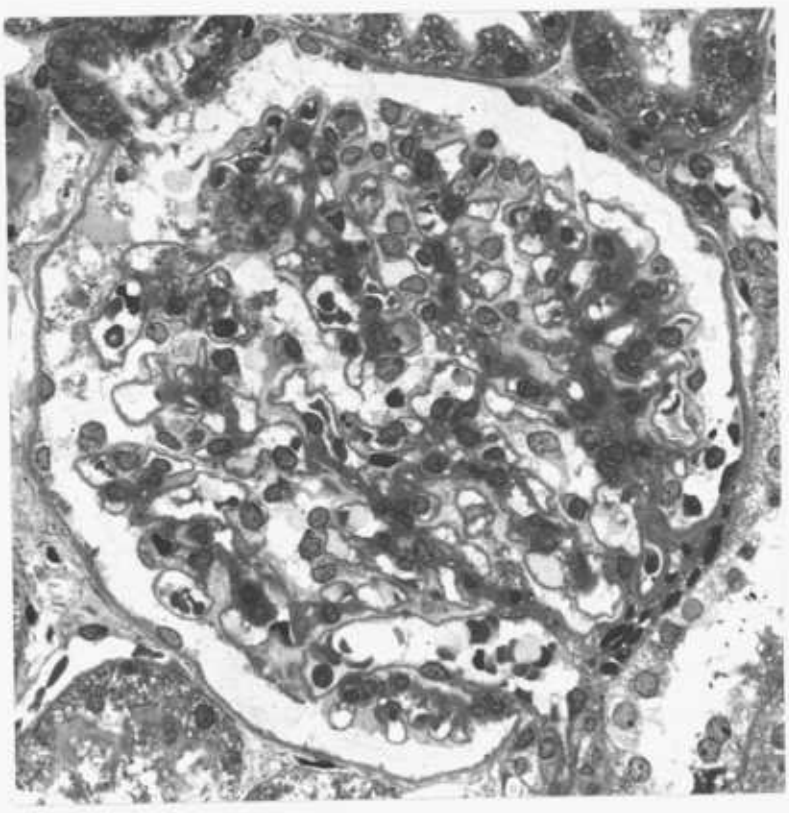
	Plane of Section	Patient 101 (C.F.)				Patient 8 (C.P.)			
		Cells				Cells			
		Epithelial (%)	Endothelial (%)	Mesangial (%)	Total No.	Epithelial (%)	Endothelial (%)	Mesangial (%)	Total No.
Glomerulus 1	0	57	25	18	72	55	25	20	68
	10μ	49	30	21	93	53	27	20	75
	20μ	40	29	31	114	50	30	20	83
	30μ	34	35	31	117	48	32	20	82
	40μ	36	31	33	116	49	33	18	87
	50μ	33	30	37	123	49	33	18	99
	60μ	37	27	36	130	45	35	20	83
	70μ	36	32	32	95	48	33	19	86
	80μ	46	29	25	106	51	29	20	83
	90μ	46	30	24	87	60	22	18	76
Glomerulus 2	0	50	31	19	113	53	29	18	72
	10μ	43	29	28	128	46	33	21	80
	20μ	41	30	29	135	48	36	16	89
	30μ	40	32	28	136	49	38	16	85
	40μ	40	32	28	130	47	34	19	96
	50μ	44	27	29	104	49	38	16	83
	60μ	47	29	24	94	50	34	16	76
	70μ	50	22	28	80	53	30	17	77
	80μ					55	27	18	69
	90μ								

Fig.30 Photomicrographs of three levels (0μ, 20μ and 60μ) from glomerulus 1 (patient 101; C.F.) from which total and differential glomerular cell counts were made. The first level (0μ) is at the periphery, and the third level (60μ) is at the 'equator' of the glomerular tuft.
Toluidine blue x 64.

Fig. 30







III. The Variation in the Proportion of Epithelial, Endothelial and Mesangial Cells in the Glomerulus With Age

In fig. 31 the percentage difference between the proportions of intracapillary (mesangial and endothelial) cells and of extracapillary (epithelial) cells in the glomeruli is compared with age. The counts were performed on sections of kidney tissue chosen from the autopsy files. In each case five glomeruli were counted. The bars represent the range in the percentage difference in each case, and the superimposed circles show the mean value. Full details of the glomerular counts are given in Table 6.

Comment

This study confirmed that there is a tendency for the proportion of epithelial (extracapillary) cells to be particularly high in younger children, although this is not invariable as is shown by the fact that this phenomenon was not seen in the glomeruli examined in the sections of kidney from the one-year-old child.

In the age range studied (1 - 12 years) the mean percentage difference between the proportions of intracapillary and extracapillary cells ranged between -17% and +10%, i.e. between an excess of extracapillary cells of 17% and an excess of intracapillary cells of 10%. In none of the glomeruli studied was the excess of intracapillary (mesangial and endothelial) cells more than 20%.

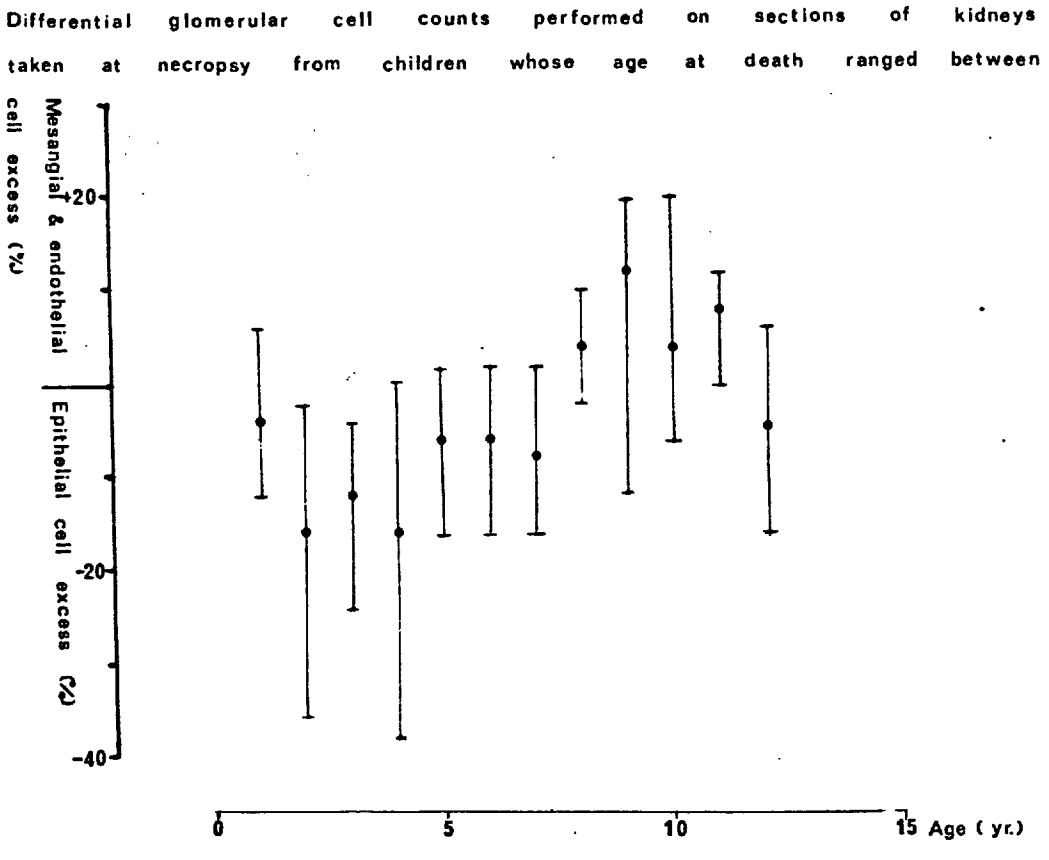


Fig. 31 In each section differential glomerular cell counts were performed on 5 randomly selected glomeruli. For each glomerulus the difference between the percentage of intracapillary (mesangial and endothelial) cells and of extracapillary (epithelial) cells was calculated. The bars in the chart represent the range of percentage differences in the 5 glomeruli examined in each case, and the dots represent the mean values. The maximum excess of intracapillary cells over extracapillary cells in any of the glomeruli examined in this age range was +20%.

Table 6 Total and Differential Glomerular Cell Counts Made on Sections of 'Normal' Kidneys Taken from Children of Various Ages at Necropsy

Autopsy Case		Glomerular Cell Counts				
		Cell Type				% Diff. between proportions of intra- and extra-capillary cells
No.	Age at death (yr.)	Epith. (%)	Endoth. (%)	Mesang. (%)	Total No.	
1	1	56	29	15	142	-12
		55	30	15	160	-10
		47	35	18	129	+ 6
		55	33	12	96	-10
		49	31	20	146	+ 2
		* 52	32	16	135	- 4
2	2	58	29	13	141	-16
		68	24	8	101	-36
		61	30	9	142	-22
		51	40	9	123	- 2
		51	34	15	96	- 2
		58	31	11	121	-16
3	3	53	32	15	154	- 6
		52	29	19	116	- 4
		62	27	11	124	-24
		60	28	12	104	-20
		55	31	14	112	-10
		56	30	14	122	-12
4	4	59	36	5	146	-18
		59	32	9	151	-18
		55	36	9	111	-10
		69	28	3	105	-38
		50	38	12	136	0
		58	34	8	130	-16
5	5	58	30	12	125	-16
		54	29	17	140	- 8
		50	34	16	162	0
		49	32	19	116	+ 2
		56	29	15	132	-12
		53	31	16	135	- 6
6	6	53	34	13	145	- 6
		58	33	9	125	-16
		53	35	12	154	- 6
		50	30	20	109	0
		49	39	12	130	+ 2
		53	34	10	127	-12

Table 6 cont/d.

Autopsy Case		Glomerular Cell Counts				
		Cell Type				% Diff. between proportions of intra- and extra-capillary cells
No.	Age at death (yr.)	Epith. (%)	Endoth. (%)	Mesang. (%)	Total No.	
7	7	56	34	10	127	-12
		58	29	13	140	-16
		51	38	11	117	- 2
		49	33	18	155	+ 2
		53	27	20	148	- 6
		54	32	14	137	- 8
8	8	48	33	19	174	+ 4
		49	34	17	125	+ 2
		45	38	17	108	+10
		46	38	16	144	+ 8
		51	30	19	113	- 2
		48	35	17	133	+ 4
9	9	43	43	14	149	+14
		54	26	20	111	-12
		40	40	20	164	+20
		41	43	16	160	+18
		44	43	13	140	+12
		44	39	17	145	+12
10	10	50	35	15	156	0
		40	44	16	129	+20
		53	32	15	152	- 6
		50	38	12	169	0
		46	38	16	124	+ 8
		48	37	14	146	+ 4
11	11	50	32	18	125	0
		44	31	25	130	+12
		44	33	23	132	+12
		47	37	16	107	+ 6
		44	39	17	124	+12
		46	34	20	123	+ 8
12	12	47	31	22	116	+ 6
		52	29	19	123	- 4
		50	33	17	150	0
		54	29	17	163	- 8
		58	26	16	113	-16
		52	30	18	133	- 4

* Mean Values

Part 2. Clinical and Histological Findings

I. Patients With Idiopathic Recurrent Haematuria

Examination of the surgical pathology files at the Hospital for Sick Children between 1967 and 1971 showed that twenty-four children in whom a clinical diagnosis of idiopathic recurrent haematuria had been made were submitted to percutaneous renal biopsy. The main clinical findings in all of these twenty-four patients are presented in Table 7. 19 (79%) were boys aged between 1 and 11 years (mean 6.2yr.) and 5 (21%) were girls aged between 3 and 11 years (mean 7.2yr.) at the onset of their symptoms. In 23 patients frank painless haematuria was the presenting symptom, and in one patient (case 16) painless haematuria was preceded by an acute nephritic syndrome characterised by transient oedema, azotaemia and hypertension. In all patients investigations including intravenous urography, and, in the majority, direct cystoscopic examination, failed to reveal any local cause of bleeding in the urogenital tract. A history of an upper respiratory tract infection preceding the first attack of haematuria had been noted in seven instances (cases 1, 2, 5, 15, 16, 18 and 23) but investigations to obtain evidence of β-haemolytic streptococcal infection, by direct bacteriological culture of a throat swab in ten instances, and by estimations of the serum A.S.O. titre in nine instances, were unrewarding apart from a marginally raised A.S.O. titre in one patient (case 18). In the majority of cases attacks of haematuria were frequent, and microscopic haematuria could be demonstrated between attacks.

Table 7. Children with Idiopathic Recurrent Haematuria. Main Clinical Findings.

Patient No.	Sex	At first attack				No. of attacks	Persist. micro. h'uria	At the time of renal biopsy				
		Age (yr.)	U.R.T.I.	A.S.O. T. (units/ml)	T.S.(βH.S.)			Duration of symptoms	B.P. (mm.Hg)	P.urea (mg/100ml)	C_c (ml/min/1.73m²)	Proteinuria (max. UA/UC ratio)
1	M	8	+	200	-ve	1	0	3m	120/75	30	98	*
2	M	10	+	200	-ve	S	+	7m	130/70	30	75	0.36
3	M	6	0	-	-	S	+	6m	95/70	22	133	0.06
4	M	4	0	-	-	S	+	3y 0m	90/50	26	164	0.50
5	F	3	+	-	-	S	+	1y 6m	110/70	30	83	0.08
6	M	9	0	-	-ve	P	+	4m	90/50	33	151	*
7	M	1	0	200	-ve	P	+	5y 0m	110/65	23	-	-
								11y 0m	-	29	109	1.8
								12y 0m	120/80	-	-	2.0
8	F	7	0	-	-	P	+	1m	110/70	34	86	0.06
9	M	8	0	-	-	P	+	9m	100/70	22	112	0.16
10	M	6	0	-	-ve	S	+	3y 0m	105/70	28	116	0.08
11	M	4	0	-	-ve	4	+	6m	130/75	-	165	0.06
12	M	10	0	-	-	S	+	5m	130/80	-	120	0.06
13	F	11	0	-	-	S	+	11m	100/60	22	149	0.17
								2y 2m	110/60	-	-	2.0
14	M	6	0	-	-	2	0	3y 0m	110/70	35	-	0.03
15	M	1	+	-	-	S	+	5y 0m	80/50	17	75	2.2
16	F	5	+	-	-	P	+	3y 0m	110/70	54	74	1.0
								4y 0m	110/70	-	-	-
17	M	4	0	-	-	S	+	4m	110/70	26	110	0.04
18	M	8	+	400	-ve	S	+	4m	90/60	32	133	0.06
19	M	11	0	200	-ve	3	+	16m	110/60	32	175	0.24
20	F	11	0	200	-ve	S	0	1y 5m	110/50	-	-	0.02
21	M	5	0	200	-ve	S	+	7m	85/55	29	265	0.09
22	M	4	0	50	-ve	S	+	1y 6m	120/70	-	-	0.17
23	M	5	+	200	-	S	+	2m	110/70	27	-	0.04
24	M	8	0	50	-	S	+	2y 6m	120/80	27	-	*

* No measurement of $^{UA}/_{UC}$ ratio but no proteinuria on repeated routine examinations

S - several; P - haematuria persistent

In one patient (case 1) only one attack of haematuria occurred prior to renal biopsy, and in another patient (case 14) only two attacks occurred over a two year period before renal biopsy. A significant family history was obtained in only one instance (case 7) in which a sibling had persistent microscopic haematuria and deafness. When first biopsied moderate proteinuria (characterised by urinary albumin/creatinine, i.e. $^{UA}/_{UC}$ ratios of 1.0 or more) was found in three patients (cases 7, 15 and 16). Slight proteinuria with $^{UA}/_{UC}$ ratios ranging between 0.1 and 1.0 was present in six patients (cases 2, 4, 9, 13, 19 and 22). In the remaining fifteen patients no significant proteinuria was found. In 12 of these $^{UA}/_{UC}$ ratios were measured as less than 0.1. In the remaining three patients (cases 1, 6 and 24), although no estimation of the $^{UA}/_{UC}$ ratios had been made, routine urinalyses on a number of occasions failed to show any proteinuria present. At the time of renal biopsy, all the patients studied were normotensive, and in none has hypertension developed subsequently during the follow-up period. In the majority no specific drug therapy was given. In three patients (cases 7, 15 and 16) all of whom exhibited moderate proteinuria and showed changes of proliferative glomerulonephritis on renal biopsy, treatment with cyclophosphamide was given. No clinical effects were noted, and repeat renal biopsies on two of them (cases 7 and 16) showed no significant alteration in the histological picture as a result of therapy.

The renal biopsy findings are summarised in table 8. The full total and differential glomerular cell counts are given in table 9. A total of 28 percutaneous renal biopsies

Table 8. Children with Idiopathic Recurrent Haematuria. Renal Biopsy Findings.

Patient No.	Biopsy	Histological Changes *	Glomerular cell counts. Difference (%) between the proportions of intra- and extracapillary cells in five glomeruli		"Glomerular Index"	Tubular Atrophy
			Range (%)	Mean (%)		
1	1	N.M.	-16 to +10	- 6	0	0
2	1	M.F.P.G.N.	- 2 to +22	+10	2	0
3	1	N.M.	-20 to + 4	- 8	0	0
4	1	M.D.P.G.N.	+14 to +28	+24	5	0
5	1	M.D.P.G.N.	0 to +46	+22	10	0
6	1	N.M.	+ 6 to +14	+10	0	0
7	1	M.D.P.G.N., F.T.A.	+20 to +30	+24	17.5	1
	2		+36 to +44	+42	24	2
	3		+36 to +48	+40	24	2
8	1	N.M.	+ 2 to +10	+ 6	0	0
9	1	N.M.	-28 to - 4	-16	0	0
10	1	N.M.	- 8 to +10	+ 2	0	0
11	1	N.M.	-24 to - 2	-12	0	0
12	1	N.M.	-10 to + 4	- 6	0	0
13	1	M.D.P.G.N.	+36 to +50	+42	20	0
	2	Early Mem. P.G.N.	-		30	0
14	1	N.M.	-14 to + 4	- 2	0	0
15	1	M.D.P.G.N., F.T.A.	+22 to +38	+30	10	1.5
16	1	M.D.P.G.N., F.T.A.	+26 to +56	+44	20.5	3
	2		+26 to +46	+36	18.5	2
17	1	N.M.	+ 8 to +18	+10	0	0
18	1	N.M.	- 8 to +10	0	0	0
19	1	M.D.P.G.N., F.G.S., F.T.A.	+10 to +60	+40	16.5	1.5
20	1	N.M.	-12 to +20	+ 4	0	0
21	1	N.M.	-14 to + 6	- 4	0	0
22	1	N.M.	-28 to -10	-20	0	0
23	1	N.M.	-10 to + 6	- 0	0	0
24	1	N.M.	-18 to + 8	- 6	0	0

* N.M. - normal morphology;
M.F.P.G.N. - mild focal proliferative glomerulonephritis;
M.D.P.G.N. - mild diffuse proliferative glomerulonephritis;
Mem.P.G.N. - membranoproliferative glomerulonephritis;
F.G.S. - focal glomerular sclerosis;
F.T.A. - focal tubular atrophy.

Table 9. Children With Idiopathic Recurrent Haematuria. Total and Differential Glomerular Cell Counts.

Patient No.	Glomerular Cell Counts				
	Epithelial cells (%)	Endothelial cells (%)	Mesangial cells (%)	Total No. of cells	Difference (%) in proportions of extra- and intracapillary cells
1	45	28	27	96	+10
	53	29	18	70	- 6
	58	27	15	67	-16
	50	3o	20	96	0
	58	20	22	87	-16
	* 53	27	20	83	- 6
2	51	31	18	112	- 2
	45	33	22	82	+10
	39	37	24	119	+22
	39	29	32	90	+22
	50	33	17	106	0
	45	33	22	102	+10
3	48	38	14	100	+ 4
	52	30	18	79	- 4
	60	24	16	86	-20
	51	31	18	116	- 2
	60	29	11	104	-20
	54	30	16	97	- 8
4	40	32	28	124	+20
	38	46	16	117	+24
	36	46	18	142	+28
	36	42	22	106	+28
	42	41	15	106	+14
	38	42	20	119	+24
5	38	35	27	124	+24
	40	36	24	107	+20
	41	30	29	91	+18
	50	28	22	78	0
	27	43	30	108	+46
	39	35	26	101	+22
6	44	42	14	110	+12
	46	31	23	96	+ 8
	47	31	22	156	+ 6
	43	30	27	87	+14
	45	39	16	75	+10
	45	35	20	105	+10

Table 9 cont/d.

Patient No.	Glomerular Cell Counts				
	Epithelial cells (%)	Endothelial cells (%)	Mesangial cells (%)	Total No. of cells	Difference (%) in proportions of extra- and intracapillary cells
7	40	31	29	181	+20
	35	42	23	167	+30
	40	40	20	116	+20
	38	39	23	146	+24
	37	38	25	183	+26
	38	38	24	155	+24
	32	36	32	188	+36
	28	35	37	240	+44
	29	28	43	181	+42
	28	34	38	182	+44
	30	34	36	152	+40
	29	34	37	188	+42
	26	45	29	103	+48
	30	26	44	174	+40
	32	34	34	149	+36
	32	37	31	148	+36
	32	38	30	125	+36
	30	36	34	140	+40
8	45	36	19	101	+10
	49	32	19	74	+ 2
	49	31	20	110	+ 2
	46	43	11	74	+ 8
	47	34	19	106	+ 6
	47	35	18	93	+ 6
9	56	29	15	106	-12
	52	31	17	75	- 4
	59	21	20	61	-18
	58	23	19	81	-16
	64	24	12	104	-28
	58	26	16	85	-16
10	45	32	23	123	+10
	54	29	17	136	- 8
	45	27	28	126	+10
	50	27	23	90	0
	52	26	22	87	- 4
	49	28	23	112	+ 2

Table 9 cont/d.

Patient No.	Glomerular Cell Counts				
	Epithelial cells (%)	Endothelial cells (%)	Mesangial cells (%)	Total No. of cells	Difference (%) in proportions of extra- and intracapillary cells
11	58	24	18	85	-16
	51	26	23	92	- 2
	53	29	18	110	- 6
	62	19	19	88	-24
	57	27	16	81	-14
	56	25	19	91	-12
12	53	23	24	132	- 6
	55	31	14	125	10
	54	30	16	122	- 8
	48	37	15	98	+ 4
	55	28	17	103	-10
	53	30	17	116	- 6
13	31	39	30	129	+38
	32	37	31	143	+36
	27	34	39	156	+46
	32	35	33	111	+36
	25	39	36	131	+50
	29	37	34	134	+42
14	48	31	21	104	+ 4
	57	28	15	98	-14
	49	32	19	112	+ 2
	52	33	15	107	- 4
	48	35	17	102	+ 4
	51	32	17	105	- 2
15	31	39	30	164	+38
	38	38	24	128	+24
	35	37	28	163	+30
	33	41	26	120	+34
	39	38	23	95	+22
	35	39	26	134	+30
16	27	24	49	127	+46
	25	34	41	119	+50
	37	29	34	104	+26
	22	33	45	125	+56
	23	34	33	96	+44
	28	31	41	114	+44
	37	33	30	89	+26
	27	43	30	135	+46
	29	35	30	85	+42

Table 9 cont/d.

Patient No.	Glomerular Cell Counts				
	Epithelial cells (%)	Endothelial cells (%)	Mesangial cells (%)	Total No. of cells	Difference (%) in proportions of extra- and intracapillary cells
	34	36	30	59	+32
	34	35	31	95	+32
	32	38	30	92	+36
17	41	34	25	104	+18
	46	39	15	74	+ 8
	45	31	24	99	+10
	45	35	20	66	+10
	46	32	22	107	+ 8
	45	34	21	90	+10
18	47	35	18	130	+ 6
	50	31	19	74	0
	54	27	19	100	- 8
	53	26	21	108	- 6
	45	35	20	88	+10
	50	31	19	100	0
19	28	40	32	109	+44
	45	40	15	99	+10
	25	44	31	106	+50
	35	37	28	96	+30
	30	40	30	110	+40
20	40	40	20	116	+20
	47	35	18	96	+ 6
	56	32	12	120	-12
	47	41	12	111	+ 6
	48	28	24	94	+ 4
	48	35	17	107	+ 4
21	50	33	17	89	0
	57	27	16	81	-14
	52	27	21	86	- 4
	55	31	14	77	-10
	47	32	21	94	+ 6
	52	30	18	85	- 4

Table 9 cont/d.

Patient No.	Glomerular Cell Counts				
	Epithelial cells (%)	Endothelial cells (%)	Mesangial cells (%)	Total No. of cells	Difference (%) in proportions of extra- and intracapillary cells
22	55	26	19	100	-10
	58	24	18	93	-16
	62	23	15	104	-24
	64	21	15	110	-28
	60	24	16	83	-20
	60	23	17	98	-20
23	47	33	20	104	+ 6
	48	30	22	109	+ 4
	50	37	13	110	0
	55	28	17	100	-10
	52	35	13	107	- 4
	50	33	17	106	-0
24	56	32	12	89	-12
	52	29	19	102	- 4
	46	38	16	69	+ 8
	59	30	11	85	-18
	54	33	13	82	- 8
	53	33	14	85	- 6

* Mean Values

were performed on the 24 patients studied. The biopsies from 16 children (cases 1, 3, 6, 8, 9, 10, 11, 12, 14, 17, 18, 20, 22, 23 and 24) showed no definite morphological abnormalities. Glomerular cell counts showed no significant differences in the proportions of epithelial, endothelial and mesangial cells present when compared with the glomerular cell counts made on sections of "normal" kidney tissue taken from autopsy cases (see fig.31). In all 16 children, the percentage differences between the proportions of intracapillary (endothelial and mesangial) cells and of extracapillary (epithelial) cells observed in the five glomeruli counted in each case, both with regard to the mean value and the range, were not significantly different from the range of values obtained for the "normal" autopsy kidney sections. In the autopsy cases these percentage differences ranged between -36% and +20%, and the mean values ranged between -14% and +10%. In the 16 children referred to in the present group the percentage differences ranged between -28% and +20%, and the mean values ranged between -20% and +10%.

