IMMUNOPATHOLOGICAL STUDIES OF ANTIGENS COMPLEXED IN HUMAN RENAL BIOPSIES

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To

my parents, my husband, and my children

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ABSTRACT

This thesis is a study of the nature of the immune complexes in renal biopsies and in the circulation in various kinds of glomerulonephritis.

There may be differences in clearance of circulating immune complexes from the circulation, dependent on the class of immunoglobulins, and the complement components present in the complex. Thus some complexes may be rather selectively eliminated from the circulation, and may be preferentially deposited in glomeruli. I have compared the immunoglobulins complexed in renal biopsies and in the circulation in various forms of glomerulonephritis, and found the composition of deposited and circulating complexes to vary with different forms of glomerulonephritis.

I have attempted to study antigens complexed in the glomeruli of renal biopsies using peroxidase labelled $F(ab')_2$ antibody prepared from the circulating complexes, but this was unsatisfactory, possibly due partly to destruction of IgM antibodies by the acid conditions needed for pepsin digestion.

A new method was developed for studying the complexed antigens using the slow equilibration of labelled IgG antibody from patients with the complexed antigens in the tissues. The incorporation of the label into the complex demonstrates that the antibody has the same specificity as that in the complex. The results show that patients with glomerulonephritis rarely show similar antigenic specificities. Their IgG antibodies, however, can be shown to react frequently with their complexes in their own kidneys. This suggests strongly that different antigens are involved in most patients and that the complexes in these diseases are deposited in antibody excess.

As one of the autologous antigens which might be involved in immune complex glomerulonephritis, I studied Tamm-Horsfall glycoprotein. The study of the renal tubular distribution of Tamm-Horsfall in renal disease on biopsied renal patients show an inverse correlation between the degree of staining of distal tubules and the presence of renal failure; the correlation with other factors was poor. In two of the 144 patients studied Tamm-Horsfall was found in the immune complexes in the glomeruli.

In summary, this work shows that there are apparently many antigens which are complexed in immune complex glomerulonephritis, and that the tissue complexes may be of different composition from those in the circulation. In addition, free antibody in the patient's serum will usually react with their own deposited complexes, demonstrating that they are present in excess of free antibody.

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PURPOSE OF THIS STUDY

Glomerulonephritis is usually mediated by immune complexes. Chronic immune complex disease requires a prolonged supply of antigen and an immune response to it. The antigen may be autologous or heterologous.

This research is to determine if the complexes in cases of glomerulonephritis are in antigen or in antibody excess, to correlate this with complement activation and disease production by the deposited complexes. I have looked at the nature of the antigen, heterologous or autologous and have also investigated how often patients with glomerulonephritis share the same antigen in the complexes deposited in the kidney.

1.1. Glomerulonephritis and Immune Complexes

Over the past seventy years immunopathological studies of glomerulonephritis have revealed two main mechanisms by which antibodies can induce glomerular injury. In one, antibodies have specificity for antigens within the glomerular basement membrane, the so-called anti-glomerular basement membrane antibodies. In the other, antibodies react with circulating non-glomerular antigen, and deposit passively as immune complexes in the glomerular filter, or glomerular basement membrane (Wilson and Dixon, 1974).

Recent studies by Van Damme et al (1978); Couser and Salant (1980) have suggested another mechanism of granular immune complex deposition in glomeruli. Free circulating antibodies can react with antigens already present or fixed in the glomerulus by mechanisms that reflect properties of the glomerulus itself and cause in situ deposition of immune complexes.

Factors influencing glomerular deposition of circulating immune complexes can be considered under two main headings, which are not completely independent of one another:

A. The intrinsic properties of the complexes themselves

1. Complexes possessing sufficient lattice structure (more than Ag_2Ab_2) are quickly removed from the circulation by the reticulo-endothelial system (Mannik et al, 1971)..

2. Complexes formed in a way that renders them ineffective in reacting with Fc and C_{3b} receptors (alklylated or reduced antibodies) are cleared more slowly and therefore accumulate in glomeruli to a greater extent than do similar complexes with intact antibodies (Haakenstad et al, 1976).

3. Complexes formed from antibodies of high avidity or antigens of high valency are more likely to accumulate in mesangial regions (Germuth et al, 1979a).

4. Complexes composed of cationic antigens and antibodies deposit along peripheral capillary loops in both subepithelial and subendothelial sites, whereas complexes containing anionic antigen do not (Gallo et al, 1980).

B. Host factors involved in localization of circulating immune complex

1. The existence of a high-pressure system and an effective filtration barrier favour the arrest of immune complexes along the inner side of the basement membrane, which is usually followed by movement into mesangial regions (McCluskey and Bhan, 1981).