In seven children (cases 2, 4, 5, 7, 15, 16 and 19) the renal biopsy showed mild proliferative changes in the glomeruli, with thickening of the mesangial stalks. The glomerular changes correspond to those of mesangial proliferative glomerulonephritis. Glomerular cell counts showed an increase in the proportion of intracapillary (endothelial and mesangial) cells. The percentage difference between the proportion of intracapillary and extracapillary cells was more than +20% (the maximum value obtained in the

glomeruli observed in sections of "normal" autopsy kidneys) in the majority of glomeruli counted in these cases, and in all but one (case 2) the mean percentage difference was also more than +20%. In case 2 the glomerular changes were very mild, the percentage difference being +22% in two glomeruli and less than +20% in the other three glomeruli counted. The "glomerular indices" in these cases ranged from 2 to 24, reflecting varying degrees of mesangial thickening. In one biopsy (case 19) very occasional sclerosed glomeruli were observed. In four biopsies (cases 7, 15, 16 and 19) there were foci of tubular atrophy (present in up to 3 out of 10 consecutive high power microscopic fields). In one child (case 13) the first biopsy showed mild proliferative changes in the glomeruli. A second biopsy was performed some fifteen months later because of an increase in the amount of proteinuria (the $^{UA}/_{UC}$ ratio rising from 0.17 to 2.6) and the finding of a persistently low serum β_{1C} globulin level. This second biopsy showed changes of an early membranoproliferative glomerulonephritis.

Comment

This study shows that the most useful clinical guide to the severity of the renal lesions present in these patients is the degree of proteinuria present. In fig.32 the mean values and the range of the percentage differences between the proportions of intracapillary (mesangial and endothelial) cells and extracapillary (epithelial) cells in the five glomeruli counted in each biopsy are correlated with the degree of proteinuria found, expressed as the maximum $^{UA}/_{UC}$ ratio

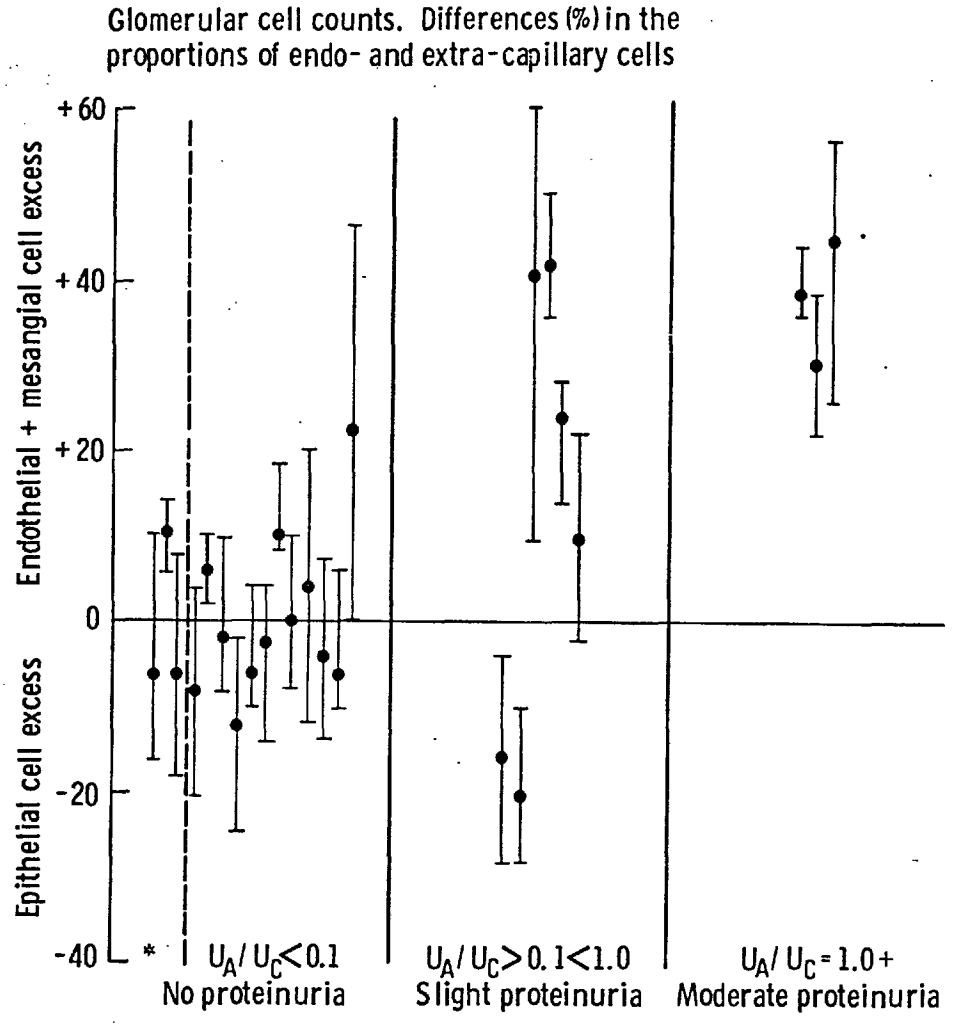


Fig. 32 Comparison of the differential glomerular cell counts made on renal biopsy specimens, with the degree of proteinuria present (expressed as the UA/UC ratio). In each biopsy differential counts were performed on 5 randomly selected glomeruli. For each glomerulus the difference between the proportions of intracapillary (mesangial and endothelial) and of extra-capillary (epithelial) cells was calculated. The bars in the chart represent the range of percentage differences in the 5 glomeruli examined in each case, and the dots represent the mean values.

obtained within three months of the date of renal biopsy (Barrett, McLaine and Soothill, 1970). $^{UA}/_{UC}$ values had been estimated at the time of biopsy in 21 patients. In one case (No. 7) in whom three biopsies were made, $^{UA}/_{UC}$ ratios were available at the time of two of the biopsies.

The patients were divided into three groups: those with moderate proteinuria ($^{UA}/_{UC}$ ratios of 1.0 or more), those with slight proteinuria ($^{UA}/_{UC}$ ratios between 0.1 and 1.0) and those with no significant proteinuria. In this last group $^{UA}/_{UC}$ ratios were less than 0.1. Three patients were also included, who, though no estimation of the $^{UA}/_{UC}$ ratio had been made, showed no proteinuria on repeated routine urinalyses.

Of the fourteen patients submitted to renal biopsy when no significant proteinuria was present, only one (case 5) exhibited significant glomerular changes. A mild diffuse proliferative glomerulonephritis was present, and the percentage difference in the proportion of intracapillary and extracapillary cells in the five glomeruli examined ranged up to +46% with a mean of +22%. In all biopsies from the other thirteen patients the percentage differences never exceeded +20% the mean values ranged only up to +10%. It is of interest that in case 5, although the $^{UA}/_{UC}$ ratio at the time of biopsy was only 0.08, slight proteinuria, with urinary protein concentrations of up to 225mg/100ml, had been recorded on a number of occasions earlier in her illness.

Of the six patients submitted to renal biopsy when slight proteinuria ($^{UA}/_{UC}$ ratio between 0.1 and 1.0) was present, two (cases 9 and 22) showed no morphological

abnormalities. Of the remaining four cases, three (cases 4, 13 and 19) showed a mild proliferative glomerulonephritis, the percentage difference in the proportions of intracapillary and extracapillary cells in the five glomeruli assessed in each case ranging up to +60% and the mean values ranging between +24 and +42%. In the remaining case in this group (case 2) mild proliferative changes were present in some glomeruli only. Of the five glomeruli in which glomerular cell counts were made, the percentage difference between the proportion of intracapillary and extracapillary cells was more than 20% in only two glomeruli (in each it was 22%) and the mean percentage difference was only +10%.

Of the three patients (cases 7, 15 and 16) with moderate proteinuria ($^{UA}/_{UC}$ ratio of 1.0 or more) when submitted to renal biopsy, all showed a diffuse proliferative glomerulonephritis. On one patient (case 7) renal biopsies were taken on two occasions. Glomerular counts made on all four renal biopsies showed percentage differences between the proportions of intracapillary and extracapillary cells of more than +20% in all the glomeruli examined. The percentage differences ranged between +22% and +56% with mean values ranging between +30% and +44%.

Histological evidence of more severe renal damage, particularly areas of tubular atrophy and associated interstitial inflammation were found only in patients with proteinuria. These changes affected the biopsies of one patient (case 19) with slight proteinuria and all three patients (cases 7, 15 and 16) with moderate proteinuria. In

addition, one patient (case 13) with slight proteinuria at the time of the first biopsy in which slight diffuse proliferative changes were present, subsequently developed a membranoproliferative glomerulonephritis which was seen in a renal biopsy made fifteen months later. It is of interest that the development of a more sinister type of glomerular lesion was coupled in this patient with an increase in the degree of proteinuria, the $^{UA}/_{UC}$ ratio rising from 0.17 to 2.0.

II. Patients With Nephritis Following Anaphylactoid Purpura

Between 1960 and 1971, 23 children with persistent urinary abnormalities following attacks of anaphylactoid purpura were submitted to percutaneous renal biopsy. The main clinical findings in these patients are shown in tables 10 and 11. 14 (61%) were boys aged between 5 and 14 years (mean 7 years) and 9 (39%) were girls aged between 4 and 12 years (mean 8.4 years) at the onset of their symptoms. In all 23 cases the clinical presentation was with a typical skin rash (Gairdener, 1948) accompanied, in the majority, by abdominal symptoms and arthritis. A preceding upper respiratory tract infection was recorded in seven instances (cases 26, 29, 30, 31, 34, 35, and 44). Investigations during the initial attacks to obtain evidence of infection with β-haemolytic streptococci resulted in direct culture of the organism from a throat swab in two out of nine patients (cases 26 and 47) and a raised serum A.S.O. titre in three out of ten patients (cases 26, 30 and 33). In seven patients (cases 28, 33, 35, 40, 43, 45 and 47) more than one attack of anaphylactoid purpura was noted, but in the remaining 15 only one attack was recorded. Haematuria was present at some stage in all 22 children, but was found during the initial illness in only 17 (83%). Frank, macroscopic haematuria was noted in seven (cases 26, 27, 30, 35, 37, 40 and 43) and in the other ten (cases 25, 28, 33, 34, 36, 39, 42, 44 and 47) haematuria was discovered on microscopy of the urine. Proteinuria was also found at some stage in all 23 patients. This was usually mild or moderate in amount, but in five children (cases 29, 30, 31, 33 and 44) proteinuria was

Table 10. Children With Anaphylactoid Purpura. Clinical Presentations

Patient No.	Sex	Presentation with Anaphylactoid Purpura									No. attacks	Total follow-up (m)	Clinical Course *	Specific Therapy
		Age (yr)	Rash	joint sympt.	U.R.T.I.	H'uria	Abdo.pain.	G.I. bleed.	T.S. (β.H.S.)	A.S.O.T.				
25	M	6	+	+	0	+	0	0	-ve	200	1	24	C.R.	St;C(18m)
26	M	14	+	0	+	+	+	0	+ve	800	1	17	P.P.;G.F.R.↓	N.
27	F	9	+	+	0	+	0	0	-ve	200	1	36	C.R.	C(12m)
28	M	5	+	0	0	+	+	+	-	-	3	27	C.R.	St.
29	F	6	+	+	+	0	+	+	-	-	2	120	C.R.	St.
30	F	7	+	+	+	+	+	+	-	400	1	18	P.P.	St;A(12m)
31	M	6	+	+	+	0	+	0	-	-	1	48	C.R.	C(15m)
32	F	7	+	+	0	0	+	0	-ve	50	1	3	P.P.H.	N
33	M	5	+	+	0	+	+	0	-	600	2	6	H.; R.F.;D.	A;C.
34	M	5	+	0	+	+	+	0	-ve	260	1	24	P.P.H.;G.F.R.↓	N
35	F	12	+	-	+	+	+	0	-	-	S	36	P.P.H.	N
36	F	12	+	0	0	+	0	0	-ve	-	1	39	C.R.	St.
37	M	5	+	0	0	+	+	0	-	-	1	120	P.P.H.	A(15m)
38	F	8	+	+	0	0	+	0	-	-	1	30	H.; R.F.	N.
39	F	4	+	+	0	0	+	0	-	-	1	84	C.R.	N.
40	M	5	+	0	0	0	+	+	-	-	S	18	R.F.; D.	St(3m)
41	M	9	+	0	0	0	+	+	-	-	1	96	P.P.H.	N.
42	M	7	+	+	0	+	+	+	-ve	70	1	25	C.R.	St(6m)
43	M	9	+	+	0	+	0	0	-	-	3	60	P.P.H.	St; C(15m)
44	F	8	+	+	+	+	0	0	-	-	1	9	P.P.	St; C.
45	M	8	+	+	0	+	0	0	-	-	3	9	P.P.H.	N.
46	M	6	+	+	0	0	+	0	-ve	50	1	2	-	N.
47	M	6	+	+	0	+	+	0	+ve	200	S	10	P.P.H.;G.F.R.↓	N.

* C.R. - clinical remission; P.P. - persistent proteinuria; P.P.H. - persistent proteinuria and haematuria; H. - hypertension; R.F. - renal failure; D - death; G.F.R.↓ - fall in glomerular filtration rate.

St. - steroids; C - cyclophosphamide; A - azathioprine; N - none. The numbers in brackets refer to the length of treatment in months (m). S - several.

Table 11. Children With Anaphylactoid Purpura. Clinical Findings at the Time of Renal Biopsy.

Patient no.	Biopsy	Interval since onset of symptoms (mth)	B.P. (mm./Hg.)	B. urea mg/100ml.	Cc (ml/min /1.73m²)	Proteinuria			Haematuria	
						mg/100ml	g/24hr.	UA/UC	Macro.	Micro.
25	1st	3	145/95	21	172	150	-	-	-	+
	2nd	23	120/70	40	114	nil	-	-	-	-
26	1st	2	130/60	29	89	250	0.6	-	+	+
	2nd	17	120/70	25	-	-	-	0.26	-	-
27	1st	9	120/80	28	130	-	0.44	-	-	-
	2nd	28	100/70	27	144	-	0.17	-	-	-
28	1st	12	90/60	30	-	-	-	-	+	+
29	1st	8	130/90	22	-	1000	-	-	-	+
30	1st	8	150/110	58	62	-	4	-	-	+
	2nd	20	130/80	28		-	0.57	-	-	-
31	1st	4	130/70	14	154	900	5.9	-	-	+
	2nd	16	110/70	-	-	nil	-	-	-	+
32	1st	1	120/60	37	-	400	-	-	-	+
33	1st	4	120/80	180	4	1000	-	-	-	+
34	1st	8	100/70	25	114	-	1.2	2.5	-	+
	2nd	24	115/60	30	-	100	-	-	-	+
35	1st	24	120/80	26	-	300	-	-	-	+
36	1st	4	120/80	32	-	300	-	-	-	+
37	1st	9	110/70	20	-	120	-	-	+	+
	2nd	80	115/75	31	200	-	2.1	-	-	+
	3rd	100	110/65	30	109	-	-	0.8	-	+
38	1st	23	190/120	195	-	100	-	2.7	-	+
39	1st	12	110/75	33	-	70	-	-	-	+
40	1st	11	160/105	76	-	900	-	-	-	+
41	1st	12	110/60	68	-	80	-	-	+	+
42	1st	7	100/70	22	-	40	-	-	-	+
43	1st	36	130/90	27	166	-	0.46	-	-	+
	2nd	48	90/60	32	185	-	0.6	-	-	+
44	1st	4	110/60	28	66	-	-	8.1	-	+
45	1st	9	110/60	21	121	-	-	0.96	-	+
46	1st	2	110/70	25	84	100	-	-	-	+
47	1st	10	100/70	31	22	-	5.6	-	-	+

heavy, and a low serum albumin concentration (less than 2.5g/100ml) was recorded.

Hypertension, with a recorded diastolic blood pressure of 90mm. of mercury or more, was seen in seven (30%) of the children studied. However, in four instances (cases 25, 29, 30 and 44) hypertension was transient, the blood pressure subsequently falling to within normal limits. In three of these cases (29, 30 and 44) transient hypertension followed treatment with corticosteroids which could reasonably be regarded as the cause. Of the other three patients with hypertension, two (cases 33 and 40) developed renal failure and died. One (case 38) presented in renal failure, and although still alive, had been the subject of only a short follow-up.

In all but ten of the children studied over-all renal function as measured by the blood urea concentration and the endogeneous creatinine clearance were consistently normal throughout the period of the study. In four patients (cases 28, 29, 30 and 42) a transient rise in the blood urea concentration was observed either during or following an attack of anaphylactoid purpura. Subsequent values in these patients were, however, normal. Two patients (cases 33 and 40) died in renal failure and one other patient (case 38) was uraemic although alive at the time of the study. In three patients (cases 26, 34, and 46), although the blood urea concentrations remained within normal limits (less than 40mg/100ml) sensitive measurements of the glomerular filtration rates using 51 Chromium - E.D.T.A. (Garnett et al., 1968) showed a

gradual and significant deterioration over the follow-up period.

Renal biopsies were performed on all 23 children at intervals ranging between one month and nine years after the onset of their symptoms. A single biopsy was performed in 15 patients (cases 28, 29, 32, 33, 35, 36, 38, 39, 40, 41, 42, 44, 45, 46 and 47); two biopsies were performed in seven patients (cases 25, 26, 27, 30, 31, 34 and 43) and three biopsies were performed in one patient (case 37). The main findings in the biopsy specimens are summarised in table 12. The full total and differential glomerular cell counts are given in table 13. In the 23 patients studied, first renal biopsies were performed at between one and 36 months (mean 9.6 months) after the onset of their symptoms. In every case histological abnormalities were present (see table 12). In one patient (case 33) the changes were of a severe proliferative glomerulonephritis with prominent "capsular crescent" formation in almost every glomerulus. In three other patients (cases 34, 38 and 47) there were changes of advanced chronic glomerulonephritis with extensive glomerular scarring, tubular atrophy and interstitial fibrosis. Because of the extent and the severity of the glomerular damage in the biopsies from these patients, it was not possible to perform differential glomerular cell counts. In the remaining 19 patients the changes in the first renal biopsies were of a proliferative glomerulonephritis. In every case differential glomerular cell counts showed an increase in the proportion of intracapillary cells in at least some of the five glomeruli counted, and in the majority all five glomeruli were abnormal. In the first biopsies from all

Table 12. Children With Anaphylactoid Purpura. Renal Biopsy Findings

Patient No.	Biopsy	Histological Changes *	Glomerular cell counts Differences (%) between the proportions of intra- and extracapillary cells in five glomeruli.		Histological Group	"Glomerular Idex"	Tubular Atrophy
			Range (%)	Mean (%)			
25	1	PGN	+28 to +58	+42	I	23.5	0
	2	PGN	+18 to +38	+26	I	10	0
26	1	PGN; FN; FTA.	+22 to +40	+30	III	26.5	3.5
	2	ACGN			IV	38	8
27	1	FPGN	+ 6 to +28	+14	I	4	0
	2	FPGN	- 6 to +24	+12	I	2.5	0
28	1	PGN	+18 to +30	+24	I	12	0
29	1	PGN; CA; FGS; FTA.	+22 to +42	+30	III	32.5	3.5
30	1	PGN; CA; FGS; FTA.	+12 to +48	+36	III	30	4
	2	PGN; CA; FGS; FTA.	+16 to +26	+22	III	33.5	4.5
31	1	PGN	+20 to +46	+38	I	18	0
	2	FGS; FTA.	-22 to + 6	- 8		12	4.5
32	1	PGN	+16 to +56	+30	I	17.5	0
33	1	PGN with C.			V	39	7.5
34	1	ACGN			III	28	3.5
	2	ACGN			III	34.5	8.5
35	1	PGN; FGS; FTA.	+24 to +48	+38	II	25.5	1
36	1	PGN; FGS; FTA.	+16 to +36	+30	II	26	2
37	1	PGN; FTA; CA.	+12 to +32	+22	II	20	1
	2	FPGN; FGS; FTA.	- 2 to +36	+16	II	12	1
	3	FGS; FTA.	- 6 to + 6	- 2		8.5	2
38	1	ACGN			IV	46.5	10
39	1	PGN	+20 to +40	+28	I	21	0
40	1	PGN; FGS; FTA.	+30 to +62	+50	II	32	1.5
41	1	PGN; FTA; CA.	+20 to +38	+30	II	32	1
42	1	PGN;	+20 to +38	+32	I	16.5	0
43	1	PGN.	+24 to +38	+34	I	14	0
	2	PGN	+22 to +48	+32	I	10	0
44	1	PGN	+10 to +48	+28	I	20	0
45	1	PGN	+34 to +50	+40	I	18	0
46	1	PGN; FGS.	+22 to +50	+35	II	20	0
47	1	ACGN			IV	38	3.5

* PGN - proliferative glomerulonephritis;
FPGN - focal proliferative glomerulonephritis;
ACGN - advanced chronic glomerulonephritis;
PGN with C - proliferative glomerulonephritis with "crescents";
FGS - focal glomerular scarring;
CA - capsular adhesions;
FN - 'fibrinoid' necrosis;
FTA - focal tubular atrophy.

Table 13. Children With Anaphylactoid Purpura. Total and Differential Glomerular Cell Counts.

Patient No.	Glomerular Cell Counts				
	Epithelial cells (%)	Endothelial cells (%)	Mesangial cells (%)	Total No. of cells	Difference (%) in proportion of extra- and intracapillary cells
25	36	29	35	121	+28
	21	36	43	129	+58
	35	27	28	158	+30
	27	29	44	133	+46
	26	30	44	200	+48
	* 29	30	41	148	+42
	41	30	29	101	+18
	31	33	36	148	+38
	36	37	27	117	+28
	38	38	24	110	+24
	40	35	25	64	+20
	37	35	28	108	+26
26	39	35	26	83	+22
	30	41	29	75	+40
	37	36	27	128	+26
	36	42	22	74	+28
	26	45	28	64	+36
	34	40	26	85	+30
27	36	41	23	113	+28
	42	33	15	106	+16
	45	24	18	105	+10
	47	32	19	110	+ 6
	46	28	20	88	+ 8
	43	32	25	102	+14
	41	35	24	127	+18
	38	32	30	116	+24
	53	27	20	119	- 6
	46	30	24	137	+ 8
	44	27	28	119	+11
	44	30	26	124	+12
28	35	36	29	188	+30
	37	25	38	128	+26
	36	44	20	117	+28
	41	40	19	119	+18
	40	38	22	114	+20
	38	36	26	133	+24

Patient No.	Glomerular Cell Counts				
	Epithelial cells (%)	Endothelial cells (%)	Mesangial cells (%)	Total No. of cells	Difference (%) in proportion of extra- and intracapillary cells
29	36	33	31	104	+28
	29	34	37	136	+42
	38	28	34	127	+24
	36	27	37	109	+28
	39	31	30	140	+22
	35	31	34	123	+30
30	28	44	28	81	+44
	44	37	19	109	+12
	34	38	30	81	+34
	26	39	35	98	+48
	31	36	33	86	+38
	32	39	29	91	+36
	40	31	29	117	+20
	38	39	23	112	+24
	42	30	28	88	+16
	37	41	22	152	+26
	40	29	31	107	+20
	39	34	27	115	+22
31	31	44	25	124	+38
	27	30	43	88	+46
	40	24	36	79	+20
	29	43	28	100	+42
	27	35	38	105	+46
	31	35	34	99	+38
	47	36	17	112	+ 6
	56	25	19	99	-12
	57	21	22	93	-14
	61	22	17	83	-22
	50	26	24	107	0
	54	26	20	99	- 8
32	32	45	24	127	+36
	42	39	19	134	+16
	22	52	26	136	+56
	42	49	9	113	+16
	37	39	24	106	+26
	35	45	20	123	+30

Table 13 cont/d.

Patient No.	Glomerular Cell Counts				
	Epithelial cells (%)	Endothelial cells (%)	Mesangial cells (%)	Total No. of cells	Difference (%) in proportion of extra- and intracapillary cells
35	38	50	12	115	+24
	34	47	19	148	+32
	28	45	27	165	+44
	27	40	33	131	+46
	26	47	27	145	+48
	31	46	23	141	+38
36	33	37	30	103	+34
	32	42	26	105	+36
	34	41	25	116	+32
	35	35	30	92	+30
	42	36	22	119	+16
	35	38	25	107	+30
37	44	27	29	112	+12
	39	31	30	131	+22
	41	25	34	122	+18
	35	39	26	145	+30
	34	41	25	140	+32
	39	32	29	130	+22
	32	38	30	141	+36
	51	31	18	140	- 2
	47	27	26	119	+ 6
	44	26	30	111	+12
	36	45	19	84	+28
	42	33	25	119	+16
	49	29	22	117	+ 2
	53	24	23	123	- 6
	53	28	19	96	- 6
	47	29	24	131	+ 6
	50	27	23	88	0
	51	27	22	111	- 2
39	30	25	45	102	+40
	33	27	40	81	+34
	37	19	44	82	+26
	40	24	36	106	+20
	40	28	32	91	+20
	36	25	39	92	+28

Table 13 cont/d.

Patient No.	Glomerular Cell Counts				
	Epithelial cells (%)	Endothelial cells (%)	Mesangial cells (%)	Total No. of cells	Difference (%) in proportion of extra- and intracapillary cells
40	24	52	24	128	+52
	25	25	50	170	+50
	24	42	34	170	+52
	35	34	31	119	+30
	19	38	43	124	+62
	25	38	36	144	+50
41	32	40	28	180	+36
	31	42	27	149	+38
	39	26	35	145	+22
	31	35	34	139	+38
	40	32	28	85	+20
	35	35	30	139	+30
42	31	44	25	122	+38
	40	33	27	135	+20
	33	35	33	132	+35
	35	36	29	120	+30
	32	33	35	147	+36
	34	36	30	131	+32
43	31	43	26	177	+38
	33	38	29	123	+34
	32	40	28	120	+36
	31	40	29	162	+38
	38	34	28	140	+24
	33	39	28	144	+34
	26	35	39	171	+48
	32	32	36	117	+36
	39	34	27	115	+22
	39	33	28	138	+22
	36	32	32	162	+28
	34	34	32	141	+32
44	45	31	24	88	+10
	26	36	38	135	+48
	37	45	18	142	+26
	39	33	28	131	+22
	34	37	29	94	+32
	36	36	28	118	+28

Table 13 cont/d.