2. Presence of C_3b receptors in human glomeruli suggests another mechanism for the arrest of immune complexes in glomeruli (Gelfand et al, 1975). Fc receptors have also been described in human glomeruli (Mizoguchi et al, 1978).

3. Abnormalities of the glomerulus itself may affect the site of localization of circulating immune complex. In rats with Heymann nephritis there is diminished mesangial accumulation of circulating immune complex (Schneeburger et al, 1980).

Glomerular deposits of immune complexes appear to be responsible for most cases of human glomerulonephritis (Wilson and Dixon, 1976). The granular immune deposits of antigen and antibody occur predominantly

at three sites in the glomerulus:

1. In the mesangium, in early lupus nephritis, Henoch-Schönlein purpura and in IgG-IgA nephropathy.

2. On the subendothelial surface of the capillary wall in the more severe cases of lupus and Type I mesangiocapillary glomerulonephritis.

3. In the subepithelial space on the outer portion of the capillary wall in post-streptococcal glomerulonephritis, and in membranous nephropathy (Couser and Salant, 1980; William and Couser, 1981).

Attempts to demonstrate that cirulating immune complexes can produce these lesions have involved infusing preformed immune complexes of different types and properties. Mesangial localization of a variety of circulating macromolecules, including circulating immune complexes, has been easy to demonstrate by immunofluorescence. The subepithelial immune deposits seen in membranous nephropathy have never been produced by infusion of preformed immune complexes (Couser and Salant, 1980). However, Germuth et al (1979b) could produce subepithelial immune deposits by using very low avidity complexes that tend to undergo intravascular dissociation. Couser and Salant (1980) demonstrated that both mesangial and subendothelial immune deposits containing exogenous antigens can also be produced by in situ mechanisms. William and Couser (1981) stated that the in situ immune complex glomerulonephritis hypothesis does not disprove an immune complex trapping mechanism in the pathogenesis of immune complex glomerulonephritis, but they considered it as an alternative mechanism, and the most acceptable explanation for the subepithelial immune deposit formation. Coi et al (1980) claimed

that the development of immune complex nephritis may be facilitated more by an impaired antibody response that fails to clear the antigen of the invading organism than by the formation of circulating immune complexes. Germuth and co-workers (1975) produced glomerulonephritis without any evidence of immune deposits in glomeruli by repeated intravenous injections of egg albumin into rabbit. O'Regan et al (1976) produced morphological and functional changes in glomeruli, without immune complex deposits, by infusion into rats of immune complexes in antigen excess.

The mechanism and mediators of tissue injury in this form of glomerular disease are unknown. Animal models suggest that both morphology and function of glomeruli may be altered by immune complexes, formed either in vitro or in vivo which do not deposit in glomeruli in amounts that may be detected by current techniques. Stilmant and co-workers (1979) noted the absence of glomerular immune deposits in one-third of his patients with circulating immune complexes and glomerulonephritis. This finding, as well as the reported absence of circulating immune complexes and immunoglobulin or complement abnormalities in patients with glomerulonephritis but without glomerular deposits (Couser and Salant, 1980), raise the possibility that the pathogenesis of immune complex glomerulonephritis might not involve immunologic mechanisms. Stachura et al (1981) detected circulating immune complexes and significant alterations of serum immunoglobulins or complement in the majority of cases of nephritis without immune deposits. In addition, the electrophoretic pattern of urinary immune complexes corresponded to those of serum immune complexes in several of these patients. Thus the passage of immune complexes into urine may account for the lack of glomerular

deposits in some cases of glomerulonephritis. Stachura et al (1981) found a correlation between the presence of circulating immune complexes, alterations of serum IgG, IgA and IgM levels, and factor B and C_3 and C_4 levels with glomerular immune complex deposition. They examined 50 consecutive renal biopsy patients for circulating immune complexes and immune complexes excreted in the urine. They found that five out of eleven patients with idiopathic glomerulonephritis with circulating immune complexes had no renal deposits. This result indicates that immune complexes that persist in the circulation and/or pass the renal glomeruli with minimal deposition may cause tissue injury. Circulating immune complexes in antibody excess were detected in all patients with membranous glomerulonephritis, and in 55% of patients with IgA nephropathy. Glomerular immune deposits were detected by immunofluorescence and electron microscopy and agarose gel zone electrophoresis was used for urinary immune complex screening (Stachura et al, 1981), These observations support the hypothesis of a circulating immune complex pathogenesis for this disease. Determination of circulating and urinary immune complexes in patients with glomerular diseases may help in clinical assessment and provide information about the pathogenesis of these diseases in man.