Patient No.	Glomerular Cell Counts				
	Epithelial cells (%)	Endothelial cells (%)	Mesangial cells (%)	Total No: of cells	Difference (%) in proportion of extra- and intracapillary cells
45	30	38	32	127	+40
	33	33	34	115	+34
	33	34	33	131	+34
	28	33	39	119	+44
	25	31	44	173	+50
	30	34	36	133	+40
46	39	30	31	141	+22
	36	29	35	138	+28
	34	38	28	143	+32
	27	35	38	144	+46
	25	34	41	150	+50
	32	33	35	143	+35

* Mean Values

but one patient (case 27) the mean percentage difference in the proportions of intracapillary and extracapillary cells in the five glomeruli counted was more than +20%. In this one case the glomerular cell count was abnormal in only one of the five glomeruli, and the proliferative changes could reasonably be described as "focal". In some other biopsies (case 28, 30, 32, 36, 37 and 44) one or two of the individual glomeruli counted showed percentage differences in the proportions of intracapillary and extracapillary cells which fell within the range seen in the control counts made on autopsy cases without evidence of renal disease. In these glomeruli, however, the percentage differences invariably tended towards the upper end of the "normal" range. In some biopsies (cases 26, 29, 30, 35, 36, 37, 40 and 46) there was focal glomerular scarring. Some glomeruli were completely hyalinized and others showed segmental areas of sclerosis of the glomerular tufts which were often associated with adhesions to Bowman's capsule, and small areas of reactive proliferation of the adjacent epithelial cells.

Further renal biopsies were performed in eight of the patients studied. In seven of these a second biopsy was performed after an interval which ranged between 12 and 21 months (mean 15.4 months) and in the other patient three biopsies were performed at intervals of 71 months between the first and second biopsies and of 20 months between the second and third biopsies. In the majority (cases 25, 27, 30, 34 and 43) there was little material difference observed between the changes in the first and subsequent biopsies. In one patient (case 26) there was a significant deterioration in the second

biopsy, whilst in two other patients (cases 31 and 37), although subsequent biopsies showed a proportion of sclerosed glomeruli and foci of tubular atrophy, other glomeruli were histologically normal. The first biopsy made in both these patients had shown a diffuse proliferative glomerulonephritis. Glomerular cell counts were performed on both the first and subsequent biopsies in six patients (cases 25, 27, 30, 31, 37 and 43). The range and the mean of the percentage differences in the proportions of intracapillary and extracapillary cells in the five glomeruli studied in each case are shown in fig. 33. In two patients (cases 31 and 37) the percentage differences in all five glomeruli counted in the second and third biopsies respectively were less than +20% and fell within the "normal" range derived from the study of the control autopsy material.

The severity of changes present in the first biopsies made in each of the 23 patients studied were assessed in terms of the "glomerular index", the extent of tubular atrophy present and also in terms of a histological grouping as described previously (see p. 70). The correlation between these various parameters is shown in fig. 34. In fig. 35 the extent of the changes present in the first renal biopsy made, assessed by histological grouping is compared with the clinical outcome in each case at the time of the study.

Comment

A number of points of interest arose from this study.

The glomerular lesion in the nephritis of anaphylactoid purpura is usually described as a "focal" glomerulonephritis

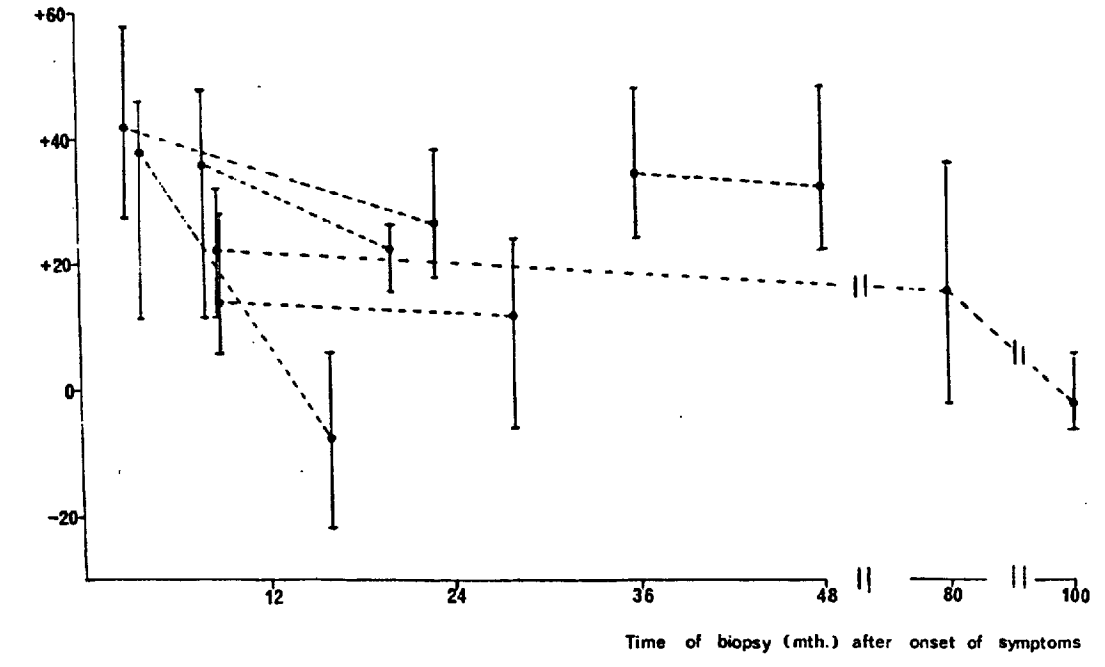


Fig. 33 In each renal biopsy, differential cell counts were performed on 5 randomly selected glomeruli. For each glomerulus the difference between the proportions of intracapillary (mesangial and endothelial) and of extracapillary (epithelial) cells was calculated. The bars in the chart represent the range of percentage differences in the 5 glomeruli examined in each case, and the dots represent the mean values. Biopsies made at different times from the same patients are joined by dotted lines.

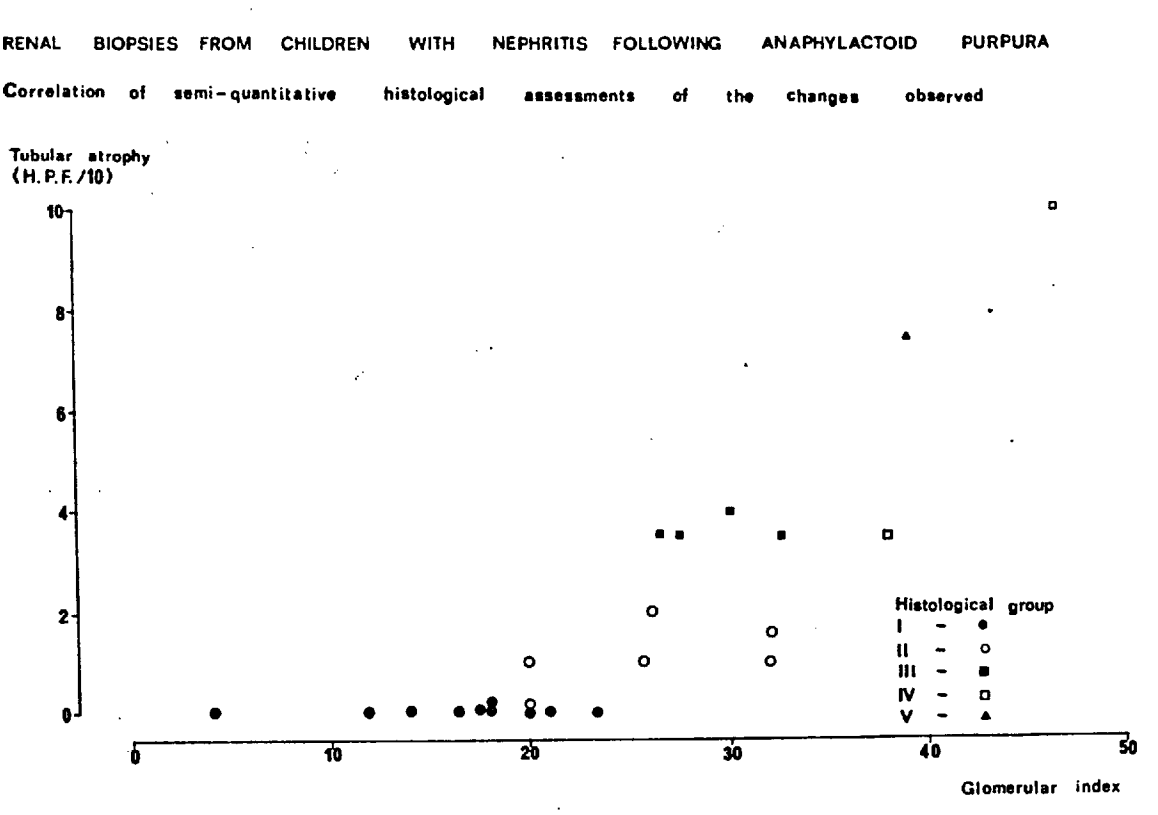


Fig. 34 This chart shows the close correlation between the two methods of semi-quantitative analysis applied to the renal biopsies from the children with anaphylactoid purpura. Each point represents the changes seen in a single renal biopsy. Those biopsies in which both glomerular and tubular damage is marked (i.e. a high 'glomerular index' and extensive tubular atrophy present) are plotted towards the top on the right. Those cases in which glomerular and tubular damage is slight (i.e. a low 'glomerular index' and little or no tubular atrophy present) appear towards the bottom on the left. Each point is also characterised in terms of the five histological groups described in the text.

CHILDREN WITH ANAPHYLACTOID PURPURA

FOLLOW-UP	HISTOLOGICAL GRADING OF THE CHANGES IN THE RENAL BIOPSY					
	Glomerulonephritis	Proliferative	Proliferative	Proliferative	Advanced chronic	With "crescents"
	Glomerular scarring	None	< 10% glomeruli	> 10% glomeruli	All glomeruli	-
	Tubular atrophy	None.	2 or less/10H. P. F.	> 2/10 H. P. F.	Extensive	-
	Complete clinical remission	○○○●●●	●	●		
	No physical abnormalities. Persistent urinary abnormalities.	○○○●	●●●	○		
	Persistent urinary abnormalities. Blood urea normal. Significant fall in G.F.R.			○○	○	
	Blood urea raised hypertension				○	
	Death		○			○

FOLLOW-UP ○ Less than 36 months ● 36 months or more

Fig. 35 This chart shows the close relationship between the subsequent clinical course and the severity of the histological changes observed on renal biopsy in the children with anaphylactoid purpura. The histological gradings shown correspond with the five histological groups described in the text.

indicating that only a proportion of the glomeruli are affected, others being morphologically normal. Counts of the proportions of the different types of cell in the glomeruli in the children studied here indicate that "focal" glomerular involvement is much less common than a diffuse glomerular lesion. The severity of the changes may, however, vary considerably from glomerulus to glomerulus in a particular biopsy, in particular glomerular scarring tends to show a focal distribution.

In all the eight patients in whom more than one biopsy was performed the indication for re-biopsying the kidney was to assess the morphological effects, if any, of treatment with immuno-suppressive drugs. In two patients (cases 31 and 37) there was morphological evidence of improvement, in one patient (case 26) definite deterioration occurred and in the remaining patients (cases 25, 27, 30, 34 and 43) the changes were essentially the same. With this small number of patients, and without controls no comment is possible with regard to the effects of immuno-suppressive therapy. However, the changes in the biopsies from cases 31 and 37 are interesting in that whilst in the first biopsies there were changes of diffuse proliferative glomerulonephritis, subsequent biopsies showed a proportion of morphologically normal glomeruli and other glomeruli either totally hyalinised or segmentally scarred. In case 37 an intermediate biopsy showed some normal glomeruli, some showing proliferative changes (as indicated by an increase in the proportion of intracapillary cells above +20% on differential glomerular cell counts) and other glomeruli which were scarred. This would seem to indicate that the

proliferative glomerular lesion seen in this condition is capable of complete resolution on the one hand, or on the other hand may heal by scarring. This latter process may result in an area of segmental scarring involving part of the glomerular tuft, or it may completely obliterate the glomerular tuft. Clinically, in these cases there was little correlation between the morphological changes in the renal biopsies and the abnormalities found in the urine. In case 31 healing of the renal lesion was accompanied by a loss of both proteinuria and haematuria. However, in case 37, despite morphological evidence of healing in the renal biopsy, both proteinuria and haematuria were still present. Furthermore, in case 25, despite the disappearance of both proteinuria and haematuria a second renal biopsy still showed changes of a diffuse glomerulonephritis.

This study also shows that useful information with regard to prognosis may be obtained from examination of the renal biopsy. Fig. 35 shows that a good correlation exists between the severity of the morphological changes seen in the first renal biopsy specimen and the eventual outcome. However, this correlation is not absolute, since one child exhibiting severe (Group III) changes in the first renal biopsy showed a complete clinical remission, with normal blood pressure and glomerular filtration rate and no abnormalities on urinalysis ten years after the onset of her symptoms. On the other hand, another child showing only Group II changes on the initial renal biopsy developed hypertension and died in renal failure 18 months after the onset of his illness.

III. Patients with a Nephrotic Syndrome

Between 1967 and 1971, 59 children with a nephrotic syndrome were successfully submitted to renal biopsy at the Hospital for Sick Children. Of these 59 patients, 42 (71%) responded to treatment with prednisolone in doses of up to 2mg/Kg body weight/day given for up to four weeks, by loss of their oedema and proteinuria (i.e. they were steroid-sensitive). The remaining 17 cases were steroid-resistent The main clinical findings in the steroid-sensitive group have been summarised on p. 142. 29 (69%) were boys aged between 1 and 12 yr. (mode 2yr.) and 13 (31%) were girls aged between 1 and 8 yr. (mode 3yr.) at the onset of their symptoms. In every case oedema was the presenting symptom, and heavy proteinuria was accompanied by hypoalbuminaemia. The selectivity of the proteinuria was measured in 23 of these patients by deriving the ratio between the clearances of $I_g G$ and albumin. Proteinuria was found to be highly selective in 12 and moderately selective in 11. In none was the proteinuria poorly selective. All the patients in this group had relapsed on three or more occasions after steroid withdrawl before renal biopsy was performed. A small minority of these children had been demonstrated to have transient microscopic haematuria, but in none was it persistent, and in none was there any history of macroscopic haematuria.

Renal biopsies were performed in these 42 steroid-sensitive nephrotic children between 1 and 10yr. (mean 3yr.) after the onset of their symptoms. The main pathological findings in this group are summarised in table 14. Full total

Table 14. Children With Steroid-sensitive Nephrotic Syndrome. Renal Biopsy Findings.

Patient No.	Biopsy	Histological Changes *	Glomerular cell counts. Differences (%) between the proportions of intra- and extra-capillary cells in five glomeruli		"Glomerular index"	Tubular atrophy
			Range (%)	Mean (%)		
48	1	M.C.	-26 to +10	- 4	0	0
49	1	M.C. (F.G.O.)	-20 to +14	-10	0	0
50	1	M.C.	-50 to -18	-22	0	0
51	1	M.C. (F.G.O.)	-28 to + 8	- 2	0	0
52	1	M.C.	-14 to 0	- 6	0	0
53	1	M.C. (F.T.D.)	-28 to +12	-12	0	0
54	1	M.C.	-14 to +12	+ 2	0	0
55	1	M.C. (F.G.O.)	- 8 to +14	+ 6	0	0
56	1	M.C. (F.G.O.)	-16 to 0	- 6	0	0
57	1	M.C.	-12 to + 2	- 6	0	0
58	1	Mes.P.G.N.	+14 to +24	+20	12	0
59	1	M.C.	-14 to +14	+ 2	0	0
60	1	M.C. (F.G.O.)	-30 to -12	-18	0	0
61	1	M.C.	- 4 to + 8	+ 2	0	0
62	1	F.G.S.	-16 to - 6	-10	10	1
63	1	F.G.S.	-16 to +20	+ 2	20	2
64	1	M.C. (F.G.O.)	-12 to +10	- 6	0	0
65	1	M.C. (F.G.O.)	-26 to + 8	- 8	2.5	0
66	1	Mes.P.G.N.	+18 to +34	+22	12	0
67	1	M.C. (F.G.O.)	- 8 to +10	0	0	0
68	1	M.C. (F.G.O.)	-12 to - 2	- 8	5	0
69	1	M.C.	0 to +12	+ 2	0	0
70	1	M.C.	-22 to + 4	-12	0	0
71	1	M.C.	-10 to + 2	- 6	0	0
72	1	M.C. (F.G.O.)	-22 to + 8	- 6	0	0
73	1	M.C.	-20 to +14	0	0	0
74	1	M.C.	-10 to + 8	- 2	0	0
75	1	M.C.	-14 to 0	- 6	0	0
76	1	M.C. (F.G.O.)	-40 to - 8	-18	0	0
77	1	M.C.	-22 to + 2	-10	0	0
78	1	M.C.	0 to +12	+ 6	0	0
79	1	M.C. (F.G.O.)	-16 to +10	- 2	2.5	0
80	1	M.C.	-10 to - 2	- 8	0	0
81	1	M.C.	-14 to + 4	- 8	0	0
82	1	M.C.	-24 to +10	- 2	0	0
83	1	M.C. (F.G.O.)	- 4 to +16	+ 6	0	0
84	1	M.C.	- 2 to +18	+ 8	0	0
85	1	M.C.	-18 to + 4	- 8	0	0
86	1	M.C.	- 8 to + 8	0	0	0
87	1	M.C.	- 8 to + 8	+ 6	0	0
88	1	M.C.	-12 to +14	+ 6	0	0
89	1	M.C.	-30 to - 6	-16	0	0

* M.C. - 'minimal changes'; M.C.(F.G.O.) - 'minimal changes' with focal glomerular obsolescence; M.C.(F.T.D.) - 'minimal changes' with focal tubular degeneration; Mes.P.G.N. - 'mesangial proliferative glomerulonephritis; F.G.S. - 'focal' glomerulosclerosis.

and differential glomerular cell counts are given in table 20. In 38 instances (90%) the biopsies showed no definite histological abnormalities in the glomeruli apart from occasional obsolescent glomeruli in some cases. Differential glomerular cell counts in these biopsies showed no significant differences in the proportions of epithelial, endothelial and mesangial cells present from those glomerular cell counts made on sections of "normal" kidney tissue taken from autopsy cases (see fig. 31). In the autopsy material the percentage differences between the proportions of intra-capillary (endothelial and mesangial) cells and of extra-capillary (epithelial) cells ranged between -36% and +20%, and the mean values between -14% and +10%. In the 38 biopsies in the present group the percentage differences between the proportions of intracapillary and extracapillary glomerular cells ranged between -40% and +18% and the mean values between -18% and +8%.

Of the four remaining biopsies in this group, two (cases 58 and 66) showed changes of mesangial proliferative glomerulonephritis and two (cases 62 and 63) showed focal glomerulosclerosis. Differential glomerular cell counts on unaffected glomeruli in the latter biopsies fell within the "normal" range, the range of percentage differences being between - 16 and +20% and the mean values being +2% and -;0%. In both the biopsies from cases 58 and 66 there were mild proliferative changes with thickening of the mesangial stalks. Glomerular cell counts showed an increase in the proportion of intracapillary cells; the percentage difference between the

proportions of intracapillary and extracapillary cells ranged between +14% and +34% with mean values of +20% amd +22% respectively.

The over-all severity of the changes present, as assessed by the "glomerular index" and by the extent of the tubular atrophy present was mild. In the great majority of the biopsies present indices for both "glomerular index" and tubular atrophy were 0. Two biopsies showed tubular atrophy in up to two out of ten randomly selected high-power fields and the glomerular index was abnormal in four biopsies with values up to 12.

The main clinical findings in the 17 patients in the steroid-resistant group are presented in tables 15 and 16. Ten were boys aged between 1 and 12 years (mean 5.4yrs.) and seven were girls aged between 2 and 8 years (mean 3.9yrs.) at the onset of their symptoms. Although all 17 patients were steroid-resistant at some stage in the course of their disease in that their proteinuria failed to disappear after four weeks therapy with prednisone or prednisolone in dosages of 2mg/Kg/day or less, one patient (case 92) responded to dosages of prednisolone in excess of 2mg/Kg/day, and also responded to cyclophosphamide in combination with steroids after failing to respond to steroids alone. A further patient (case 93) although initially steroid-sensitive later became steroid-resistant. Cyclophosphamide therapy in this patient produced a dramatic fall in urinary protein excretion, but proteinuria did not entirely disappear. Four other patients (cases 96, 97, 101 and 103) whilst showing no response to steroids, exhibits marked

Table 15. Children With Steroid-resistant Nephrotic Syndrome. Main Clinical Findings.

Patient No.	Sex	Age at onset	Total follow-up time	Specific Therapy (other than corticosteroids)	Clinical Course
90	F	2	31	Cyclophosphamide	Remission with cyclophosphamide. One relapse which also responded to steroids and cyclophosphamide
91	F	2	3	Cyclophosphamide	Clinical Remission
92	F	3	30	Cyclophosphamide	Relatively steroid-resistant only. 3 relapses whilst on steroids, responded to dosage increase. 2 further relapses responded dramatically to cyclophosphamide.
93	F	2	23	Cyclophosphamide	Initially steroid-sensitive but became steroid resistant Responded to cyclophosphamide, proteinuria all but disappearing.
94	M	2	120	Cyclophosphamide	Persistent heavy proteinuria. Unaffected by treatment. Physically fairly well; no diminution of renal function.
95	M	4	22	Cyclophosphamide	Physically well hut persistent proteinuria unaffected by treatment. No diminution in renal function.
96	M	7	48	Cyclophosphamide	Marked diminution in proteinuria during cyclophosphamide therapy.
97	F	2	53	Cyclophosphamide	Marked diminution in proteinuria during cyclophosphamide therapy.
98	M	4	29	Cyclophosphamide and Azathioprine	Persistent heavy proteinuria unaffected by treatment, latterly developed hypertension.
99	M	9	24	Cyclophosphamide and Azathioprine	Persistent heavy proteinuria unaffected by treatment. Developed hypertension
100	F	8	29	Cyclophosphamide	Persistent heavy proteinuria unaffected by treatment. Development of hypertension and renal failure.
101	M	5	30	Cyclophosphamide and Azathioprine	Marked diminution in proteinuria during immunosuppressive therapy.
102	F	8	31	Cyclophosphamide and Azathioprine	Persistent heavy proteinuria unaffected by treatment. Death in renal failure.

Table 15 cont/d.

Patient No.	Sex	Age at onset	Total follow-up time	Specific Therapy (other than corticosteroids)	Clinical Course
103	M	6	61	Cyclophosphamide	Marked diminution in proteinuria during cyclophosphamide therapy.
104	M	1	12	Cyclophosphamide and Azathioprine	Proteinuria unaffected during immunosuppressive therapy, but some weeks later, spontaneously disappeared.
105	M	12	6	Cyclophosphamide	Persistent heavy proteinuria unaffected by treatment.
106	M	6	4	None	———

Table 16. Children With Steroid-resistant Nephrotic Syndrome. Clinical Findings at the Time of Renal Biopsy.

Patient No.	Biopsy	Time since onset (m)	Investigations											
			B.P.	P. Urea (mg/100ml)	Cc (ml/min/1.73m²)	Proteinuria				Haematuria †	S. Albumin (g/100ml)	cholesterol (mg/100ml)	T.S. (β H.S.)	A.S.O.T. (units/ml)
						mg/100ml.	g/24hr.	UA/UC	Select-ivity.*					
90	1	27	-	60	81	1000	2	-	M	0	1	600	-ve	-
91	1	1	100/60	21	-	500	-	6.9	H	0	1.0	295	-	-
92	1	10	120/60	51	-	-	12	12.6	H	0	2.0	380	-	-
93	1	16	130/90	20	202	-	-	10.6	H	0	1.8	-	-	1200
94	1	14	140/90	16	-	1200	-	-	-	0	•↓	600	-	-
	2	96	100/60	27	200	-	-	11.8	-	0	-	340	-	-
95	1	3	-	19	89	-	1.3	10.8	-	0	↓	390	-	-
96	1	1	130/90	110	-	1000	2.7	-	M	0	1.7	-	-ve	800
97	1	20	110/60	15	120	-	3.9	-	P	m	1.5	645	-	100
	2	32	90/60	20	150	-	-	0.5	-	0	3.9	275	-	-
98	1	3	120/80	18	112	1000	-	-	M	m	1.5	-	-	200
99	1	8	150/90	-	115	-	-	8.8	-	M	1.8	-	-ve	200
100	1	20	140/90	77	52	-	7.8	-	-	m	2.9	480	+ve	200
101	1	20	110/70	25	193	-	4.8	8	M	m	1.8	-	-	-
102	1	4	115/85	16	135	-	3.6	20	P	m	0.9	519	-ve	50
103	1	30	140/100	23	171	-	1.2	5	-	0	0.7	578	+ve	800
104	1	3	90/70	36	234	-	4.3	29	P	m	↓	359	-ve	50
105	1	2	140/90	43	171	-	-	7.3	P	m	1.9	-	-	200
106	1	4	120/50	25	149	-	3.6	5.9	M	m	1.2	425	-ve	50

* H - highly selective; M - moderately selective; P - poorly selective.

† M - macroscopic haematuria; m - microscopic haematuria.

•↓ - low serum albumin on serum electrophoresis.

falls in urinary protein excretion whilst on immunosuppressive drugs. In none of these four patients, however, did the proteinuria entirely disappear.

All 17 cases presented with heavy proteinuria, hypoalbuminaemia and oedema. Their serum cholesterol was measured in 12 of the 17 patients and in all instances it was raised. In the majority the onset of their symptoms was insidious, although a mild preceding upper respiratory tract infection was noted in three patients (cases 90, 91 and 98). Three other patients presented with an acute nephritic syndrome, characterised by the sudden onset of oedema, haematuria and proteinuria following an upper respiratory tract infection. In one patient (case 103) bacteriological culture of a throat swab isolated β-haemolytic streptococci of Lancefield group A. A subsequent serum A.S.O. titre in this patient was more than 800 units/ml. One other patient (case 95) was an example of the nail-patella syndrome. Raised serum A.S.O. titres were recorded in three patients (cases 93, 95 and 103) at some stage during their disease. Persistent microscopic haematuria on routine urinalysis was found in 7 patients (cases 98, 99, 100, 102, 104, 105 and 106). In two patients (cases 101 and 97) microscopic haematuria was present initially, but later disappeared, and in both instances this change was associated with a marked diminution in the amount of protein excreted in the urine. Two other patients (cases 93 and 101) developed haematuria whilst on cyclophosphamide therapy and in both cases this was associated with a haemorrhagic cystitis developing as a result of the drug therapy.