Autologous immune complex glomerulonephritis pathogenesis was initially described by Edgington et al (1968), who demonstrated deposition of immune complexes along the glomerular basement membrane consisting of an antigen present in the brush border of proximal tubulues of rat kidney and an antibody directed against this antigen. They induced this glomerulopathy by immunisation of rats with either a crude antigen preparation from the brush border, commonly referred to as FxIA, or with

purified nephritogenic antigen. In the first week following immunisation, free circulating autologous anti-FxIA antibody was present in the serum of diseased rats. Fleuren et al (1980) studied the pathogenic role of this free circulating anti- FxIA antibody in autologous immune complexes. The results of these studies indicate that free circulating autologous anti-FxIA antibody plays a pathogenic role in autologous immune complexes and the anti-FxIA antibody is able to bind to the GBM. Therefore, immune aggregates in the glomerular basement membrane (GBM) in this disease might result from in-situ binding of autologous antibody to FxIA-like antigen present in GBM.

1.2. Immune Complexes

Immune complexes are formed after an immune response to an antigen which persists after specific antibody has been produced. This process can be of benefit to the host as it results in the neutralisation or elimination of the antigen. Immune complexes, however, under certain conditions, may localise in vascular structures initiating a pathological reaction. The localisation may result either from formation of the complexes at the site, as in the Arthus reaction, or from deposition of circulating immune complexes in multiple sites which is usually referred to as immune complex disease (Theofilopoulos and Dixon, 1980).

The harmful effects of immune complexes were first suggested by Von Pirquet (1911) through his studies on the toxic factors produced in the onset and course of serum sickness. Dixon et al (1961) demonstrated that the onset of glomerulonephritis and generalised vasculitis coincides with the appearance of soluble immune complexes

in the circulation of one-shot serum sickness in rabbits, and a decrease in the complement levels in the serum and deposition of immune complexes at the sites of injury.

1.2.1. Immune complex formation

When a polyvalent antigen solution is mixed in correct proportion with a potent antiserum a precipitate is formed; if more antigen is added an optimum will be reached after which less or no precipitate will form. At this point the supernatant contains soluble complexes of antigen (Ag) and antibody (Ab) mostly of composition Ag_4Ab_3, Ag_3Ab_2 and Ag_2Ab. Between these extremes the cross-linking of antigen and antibody will give rise to three dimensional lattice structures which coalesce to form large precipitating aggregates (Roitt, 1980). The amount of precipitate varies with the proportion of the reagents. In antibody excess the complexes formed are insoluble. In antigen excess the precipitate tends to dissolve forming soluble complexes (Roitt, 1980). Depending on the nature of the antigenic determinants the antigen-antibody bond may be mainly electrostatic. Alteration of the pH of immune complex solution then results in dissociation and re-association of antigen and antibody as antigen-antibody interation is reversable. Dissociation usually occurs at extremes of pH which make the proteins either all positively or all negatively charged, thus making them lose their capacity for attraction. The factors that determine the formation of immune complexes are:

i. Nature of antigen-antibody bond and lattice formation

The interaction of an antigenic determinant with the antibody binding site is a noncovalent bond and has been postulated to include hydrophobic, hydrogen bonds and Van der Waals forces (Kabat, 1976). Due

to these bonds, the free energy of the antigen-antibody bond favours immune complex formation when an antigen and specific antibodies are present together in solution. Monovalent antigens do not form lattices when they combine with their corresponding antibodies and the formed complexes will remain in the circulation for a long time, while multivalent antigens, e.g. polysaccharides and proteins combine with their specific antibodies to form a network or lattices (Weigle, 1961; Haakenstad and Mannik, 1977).

ii. The association constant for the union of antibody and antigen

Dissociation of some of the immune complex molecules into free antigen and free antibody occurs if the association constant is lowered by high or low pH, high salt concentration, heat or drugs. Dissociation of the non-ionisable complexes can occur in the presence of free hapten (Theofilopoulos and Dixon, 1980).

iii. The size of immune complex formed

Very large immune complexes which form in antibody excess are insoluble and are capable of activating the complement system through C_1 activator; therefore they can initiate the inflammatory process. On the other hand, complexes formed in antigen excess are soluble and small in size but cannot fix complement and consequently cannot initiate an inflammatory process. The intermediate sized immune complexes are soluble and circulate and disseminate easily (Theofilopoulos and Dixon, 1980).

iv. Duration of antigen exposure

If the duration of antigen exposure is limited, tissue injury as well as the other clinical manifestations are transient, e.g. one-shot serum

sickness. In contrast, if the antigen remains for a long time in the circulation or if there is a continuous supply of antigen for a considerable period, as in chronic infections or with autoantigens, the manifestations are persistent, e.g. chronic serum sickness (Wilson and Dixon, 1970).

v. The state of the phagocytic system of the host

The fate of circulating immune complexes is determined by the host phagocytic system, represented both by circulating leucocytes and by the reticuloendothelial system. Overload of the system or the blockage of Fc and complement receptors on mononuclear phagocytic cells may be important factors in allowing more complexes to circulate. If these circulating complexes are in antibody excess, they can activate complement and precipitate immune complex disease (Wilson and Dixon, 1970).

In conclusion, in order for immune complex disease to occur:

1. There must be persistence of complex formation.
2. The complexes must activate the complement system and hence become subjected to phagocytosis, which releases lysosomal enzymes.
3. The complexes which cause disease must be in excess of those phagocytosed.
4. There must be a considerable source of antigen and a good immune response so that antibody excess occurs, and there is a surplus of complexes over those the reticuloendothelial system removes.

vi. Haemodynamic factors and anatomy of particular sites

Immune complex deposition seems to depend greatly on haemodynamic factors. The glomerulus, choroid plexus, synovium, skin and uveal tract sustain a high degree of blood-flow per unit mass of tissue, so they are exposed to, and can trap, large quantities of immune complexes in their vascular walls (Theofilopoulos and Dixon, 1980).

1.2.2. Function of immune complexes

Once antibody combines with its antigen it can assume biological functions of which it was previously incapable, e.g. it interacts with serum components such as those of the complement system and with cellular Fc receptors (Theofilopoulos and Dixon, 1980).

i. Involvement of complement systems

Immune complexes activate the complement system through both the classical and alternative pathways (Müller-Eberhard, 1975). Once this system is activated, several biological activities are generated that play a role in immune complex-induced disease. Complement components which interact with and bind to immune complexes are the early components of the classical pathway, C1, C4, C2 and C3. In addition, if alternative pathway activation occurs, factor B and C_3b of the amplification loop may also be present in significant amounts. The quantities of the complement components depend on the degree of activation of the pathways occurring, but may represent more than 50% of the protein in some immune complexes (Jones and Orlans, 1981). The addition of fresh normal serum to an immune precipitate may result in its solubilisation (Takahashi et al, 1977). It has been reported that complement activation by the immune complex leads to the interposition

of C_3b molecules into the antigen-antibody lattice leading to its rearrangement into smaller and therefore more soluble complexes. This process was recognised in the course of experiments on the fate of immune complexes attached through a C_3b receptor, to the surface of B lymphocytes. It was found that in the presence of normal serum such complexes were released, and were no longer capable of binding to the surface of fresh B lymphocytes, or other C_3b receptor bearing cells (Takahashi et al, 1977). It is now obvious that the release mechanism is the same process as the solubilisation of antigen-antibody precipitates. It has been shown that in rabbits with acute serum sickness, depletion of complement by cobra venom factor delays the removal of glomerular-bound radiolabelled antigen (Bartolotti and Peters, 1978). Cochrane and Janoff (1974) showed that complement and neutrophils are required for tissue damage occurring in the Arthus reaction. However, neither depletion of complement nor that of neutrophils prevents the development of glomerular lesions.

ii. Activation of other plasma enzyme systems

Simpson et al (1973) suggested that the interaction of immune complexes with Hageman factor (factor XII) accounts for the intravascular coagulation and fibrinolysis that follows the administration of antigen to immunised rabbits. In these animals, platelet and fibrinogen levels fall immediately. If Hageman factor is directly activated by immune complexes, then activation of the kinin system could be expected in addition to the activation of coagulation and fibrinolytic systems (Kaplan and Austen, 1972). Cochrane et al (1972), however, could not find any evidence for direct activation of Hageman factor by immune complexes.

iii. Interaction of immune complexes with cell surfaces and the biologic effects of this interaction

Immune complexes modulate cellular immune responses by interacting with B and T cells having Fc, complement and/or antigen receptors. Via such interaction, immune complexes may suppress or augment immune responses (Haakenstad and Mannik, 1977).

Immune complexes containing particular antigens, such as bacteria and red blood cells, have been used to examine the interaction of immune complexes with mononuclear phagocytes and with neutrophils (Cruchaud and Unanne, 1971). Binding sites specific for the Fc fragment of IgG were found on the cell membrane.

Ishizaka et al (1970) demonstrated that human basophils and mast cells have IgE receptors on their surface, and that IgE myeloma proteins would adhere to the surface of basophils and mast cells. The receptor on the surface of the basophil and mast cells were specific for the Fc portion of the IgE molecule.