In all but seven of the 17 children studied, over-all renal function as measured by the blood urea concentration and the endogenous creatinine clearance was normal throughout the period of study.

In three patients (cases 90, 92 and 96) transient azotaemia occurred either whilst on steroid therapy or during periods of hypovolaemia due to severe oedema. In four patients (cases 99, 100, 102 and 105) a progressive fall in glomerular filtration rate occurred. In two (cases 100 and 102) the fall was marked and one patient (case 102) death in renal failure occurred.

Hypertension with a recorded diastolic blood pressure of 90mm. of mercury or more was seen in nine patients (cases 93, 94, 96, 98, 99, 100, 102, 103 and 105). However, in four (cases 93, 94, 96 and 103) hypertension was transient, and followed treatment with corticosteroids. In the other five (cases 98, 99, 100, 102 and 105) the hypertension was sustained, and was severe (110mm. of mercury or more) in cases 98, 99, 100 and 102, but only mild (90mm. of mercury) in case 105.

Renal biopsies were performed on all 17 children at intervals ranging between one month and eight years after the onset of their symptoms. A single biopsy was performed in 15 patients, and two biopsies were made in the remaining two (cases 94 and 97). The main pathological findings in the biopsy specimens from the children with steroid-resistant nephrotic symptoms are summarised in table 17. Full total and differential glomerular cell counts are given in table 21. Numerically the largest group were the seven whose biopsies showed changes of

Table 17. Children With Steroid-resistant Nephrotic Syndrome. Renal Biopsy Findings.

Patient No.	Biopsy	Histological Changes *	Glomerular cell counts Differences (%) between the proportions of intra- and extra-capillary cells in five glomeruli.		"Glomerular index"	Tubular atrophy
			Range (%)	Mean (%)		
90	1	M.C. (F.G.O.)	-18 to - 4	- 8	0	0
91	1	Mes.P.G.N.	+ 4 to +56	+26	10	0
92	1	M.C. (F.T.A.)	-24 to -14	-22	0	2
93	1	F.G.S. (early)	- 6 to +10	+ 2	10	2
94	1	F.G.S. (early)	-10 to +10	0	6	0
	2	F.G.S. (early)	-12 to +20	+ 6	10	2
95	1	M.C.	-18 to + 4	- 8	0	0
96	1	F.G.S. (early)	-22 to +16	+ 4	2	0
97	1	M.P.S.G.N.	+22 to +40	+30	12	1.5
	2	M.P.S.G.N.	+ 4 to +40	+20	10	1
98	1	F.G.S. (advanced)			27	6
99	1	F.G.S. (advanced)			37	6
100	1	Mem.P.G.N.			22.5	3.5
101	1	Mes.P.G.N.	+20 to +46	+34	11	0
102	1	F.G.S. (advanced)			32.5	2
103	1	F.G.S. (early)	+ 6 to +10	+ 8	12.5	3
104	1	Mes.P.G.N.	+16 to +44	+34	14	0
105	1	Mem.P.G.N.			40	0
106	1	Mes.P.G.N.	+26 to +44	+40	27	0

* M.C. - "minimal changes";
M.C. (F.G.O.) - "minimal changes" with focal glomerular obsolescence;
M.C. (F.T.A.) - "minimal changes" with focal tubular atrophy;
F.G.S. - focal glomerulosclerosis;
M.P.S.G.N. - mild proliferative and sclerosing glomerulonephritis;
Mes.P.G.N. - 'mesangial' proliferative glomerulonephritis;
Mem.P.G.N. - membranoproliferative glomerulonephritis.

focal glomerulosclerosis (cases 93, 94, 96, 98, 99, 102 and 103). In four instances biopsy showed changes of mesangial proliferative glomerulonephritis (cases 91, 101, 104 and 106). The biopsies from three patients (cases 90, 92 and 95) showed no definite histological abnormalities in the glomeruli, apart from occasional obsolescent glomeruli in the biopsy from case 90. In two patients (cases 100 and 105) renal biopsies showed changes of membranoproliferative glomerulonephritis and in one patient (case 97) the renal biopsy changes were of a mild proliferative and sclerosing glomerulonephritis. In three of the biopsies from patients with focal glomerulosclerosis (cases 98, 99 and 102) and in the two biopsies from patients with membranoproliferative glomerulonephritis (cases 100 and 105) the extent and severity of the glomerular lesions made it impossible to perform differential glomerular cell counts. Glomerular cell counts performed on biopsies from the remaining four patients with focal glomerulosclerosis and the three patients with "minimal change" lesions showed no increase in the proportion of intracapillary cells. The percentage differences between the proportions of intracapillary and extracapillary cells in the glomeruli in these cases ranged between -24 and +20% with mean values between -22 and +8%. Glomerular cell counts made on the five other patients (four with mesangial proliferative glomerulonephritis and one with mild proliferative and sclerosing glomerulonephritis) were all abnormal with an excess of intracapillary cells present in the glomeruli. The percentage differences in the proportions of intracapillary and extracapillary cells ranged between +4 and +56% with mean values ranging between +20% and +40%

The over-all severity of the histological changes present assessed in terms of the "glomerular index" and the extent of the tubular atrophy present varied widely. The "glomerular index" ranged between 0 and 40 and tubular atrophy was present between 0 and 6 out of ten randomly selected high-power fields.

Comment

This study confirms again the excellent correlation between the presence of definite histological abnormalities in the renal glomeruli on renal biopsy and a lack of response to treatment with corticosteroids in children with a nephrotic syndrome. Table 18 shows that of the 42 children whose proteinuria disappeared with corticosteroid therapy, only four exhibited unequivocal histological abnormalities on renal biopsy. Conversely, of the 17 children failing to respond to corticosteroid all but three showed definite morphological changes in the glomeruli on renal biopsy.

In the steroid-resistant children useful prognostic information was obtained from a study of the biopsy specimen. The largest group were the seven children with changes of focal glomerulosclerosis on renal biopsy. The biopsies in four showed "early" changes with only occasional affected glomeruli, and slight, focal tubular atrophy. One of these children had been steroid-sensitive at first, and only subsequently became steroid-resistant. Three of the four children showed a marked diminution in the amount of protein excreted in the urine on treatment with cyclophosphamide. Proteinuria did not entirely disappear however, and the follow-up period in these three

Table 18. The Histological Classification of the Renal Biopsy Appearances in the Children with a Nephrotic Syndrome Compared with their Response to Steroid Therapy.

Histological Changes	Steroid-Sensitive	Steroid-Resistant
MINIMAL CHANGES		
(i) No histological abnormalities	24	1
(ii) With focal glomerular obsolescence	13	1
(iii) With focal tubular atrophy	1	1
FOCAL GLOMERULOSCLEROSIS		
(i) Focal and segmental glomerulosclerosis	1	7
(ii) Focal glomerular obliteration and significant tubular atrophy	1	0
PROLIFERATIVE GLOMERULONEPHRITIS		
(i) Diffuse exudative	0	0
(ii) Mesangial	2	4
(iii) Proliferative and sclerosing	0	1
(iv) With crescents	0	0
(v) Membranoproliferative	0	2
MEMBRANOUS NEPHROPATHY	0	0
ADVANCED CHRONIC GLOMERULONEPHRITIS	0	0
TOTAL	42	17

children ranged from only 23 to 61 months, so that the long-term outcome is not clear. The fourth child in this group, although not responding to any form of treatment has survived for ten years in relatively good health and shows no diminution in glomerular filtration rate.

The biopsies in the three remaining children with focal glomerulosclerosis showed much more severe histological changes. The majority of glomeruli were affected and there was widespread tubular atrophy and interstitial fibrosis. Follow-up in these three children ranged from 24 to 31 months. All three developed hypertension, two developed severe renal failure with marked diminution in glomerular filtration rate, and one has died.

Four children showed changes of mesangial proliferative glomerulonephritis in renal biopsies. The prognosis in these children appeared favourable despite their lack of response to steroids. In two, complete remission of their proteinuria occurred. In one of these proteinuria disappeared while on cyclophosphamide therapy. In the other the remission occurred after cyclophosphamide therapy had been terminated because of an apparent lack of effect. In another case marked diminution in the amount of protein excreted in the urine occurred during cyclophosphamide therapy although some proteinuria persisted. In the fourth patient in this group the follow-up period of four months was too short for comment.

Three children showed no definite histological changes in renal biopsies. One of these was an example of the nail-patella syndrome, and this child's proteinuria showed no response to treatment. The other two children both exhibited

features in common with the children in the steroid-sensitive group. One child was only relatively steroid-resistant, her proteinuria responding to increased doses of prednisolone. Both children responded to cyclophosphamide. In addition, in both children the urinary protein excretion showed a moderately or highly selective pattern.

Two children showed changes of membranoproliferative glomerulonephritis on renal biopsy. In neither was there any response to treatment either with steroids or cyclophosphamide. One child developed hypertension and progressive renal failure.

One child showed changes of proliferative and sclerosing glomerulonephritis on renal biopsy. The changes were mild, only occasional sclerosed glomeruli being present, in two renal biopsies performed at 20 and 32 months after the onset of the child's symptoms. The total follow-up period in this child is 53 months. Although her proteinuria has not entirely disappeared, a marked diminution in urinary protein excretion occurred during therapy with cyclophosphamide.

Table 19 illustrates the correlation found in this study between the occurrence of <u>persistent</u> microscopic haematuria and the renal biopsy changes in children with steroid resistent nephrotic syndrome. Haematuria occurred at some stage in 11 of the 17 children studied in this group. However, in one child with mild proliferative and sclerosing glomerulonephritis, microscopic haematuria was only present initially, and did not persist and in two other children haematuria developed only as a result of a haemorrhage cystitis due to cyclophosphamide therapy. The eight remaining children showed persistent microscopic haematuria developing during their

Table 19. The Correlation of the Histological Changes Seen on Renal Biopsy, and the Incidence of Persistent Haematuria in Children with Steroid-resistant Nephrotic Syndrome.

Histological Changes		Persistent Microscopic Haematuria	
		Present	Absent
Minimal changes		0	3
Focal glomerulosclerosis	"Early"	0	4
	"Advanced"	3	0
Proliferative glomerulonephritis	Mesangial	3	1
	Membranoproliferative	2	0
	Proliferative and sclerosing	0	1*
Membranous nephropathy		0	0
Advanced chronic glomerulonephritis		0	0
	Total	8	9

* Haematuria initially, later disappearing.

disease and continuing throughout. All eight showed either focal glomerulosclerosis or proliferative glomerulonephritis on renal biopsy. Three showed changes of mesangial proliferative glomerulonephritis, but the other five all showed much more sinister glomerular lesions. In three there were changes of advanced focal glomerulosclerosis, and in two there were changes of membranoproliferative glomerulonephritis, thus illustrating the potential seriousness of this clinical finding. It is also of interest that in the children with renal biopsy changes of focal glomerulonephritis, the incidence of persistent microscopic haematuria exactly matched the severity of the histological changes. The four cases with mild "early" lesions showed no haematuria, whilst all three with severe "advanced" changes had persistent microscopic haematuria.

Table 20. Children with Steroid-sensitive Nephrotic Syndrome. Total and Differential Glomerular Cell Counts.

Patient No.	Glomerular Cell Counts				
	Epithelial cells (%)	Endothelial cells (%)	Mesangial cells (%)	Total No. of cells	Difference (%) in the pro-proportions of intra- and extracapillary cells
48	53	29	18	107	- 6
	47	22	31	108	+ 6
	45	30	25	127	+10
	53	26	21	103	- 6
	63	23	14	108	-26
	* 52	26	22	111	- 4
49	60	23	17	70	-20
	54	31	15	107	- 8
	58	25	17	80	-16
	43	35	22	108	+14
	60	20	20	74	-20
	55	27	18	88	-10
50	63	20	17	106	-26
	59	26	15	125	-18
	75	16	9	144	-50
	64	22	14	137	-28
	66	22	12	100	-32
	66	21	13	122	-22
51	46	41	13	81	+ 8
	52	28	20	90	- 4
	64	22	14	69	-28
	46	35	19	102	+ 8
	47	32	21	104	+ 6
	51	32	17	89	- 2
52	55	27	18	125	-10
	57	23	20	115	-14
	49	33	18	111	+ 2
	50	33	17	91	0
	52	31	17	96	- 4
	53	29	18	108	- 6
53	57	28	15	76	-14
	52	28	20	49	- 4
	49	32	19	77	+ 2
	64	25	11	52	-28
	44	31	25	52	+12
	56	25	19	87	-12

Table 20 cont/d.

Patient No.	Glomerular Cell Counts				
	Epithelial cells (%)	Endothelial cells (%)	Mesangial cells (%)	Total No. of cells	Difference (%) in the proportions of intra- and extracapillary cells
54	44	36	20	124	+12
	50	32	18	146	0
	57	31	12	88	-14
	49	30	21	110	+ 2
	46	30	24	123	+ 8
	49	32	19	118	+ 2
55	43	30	27	207	+14
	47	28	25	175	+ 6
	45	33	22	169	+10
	54	28	18	93	- 8
	45	31	24	145	+10
	47	30	23	157	+ 6
56	53	30	17	102	- 6
	50	38	12	133	0
	58	30	12	84	-16
	51	38	11	101	- 2
	55	29	16	105	-10
	53	33	14	105	- 6
57	49	33	18	84	+ 2
	50	29	21	124	0
	58	27	15	111	-16
	51	32	17	108	- 2
	56	32	12	94	-12
	53	30	17	104	- 6
58	39	40	21	150	+21
	38	37	25	161	+24
	38	34	28	141	+24
	43	36	21	110	+14
	41	36	23	214	+18
	40	37	23	155	+20
59	45	32	23	101	+10
	52	30	18	121	- 4
	57	25	18	89	-14
	48	28	24	140	+ 4
	43	33	24	157	+14
	49	30	21	122	+ 2

Table 20 cont/d.

Patient No.	Glomerular Cell Counts				
	Epithelial cells (%)	Endothelial cells (%)	Mesangial cells (%)	Total No. of cells	Difference (%) in the proportions of intra- and extracapillary cells
60	64	23	13	82	-28
	56	28	17	89	-12
	65	27	8	87	-30
	56	26	18	68	-12
	56	27	17	66	-12
	59	26	15	79	-19
61	47	31	22	88	+ 6
	46	34	20	98	+ 8
	52	27	21	56	- 4
	51	32	17	98	- 2
	50	24	26	78	0
	49	30	21	83	+ 2
62	58	24	18	131	-16
	53	30	17	135	- 6
	55	25	20	147	-10
	54	27	19	129	- 8
	53	26	21	107	- 6
	55	26	19	130	-10
63	40	42	18	153	+20
	58	26	16	140	-16
	58	29	13	106	-16
	46	30	24	127	+ 8
	44	39	17	122	+12
	49	33	18	130	+ 2
64	56	27	17	70	-12
	45	35	20	84	+10
	53	34	13	79	- 6
	54	31	15	83	-12
	56	29	15	77	- 8
	53	31	16	79	- 6
65	50	30	20	133	0
	46	33	21	83	+ 8
	63	25	12	94	-26
	54	28	18	98	- 8
	58	26	16	100	-16
	54	28	18	101	- 8

Table 20 cont/d.

Patient No.	Glomerular Cell Counts				
	Epithelial cells (%)	Endothelial cells (%)	Mesangial cells (%)	Total No. of cells	Difference (%) in the proportions of extra- and intracapillary cells
66	44	35	21	136	+12
	33	37	30	145	+34
	34	35	31	163	+32
	43	32	25	150	+14
	41	31	28	123	+18
	39	34	27	143	+22
67	45	32	23	130	+10
	50	31	19	146	0
	39	28	23	113	+ 2
	53	28	19	113	- 6
	54	29	17	115	- 8
	50	30	20	123	0
68	55	25	20	114	-10
	56	26	18	83	-12
	51	34	15	130	- 2
	52	31	17	108	- 4
	56	29	15	117	-12
	54	29	17	110	- 8
69	48	32	20	136	+ 2
	44	38	18	156	+12
	50	34	15	97	0
	48	33	19	121	+ 4
	50	35	15	125	0
	49	33	18	127	+ 2
70	52	37	11	75	- 4
	60	28	12	100	-20
	48	32	20	92	+ 4
	60	25	15	57	-20
	61	26	13	67	-22
	56	30	14	78	-12
71	54	36	10	120	- 8
	55	31	14	94	-10
	49	33	18	67	+ 2
	51	31	18	49	- 2
	55	32	13	79	-10
	53	32	15	82	- 6

Table 20 cont/d.

Patient No.	Glomerular Cell Counts				
	Epithelial cells (%)	Endothelial cells (%)	Mesangial cells (%)	Total No. of cells	Difference (%) in the proportions of intra- and extracapillary cells
72	46	31	23	80	+ 8
	52	25	23	85	- 4
	48	28	24	105	+ 5
	57	23	20	99	-14
	61	22	17	83	-22
	53	24	23	90	- 6
73	52	29	19	78	- 4
	60	14	26	77	-20
	43	30	27	90	+14
	45	30	25	160	+10
	48	28	24	92	+ 4
	50	26	24	99	0
74	52	32	16	115	- 4
	49	33	18	120	+ 2
	46	29	25	105	+ 8
	51	30	19	112	- 2
	55	31	14	121	-10
	51	31	18	115	- 2
75	50	29	21	100	0
	52	26	22	136	- 4
	57	21	22	104	-14
	52	20	28	116	- 4
	52	30	18	86	- 4
	53	25	22	108	- 6
76	56	19	25	106	-12
	70	15	15	82	-40
	54	20	26	82	- 8
	59	21	20	113	-18
	72	14	14	81	-14
	62	18	20	93	-18
77	49	30	21	104	+ 2
	61	21	18	79	-22
	50	33	17	108	0
	56	27	17	139	-12
	61	27	12	114	-22
	55	27	18	109	-10

Patient No.	Glomerular Cell Counts				
	Epithelial cells (%)	Endothelial cells (%)	Mesangial cells (%)	Total No. of cells	Difference (%) in the proportions of intra- and extracapillary cells
78	44	36	20	160	+12
	48	32	20	145	+ 4
	50	28	22	115	0
	45	30	25	141	+10
	50	26	24	154	0
	47	30	23	143	+ 6
79	45	35	20	66	+10
	58	21	21	72	-16
	52	30	18	76	- 4
	49	26	25	97	+ 2
	50	35	15	100	0
	50	29	20	82	- 2
80	55	30	15	82	-10
	51	25	24	96	- 2
	52	30	18	76	- 4
	55	22	23	104	-10
	55	27	18	122	-10
	54	27	19	96	- 8
81	56	27	17	120	-12
	48	34	18	157	+ 4
	57	24	19	127	-14
	51	31	18	153	- 2
	55	30	15	146	-10
	54	29	17	141	- 8
82	46	28	26	90	+ 8
	56	25	19	108	-12
	62	19	19	87	-24
	45	35	20	78	+10
	46	32	22	112	+ 8
	51	28	21	93	- 2
83	45	39	16	182	+10
	50	30	20	160	0
	52	31	17	156	- 4
	46	35	19	160	+ 8
	42	37	21	154	+16
	47	34	19	162	+ 6

Table 20 cont/d.

Patient No.	Glomerular Cell Counts				
	Epithelial cells (%)	Endothelial cells (%)	Mesangial cells (%)	Total No. of cells	Difference (%) in the proportions of intra- and extracapillary cells
84	41	34	25	133	+18
	51	29	20	101	- 2
	45	35	20	110	+10
	48	31	21	91	+ 4
	45	38	17	87	+10
	46	33	21	104	+ 8
85	48	35	17	124	+ 4
	56	30	14	138	-12
	49	38	13	126	+ 2
	56	29	15	127	-18
	59	26	15	106	-18
	54	31	15	124	- 8
86	48	34	26	142	- 4
	54	24	25	111	- 8
	46	28	24	93	+ 8
	50	32	25	115	0
	51	18	22	82	- 2
	50	27	23	109	0
87	41	35	24	135	+18
	42	35	23	136	+16
	51	35	14	141	- 2
	54	26	20	85	- 8
	45	35	20	160	+10
	47	33	20	131	+ 6
88	49	32	19	110	+ 2
	43	40	17	100	+14
	56	35	9	92	-12
	45	40	15	119	+10
	43	32	24	115	+12
	47	35	18	107	+ 6
89	60	31	9	95	-20
	56	31	13	96	-12
	65	25	10	107	-30
	53	32	15	82	- 6
	56	33	11	83	-12
	58	31	11	92	-16

* Mean Values

Table 21. Children with Steroid-resistant Nephrotic Syndrome. Total and Differential Glomerular Cell Counts.

Patient No.	Glomerular Cell Counts				
	Epithelial cells (%)	Endothelial cells (%)	Mesangial cells (%)	Total No. of cells	Difference (%) in the proportions of intra- and extracapillary cells
90	53	30	17	100	- 6
	52	29	19	96	- 4
	59	26	15	76	-18
	51	30	19	107	- 2
	54	28	18	111	- 8
	* 54	29	17	98	- 8
91	39	35	26	134	+22
	40	32	28	173	+20
	48	26	26	119	+ 4
	22	40	38	144	+56
	32	36	32	156	+36
	37	33	30	145	+26
92	62	21	17	133	-24
	57	25	18	89	-14
	62	26	12	102	-24
	62	24	14	102	-24
	62	26	12	120	-24
	61	24	15	109	-22
93	48	32	20	100	+ 4
	45	32	23	110	+10
	52	32	16	101	- 4
	53	30	17	83	- 6
	46	28	26	104	+ 8
	49	31	20	100	+ 2
94	55	28	17	-106	-10
	50	30	20	90	0
	48	36	16	104	+ 4
	45	41	14	110	+10
	50	32	18	103	0
	50	33	17	103	0
	40	33	27	132	+20
	43	39	18	81	+14
	51	37	12	79	- 2
	43	42	16	136	+15
	56	34	10	87	-12
	47	37	16	103	+ 6

Patient No.	Glomerular Cell Counts				
	Epithelial cells (%)	Endothelial cells (%)	Mesangial cells (%)	Total No. of cells	Difference (%) in the proportions of intra- and extracapillary cells
95	49	37	14	62	+ 2
	55	26	19	58	-10
	58	33	9	58	-16
	59	28	13	71	-18
	48	26	26	54	+ 4
	54	30	16	61	- 8
96	42	38	20	107	+16
	63	28	11	65	-22
	41	32	27	92	+18
	48	34	18	99	+ 4
	49	33	18	70	+ 2
	48	33	19	87	+ 4
97	30	44	26	115	+40
	36	32	32	124	+28
	39	33	28	95	+22
	35	36	29	104	+30
	36	34	30	127	+28
	35	36	29	113	+30
	40	32	28	126	+20
	43	38	19	119	+14
	39	29	32	133	+22
	30	38	31	128	+40
	48	19	33	88	+ 4
	40	31	29	119	+20
101	34	32	34	152	+32
	39	35	26	119	+22
	40	32	28	133	+20
	27	43	30	111	+46
	27	43	30	126	+46
	33	37	30	128	+34
103	46	33	21	80	+ 8
	45	34	21	88	+10
	47	31	22	121	+10
	47	30	25	80	+ 6
	46	29	25	112	+ 8
	46	31	23	96	+ 8

Table 21 cont/d.

Patient No.	Glomerular Cell Counts				
	Epithelial cells (%)	Endothelial cells (%)	Mesangial cells (%)	Total No. of cells	Difference (%) in the proportions of intra- and extracapillary cells
104	32	32	36	137	+36
	28	36	36	140	+44
	35	31	34	111	+30
	42	40	18	84	+16
	28	34	34	111	+44
	33	35	32	117	+34
106	25	32	43	176	+50
	33	32	35	166	+34
	37	30	33	128	+26
	28	34	38	151	+44
	28	32	40	218	+44
	30	32	38	167	+40

* Mean Values

SECTION 4

DISCUSSION AND CONCLUSIONS

Quantitative and semi-quantitative assessments of the pathological abnormalities present in renal biopsy specimens have a number of advantages. In particular, by quantifying the extent of the morphological changes present, objective comparisons can be made between different cases and also between different biopsies made at intervals from a single patient during the course of their disease. In addition to the morphological classification of the pathological changes encountered in the renal biopsy specimens studied in this thesis, each specimen was assessed quantitatively in terms of the extent of glomerular and tubular damage present, and also where possible with regard to the number and distribution of the cells present in the renal glomeruli.

Previous reports of histological methods of quantitative assessment of the number and distribution of the nuclei in the glomeruli in renal biopsy specimens are few. Brun et al. (1965) and Suc et al. (1967) performed total counts of the glomerular nuclei seen in histological sections of renal biopsy specimens. In the study of Brun et al. counts were performed on the projected image of each glomerulus. Suc et al., on the other hand, performed glomerular counts on photomicrographs of each glomerulus. Results in each case were expressed as numbers of nuclei per unit area of the glomerulus. In addition counts of the nuclei within a standard area (in each case 625 μ^2) in different parts of a single glomerulus were performed in both studies in order to assess localised areas of nuclear aggregation and hypercellularity which might

not be apparent if the total count was 'normal'. Brun et al. (1965) were able to show in renal biopsies from patients with rheumatoid arthritis that whilst there was no over-all increase in the total number of nuclei in the glomeruli, localised areas of hypercellularity within the glomerular tufts were present. Suc et al. (1967) obtained similar results in cases of 'lobular' glomerulonephritis. They attributed the localised hypercellularity in these cases to an increase in cells in the centrilobular and intercapillary regions, but they did not make differential counts of the various types of cells involved. Such differential counts of endothelial, epithelial and mesangial cells have been made, however, by Iidaka, McCoy and Kimmelstiel (1968) and Kawano et al. (1969) on glomeruli in renal biopsy specimens from patients with diabetic glomerulosclerosis and post-acute proliferative focal glomerulonephritis, and these studies resemble most closely the methods employed in the present work. These authors performed differential counts on camera lucida drawings. In the present study differential nuclear counts were performed by direct microscopy using an oil-immersion (x100) objective. In order to establish the validity of this method as applied to the renal biopsies studied, a number of preliminary investigations were necessary.