Immune complexes constitute one of the many substances that interact with platelets to cause aggregation and release of platelet constituents including thrombin, esterase enzyme and adenosine diphosphate (ADP). This explains the obliterative endarteritis found so frequently in the vessels involved in immune complex deposition (Pariyanonda and Mowbray, 1971).

iv. The effect of immune complexes on the immune response

The administration of antigen to experimental animals in the form of an immune complex has long been known to modify the responses to that antigen (Uhr and Möller, 1968). Immune complex present in antigen excess usually enhances the antibody response, while its presence in antibody excess suppresses the antibody response.

1.2.3. Detection of immune complexes

Both autologous and heterologous antigens can trigger immune responses resulting in immune complex disease. Because circulating immune complexes play such an important part in many disease, including autoimmune disease, neoplastic disease and infectious disease, the demonstration of immune complexes in tissue and biologic fluids has a very important role for diagnostic purposes. In tissues the deposition of immune complexes is established by histological or histochemical methods. Histologically, the patterns of injury in pathological tissue may resemble those in experimental animals induced to develop immune complex disease (Wilson and Dixon, 1976). Techniques used for detection of tissue bound immune complexes are immunohistochemical, immunofluorescence, assay for immunoglobulin and in some cases assay for specific antigen (Wilson and Dixon, 1976). Various methods have been devised to detect immune complexes in biological fluids. These techniques are based on the physical properties of immune complexes, their specific recognition by free molecules such as complement component, rheumatoid factor and conglutinin, or their interaction with receptors on various cells for the Fc part of immunoglobulins or for C_3b (Lambert et al, 1978).

1.3. Groups of Immune Complex-mediated Glomerulonephritis

I have used the WHO classification for glomerulonephritis (Churg, 1982).

1.3.1. Diffuse endocapillary proliferative glomerulonephritis

This disease usually occurs during bacterial infection, mainly streptococcal (James, 1972). Clinical, bacteriological and serological data indicate that post-infectious glomerulonephritis is produced by immunological mechanisms (Albini et al, 1979). Acute post-streptococcal glomerulonephritis will be described as the commonest example of this type of glomerular disease in which the agent is known.

1.3.1.1. Acute post-streptococcal glomerulonephritis

This type of renal disease is characterised by acute proliferative and exudative lesions most probably due to immune complex deposition in the glomeruli. The disease usually follows infection with group A, β-haemolytic streptococci (Leaf and Cotran, 1980). It occurs mostly in children, but it may affect any age. There is usually a gap of about fourteen days between the onset of the streptococcal infection and the development of a renal lesion (de Wardener, 1973). The antigens involved in this type of nephritis are most likely to be products of the infecting organism itself; but evidence for the presence of streptococcal components within the glomeruli is still a point of discussion. Treser et al (1970), using fluorescein-labelled antisera to purified streptococcal membrane, stated that the streptococcal antigen was part of the membrane structure of the streptococcal cell. Zabriskie (1971) failed to demonstrate any streptococcal antigens in any of his patient's biopsies. The reason might be that these antigens are presum-

ably present in the circulation only for a short time and the antigenic sites in the glomerular deposits would quickly be covered by the excess of antibody. The delayed method, used in this thesis, for detection of tissue bound antigen using patients' labelled globulins might overcome this problem as it allows enough time for a new equilibrium between the labelled antibody and the antigen complexed in the tissue to occur. In this case the labelled antibody could detect the complexed antigen in the tissue even if it is covered by excess antibody:

$$AgAb \rightleftharpoons Ag + Ab$$

$$Ag + Ab + Ab^* \rightleftharpoons AgAb^* + Ab$$

Clinical picture

The usual presentation is with haematuria which often produces smokey urine (Kaplan et al, 1970; Poon-King et al, 1967). Some oedema is usual. Proteinuria varies from slight to massive proteinuria and a nephrotic syndrome may result (James, 1972); Leaf and Cotran, 1980; Kaplan et al, 1970; Poon-King et al, 1967). Hypertension is usually mild to moderate and responds quickly to the diuresis accompanying improvement in renal function (James, 1972); oliguria may occur but is usually of short duration.

Pathology

Light microscopy

The glomeruli are uniformly affected. They show hypercellularity due to proliferation of the mesangial cells, swelling of the endothelial cells, and infiltration with aggregates. Polymorph-nuclear leucocytes are also usually present. There is an increase in the mesangial matrix

(Burkholder, 1974). The epithelial cells are not usually involved in the proliferative process; crescents are uncommon unless the case is severe (Heptinstall, 1974; Burkholder, 1974). The capillary basement membrane usually shows no thickening but occasionally may be focally thickened. Protein materials, casts, red blood cells and polymorphnuclear leucocytes are often present within the tubules. Hyaline or granular casts are sometimes seen. Some degree of oedema can sometimes be seen in the interstitial tissue. Arteries and arterioles show no change or minimal change (Heptinstall, 1974).

Immunofluorescence and immunoperoxidase

Granular deposits of IgG and C3 are often present along the glomerular basement membrane and mesangium (Heptinstall, 1974; Andres et al, 1978). Heptinstall (1974) stated that C3 staining usually appears stronger and more constant than IgG. Streptococcal antigens have been identified in some cases in the course of the disease (Zabriski, 1971).

Electron microscopy

Subepithelial electron dense granular deposits, usually referred to as humps, are the most prominent feature of this disease (Heptinstall, 1974).

Serological findings

Serum complement concentrations are reduced with the onset of the disease, but usually return to normal within four to eight weeks (Cameron, 1979). Rodriguez-Iturbe et al (1980) have reported circulating immune complexes during the acute stage. Antistreptolysin O (ASO), antistreptokinase (ASK), antihyaluronidase (AH), and antideoxyribonuclease (AINase) are antibodies to the haemolytic streptococcus which

are frequently identified in the serum during the acute stage; elevated titres of one of these antibodies is a useful indicator of recent streptococcal infection (Taranta and Uhr, 1971). These antibodies apparently give little or no protection against the infection (Cameron, 1979). Antibodies formed in response to streptococcal M proteins as well as to extracellular antigens of the streptococcus and the cellular antigen, give prolonged immunity (Kassirer and Schwartz, 1961). Antibodies to streptococcal M protein develop slowly and may remain in the circulation for many years.

1.3.1.2. Diffuse crescented glomerulonephritis

This type of glomerulonephritis was first described by Ellis (1942). It occurs in adults (Stilmant et al, 1979) and is characterised by rapid development of irreversible renal failure and uraemia which often leads to death within weeks or months. The pathogenic mechanism of renal lesion may be mediated by immune complexes, antiglomerular basement membrane antibody or by unknown mechanisms (Glassock, 1979). The disease may occur as an idiopathic entity or as part of other conditions such as systemic vasculitis, Wegener's granuloma, systemic lupus erythematosus, acute post-streptococcal glomerulonephritis, polyarteritis nodosa and Henoch-Schönlein purpura (Heptinstall, 1974; Arieff and Pinggera, 1972; Stilmant et al, 1979; Cameron, 1979).

Clinical picture

There is a sudden onset of oliguria or anuria, often with little or no preceding malaise (Cameron, 1979). Some patients may present with peripheral oedema (Arieff and Pinggera, 1972). Proteinuria and microscopic haematuria are often present also. Hypertension may occur (Leaf

and Cotran, 1980; Stilmant et al, 1979). Usually a nephrotic syndrome occurs over a few weeks to months, and the renal function often deteriorates rapidly.

Pathology

Light microscopy

The essential histopathological feature is the presence of epithelial crescents in 70% or more of the glomeruli (Arieff and Pinggera, 1972; Leaf and Cotran, 1980). It is thought that the crescents are formed partly by the proliferation of the parietal epithelial cells and partly by macrophages (Schiffer and Michael, 1978). The epithelial proliferation may be due to the reaction of the epithelial cells to the blood components such as fibrin and red blood cells which are exuded into the capsular space (Heptinstall, 1974; Andres et al, 1978). When this disease occurs in post-streptococcal glomerulonephritis, the glomerular changes are uniform and there is moderate to marked proliferation of mesangial cells (Andres et al, 1978; Heptinstall, 1974). Adhesions between the glomerular capillary tufts and the capsular crescents are common (Burkholder, 1974). Epithelial proliferation and hyalin droplet degeneration are usually seen in the proximal convoluted tubules. Casts and RBCs are also present in the tubular lumen and interstitial oedema is common (Heptinstall, 1974; Andres et al, 1978).

Immunofluorescence and immunoperoxidase

The immunofluorescent findings depend on the underlying mechanisms which precipitated the disease. In patients in whom antiglomerular basement membrane antibody is the mechanism, diffuse linear deposits

of IgG alone or with C3 have been found (Cameron, 1979, Arieff and Pinggera, 1972; Burkholder, 1974; Andres et al, 1978). Granular pattern of IgG and C3 may be seen along the glomerular capillary walls with or without IgM. In another group of patients no immunoglobulin can be detected in the glomeruli (Stilmant et al, 1979; Arieff and Pinggera, 1972; Andres et al, 1978).

Serological findings

Anti-GBM antibody will be found if the lesion is due to this. Circulating immune complexes may be found in lesions which develop as a result of deposition of immune complexes (Glassock, 1979), in which case the immunoglobulin deposition in the capillary walls is granular rather than linear.