To confirm the reliability of glomerular nuclear cell counts made by direct microscopy, comparisons were made between those counts made by this method on paraffin sections and those made on photomicrographs of epoxy-resin sections made from the same biopsies. Comparisons were made in this way on 24 biopsies, in which three or more glomeruli were

present in the epoxy-resin sections. The counts were made on separate occasions and comparisons between the two sets of results were deferred until all were completed. Very accurate glomerular nuclear counts were possible on the epoxy-resin preparations, both because of the high degree of histological detail afforded by this method of preparation (Eastham and Essex, 1969) and because photomicrographs were used for the counts. This technique could be used, therefore, as a reference method to evaluate the counts made by direct microscopy on the paraffin sections. Average values for the total number of glomerular nuclei, and for the percentages of epithelial, endothelial and mesangial cells were calculated on each case in all the glomeruli present in the epoxy-resin sections and in five glomeruli in the paraffin sections. The average total number of nuclei per glomerulus differed by the two methods, there being invariably fewer nuclei present per glomerulus in the thinner epoxy-resin sections. On the other hand, there was an extremely good correlation ($r = 0.97$) between the average percentages of epithelial, endothelial and mesangial cells per glomerulus by the two methods. This correlation was regarded as evidence of the accuracy of the counts made by direct microscopy on the paraffin sections.

The thickness of the section would seem on empirical grounds to affect the total number of cells seen in particular sections of a glomerulus. Brun et al. (1965) stated that the thickness of the section was not important up to about 9μ thickness, since the maximum diameter of the glomerular cell nuclei measured only 8 - 10μ. These authors found no

significant difference in the total nuclear counts made on three serial sections of the same glomeruli is cut at different section thickness all less than 10u. In the present study it was found that the average total glomerular nuclear counts performed on thin (0.5μ) sections was invariably less than the average total glomerular nuclear counts made on 2μ paraffin sections. However, the results of the differential counts were in any case expressed as percentages of the total count in order to avoid theoretical errors in the counts due to section thickness. With regard to the counting by direct microscopy of glomerular nuclei in paraffin sections, it was found that in order to ascertain accurately the position of a nucleus relative to the basement membrane and the mesangium, thin sections (about 2μ thickness) were necessary, a point recognised by Iidaka, McCoy and Kimmelstiel (1968). In some cases it was necessary to recut the blocks to achieve sections suitable for counting. In addition, accurate differential counts required the use of sections stained by periodic acid-Schiff with a nuclear counterstain. Such counts proved impossible in most instances on sections stained by haematoxylin and eosin.

Total and differential glomerular nuclear counts were made in each instance where the plane of the section went through the 'equator' of the glomerular tuft rather than at its periphery. In fact, by examining photomicrographs of 'step' sections taken at intervals through glomeruli in epoxy-resin-embedded material, it was shown that the plane of the section had little effect on the relative proportions of endothelial

epithelial and mesangial cells except right at the periphery of the tuft. This finding, confirmed both in morphologically normal glomeruli and in those showing mild 'proliferative' changes, was in agreement with that of Iidaka, McCoy and Kimmelstiel (1968) who performed similar counts on sections taken at different levels through glomerular tufts in their material.

Although the differential glomerular nuclear counts were performed in basically similar ways in the studies of Iidaka, McCoy and Kimmelstiel, 1969 and Kawana et al., 1969, their results regarding the relative proportions of endothelial, peithelial and mesangial cells in the glomeruli in their control studies varied quite considerably from those found in the present study. In particular, the proportions of intracapillary cells (endothelial and mesangial cells) tended to be far higher. However, the great majority of the patients from whom the biopsies were obtained in these studies were adults. It is common experience amongst those used to dealing with renal biopsies from children, particularly young children, that the proportion of epithelial cells in the glomeruli tends to be greater than in adults (White, 1970). Jones (1951), in describing various features of normal glomeruli as part of a histological study of inflammation and repair in the glomerulus, performed differential counts of the epithelial, endothelial and interstitial (mesangial) cells in the glomeruli from 12 normal kidneys taken from subjects up to 30 yr. of age. In each case, as in the present study, five representative glomeruli were examined and the average percentage of each cell type were calculated. It is not entirely clear from Jones'

description whether only nuclei in the glomerular tuft were counted, or whether the epithelial cells numbered included those lining Bowman's capsule. In any case, even in this relatively small number of cases it was clear that the percentage of epithelial cells was much greater in children in the age range dealt with in the present study. Since it was necessary both to establish the relationship of age to the relative proportions of the various types of nuclei in the glomeruli and to provide 'control' material from morphologically normal material, differential glomerular nuclear counts were performed on kidneys obtained at autopsy from children dying from non-renal causes whose age at death spanned the age range of the children in the present study. Cases dying with cyanotic congenital heart disease were also excluded in view of the finding of glomerular abnormalities in the kidneys from such patients (Spear, 1960). Differential glomerular nuclear counts were performed on five randomly selected glomeruli from each of twelve cases to cover the age range 1 - 12 years. In this age range, in none of the glomeruli studied was the excess of intracapillary (mesangial and endothelial cells) more than 20% and this figure was used to define the upper limit of the 'normal' range. In fact in the majority of glomeruli, particularly those from younger children the proportion of extracapillary (epithelial) cells tended to be the greater, the excess ranging up to 38%.

Measurements of over-all glomerular and tubular damage were made in the present study as previously in cases of persistent glomerulonephritis in adults (Risdon, Sloper and de Wardener, 1968). Glomerular lesions were assessed by

deriving a "glomerular index" from an estimation of the proportion of each of ten glomeruli occupied by abnormal material staining with periodic acid-Schiff: tubular lesions were assessed in terms of the number out of ten microscopic fields in the cortex of each specimen which contained atrophic tubules. For comparisons of such quantitative assessments of the histological abnormalities present in different renal biopsies to be valid it is necessary that the changes present in a particular specimen should be representative of the lesions present in the kidney as a whole, and that the method used to quantitate the abnormalities seen should be reproduceable.

A number of previous studies involving comparisons of randomly sampled needle biopsy specimens taken from kidneys at autopsy with conventional large wedge sections from the same specimens (Muehrcke, Kark and Pirani, 1955; Sala, 1957; and Kellow et al., 1959), have demonstrated that small biopsy specimens adequately reflect the changes present in diseases which affect the kidney diffusely. In the present study only cases in which the renal biopsy specimen obtained contained ten or more glomeruli were included. Although Muehrcke, Kark and Pirani (1955) considered biopsies with five or more glomeruli present adequate for diagnosis, both Sala (1957) and Parrish and Howe (1955) considered ten glomeruli a suitable minimum for adequate histological assessment. The 'glomerular index' on which the quantitative assessments of glomerular damage were made, was therefore based on an examination of ten glomeruli in each case.

In the previous study (Risdon, Sloper and de Wardener, 1968) the reproducibility of the histological techniques used here was established by comparing the gradings made after presenting each specimen, labelled with a random number, 'blind' to two independant observers. In the present study the biopsies were examined by only one person. However, each specimen was examined 'blind' without knowledge of any pertinent clinical or other data, on two separate occasions some weeks apart. In each instance the gradings on ten consecutive glomeruli were added together and a mean calculated between the findings on the two separate occasions, this figure being the 'glomerular index'. Similarly the number of consecutive high-power microscopic fields (x 40 objective) in the cortex of each specimen out of ten examined in which unequivocal tubular atrophy was seen was assessed on the two separate occasions, and where these assessments differed a mean was taken. Agreement on both the glomerular and the tubular assessments on the two occasions was in fact close (see tables 1 and 2). The largest difference between the sums of the glomerular gradings in a particular case was seven and the mean difference only 1.24. With regard to the tubular changes, assessment of the number of fields showing tubular atrophy differed by three out of ten in two specimens, by two out of ten in ten specimens and by one out of ten in twelve specimens. In the remaining 91 biopsies agreement was complete.

Of the renal biopsies studied in this thesis, the largest group were from children presenting with a nephrotic syndrome. Classification of the morphological changes encountered in the biopsy specimens was based on that used by

the International Study of Kidney Disease in Children (Churg et al., 1970).

41 of the 59 biopsies studied in this group showed no definite glomerular abnormalities on light microscopy, beyond the presence of occasional obsolescent glomeruli. The lack of structural changes was reflected in the semi-quantitative assessments of glomerular and tubular damage. The "glomerular indices" were 0, apart from four cases in which occasional obsolescent glomeruli were present where the indices ranged between 2.5 and 15. In none of the biopsies was any tubular atrophy seen. Differential glomerular cell counts performed on these specimens all fell within the "normal" range encountered in the control material. It is of interest that on reviewing the original histological reports made on these 41 biopsy specimens, all by pathologists experienced in examining renal biopsies, histological diagnoses of "focal" proliferative glomerulonephritis had been made on 11 (27%). Differential glomerular cell counts made on these cases differed in no significant way from the remainder on whom a diagnosis of normal glomerular morphology ("minimal changes") had been made, thus illustrating the value of this technique in improving diagnostic accuracy. As in the recent studies of Churg et al., 1970 and of White et al., 1970, a very good correlation was found in the present series between the lack of glomerular abnormalities in the renal biopsy specimens and remission of proteinuria with steroid therapy. This correlation was not, however, absolute. Four of the 42 children responding to steroid therapy showed definite morphological abnormalities

in the glomeruli, whilst three of the 17 children resistant to steroid therapy lacked histological changes in the glomeruli. Of the three steroid-resistant cases one was an example of the nail-patella syndrome (Brixey and Burke, 1950). Descriptions of the histological features of this type of hereditary nephritis are sparse, but a few histological studies exist (Brixey and Burke, 1950; Muth, 1965 and Leahy, 1966). Renal biopsies performed early in the course of the disease in one patient by Muth, and in another by Leahy showed only,relatively minor changes analogous to those encountered in the patient studied here. However, it is clear from the autopsy studies that the renal disease is progressive and that a chronic glomerulonephritis ultimately develops. The remaining two cases, though steroid-resistant by strict definition, had features in common with the steroid-sensitive group. One child was only relatively steroid-resistant, three relapses which occurred during steroid therapy responding to dosage increases, and both children responded dramatically to treatment with cyclophosphamide, completely losing their proteinuria. In both cases differential urinary protein clearance estimations showed the highly selective pattern normally seen in steroid-sensitive patients.

Of the four steroid-responders with glomerular abnormalities, two showed changes in the renal biopsy interpreted as mesangial proliferative glomerulonephritis, and two showed focal glomerulosclerosis. In the biopsies from the two children with mesangial proliferative glomerulonephritis, the glomerular changes were mild, with only a slight excess of mesangial matrix. Semi-quantitative assessments of the

histological abnormalities seen showed a "glomerular index" of 12, and no tubular atrophy present in both biopsy specimens. Differential glomerular cell counts showed an average excess of intracapillary over extracapillary cells of +20% and +22%, but in both cases some of the five glomeruli studied showed differential cell counts within the "normal" range. Churg et al. (1970) also noted very occasional cases of mesangial proliferative glomerulonephritis who apparently responded to steroid therapy. The significance of such cases is difficult to assess. Clinically the two patients studied here differed in no way from the other cases of steroid-sensitive nephrotic syndrome with no glomerular abnormalities on renal biopsy. In both cases relapses occurred on a number of occasions after steroid withdrawl.

In the biopsies from the two steroid-sensitive cases with focal glomerulosclerosis, many glomeruli were normal, but a proportion were completely fibrosed and others showed segmental areas of sclerosis of the glomerular tufts. In both instances occasional areas of tubular atrophy were also present. Semi-quantitative histological assessments of these structural changes showed "glomerular indices" of 10 and 20 and tubular atrophy in one and two out of ten high-power (x 40) microscopic fields examined. Differential glomerular cell counts performed on five unscarred glomeruli in these two biopsies showed counts within the "normal" range. Both Churg et al. (1970) and White et al. (1970) also noted occasional cases with similar histological changes who responded, at any rate initially, to treatment with steroids, although, as in the present series, the

majority of such cases were steroid-resistant. The long term outcome in cases of focal glomerulosclerosis responding to steroid therapy is difficult to assess. One case studied here although initially steroid-sensitive later became resistant to steroid therapy, and it is therefore conveivable that the two steroid-sensitive cases might eventually follow a similar course. Follow-up studies (©hurg et al., 1970; White et al. 1970) indicate that this type of glomerulopathy tends to be progressive, leading eventually to renal failure. Of the ten patients with this lesion studied here, two developed severe renal failure and one of these died. However, the rate of progress seems to be extremely variable, for example, one patient was followed for ten years and although his proteinuria was resistant to steroid therapy, he remained in fairly good health, and whilst unequivocal focal glomerulosclerosis was found on renal biopsy, the changes were mild and showed no progression on serial biopsies made at intervals throughout his illness. On the other hand, another child with focal glomerulosclerosis developed renal failure and died only 31 months after the onset of her symptoms. It is also unclear whether focal glomerulosclerosis is always progressive or whether the course of the disease may arrest either spontaneously or as a result of treatment. An important finding in the present series was that three children with focal glomerulosclerosis resistant to steroids showed a marked diminution in the amount of protein excreted in the urine as a result of treatment with cyclophosphamide. All three showed only mild, focal lesions on biopsy, the majority of the glomeruli

being morphologically normal. "Glomerular indices" in these three cases ranged between 2 and 10, and tubular atrophy was present in between 0 and 2 out of ten randomly selected high-power (x 40) microscopic fields. Differential glomerular cell counts on five unscarred glomeruli in each biopsy fell within the normal range. On the other hand, three other children unresponsive either to steroids or cyclophosphamide showed severe and extensive glomerular lesions with widespread tubular atrophy. "Glomerular indices" in these three biopsies ranged between 27 and 37 and tubular atrophy was present in between 2 and 6 out of ten high-power (x 40) microscopic fields. Differential glomerular cell counts on these three biopsies were impossible owing to the extensive glomerular scarring present. All three became hypertensive during the follow-up period; two developed severe renal failure, and one died.

The cases of focal glomerulosclerosis appear to form a much less homogeneous group than the steroid-sensitive nephrotics with "minimal change" lesions, and in view of the varied clinical course it would seem unwise to regard these cases as necessarily forming a distinct group. Although the sclerotic lesions represent irreversible damage to the glomeruli, and their progressive development accounts for the gradual progress of the disease to renal failure, there is no reason on morphological grounds to suggest that a single pathogenic mechanism is responsible for them.

The present study confirms the prognostic importance of <u>persistent</u> haematuria in children with nephrotic syndrome. Persistent haematuria, usually detected only on microscopic

examination of the urine deposit was found only in children with definite glomerular abnormalities on renal biopsy. Of the 17 children resistant to steroid therapy, eight showed microscopic haematuria which persisted throughout the follow-up period. Of these, three showed changes of mesangial proliferative glomerulonephritis, but the remainder showed more sinister glomerular lesions. In three, renal biopsy showed advanced focal glomerulosclerosis, and in the other two, changes of membranoproliferative glomerulonephritis were present.

Since the majority of unselected children with a nephrotic syndrome are steroid-responsive and a very good correlation has been shown between this clinical finding and the presence of a "minimal change" type of lesion on renal biopsy, the advisability of routine renal biopsy as part of the investigation of these children has been questioned (Cameron, 1968). It seems reasonable to manage such cases by a trial of steroid therapy in the first instance, and to biopsy only those patients who fail to respond, when information of prognostic significance can almost always be obtained. For example, cases of mesangial proliferative glomerulonephritis, in whom the prognosis is likely to be good can be separated from cases of membranoproliferative glomerulonephritis, or focal glomerulonephritis where the reverse is liable to be true. There is, however, evidence that the high-dosage steroid therapy normally given to these children may be especially liable to lead to the complications of steroid treatment in children with membranoproliferative glomerulonephritis (Cameron et al., 1970). Where other clinical evidence exists

which might lead to the suspicion of this diagnosis, such as a history of preceding acute nephritic syndrome, a persistently low serum β_{1C} globulin level, or the presence of persistent haematuria, early renal biopsy before a trial of steroids would seem to be the best course.

Many previous reports of the histological changes observed in renal biopsy specimens from patients with nephritis following anaphylactoid purpura, or with idiopathic recurrent haematuria, have stressed the focal nature of the glomerular lesions encountered, describing in many of these cases a proliferative glomerulonephritis in which only a proportion of the glomeruli are involved, the remainder being spared (Vernier et al., 1958; Bergstrand, Bergstrand and Bucht, 1960; Ross, 1960; Dodge et al., 1961, Kobayashi, Wada and Kifune, 1961; Joekes, Pugh and Prior, 1962; Bodian et al., 1965; Todd and Bouton, 1966; Ferris et al., 1967). Differential counts of the glomerular cells in the renal biopsy specimens from children with these clinical presentations in the present study suggest that a focal glomerulonephritis is comparitively uncommon. Of the 47 biopsies studied from children with recurrent haematuria and anaphylactoid purpura, differential glomerular cell counts were performed in 43, and of these proliferative glomerulonephritis was confirmed in 27. In each instance differential counts were performed on five randomly selected glomeruli and in 16 (59%) an abnormal excess (more than +20%) of intracapillary cells was found in all five glomeruli examined. Of the remaining 11 cases, in six instances, a similar abnormal excess of intracapillary cells was found in all

but one of the five glomeruli examined, and in these cases in the differential cell count in the remaining one glomerulus invariably showed an excess of intracapillary cells towards the upper end of the range found in "normal" controls. These results suggest that the "focal " glomerulonephritis described in such patients is comparatively uncommon, and particularly since no clinical differences were apparent between patients showing a "diffuse" as opposed to a "focal" glomerulonephritis, it seems reasonably to conclude that the difference is one of degree, and does not warrant a separate category of classification. It is important to note, however, that in the cases of nephritis following anaphylactoid purpura, glomerular scarring may often exhibit a "focal" and "segmental" distribution, sparing some glomeruli and involving only parts of the glomerular tufts in others. Assessment of the severity and extent of such glomerular scarring is of considerable value in assessing the prognosis in individual patients as is shown by the good correlation found in this study between the histological changes in the renal biopsy specimens and the clinical outcome. This view of the value of renal biopsy in patients with anaphylactoid purpura is supported by the study of Meadow et al., (1971).

A striking finding in the present study, with regard to the children presenting with symptomless haematuria, is the high proportion of patients (16 out of 24; 67%) showing no morphological abnormalities on renal biopsy. Differential glomerular cell counts performed on the biopsy specimens from these patients showed no significant differences from those in "normal" controls, a finding which is in striking contrast to

the description by Bodian et al. (1965) of a focal and segmental glomerulonephritis in all the biopsy specimens examined from a similar group of children.

A more recent clinico-pathological study of the renal biopsy changes in children with symptomless haematuria (Glasgow, Moncrieff and White, 1970) however, showed a similar high proportion of patients (37 out of 47) in whom the glomeruli were essentially morphologically normal. These authors also drew attention to the higher proportion of children with proteinuria as well as symptomess haematuria showing a proliferative glomerulonephritis on renal biopsy. In the present study comparisons between the presence of proliferative changes in the glomeruli, based on differential glomerular cell counts, and the degree of proteinuria present at the time of biopsy, based on the urinary albumin/creatinine ratio, showed an even more striking correlation. Only one of the eight patients exhibiting proliferative changes on renal biopsy showed no significant proteinuria at the time of biopsy, and even in this patient, proteinuria had been demonstrated previously. Many previous reports have emphasised the usually very good prognosis in children presenting with idiopathic recurrent haematuria (Wyllie, 1955; Ross, 1960; Livaditis and Eriksson, 1962; Ayoub and Vernier, 1965; McConville, West and McAdams, 1966; Johnson and Schuler, 1969), and since the likelihood of finding pathological changes can be accurately predicted by measuring urinary protein excretion, there seems little justification for submitting such children to renal biopsy unless significant proteinuria can be demonstrated.

Children with both proteinuria and haematuria appear to form a less homogeneous group. One of the patients studied here developed membranoproliferative glomerulonephritis, and two of the patients studied by Glasgow, Moncrieff and White (1970) exhibited similar changes. It seems clear that a minority of patients with membranoproliferative glomerulonephritis may present with recurrent haematuria. All three of the patients in addition showed significant proteinuria and persistently low β_{1C} globulin levels. Renal biopsy appears to be the best way of differentiating these more severe glomerular lesions. It is probable that the prognosis is good in the children showing only a mild proliferative glomerulonephritis on renal biopsy. None showed any evidence of renal functional impairment over the follow-up period. However, in three of the biopsies studied in this group there was histological evidence of more permanent damage, in the form of foci of tubular atrophy and sclerosed glomeruli. In none were the changes extensive and whilst they may merely be the result of scarring after an initially more severe form of glomerulonephritis, it is also possible that they represent a progressive glomerular disease analagous to focal glomerulosclerosis. It would seem important to submit such cases to extended follow-up, possibly with repeated renal biopsies, in order to clarify the outcome.

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Urographic Changes in Acute Papillary Necrosis in the Rat

Summary: Intravenous high-dosage urography was performed in rats which had renal papillary necrosis induced with ethyleneimine or renal tubular necrosis produced with mercuric chloride. In both groups, nephrograms were abnormally persistent. In animals treated with ethyleneimine dense selective opacification of the necrotic renal pyramid occurred. It is suggested that this selective opacification may be a valuable radiological sign of recent renal papillary necrosis.

INTRODUCTION

Until recently intravenous urography was thought to be contraindicated in acute renal failure because it was considered dangerous and of little clinical value (Emmett, 1964). By using contrast media such as diatrizoate in high doses, however, information of diagnostic and prognostic significance may be obtained by this technique in renal failure (Schwartz *et al.*, 1963; Chrispin, 1968; Mahaffy *et al.*, 1968; Kelsey Fry and Cattell, 1970).

The present study stems from finding urographic abnormalities in infants suffering acute renal damage following severe gastroenteritis (Chrispin *et al.*, 1970). In these three patients intravenous urography revealed nephrograms that were abnormally persistent and prolonged selective opacification of the renal pyramids. Repeat urograms on two of these infants some months later showed loss of renal papillae. To investigate this matter further we decided to induce renal papillary necrosis in rats to see if similar urographic changes could be produced. Our findings confirm that persistent opacification of the renal papilla occurs as a result of renal papillary necrosis. No such abnormal opacification of the renal papilla occurs where there is acute tubular necrosis alone.

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Materials and Methods

White male Wistar rats weighing between 250 and 300 g. were used throughout. The animals were divided into two groups and treated as follows:

Group A (eight animals).—Ethyleneimine (between 0·6 and 1·0 ml./kg. body weight of a 1% solution) was administered by intraperitoneal injection to each animal once only.

Group B (seven animals).—Mercuric chloride (between 1·5 and 1·8 mg./kg. body weight of the mercuric ion) was administered by intraperitoneal injection to each animal once only.

Several untreated animals were used as controls.

Radiological studies were performed at one- and four-day intervals after injection of the nephrotoxins. The animals were anaesthetized with sodium pentobarbitone (25 mg./kg. body weight) given by intraperitoneal injection and augmented by ether inhalation. Hypaque 65% (sodium and methylglucamine diatrizoate, Bayer) in a dose of between 2 and 2·4 ml./kg. body weight was injected into a femoral vein, radiographs being taken at 1, 3, 10, and 45 minutes after the injection. In addition further films were made at 90 minutes in some of the animals in group A. Each animal was subsequently killed and the kidneys were removed. These were examined macroscopically and then fixed in buffered formalin (*p*H 7·0) for 48 hours and secondarily fixed in mercuric chloride-formalin for six hours. The tissue was then embedded in paraffin wax and sections of 3-4 μ thickness were prepared. These were stained by Ehrlich's haematoxylin and eosin, periodic acid-Schiff, and Martius scarlet blue (M.S.B.).

Pathological Changes in the Kidneys

Animals Treated with Mercuric Chloride

Macroscopic.—The kidneys were swollen and there was pronounced yellowish-brown pallor of the cortex, with considerable congestion of the medulla.

Microscopical.—In animals killed one and four days after administration of mercuric chloride extensive areas of acute tubular necrosis were observed in the renal cortex. These principally affected the proximal convoluted tubules and were more pronounced in the rats killed at four days; the changes did not affect the tubules in the medulla. The glomeruli were engorged but showed no other abnormality.

Animals Treated with Ethyleneimine

Macroscopic.—The kidneys were swollen and intensely congested. In animals killed one day after injection of ethyleneimine transection of the kidney showed the renal pyramid to be dark red and haemorrhagic. At four days necrosis of the pyramid was obvious macroscopically. The renal papilla was a pale creamy buff colour, and at the base of the pyramid there was a sharp line of demarcation outlining the necrotic zone.

Microscopical.—The changes were confined to the medulla; the cells lining the collecting ducts and loops of Henle were frankly necrotic in the kidneys removed both one and four days after the administration of ethyleneimine. At four days the majority of cells were desquamated, and at the edge of the infarcted area there was a zone of acute inflammation.

Radiological Findings

These are summarized in the Table. In rats treated with mercuric chloride excretion of contrast medium was not visible one day after injection of the nephrotoxin. At four days a nephrogram of even intensity was evident in films taken one minute after injection of diatrizoate, and this was still easily seen in the films taken at 45 minutes; no opacification of the ureter or urinary bladder was observed. This was in contrast to the findings in the untreated animals, in which the nephrogram had faded at 45 minutes.

In rats treated with ethyleneimine there was a persistent nephrogram as well as selective opacification of the renal pyramid. This was apparent in films taken at 3 and 10 minutes after injection of diatrizoate, was intensified at 45 minutes, and persisted until 90 minutes in some animals. Filling of the urinary bladder was occasionally seen in films taken at 10 minutes, but more often it was not apparent until 45 minutes.

Discussion

Our radiographic findings in the control rats and in those treated with mercuric chloride are essentially similar to those of Chamberlain and Sherwood (1967). In the animals treated with ethyleneimine there was also a persistent nephrogram, but dense selective opacification of the renal pyramid also occurred (Fig. 1). These results are very similar to those we described in young infants with renal impairment following acute gastroenteritis (Chrispin *et al.*, 1970), and it seems reasonable, particularly in view of later urograms showing loss of renal papillae, to attribute the radiological appearances in these children to recent papillary necrosis.