1.3.2. Diffuse mesangial proliferative glomerulonephritis (MPGN) (Lobular Stalk Thickening)

The histologic appearance in this condition is characterised by proliferation, exudation and sclerosis in the mesangial area (Cameron, 1979). This histological picture is similar to the healing stage of acute post-streptococcal glomerulonephritis but evidence of streptococcal infection is lacking in the majority of patients (Cameron, 1979; Migone et al, 1980).

Clinical picture

The common presentation is recurrent proteinuria and haematuria (White et al, 1970; Cameron, 1979). A few patients may present with a nephrotic syndrome (Migone et al, 1980), but most patients show a good recovery (Cameron, 1979).

Pathology

Light microscopy

The histologic appearance is characterised by proliferation, expansion and sclerosis in the mesangial area (Heptinstall, 1974). The proportion of cells to matrix is variable (Cameron, 1979) and minor extension of the mesangium onto the capillary walls may be seen. The capillary walls are almost normal and this forms the distinction between MPGN and MCGN. The lesion is diffuse, affecting all the glomeruli but may vary in severity. Areas of focal and segmental proliferation may be seen in some patients (Cameron, 1979).

Immunofluorescence and immunoperoxidase

Diffuse mesangial deposits are found containing immunoglobulins and complement specially IgG with or without C3. In a few patients there may be no visible deposits. The cause may be that immune complexes that persist in the circulation and/or pass through the glomeruli with minimal or no deposition may cause tissue injury (Migone et al, 1980). In many cases, IgA predominates, a syndrome usually considered separately and designated IgA nephropathy (Berger, 1969; Lowance et al, 1973; Davies et al, 1973; Sisson et al, 1975). Recently a primary mesangial proliferative glomerulonephritis with IgM predominant on immunofluorescence has been described and considered by the authors as a distinct clinical and immunopathological entity (Cohen et al, 1978; Bhasin et al, 1978). The aetiology and pathogenesis of this entity are unknown (Lawler et al, 1980). Germuth and Rodriguez (1979) have shown experimentally that small soluble complexes are located predominantly in peripheral capillary basement membranes, while intermediate, less soluble complexes are in

variably found in the mesangial region. IgM has a very big molecular weight and thus its size precludes formation of small soluble complexes. They also noted that it is only in extremely large antigen excess that intermediate, less soluble complexes are formed with IgM. Although the source of antigens in this disease is unknown, it is possible that those which preferentially generated an IgM immune response, e.g. the common antigen enterobacteria are involved, or abnormalities of the host immune system (Albini et al, 1979).

Electron microscopy

Electron dense deposits are not usually present in the mesangium (Burkholder, 1974). There is an increase in the mesangial matrix. The glomerular basement membrane is usually normal in thickness.

Serological findings

Serum C3 levels are usually normal (White et al, 1970): circulating immune complexes have been detected in some patients (Woodroff and Wilson, 1977).

1.3.3. Diffuse mesangiocapillary glomerulonephritis (MCGN) (Membranoproliferative glomerulonephritis)

This disease was mentioned by Addis and Bell as chronic latent glomerulonephritis (Burkholder, 1974). Mixed membranous and proliferative glomerulonephritis was the name given to the disease by Burkholder (1970). Hypocomplementaemic chronic glomerulonephritis was the name given by Michael et al (1971) and West et al (1965) to describe the persistent deficiency of C3 accompanying this condition. Finally it was called membranoproliferative glomerulonephritis, the name which

provided a clear description of the features of this disease (Burkholder, 1970; Burkholder et al, 1973; Herdman et al, 1970).

The aetiology of this disease is unknown and no causal antigens are recognised. The cause of hypocomplementaemia could be diminished synthesis of C3 (Ogg et al, 1968; Cameron et al, 1970; Colten et al, 1973), as they proved that the liver tissue obtained from these patients did not synthesise C3. Failure of opsonisation because of complement deficiency resulted in inadequate clearance of antigen-antibody complexes by the reticuloendothelial system. Complexes then tend to persist longer in the circulation and deposit in the kidney. A simpler explanation is that the complement deficiency predisposes to immune complex nephritis by rendering the patient more susceptible to infection.

The association between persistent hypocomplementaemia (low C3) and mesangiocapillary glomerulonephritis was first recognised by West et al (1965) and Williams et al (1973). Estimation of serum complement components shows that the classical early components C1, C4 and C2 are usually normal, but C3 is low (Peters et al, 1972). Moreover, the serum from patients with mesangiocapillary glomerulonephritis, when incubated with normal human serum, causes C3 breakdown. This property has been attributed to the so-called C3 nephritic factor (C3NeF) in the serum. Williams et al (1973) established that C3NeF causes C3 breakdown in serum by activating the C3b feedback cycle and that the reaction does not require the classical early complement

components. C3NeF is an IgG antibody to the alternative pathway convertase (C3bB) which stabilises it and prevents its inactivation. As a consequence of this C3 is continuously split, and the large amount of C3b generated inhibits C3 synthesis. So the low C3 level in serum of these patients is partly caused by the increased consumption by the alternative pathway convertase, but largely by C3b mediated suppression of synthesis. It seems that the in vivo action of C3NeF results in depression of at least one of the properdin (alternative pathway) factors, since sera from patients with mesangiocapillary glomerulonephritis are usually without effecton highly purified C3 (Vallota et al, 1971; Peters et al, 1972).