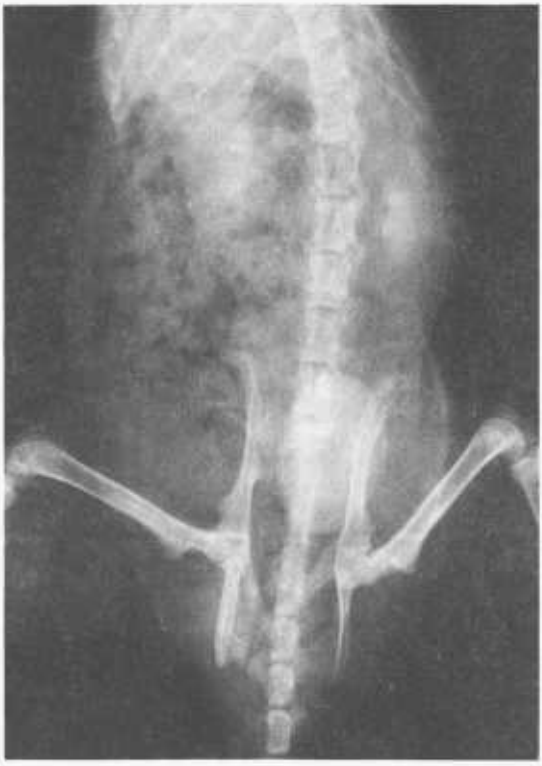

FIG. 1.—Film taken 45 minutes after injection of diatrizoate in an animal 4 days after treatment with ethyleneimine. Note selective opacification of renal pyramid.

The selectively opacified area in the urogram exactly matches the zone of necrosis in the renal pyramid seen in the excised kidney (Fig. 2). The increased density in the pyramid reflects an increase in the amount of contrast medium it contains. The necrotic tubules are denuded of lining epithelial cells, thus increasing their cross-sectional area and the volume of contrast medium they contain at any given moment. The persistent nephrogram effect in the ethyleneimine-treated animals probably results from a "leakage" of contrast medium from the damaged tubules in the pyramid into the interstitial tissues, where a constant reabsorption into the blood vessels at the edge of the necrotic zone would lead to its subsequent recirculation and re-excretion by the kidney (see Fig. 3).

Summary of Radiological Findings

	Days After Injection of Nephrotoxin	Time Radiographs Taken After Injection of Diatrizoate				
		1 Minute	3 Minutes	10 Minutes	45 Minutes	90 Minutes
Normal		Nephrogram	Nephrogram with outlining of tip of renal papilla and ureter	Nephrogram beginning to fade. Filling of bladder	Nephrogram gone. Faint outlining of renal papilla and ureter	
Mercuric chloride	1	No excretion of contrast medium				
	4	Nephrogram	Uniform nephrogram with no filling of ureter	Persistent nephrogram	Nephrogram still apparent. No filling of bladder	
Ethyleneimine	1	Nephrogram	Nephrogram	Nephrogram persisting. Some selective opacification of renal pyramid	Selective opacification of renal pyramid. Filling of bladder	
	4	Nephrogram	Nephrogram with slight selective opacification of renal pyramid	Nephrogram persisting. Selective opacification of renal pyramid	Nephrogram persisting. Dense selective opacification of renal pyramid. Filling of urinary bladder	Opacification of renal pyramid still present though faded. Filling of urinary bladder

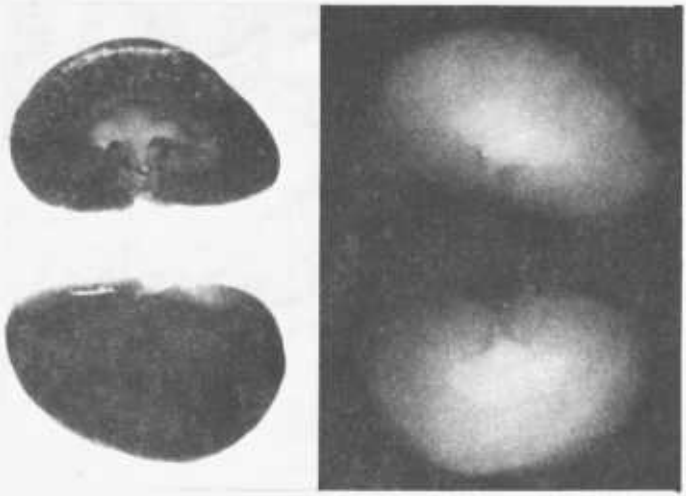

FIG. 2.—Left: Isolated rat kidneys 4 days after administration of ethyleneimine. Kidneys were removed and *x*-rayed after animal had received diatrizoate by intravenous injection. Right: Transected kidney from same animal. Note how area of opacification in radiograph corresponds to area of necrosis in renal pyramid.

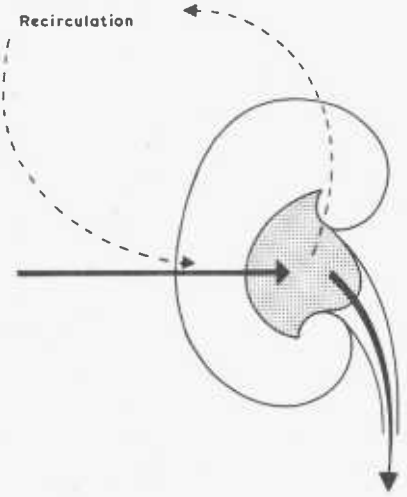

FIG. 3.—Diagram showing continuous recirculation of proportion of contrast medium excreted by kidney after it has "leaked" from damaged tubules in necrotic renal papilla.

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RELATIONSHIP BETWEEN RENAL FUNCTION AND HISTOLOGICAL CHANGES FOUND IN RENAL-BIOPSY SPECIMENS FROM PATIENTS WITH PERSISTENT GLOMERULAR NEPHRITIS

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Summary The pathological changes in 50 percutaneous renal-biopsy specimens from patients with persistent glomerular nephritis have been analysed. Techniques were developed which allowed glomerular and tubular lesions to be assessed quantitatively. A highly significant correlation was found between the extent of tubular damage and creatinine clearance, plasma-creatinine, and the ability to concentrate and to acidify the urine. A less significant correlation was found between the extent of the structural changes in the glomeruli and creatinine clearance, plasma-creatinine, and the ability to concentrate the urine. In some patients with inconspicuous glomerular changes but severe tubular damage, the creatinine clearance was grossly reduced; conversely, in some patients with extensive glomerular changes but little or no tubular damage, the creatinine clearances were normal. It is concluded that in persistent glomerular nephritis the glomerular filtration-rate is more affected by changes in the tubules than by changes in the glomeruli. Degenerative changes in the renal arterioles tended to occur significantly more often with increasing age and with increasing diastolic blood-pressure. The severity of the structural changes in the glomeruli

correlated significantly with the presence of hyaline arteriolar change; the extent of tubular atrophy correlated significantly with the presence of fibroelastic intimal hyperplasia.

Introduction

ALTHOUGH the diagnostic value of renal biopsy is well recognised, few attempts have been made to correlate the extent of the histological abnormalities with the changes in renal function. We have sought to make such a correlation in 50 cases of persistent glomerular nephritis; in 38 cases, renal-biopsy specimens contained unequivocally abnormal eosinophilic glomerular deposits. It was necessary for the purpose of this analysis to develop techniques which would allow the separate assessment of tubular and glomerular lesions. Applying these techniques, we found that the greatest diminution in renal function tended to be in patients with extensive tubular atrophy, irrespective of the severity of the glomerular lesions.

Patients

Selection of Cases

Of a series of 290 patients on whom renal biopsy was done persistent glomerular nephritis (de Wardener 1961) was diagnosed in 70, all of whom had proteinuria. None of these 70 patients had acute glomerular nephritis, and in none was there clinical or histological evidence of systemic lupus erythematosus, polyarteritis nodosa, diabetes mellitus, or amyloidosis. 20 of these 70 cases were excluded from the present study, 9 because no estimation had been made of the creatinine clearance, and 11 because fewer than ten glomeruli were found in the biopsy specimen. The remaining 50 cases were divided into two categories.

Category 1.—38 patients, renal-biopsy specimens from whom contained unequivocally abnormal eosinophilic deposits in the glomerular tufts. This change was associated in 14 instances with some increase in the number of nuclei in parts of the glomerular tufts (" proliferative change "), and in 2 instances there was also an increase in the number of capsular cells, forming capsular " crescents ". 20 of these patients had a nephrotic syndrome, with heavy proteinuria, hypoalbuminæmia, and œdema.

Category 2.—12 cases of the nephrotic syndrome with normal or only very slightly abnormal glomeruli were termed " minimal change glomerular nephritis ".

Techniques

Renal Biopsy

Renal-biopsy specimens were obtained by percutaneous puncture, using a Vim-Silverman needle, and immediately fixed in Heidenhain's susa. They were embedded in paraffin wax and serial sections were prepared between 3 and 4 μ in thickness. Sections were routinely stained by Mayer's

hæmalum and eosin, periodic-acid/Schiff, van Gieson's mixture, Mallory's phosphotungstic-acid/hæmatoxylin, Moore's elastic stain, and by congo-red and cresyl-violet for amyloid. Other stains were used where indicated.

Renal Function

Endogenous creatinine clearance.—Creatinine clearance was estimated twice in all 50 cases. Twenty-four-hour specimens of urine were collected, and single samples of blood were taken during the period of collection. Creatinine concentrations in plasma and urine were estimated with an ' AutoAnalyzer '.

Ability to concentrate the urine.—The ability to concentrate the urine was measured in 38 of the patients. 5 units of vasopressin tannate in oil were given in the late afternoon, after which all fluids were withheld until the completion of the test the following morning. During the period of the test, each specimen of urine was collected separately until 8 A.M. of the following morning, when the bladder was emptied for the last time. The osmolality of the urine was measured with a Fiske osmometer. The highest osmolality obtained was taken to represent the maximum ability to concentrate the urine.

Ability to acidify the urine.—The ability to acidify the urine was measured in 36 patients. Urine samples were collected for estimation of the urinary pH between four and six hours after the oral administration of ammonium chloride (0·1 g. per kg. body-weight). In those patients who had a plasma bicarbonate concentration of less than 20 meq. per kg., ammonium chloride was not given, but the pH of a number of random urine samples was measured. The lowest pH achieved was taken to represent the maximum ability to acidify the urine.

Urinary protein excretion.—Twenty-four-hour urinary protein excretion was estimated in 43 of the patients studied by a conventional biuret estimation, after protein precipitation with trichloroacetic acid in a final dilution of 10%.

Assessment of the Histological Changes

Changes in the glomeruli.—In each biopsy specimen, ten consecutive glomeruli were examined for the presence of eosinophilous deposits. Each glomerulus was graded from 1 to 5 in terms of the extent of these deposits. Where deposits entirely obliterated the glomerulus a grading of 5 was made. If deposits occupied more than three-quarters of the glomerulus, the grading was 4; if more than half but less than three-quarters, the grading was 3; if more than a quarter but less than a half, the grading was 2; if less than a quarter, the grading was 1. If the glomerulus was considered normal the grading was 0. These gradings were made independently by two of us on slides which had been labelled with random numbers. In each instance the gradings on ten consecutive glomeruli were added together, and a mean was calculated between the findings of the two observers, the figure being termed the " glomerular index ". These indices ranged between 0 and 47. The largest difference between the sums observed in individual cases was 8, and the mean difference was 2·8. The glomerular index was less than 10 in the biopsy specimens of the 12 patients with

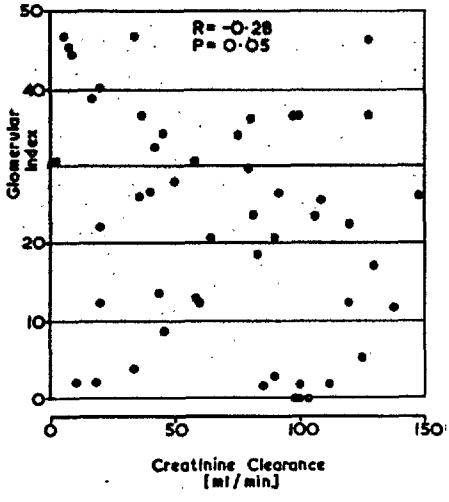

Fig. 1—Correlation between creatinine clearance and glomerular index.

minimal change glomerular nephritis. In 2 of these there was a single glomerulus which had undergone fibrous obliteration.

Changes in the tubules.—Atrophic tubules were characterised by significant thinning of the epithelial cells. Such tubules were sometimes dilated by eosinophilic colloid but more often they were narrowed, empty, and separated from each other by interstitial connective tissue which was usually collagenous. In some instances the nuclei of tubular epithelium were pyknotic, and in other instances adjacent tubular epithelial cells contained mitotic figures. The extent of tubular atrophy was assessed by two of us independently. Ten consecutive microscopic fields were examined in the cortex of each specimen, with the aid of a $\times 40$ objective. The extent of tubular damage was assessed by the number of microscopic fields in which there was unequivocal tubular atrophy. When the assessments of the two examiners differed, a mean was taken. Agreement was close: the number of fields in which the two examiners considered atrophy to be present differed by 3 out of 10 in 4 specimens, by 2 out of 10 in 11, and by 1 out of 10 in 15.

Changes in the arterioles.—The two most conspicuous lesions were " hyaline " change and fibroelastic intimal hyperplasia. Numerous sections from each specimen were examined for the presence or absence of these lesions. Hyaline deposits stained red with eosin and periodic-acid/Schiff and yellow with van Gieson. These lesions were usually chronic (orange to phosphotungstic-acid/hæmatoxylin) and thus probably lacking in " fibrinoid " material.

Statistical Analysis

The results of correlations relating " glomerular index " and tubular atrophy with various tests of renal function were

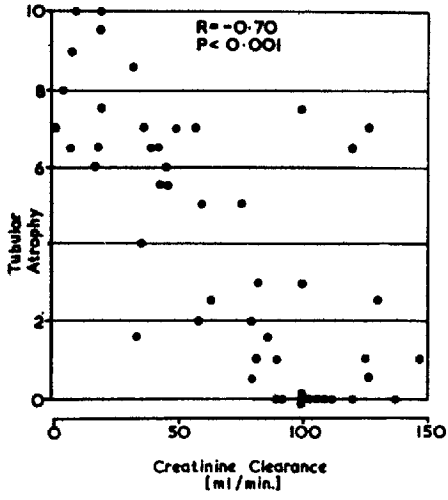

Fig. 2—Correlation between creatinine clearance and tubular atrophy (per 10 microscope fields).

analysed by determining Spearman's coefficient of rank correlation (*Documenta Geigy* 1962).

The relationships between the arteriolar changes observed in the biopsy specimens, and the patients' age and diastolic blood-pressure, glomerular index, and tubular atrophy were analysed by Wilcoxon's test for two samples (*Documenta Geigy* 1962).

Results

(*Figs. 1 and 2, tables I and II*)

There was a statistically highly significant correlation between the extent of tubular damage and creatinine

TABLE I—SPEARMAN'S RANK CORRELATIONS

Comparison between	Spearman's coefficient of rank correlation (*R*)	P
Glomerular index and plasma creatinine ..	+0·35	0·02>P>0·01
Tubular atrophy and plasma creatinine ..	+0·77	P<0·001
Glomerular index and ability to concentrate the urine	−0·45	0·01>P>0·001
Tubular atrophy and ability to concentrate the urine	−0·54	P=0·001
Glomerular index and ability to acidify the urine	+0·014	Not significant
Tubular atrophy and ability to acidify the urine	+0·501	0·01>P>0·001
Glomerular index and urinary protein excretion	−0·22	Not significant
Tubular atrophy and urinary protein excretion	−0·01	Not significant

clearance, plasma-creatinine, or ability both to concentrate and to acidify the urine.

There was a less significant correlation between the extent of the structural changes in the glomeruli and creatinine clearance, plasma-creatinine, and ability to concentrate urine.

The extent of the glomerular changes was not significantly correlated with either ability to acidify urine or with urinary protein excretion; nor was the extent of tubular atrophy correlated with urinary protein excretion.

Both fibroelastic intimal hyperplasia and hyaline

TABLE II—WILCOXON'S TESTS

Comparison	Wilcoxon's test for two samples (no. in samples)		P
Hyaline change and age	19	31	0·05
Fibroelastic intimal hyperplasia and age	22	28	0·01
Hyaline change and diastolic blood-pressure	19	31	0·02
Fibroelastic intimal hyperplasia and diastolic blood-pressure	22	28	0·05
Hyaline change and glomerular index	19	31	0·01
Fibroelastic intimal hyperplasia and glomerular index	22	28	0·1
Hyaline change and tubular atrophy	19	31	0·1
Fibroelastic intimal hyperplasia and tubular atrophy	22	28	0·05

arteriolar change were significantly correlated with age and with diastolic blood-pressure.

There was a significant correlation between fibroelastic intimal hyperplasia and the extent of tubular atrophy, and between the presence of hyaline arteriolar change and the extent of glomerular changes, but no correlation between fibroelastic intimal hyperplasia and the extent of glomerular changes, nor between hyaline arteriolar change and the extent of tubular atrophy.

Discussion

This report stems from an analysis of biopsy findings and renal function in 290 patients with diseases of the kidney. It became clear that renal function was more closely related to the structural changes observed in the tubules than to those seen in the glomeruli. The validity of this impression was tested in 50 of the 70 cases of persistent glomerular nephritis, 20 patients being excluded for reasons given above.

Glomerular lesions were assessed by the derivation of a glomerular index from an estimation of the proportion of each of ten glomeruli occupied by abnormal eosinophilous

deposits: tubular lesions were assessed in terms of the number out of ten microscopic fields in the cortex of each specimen which contained atrophic tubules. In a disease such as glomerular nephritis, in which the lesions tend to affect the kidney diffusely, the changes present in specimens obtained by needle biopsy are probably reasonably representative of those in the kidney as a whole (Kellow et al. 1958).

We found a close and statistically highly significant correlation between the extent of the tubular changes and the degree of functional deterioration measured by creatinine clearance, plasma-creatinine concentration, and the ability of the kidney to concentrate and to acidify the urine. Comparable data in persistent glomerular nephritis are lacking, although in their series of cases of acute glomerular nephritis, Hutt, Pinniger, and de Wardener (1958) noted the possible significance of tubular lesions.

In our series there was a significant correlation between the extent of the eosinophilous deposits in the glomeruli and the creatinine clearance, plasma-creatinine, and the ability of the kidney to concentrate the urine. The correlations between these functions and the glomerular changes were less striking than those found between the extent of the tubular lesions and alterations in renal function. For example, the correlation coefficient (r) between creatinine clearance and the glomerular index was $-0{\cdot}28$ (fig. 1), whereas that between creatinine clearance and tubular atrophy was $-0{\cdot}7$ (fig. 2). Again, the correlation coefficient (Spearman) between plasma-creatinine and glomerular index was $+0{\cdot}35$, while that between plasma-creatinine and tubular atrophy was $+0{\cdot}77$ (table I).

These findings suggest that in glomerular nephritis the structural damage in the tubules may have much more effect on the glomerular filtration-rate than does the structural damage in the glomeruli. This conclusion is not so paradoxical as it might at first appear, since it seems likely that creatinine clearance, while an adequate measure of the glomerular filtration-rate in the normal kidney, is not necessarily so in the abnormal kidney. For example, lissamine-green, which like creatinine and inulin tends to pass down normal renal tubules without being reabsorbed or secreted, is in fact reabsorbed from the damaged proximal convoluted tubules in rats rendered anuric with mercuric chloride (Bank et al. 1967). It is possible, therefore, that in some renal diseases the apparent glomerular filtration-rate, as measured with creatinine, may be much lower than the true rate of filtration through the glomeruli, because creatinine is

leaking from the lumina of tubules into the interstitial spaces and into the blood.

The relation of the creatinine clearance to both the extent of tubular atrophy and changes in the glomeruli is illustrated in fig. 3. In this figure there are three combinations of glomerular and tubular damage. In six biopsies the glomerular changes were slight (glomerular index <13) whereas tubular lesions were extensive, atrophy being present in 5 or more out of the 10 microscopic fields examined; creatinine clearance ranged between 10 and 60 ml. per minute. In these patients, therefore, the diminution in creatinine clearance could well have been due principally to tubular damage. In 1 of these patients a second renal biopsy was done twenty months later at a time when the creatinine clearance had returned to normal—i.e., it had risen from 19 to 112 ml. per minute. In the second specimen the glomerular changes were still inconspicuous, whereas tubular atrophy was far less striking than it had been in the first biopsy. Independent assessment of the extent of tubular atrophy present in the two biopsies from this patient made by two

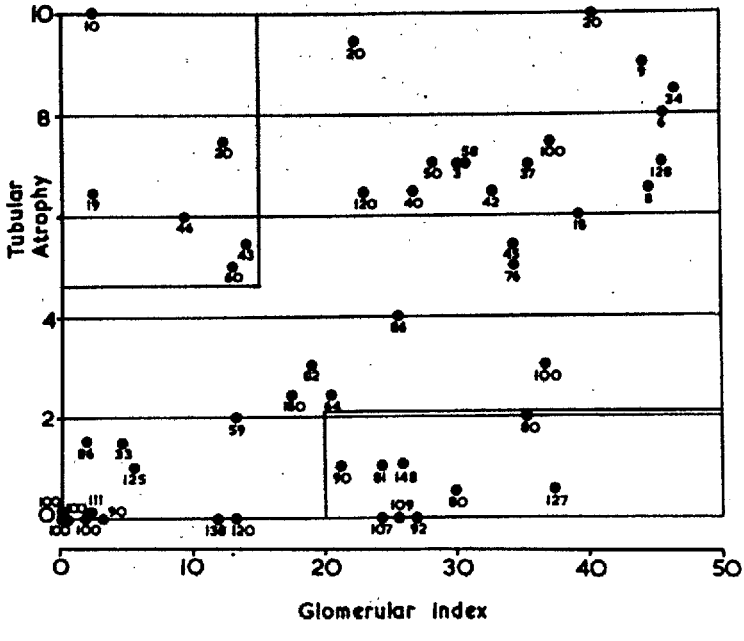

Fig. 3—Relationship between glomerular index, tubular atrophy (per 10 microscope fields), and creatinine clearance (ml. per minute).

The rectangles in the top-left and bottom-right corners enclose the points of especial interest (see text).

of us were 0 and 1 (mean 0·5) microscopic fields out of 10 in the second specimen, as opposed to 6 and 7 (mean 6·5) out of 10 in the first.

In nine other biopsy specimens glomerular changes were severe (glomerular index 20–40) but tubular damage was slight, atrophy being present in 2 or less out of 10 microscopic fields examined; creatinine clearance was above 80 ml. per minute. This suggests that even extensive glomerular deposits may not significantly interfere with glomerular filtration.

In fig. 3, the largest group consisted of 35 cases in which tubular and glomerular lesions were of about the same severity. In most of these cases creatinine clearance tended to be low when tubular and glomerular lesions were severe, and to be within normal limits when they were not. In these cases the creatinine clearance seemed essentially to be a measure of damage to the kidney as a whole. There were, however, 3 cases in which, although the creatinine clearances were more than 100 ml. per minute, the renal-biopsy specimen contained conspicuous lesions both in tubules and in glomeruli. The discrepancy between structure and function in these instances is difficult to explain.

With regard to the changes observed in the renal arterioles, these showed, as expected, a significant correlation with both age and diastolic blood-pressure. It was possible to correlate the glomerular lesions with the presence of hyaline arteriolar change, and the tubular lesions with the presence of fibroelastic intimal hyperplasia. The first correlation is understandable on the assumption that in glomerular nephritis the pathogenic mechanisms responsible for the deposition of eosinophilous material in the arteriolar wall and glomerular capillary are the same. The relationship between fibroelastic intimal hyperplasia and tubular atrophy is more obscure.

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Renal dysplasia

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Renal dysplasia

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Part I A clinico-pathological study of 76 cases

SYNOPSIS The clinical and pathological findings in 150 children submitted to partial or total nephrectomy have been reviewed. Histological examination of the kidney removed at operation showed evidence of renal dysplasia in 76 (51%). These 76 patients were divided into three main groups on the basis of the pathological changes found in the kidney and the associated urinary tract anomalies. In group 1, gross cystic renal dysplasia was associated with absence or atresia of the renal pelvis and ureter. In group 2, renal dysplasia was segmental; the ureter, although patent, had some anatomical or functional abnormality which resulted in urinary stasis or reflux. In many of these patients dysplasia was confined to the upper pole of a 'duplex' kidney which was drained by an ectopic ureterocele. In group 3, renal dysplasia was associated with obstruction of the lower urinary tract, most commonly by posterior urethral valves. In group 1 dysplasia was total, involving the whole kidney, whilst in groups 2 and 3 dysplasia tended to be segmental; in the majority some normal renal tissue was present. Pyelonephritis was a very common complication, but was present only in patients from groups 2 and 3, in whom a lumen was present in the draining ureter, and not in patients from group 1 in whom the ureter was atretic or absent, and the kidney not functioning. It appears that urinary obstruction, stasis, or reflux are the principal factors predisposing to and promoting pyelonephritis in dysplastic kidneys. There seems to be no reason to suppose that dysplastic renal tissue is abnormally susceptible to infection since pyelonephritic changes were lacking in those cases in which dysplasia was most severe.

Renal dysplasia is the abnormal, disorganized development of renal parenchyma due to anomalous differentiation of metanephric tissue. Histologically the normal renal architecture is distorted; the glomeruli, tubules, and collecting ducts are deficient in number, appear morphologically immature, and often undergo cystic changes (Kissane, 1966). The extent of the abnormality varies from a grossly disorganized multicystic dysplasia involving the whole kidney to a less severe segmental change in which part of the kidney is unaffected. Osathanondh and Potter (1964), in a study of the pathogenesis of polycystic kidneys based on microdissection, concluded that cystic dysplasia was due to diminished branching of the ampullary portion of collecting ducts derived from the ureteric bud, with resulting cyst formation and failure to induce normal nephron formation in the metanephric blastema. The cause of inhibition of ampullary activity in these cases is unknown, but it has been suggested that the frequent association of urinary tract obstruction may be an important factor (Bernstein, 1968). The present study was undertaken in order to assess the frequency of renal dysplasia in kidneys surgically resected in children and to correlate the clinical and pathological changes found. Pyelonephritis complicating renal dysplasia was found to be confined to those cases in which the lumen of the draining ureter was patent.

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Patients

The pathological material has been reviewed from 150 consecutive cases of children submitted to total or partial nephrectomy at the Hospital for Sick Children between 1962 and 1969. The case records of these children were also examined in order to correlate the clinical and histopathological findings. Seventy-three were boys and 77 girls, ranging in age between 1 week and 12 years 6 months at the time of operation. The kidney tissue removed was fixed in buffered formalin (*p*H 7·0) and between one and five blocks of tissue from each specimen were embedded in paraffin wax. Sections of 5 μ thickness were cut and stained routinely by Ehrlich's haemotoxylin and eosin (H & E) and, in the majority of cases, by van Gieson's mixture. Other stains were also used when necessary.

The patients fell into six categories (Table I). The main clinical findings and pathological abnormalities, particularly the associated anomalies found in the ureters and lower urinary tracts, are shown. 'Hypoplastic' kidneys were congenitally small; the five examples examined all weighed less than 9 g. Gross multicystic kidneys were those in which the kidney consisted of a disorderly non-reniform mass of cysts of varying size, in which no normal renal parenchyma was recognizable. Partially cystic kidneys were those in which the structural disorganization and cystic change was somewhat less marked and in which some renal parenchyma was present.

Assessment of the Histological Changes

In each case the histological sections were examined for evidence of renal dysplasia and pyelonephritis.

RENAL DYSPLASIA

The histological diagnosis of renal dysplasia

Category	*Pathological Abnormalities*				*Uni-lateral*	*Bi-lateral*	*Sex*		*Age at Operation Range (median)*	*Operation Performed*	*No. of Patients*
	Kidney	*Ureter*	*Lower Urinary Tract*	*Other*			*M*	*F*			
1	Gross multi-cystic	Atresia (10)[1] Absence (4)		Rectal agenesis (1) Non-development of abdominal wall (1) Crossed renal ectopia (1)	14	0	7	7	1 wk to 11 yr 10 mth (3½ mth)	Unilateral total nephrectomy	14
2	'Duplex'	Ectopic lower end of upper pole ureter (62) Associated with ureterocele (55)			57	10	16	51	1 wk to 8 yr 8 mth (7 mth)	Upper pole heminephrectomy (52)[2] Unilateral total nephrectomy (15)	67
3	Partially cystic (2) or 'hypoplastic' (5)	Ectopic lower end of ureter (2) Ureterocele (2) Vesicoureteric reflux (3)			7	0	5	2	1 wk to 8 yr 7 mth (3 yr)	Unilateral total nephrectomy	7
4	Hydronephrosis	Hydroureter	Posterior urethral valves (17) Urethral atresia (1)		0	18	18	0	1 wk to 5 yr 7 mth (6 wk)	Unilateral total nephrectomy	18
5	Hydronephrosis	Pelvi-ureteric obstruction			17	0	13	4	2 mth to 12 yr 6 mth (3 yr 6 mth)	Unilateral total nephrectomy	17
6	Severe pyelonephritis	Ectopic lower end of ureter (3) Congenital megaureter (2) Vesico-ureteric reflux (3)		Calculus formation (16) Rectal agenesis (3)	27	0	15	12	1 mth to 11 yr 8 mth (3 yr 8 mth)	Unilateral total nephrectomy	27

Table I *Main clinical and pathological findings in 150 children submitted to partial or total nephrectomy*

[1]The numbers in brackets refer to numbers of patients. [2]Nine of these patients subsequently had the remaining lower pole removed.

depended primarily upon disorganization of the renal parenchyma, and the presence of 'primitive' ducts. These structures are lined by columnar epithelium and surrounded by concentric mantles of cellular mesenchyme (Fig. 1). They are thought to be derived from the branches of the ureteric bud (Ericsson and Ivemark, 1958a). Often they are aggregated, forming nodular collections (Fig. 2). A case was classified as dysplastic only when these features were present. Foci of metaplastic cartilage (Fig. 3) were also regarded as definite evidence of renal dysplasia, but these were present in only a proportion of the cases showing parenchymal disorganization and 'primitive' ducts. Other so-called 'dysplastic' structures sought were 'foetal' glomeruli (Pasternack, 1960), 'primitive' tubules and ductules (Ericsson and Ivemark, 1958a), and tubular and glomerular cysts (Rubenstein, Meyer, and Bernstein, 1961). These are illustrated in Figures 4 and 5.

PYELONEPHRITIS

Histological evidence of pyelonephritis sought was the presence of conspicuous acute or chronic inflammation of the renal interstitial tissues and of the mucosa lining the renal pelvis (Figs. 6 and 7). Periglomerular fibrosis, glomerular sclerosis, and foci of tubular atrophy (Fig. 8) were almost invariably present in the inflamed areas.

Results

These are summarized in Tables II, III, and IV and in Figure 10.

Histological evidence of renal dysplasia was present in 76 (51%) of the 150 patients studied (Table II). These 76 patients were divided into three main groups (Table III) on the basis of the pathological changes found in the kidneys and the associated anomalies in the urinary tract.

Pyelonephritis was a common complication in the cases with renal dysplasia; histological

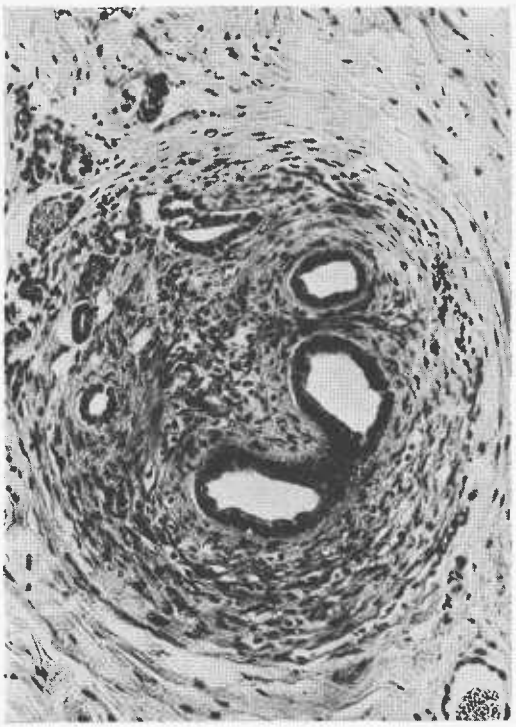

Fig. 1 *Renal dysplasia. A 'primitive' duct lined by columnar epithelium and surrounded by concentric rings of mesenchymal cells. H. & E. × 20.*

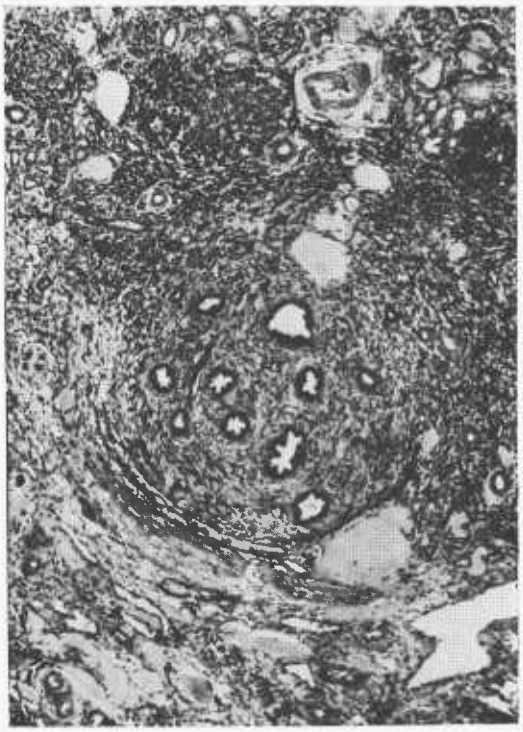

Fig. 2 *Renal dysplasia. A group of 'primitive' ducts aggregated to form a nodular collection. H. & E. × 10.*

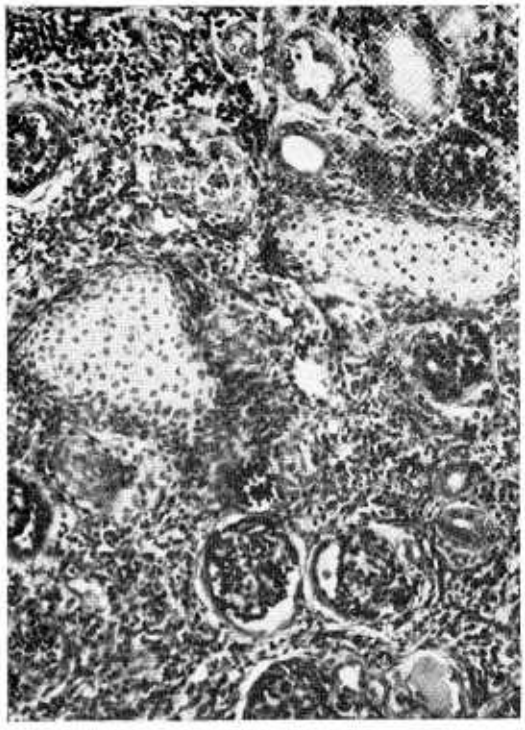

Fig. 3.

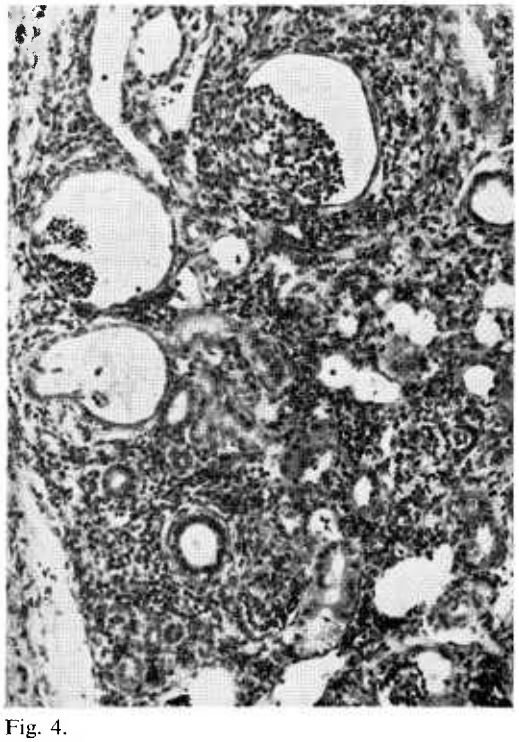

Fig. 4.

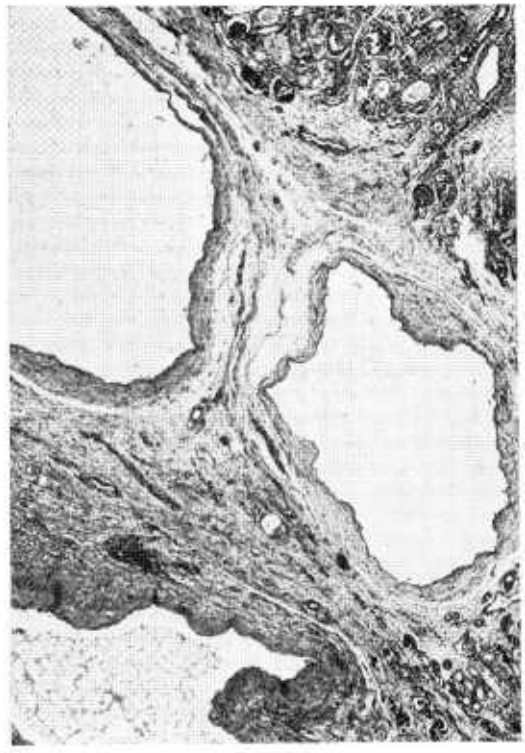

Fig. 5.

Fig. 3 *Renal dysplasia. Bars of metaplastic cartilage and 'foetal' glomeruli. H. & E.* × *40.*

Fig. 4 *Renal dysplasia. 'Primitive' tubules and ductules and glomeruli. Glomerular cysts are also present. H. & E.* × *20.*

Fig. 5 *Renal dysplasia. Large fibrous-walled cysts. H. & E.* × *10.*

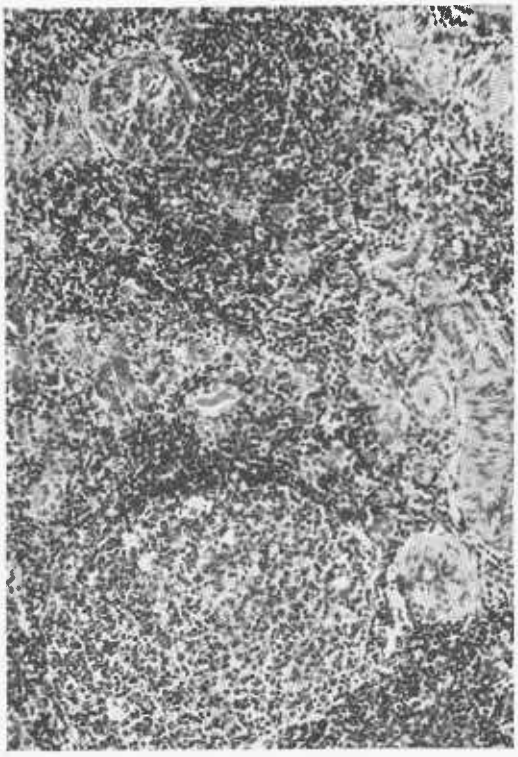

Fig. 6.

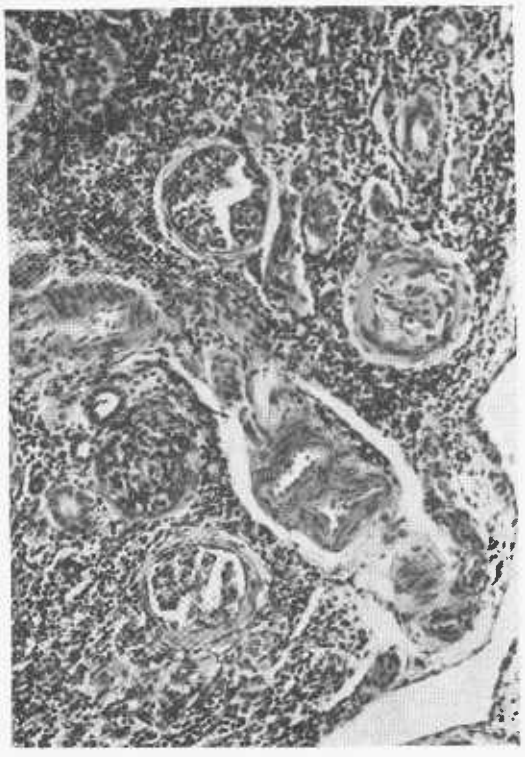

Fig. 8.

Fig. 7.

Fig. 6 *Pyelonephritis. Marked interstitial chronic inflammation with lymphoid follicle formation. H. & E. × 40.*

Fig. 7 *Pyelonephritis. Chronic inflammation of the renal pelvis. H. & E. × 10.*

Fig. 8 *Pyelonephritis. Periglomerular fibrosis and glomerular sclerosis in an area of chronic inflammation. H. & E. × 40.*

evidence of this condition was present in 58 (76%), but was found only in patients in groups 2 and 3 (Table IV). Two patients in group 1 had clinical evidence of urinary tract infection (pyuria and bacteriuria) but in both the opposite kidney was abnormal; in one intravenous pyelography showed a small kidney with clubbed calyces, and in the other hydronephrosis due to stenosis of the pelvi-ureteric junction was found.

The blood urea concentration, which in each case was measured a few days before operation, was often raised in all three groups (Fig. 9). In group 1 the urea concentration was normal or moderately raised in all but two cases in which the levels were more markedly raised (141 and 153 mg/100 ml). In these instances there were marked abnormalities in the opposite kidney. In group 2, one case had a urea level of 150 mg/100 ml. This child died two days after operation. In the remainder the initial urea level was often raised, but in every case fell to less than 35 mg/100 ml following operation. In group 3 the blood urea was usually raised and was less than 40 mg/100 ml in only six cases.

Mortality was highest amongst patients in group 3, five of whom died of renal failure between three weeks and 30 months after operation. In group 1, one patient died of renal failure. This child had severe hydronephrosis of the contralateral kidney due to stenosis of the pelvi-ureteric junction. In group 2, one child died in the post-operative period. He had severe bilateral hydronephrosis due to an obstructing ectopic ureterocele in the urinary bladder.

Patient Category	*Main Pathological Abnormalities*	*No. with Renal Dysplasia*	*Total No. of Patients*
1	Gross multicystic dysplasia	14	14
2	'Duplex' kidney with double ureters	38	67
3	Partially cystic or 'hypoplastic' kidney	7	7
4	Hydronephrosis due to lower urinary tract obstruction	17	18
5	Hydronephrosis due to pelvi-ureteric stenosis	0	17
6	Severe pyelonephritis	0	27
	Total	76	150

Table II *Number of patients in each category showing histological evidence of renal dysplasia*

Renal Dysplasia	*Pyurial and Bacteriuria*	*Histological Evidence of Pyelonephritis*
Group 1 (14 cases)	2	0
Group 2 (45 cases)	31	44
Group 3 (17 cases)	13	14

Table IV *Incidence of pyelonephritis in the three groups of patients with renal dysplasia*

Discussion

The criteria on which the histological identification of renal dysplasia is based are unsatisfactory. The diagnosis depends on the recognition of various 'dysplastic' structures in the kidney including 'primitive' ducts and tubules (Ericsson and Ivemark, 1958a), metaplastic cartilage

Group	*Pathological Abnormalities*		*Sex*		*Age at Operation Range (median)*	*Nephrectomy*		*Side*		*Follow up (mth) Range (mean) No. of Cases*	*Total No. of Patients*
	Kidney	*Ureter and Lower Urinary Tract*	*M*	*F*		*Total*	*Partial*	*R*	*L*		
1	Gross multicystic	Atresia of ureter (10) Absence of ureter (4)	7	7	1 wk to 11 yr 10 mth (3½ mth)	14	0	5	9	2 to 60 (20) 13 cases	14
2	'Duplex' (38)[1]	Ectopic insertion of upper pole ureter (38) Ectopic ureterocele (36)	13	32	1 wk to 8 yr 8 mth (9 mth)	16	30	29	17	30 to 140 (38) 41 cases	45
	'Hypoplastic or partially cystic (7)	Ectopic insertion of ureter (2) Ureterocele (2) Vesico-ureteric reflux (3)					1 (bilat)	1 (bilat)			
3	Hydronephrosis	Hydroureter (17) Posterior urethral valves (16) Urethral atresia (1)	17	0	1 wk to 5 yr 7 mth (6 wk)	17	0	9	8	1 to 82 (28) 17 cases	17

Table III *Main pathological findings in patients with renal dysplasia*

[1]The numbers in brackets refer to numbers of patients.

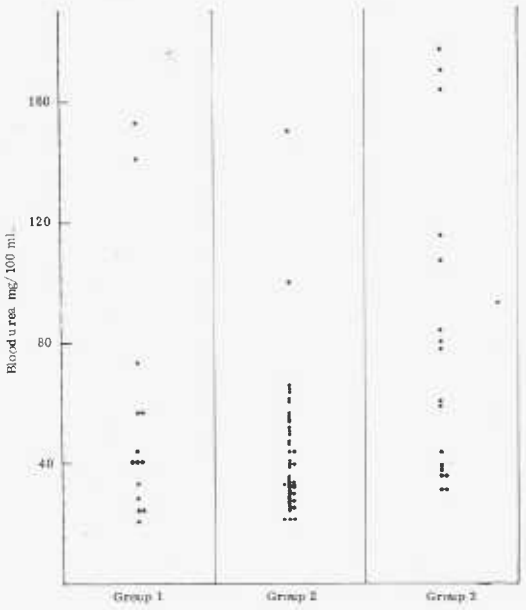

Fig. 9 *Blood urea estimations immediately before operation in the three groups of patients with renal dysplasia.*

Fig. 10 *Hydronephrosis. Flattening of the renal papilla and atrophy of the medulla. The collecting ducts are separated by fibrous connective tissue. H. & E. × 10.*

(Bigler and Killingsworth, 1949), 'foetal' glomeruli (Pasternack, 1960), and renal cysts (Rubenstein *et al*, 1961). However, Bernstein (1968) has demonstrated in the kidneys of young children that an apparently 'primitive' morphology may be induced in glomeruli, tubules, and ductules as a result of ischaemic damage, or even by scarring following renal biopsy, without there being any suggestion of maldevelopment. For these reasons, in the present study, the histological diagnosis was based on the presence of structural disorganization of the renal parenchyma as a result of abnormal development, and on the presence of 'primitive' ducts of the type described by Ericsson and Ivemark. These structures are thought to represent branches of the ureteric bud, and are not seen in conditions other than renal dysplasia (Bernstein, 1968). Bars of metaplastic cartilage have also been regarded as definite evidence of renal dysplasia (Bigler and Killingsworth, 1949), but since they are not always present even in obviously dysplastic kidneys, their presence was regarded more as useful confirmation of the diagnosis. Dysplastic changes may sometimes be confused with those due to hydronephrosis, particularly when the two conditions coexist. Flattening of the renal papillae as a result of back pressure causes a tangential rather than a radial alignment of the collecting ducts. The ducts become separated by fibrous connective tissue which may condense around the tubules and mimic mantles of mesenchyme (Fig. 10). Such cases may account for descriptions of so-called pure medullary dysplasia (Gwinn and Landing, 1968). In the cases studied it was usually evident that hydronephrosis primarily produced atrophic changes in the medulla, the cortex becoming thin only at a late stage. In renal dysplasia the cortex was disorganized and usually markedly thinned by comparison with the adjacent medulla. 'Primitive' ducts were often aggregated together and formed nodules (Fig. 2), a feature not seen in pure hydronephrosis.

In the present series, histological evidence of renal dysplasia was found in 51% of cases. Consideration of the pathological changes in the kidneys and the associated urinary tract abnormalities allowed their division into three groups (Table III). In group 2 cases of 'duplex' kidney with double ureters were included with cases in which the kidneys were 'hypoplastic' or partially cystic. This was considered reasonable,

since in all the dysplastic changes were segmental, and in all the ureter draining the dysplastic kidney, whilst possessing a lumen, showed some structural or functional abnormality which resulted in urinary stasis or reflux.

Elevation of the blood urea concentration was seen in a proportion of patients from all three groups, but was most common in those in group 3 with obstruction of the lower urinary tract. In fact, in most cases in group 3 the initial blood urea level had been higher, but the establishment of bilateral ureterostomies or nephrostomies produced a fall in the blood urea. Nephrectomies were performed when urine formation was so slight as to suggest little useful renal function. In all cases in group 2 with raised blood urea concentrations, ectopic ureteroceles (Berdon, Baker, Becker, and Uson, 1968) were present in the urinary bladder. The fact that urea levels fell after operation suggests that the initial elevation may have been due to urinary obstruction by the ureterocele. In group 1, the blood urea levels were markedly raised in two cases in which abnormalities were also present in the contralateral kidney and moderately raised (57 and 74 mg%) in two others. Both of these were young infants, less than 2 weeks old. The raised blood urea level in these cases may reflect a relative inability of the opposite 'normal' kidney to cope as a result of some functional immaturity.

The mortality was highest in patients in group 3, five of whom died of renal failure. This was not surprising since they all presented with bilateral hydronephrosis and hydroureters and the resulting renal damage was sufficient to destroy entirely the function in at least one kidney.

Pyelonephritis was a very common complication in those patients with renal dysplasia. Histological evidence of pyelonephritis was found in 76% of the cases. This association has also been emphasized by others (Marshall, 1953; Ericsson and Ivemark, 1958b; Pasternack, 1960). These authors concluded that dysplastic renal tissue was abnormally susceptible to infection in much the same way as areas of scar tissue in the kidney (Rocha, Guze, Freedman, and Beeson, 1958). Marshall (1968) even suggested that most cases of pyelonephritis in the adult arise from infection of areas of renal dysplasia. In the present study, although pyelonephritis was common, it occurred only in patients in groups 2 and 3 in whom a lumen was present in the draining ureter, and not in group 1 in whom the ureter was absent or atretic, and the kidney was not functioning. Two patients in group 1 had clinical evidence of a urinary tract infection, but in both there were marked abnormalities of the opposite kidney. Such a finding is not uncommon in cases of cystic renal dysplasia (Pathak and Williams, 1964). In both these cases histological evidence of pyelonephritis was lacking in the resected dysplastic kidney. In any case, pyuria and bacteruria in the voided urine could hardly have arisen in this kidney since it was effectively isolated by an atretic ureter.

It appears that a lumen is necessary in the draining ureter for infecting organisms to gain access to the dysplastic kidney. Structural or functional abnormalities in the ureter or lower urinary tract causing urinary stasis or reflux seem to be the most important factors in promoting pyelonephritis in dysplastic kidneys as in other situations. There is no reason to conclude that dysplastic renal tissue is abnormally prone to infection since pyelonephritis was absent in those cases in group 1 in which dysplasia was most widespread and severe. Absence or atresia of ureter in these cases may be a secondary phenomenon. Dysplasia was marked and affected the whole kidney and it seems unlikely that urine formation could have occurred at any stage in its development. It is possible that the maintenance of a lumen in the developing ureter requires the passage of urine and in its absence the lumen either does not develop or even the ureter may disappear.

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Renal dysplasia

Part II A necropsy study of 41 cases

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Part II A necropsy study of 41 cases

SYNOPSIS. The pathological findings at necropsy have been reviewed in 121 children with congenital malformations of the kidney and lower urinary tract. Histological examination of the kidneys revealed evidence of renal dysplasia in 41 (34%). Comparison of these 41 cases with other cases in which renal dysplasia was found in kidneys removed surgically showed a much higher incidence of bilateral involvement and of other associated major congenital abnormalities. Histological evidence of pyelonephritis was less common except in those cases in which renal dysplasia was associated with lower urinary tract obstruction. A total of 34 children with bilateral hydronephrosis and hydroureters due to congenital urethral obstruction was found, and in these cases severe degrees of renal dysplasia were present only in those dying in the first two months.

Many previous reviews have been made of multicystic and 'hypoplastic' renal dysplasia (Elkstrom, 1955; Spence, 1955; Coppridge and Ratliff, 1958; Parkkulainen, Hjelt, and Sirola, 1959; Vellios and Garrett, 1961) but rather less emphasis has been given to renal dysplasia associated with congenital lower urinary tract obstruction (Pathak and Williams, 1964; Rattner, Meyer, and Bernstein, 1963). For this reason a separate quantitative study was made of the incidence and degree of renal dysplasia present in the 34 children with obstruction of the lower urinary tract included in the present series.

Patients

A review of the necropsy records at the Hospital for Sick Children for the period between 1954 and 1969 revealed 157 congenital malformations of the kidney and lower urinary tract occurring in 121 children. Eighty-four were boys and 37 girls ranging between 1 day and 13 years 6 months at death. The various anomalies encountered are shown in Table I. In each case histological sections from the kidneys were examined. Blocks of kidney tissue had been fixed in buffered formalin (*p*H 7·0) and embedded in paraffin wax. Sections were cut of 5 μ and stained routinely by Ehrlich's haematoxylin and eosin (H and E); other stains were employed where necessary.

Assessment of the Histological Changes

In each case the histological sections were

Classification	*No. of Cases*
1 *Anomalies of Structure*	
(a) Agenesis	15
(b) Polycystic disease	10
(c) Simple renal 'hypoplasia'	12
(d) Renal dysplasia	41
2 *Anomalies of Position or Shape*	
(a) Renal ectopia	9
(b) Renal fusion	10
(c) 'Duplex' kidney	11
3 *Anomalies of Lower Urinary Tract*	
(a) Extrophy of the bladder	5
(b) Duplication of the bladder	1
(c) Rectourethral fistula	6
(d) Megacystic-megaureter without obstruction	3
(e) Congenital urethral obstruction	34
Total	157

Table I *Classification of 157 congenital abnormalities of the kidneys and lower urinary tract found at necropsy in 121 children*

examined for evidence of renal dysplasia and pyelonephritis using the criteria described previously (Risdon, 1970).

In addition a separate quantitative assessment of the degree of renal dysplasia present in the kidneys from the 34 children with bliateral hydronephrosis and hydroureters due to congenital lower urinary tract obstruction was made as follows: O = where there was no histological evidence of renal dysplasia. + = where occasional foci of renal dysplasia were seen, but most of the renal parenchyma was normally differentiated (Fig. 1). ++ = where renal dysplasia was segmental, with areas of normally and anomalously differentiated parenchyma present (Fig. 2). +++ = where renal dysplasia was total, but some separation of the renal parenchyma into cortex and medulla was discernible. Cystic changes tended to be prominent in the subcapsular zone (Fig. 3). ++++ = where dysplasia was total and complete disorganization of the renal parenchyma was associated with marked cystic changes (Fig. 4).

In 33 of the 34 cases sections from both kidneys were examined. In the remaining case, unilateral renal agenesis was present, and a section from the single kidney was examined.

Results

Histological evidence of renal dysplasia was present in 41 (34%) of the cases studied and was bilateral in just over half of them (23 cases).

The 41 children with renal dysplasia were divided into three groups (Table II) on the basis of the pathological changes in the kidney and the associated anomalies in the draining urinary tract as described previously (Risdon, 1970). Dysplasia was bilateral in 23 (63%) and additional congenital abnormalities were frequently present both in the urogenital system and also in other systems, particularly the cardiovascular and gastrointestinal systems. These are summarized in Table III.

Histological evidence of renal infection or pyelonephritis was present in two of nine cases from group 1, three of the 10 cases from group 2, and in 17 of the 22 cases from group 3.

Of the two cases affected in group 1, in the first, evidence of pyelonephritis was confined to the contralateral non-dysplastic kidney, in which there was hydronephrosis due to mid-ureteric obstruction. In the other case, there was focal abscess formation in the cystic dysplastic kidney and a pyonephrosis in the other kidney due to pelvi-ureteric stenosis. In group 2, the three cases

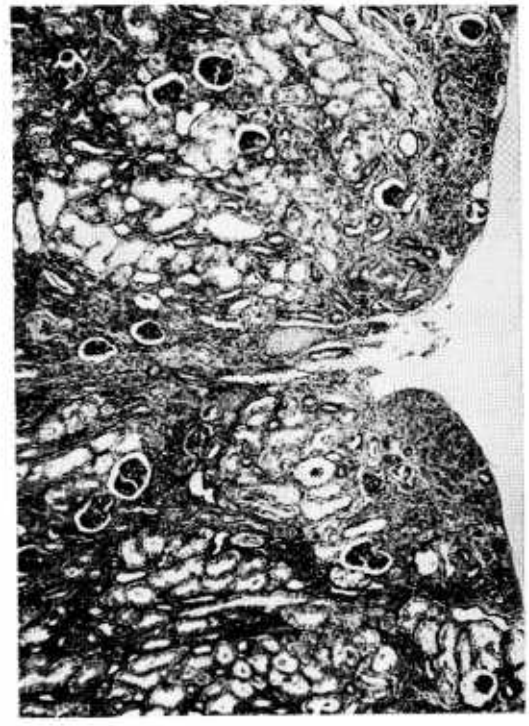

Fig. 1 *Renal dysplasia (group 3). Grade + changes. A focal dysplastic area is shown, but most of the renal parenchyma is normally differentiated. H & E × 15.*

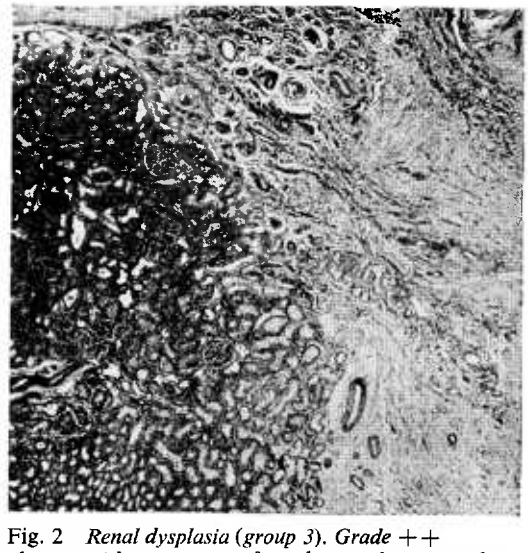

Fig. 2 *Renal dysplasia (group 3). Grade ++ changes. Adjacent areas of renal parenchyma, on the left showing normal development and on the right dysplasia. H & E × 10.*

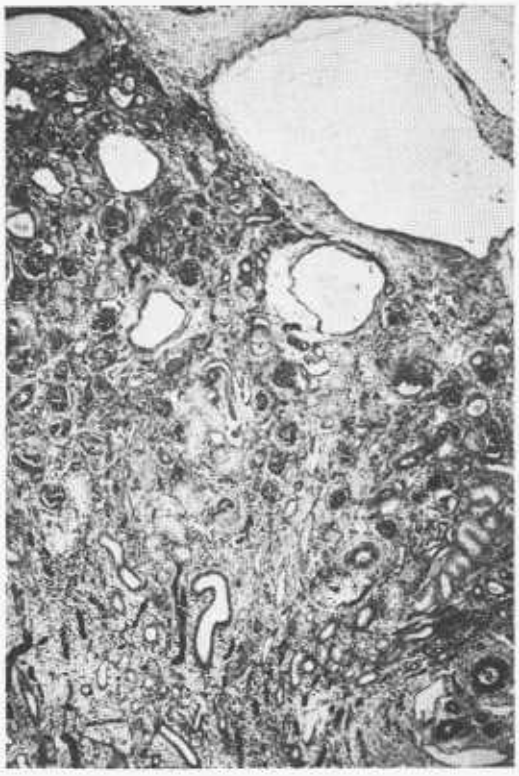

Fig. 3 *Renal dysplasia (group 3). Grade +++ changes. Renal dysplasia is total but some differentiation of cortex and medulla is seen. Sub-capsular cyst formation is prominent. H & E × 10.*

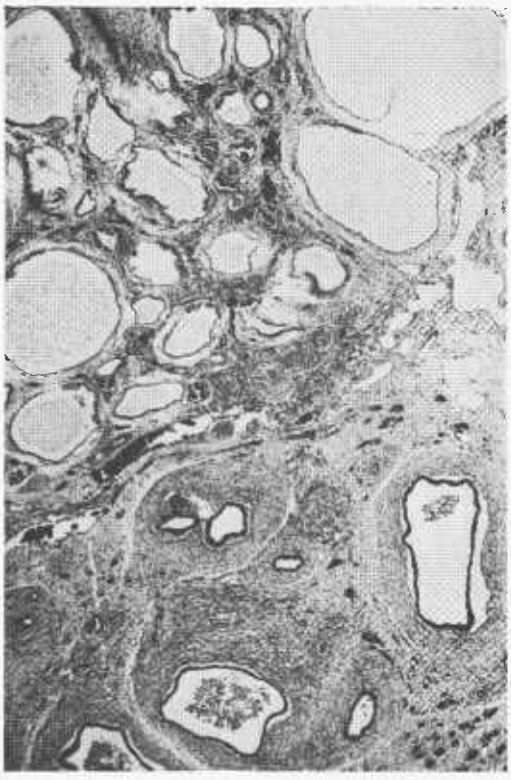

Fig. 4 *Renal dysplasia (group 3). Grade ++++ changes. There is complete disorganization of the renal parenchyma and cystic changes are marked. H & E × 10.*

Dysplasia	*No. of Patients*	*Unilateral Involvement*	*Pathological Abnormalities*		*Bilateral Involvement*	*Pathological Abnormalities*	
			Kidney	*Ureter and Lower Urinary Tract*		*Kidney*	*Ureter and Lower Urinary Tract*
Group 1	9 (M6; F3)	6	Gross multicystic	Absence of ureter (1)[1] Atresia of ureter (3) Gross narrowing of ureter (2)	3	Gross multicystic	Bilateral atresia of ureters (2) Absence of one ureter and atresia of contra-lateral ureter (1)
Group 2	10 (M5; F5)	6	'Duplex' (1) 'Hypoplastic' or partially cystic (5)	Ectopic insertion of upper pole ureter (1) Ureterocele (1) Ectopic insertion of ureter (2) hydroureter (2)	4	'Hypoplastic' or partially cystic (4)	Ectopic insertion of one ureter; the opposite ureter hypoplastic (1) Bilateral hypoplastic ureters (1) Bilateral hydroureters (1)
Group 3	22 (M22)	6[2]	Hydronephrosis (6)	Hydroureter (6) Posterior urethral valves (5) Urethral stenosis (1)	16	Hydronephrosis (16)	Hydroureters (16) Posterior urethral valves (12) Urethral stenosis (2) Urethral atresia (1)

Table II *Main pathological findings in kidneys and draining urinary tract in three groups of cases with renal dysplasia.*

[1]The numbers in brackets refer to the numbers of patients.

[2]This figure refers to the incidence of unilateral renal dysplasia. In this group, hydronephrosis was invariably bilateral with the exception of one case where agenesis of one kidney was present.

Renal Dysplasia	*Associated Anomalies in the Urogenital System*		*Associated Anomalies in Other Systems*			
	Kidney and Ureter	*Other*	*Cardiovascular*	*Gastrointestinal System*	*Central Nervous System*	*Other Systems*
Group 1 (9 cases)	Contralateral renal agenesis (1)	Extrophy of bladder (1)	Patent ductus arteriosus (3)	Imperforate anus (2) Tracheoesophageal fistula with oesophageal atresia (2) Anorectal agenesis (2) Duodenal atresia (1) Exomphalos (1) Annular pancreas (1) Agenesis of gall-bladder (1)	Arnold-Chiari malformation (1) Meningo-myelocoele (1)	Harelip (1)
	Contralateral hydronephrosis due to ureteric stenosis (2) Ectopia of dysplastic kidney (1) Renal fusion (1)	Rectovesical fistula (1) Bicornuate uterus (1)	Atrial septal defect (2) Coarctation of the aorta (1) Ventricular septal defect (1)			
Group 2 (10 cases)	Contralateral hydronephrosis due to ureteric stenosis (1)		Ventricular septal defect (1)	Tracheoesophageal fistula with oesophageal atresia (1) Hiatus hernia (1) Intrahepatic biliary atresia (1)		Spina bifida occulta (1)
Group 3 (22 cases)	Unilateral renal agenesis (1)			Tracheoesophageal fistula with oesophageal atresia (1) Rectal agenesis (1)		'Prune belly' syndrome (4)

Table III *Associated congenital anomalies in the urogenital and other systems in the three groups of cases with renal dysplasia*

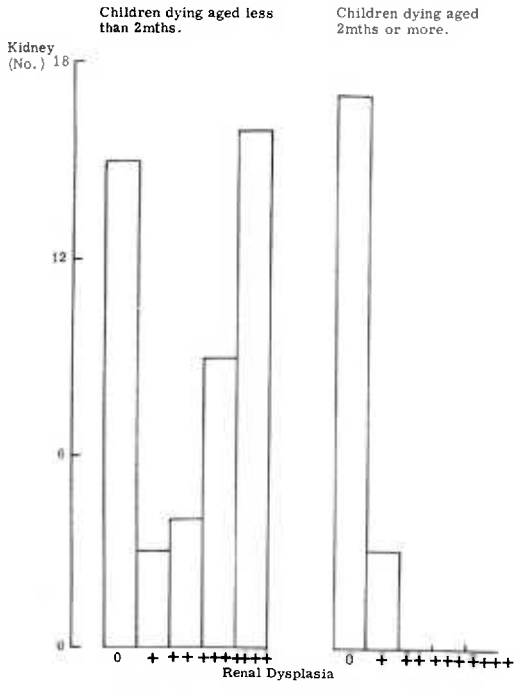

Fig. 5 *Histological grading of the degree of renal dysplasia present in sections from each kidney in the 34 cases with congenital lower urinary tract obstruction correlated with the age at death.*

with histological evidence of pyelonephritis were aged 6 weeks or more at the time of death, whereas the seven cases in which pyelonephritis changes were absent were aged 19 days or less at death.

Of the 34 cases with bilateral hydronephrosis and hydroureters due to congenital lower urinary tract obstruction, histological evidence of renal dysplasia was present in 22 (65%). Severe degrees of renal dysplasia (++, +++, or ++++) were present only in those children dying in the first two months (Fig. 5).

Discussion

In the previous account of renal dysplasia in surgically resected kidneys in children (part I) the cases were divided into three main groups according to the pathological changes in the kidney and the associated anomalies in the draining urinary tract. Group 1 comprised cases of gross multicystic renal dysplasia associated with absence or atresia of the ureter and renal pelvis (Fig. 6). In group 2 renal dysplasia was segmental, and the draining ureter, whilst it possessed a lumen, showed some structural or functional abnormality which interfered with urinary drainage (Figs. 7 and 8). In group 3, renal dysplasia was associated with bilateral hydronephrosis and hydroureter due to lower

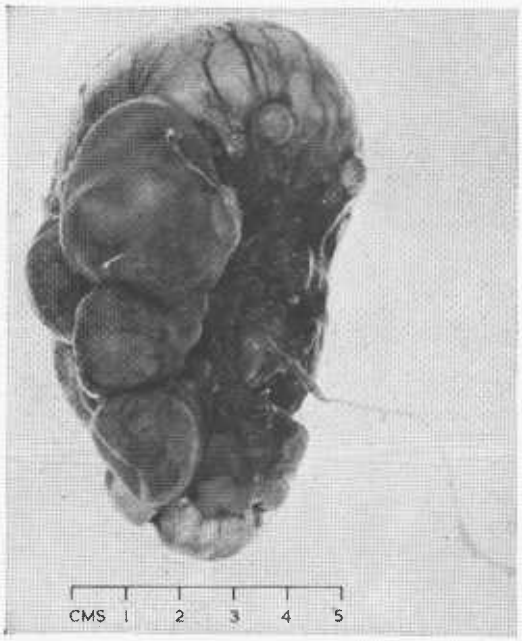

Fig. 6 *Renal dysplasia (group 1). Gross multicystic renal dysplasia with atresia of the draining ureter.*

urinary tract obstruction (Fig. 9). A similar grouping was used to classify the cases in the present survey of postmortem cases, and this emphasized important differences in the relative incidence of cases placed in the various groups in the two series. In two cases of unilateral multicystic dysplasia discovered at necropsy, the draining ureter, although grossly narrowed and hypoplastic, was not entirely atretic. These cases were included in group 1 because the changes in the kidney were entirely similar to the other cases in the group in that the cystic changes were gross and no normal renal parenchyma was discernible. Four cases included in group 3 in the postmortem series had congenital absence of the abdominal muscles, and were examples of the so-called 'prune belly' syndrome (McGovern and Marshall, 1959). All four had bilateral hydronephrosis and hydroureters and severe renal dysplasia. In three there was urethral obstruction due to urethral atresia or stenosis, but in the fourth case, although the posterior urethra was dilated, no definite mechanical obstruction was demonstrated. Inclusion of this case in group 3 seemed justified, since in all other respects the findings were indistinguishable.

Amongst the surgical cases, the numerically largest category were those in group 2 with 'duplex' kidneys in which the upper pole was dysplastic. Only one such case was present in the necropsy series. In the whole survey of postmortem cases there were 11 instances of 'double kidneys' with pelvic duplication, but in none of these was there any parenchymal dysplasia. It is of interest that in all of these the double ureters fused to form a single structure before insertion into the bladder, and the ureteric orifice was normally situated in the trigone. In contrast, 'duplex' kidneys with dysplasia of the upper pole invariably had two separate ureters draining the upper and lower poles. In every case fully investigated the ureter draining the dysplastic pole was inserted ectopically into the bladder, and in the majority of cases this was associated with a ureterocele (Berdon, Baker, Becker, and Uson, 1968).

Cases of congenitally small 'hypoplastic' dysplastic kidneys, and partially cystic dysplastic kidneys placed in group 2, were slightly more common in the postmortem series. As in the surgical cases, structural abnormalities of the draining ureter were almost universal, being present in all but one case. The ureteric abnormalities were similar, and included ectopic insertion into the bladder, ureterocele, ureteric dilatation without obstruction, and ureteric hypoplasia.

In contrast to the surgical series in which all the cases placed in groups 1 and 2 had unilateral renal dysplasia, seven of the 19 postmortem cases in groups 1 and 2 showed bilateral involvement. In addition there were major congenital abnormalities in other systems in 10 instances. These anomalies, which are summarized in Table III, principally involved the cardiovascular and gastrointestinal systems, a finding also noted by Rubenstein, Meyer, and Bernstein (1961). These findings in groups 1 and 2 constituted the main difference between the surgical and necropsy series. Involvement of both kidneys or the presence of other major and often multiple congenital abnormalities were not compatible with survival.

In three of the cases placed in groups 1 and 2 in the necropsy series there was hydronephrosis of the contralateral kidney due to ureteric stenosis. In two the stenosis was at the pelviureteric junction and in the third the stenosis was at the junction of the upper and middle thirds of the ureter. Similar findings were present in two of the surgical cases. This type of abnormality in the opposite kidney is an important and not uncommon finding in cases of multicystic renal dysplasia, as has been emphasized by Pathak and Williams (1964). Early diagnosis of the contra-

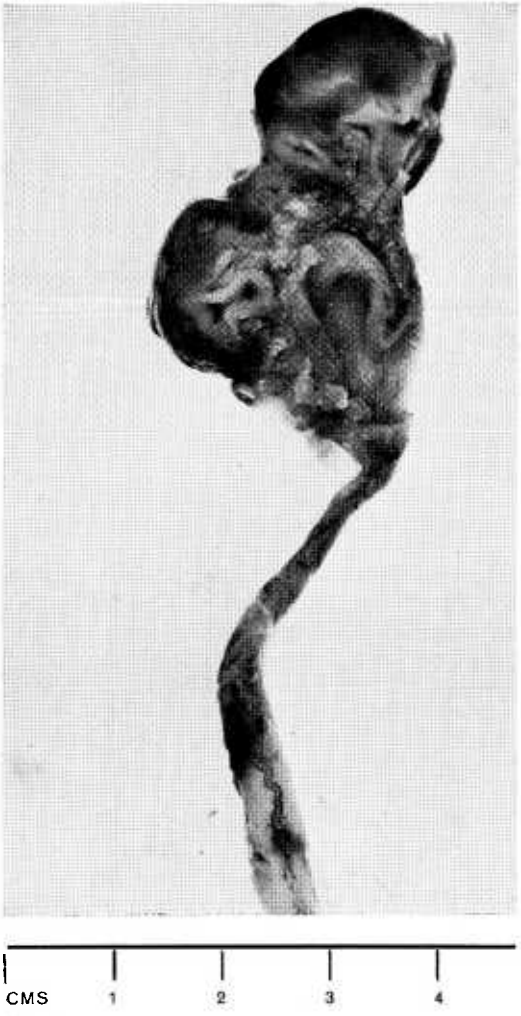

Fig. 7.

Fig. 7 *Renal dysplasia (group 2). Congenitally small ('hypoplastic') kidney with a central dysplastic segment.*

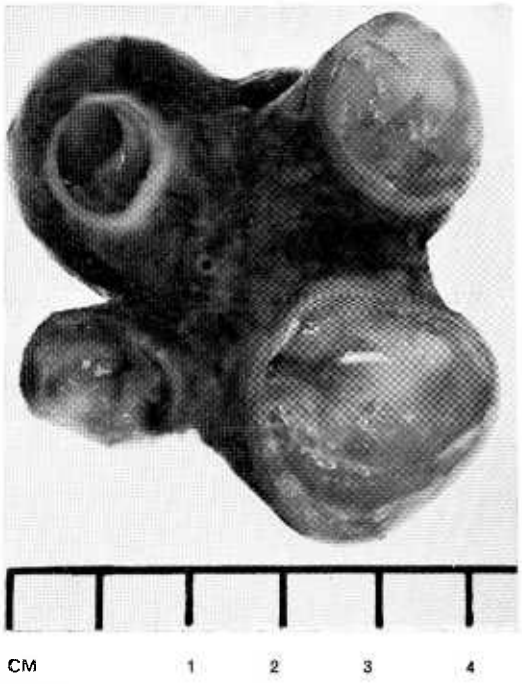

Fig. 8.

Fig. 8 *Renal dysplasia (group 2). Partially cystic renal dysplasia. The cysts are separated by renal parenchymal tissue.*

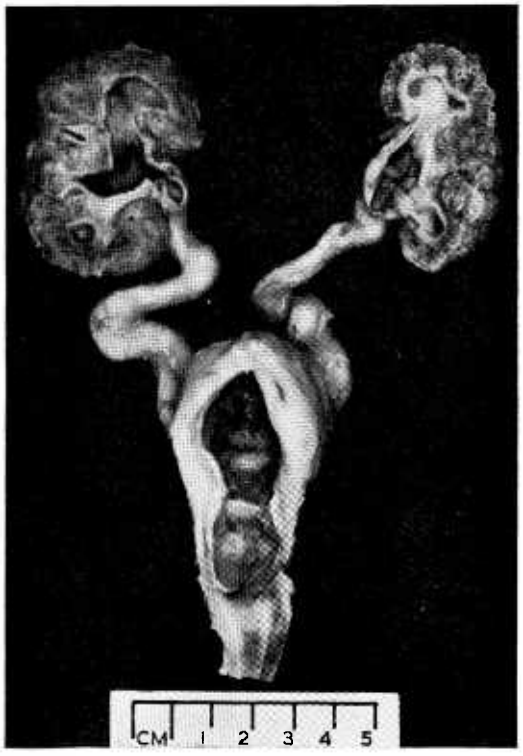

Fig. 9.

Fig. 9 *Renal dysplasia (group 3). There is obstruction of the posterior urethra by valves and bilateral hydronephrosis and hydroureters. A 'stag horn' calculus is present on the left.*

lateral hydronephrosis is vital if progressive destruction by back-pressure and ascending infection is to be avoided in this single functional kidney.

The pattern of secondary pyelonephritis differed somewhat between the surgical and the necropsy series. None of the surgically resected dysplastic kidneys placed in group 1 showed histological evidence of pyelonephritis. Of the cases in group 1 found at necropsy, one case showed evidence of infection in the dysplastic kidney; scattered abscess formation and focal pyogenic inflammation were present. However, in this case infection was clearly blood-borne, since a pyonephrosis due to pelvi-ureteric obstruction was found in the opposite kidney. This was the only instance of infection in a multicystic dysplastic kidney in the 23 cases in both series in which the draining ureter was atretic or absent. This finding emphasizes the importance of ascending infection in the pathogenesis of pyelonephritis complicating renal dysplasia (Risdon, 1970). Histological evidence of pyelonephritis was less common in the cases in group 2 in the present series, only three of the 10 cases being affected, whereas all but one of the 45 surgical cases in this group showed pyelonephritic changes. This discrepancy probably reflects the difference in ages in the two series. The three cases in group 2 in the necropsy series were all aged 6 weeks or more at the time of death whereas the seven without pyelonephritic changes were aged 19 days or less. These seven cases all had either bilateral renal dysplasia or other major congenital abnormalities, these factors being responsible for their early death.

In all the cases found at necropsy to have bilateral hydronephrosis and hydroureters due to congenital lower urinary tract obstruction, including the cases of 'prune belly' syndrome, death had resulted from renal failure. This was variously due to a combination of pyelonephritis, renal atrophy due to hydronephrosis, and renal dysplasia. In order to assess the importance of renal dysplasia in this context, quantitative assessment was made in all cases of the degree of renal dysplasia in sections from both kidneys. Histological changes of renal dysplasia were found in 22 (65%) of the 34 cases, and was bilateral in 16. This compares with an incidence of 10 out of 21 (eight bilateral) in a similar necropsy survey by Rattner *et al* (1963). Apart from the six cases in which only one kidney showed dysplastic changes, quantitative assessment showed that the degree of renal dysplasia present often varied in the two kidneys. In three instances severe renal dysplasia was present in one kidney and absent in the contralateral kidney. The occurrence of such marked variation in the degree of dysplasia in the two kidneys probably explains the much higher incidence of renal dysplasia in the surgical cases with congenital lower urinary tract obstruction, all but one of which were affected. In these, unilateral nephrectomy was performed to remove a non-functioning kidney. They were, therefore, highly selected in terms of disparate function on the two sides, and renal dysplasia might reasonably be expected to be commoner in the resected organ. Correlation of the age at death in the cases in this group coming to necropsy with the degree of renal dysplasia found in the kidneys showed that severe degrees of dysplasia were associated with early death, before the age of 2 months. Treatment of ascending infection and relief of the obstruction should not be expected to affect the outcome when both kidneys are severely deformed, but, as Rattner and his colleagues have emphasized, in other cases early diagnosis with surgical intervention and treatment of infection may be successful in preventing further progressive renal damage.

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