Clinical picture

Early stages of this disease may go undetected by the presence of asymptomatic proteinuria, microscopic haematuria or both. Most cases present with severe oedema and haematuria and some degree of renal impairment (Burkholder, 1974; Cameron et al, 1970). Other groups may present with the complete picture of the nephrotic syndrome (Habib et al, 1973; Magil et al, 1979). This disease seems to occur in the age group under 30 years.

Pathology

Light microscopy

This is characterised by the presence of electron dense deposits in the subendothelial space (Habib et al, 1973; Andres et al, 1978). Usually all glomeruli are affected and there is thickening of the capillary walls with proliferation of the mesangial cells and an increase

in the mesangial matrix, with narrowing of capillary lumina. Occasionally, polymorphonuclear leucocytes may be present within the glomerular tufts. A few epithelial crescents are sometimes seen (Andres et al, 1978; Habib et al, 1973). The blood vessels usually appear normal at this stage unless hypertension occurs, and the interstitial tissue may contain chronic inflammatory cells (Heptinstall, 1974).

Immunofluorescence and immunoperoxidase

Granular deposits or C3 along the glomerular basement membrane are the most striking feature. Immunoglobulins, especially IgG and IgM are present (Burkholder, 1974; Heptinstall, 1974; Habib et al, 1973).

Electron microscopy

Granular dense deposits are present on the endothelial layer of the basement membrane and in the mesangial region (Habib et al, 1973; Magil et al, 1979). There may be interposition of mesangial cell cytoplasm between the glomerular basement membrane and the endothelial cell wall (Magil et al, 1979; Heptinstall, 1974; Andres et al, 1978).

1.3.4. Dense deposit glomerulonephritis (DDGN)

This is a disease induced by the activation of the alternative complement pathway and possibly mediated by antigen-antibody complexes. The mechanism of C3NeF has been discussed before.

Light microscopy

All glomeruli are enlarged and show diffuse thickening of the basement membrane and hypercellularity of varying severity (Davis et al, 1978).

Epithelial crescents are usually more frequent than in mesangiocapillary GN. Neutrophils may be seen inside the glomeruli. When the deposits occupy the whole length of the glomerular capillary walls it is seen like a ribbon (Habib et al, 1973).

Immunofluorescence and immunoperoxidase

C3 is present in all cases along the glomerular and tubular basement membrane; mesangial deposits of C3 are also seen (Habib et al, 1973; Davis et al, 1978). Immunoglobulins are usually absent (Heptinstall, 1974). But Habib et al (1973) and Davis et al (1978) reported the presence of IgM, IgG and IgA in some patients. The cause of absence of undetectable immunoglobulins in some cases might be due to extensive deposition of C3 on the determinants of immunoglobulins and early complement components or because the immune complexes are only intermittently present in the circulation and, in their absence, degradation of determinants of previously deposited complexes occurs.

Electron miscroscopy

Homogenous dense deposits are present along the basement membrane of the glomeruli and tubules and Bowman's capsule (Habib et al, 1973; Davis et al, 1978). Mesangial deposits, cellular proliferation and increase in matrix are usually found (Habib et al, 1973; Davis et al, 1978).

Serological findings

Serum complement levels are reduced in the majority of patients with MCGN and dense deposit GN. C3 levels are decreased in the course of the disease in over 75% of patients and to a greater degree in

dense deposit GN than Type I MCGN (Ooi et al, 1976; Habib et al, 1973; Davis et al, 1978). Clq, C4 and C1 may be decreased in Type I MCGN. The early-acting components are usually normal in dense deposit GN (Lebowitch et al, 1980). C3 nephritic factor (C3NeF) is found in MCGN and in dense deposit GN (Ooi et al, 1976). Circulating immune complexes may be found in both types (Ooi et al, 1977).

1.3.5. Focal proliferative glomerulonephritis

This lesion may occur either as a specific isolated renal condition often with mesangial IgA deposits called Berger's disease (Berger, 1969), or it is associated with systematic disease such as systemic lupus erythemat osus (SLE), Henoch-Schönlein purpura, polyarteritis nodosa or sub-acute bacterial endocarditis.

Berger's disease (IgA, or IgA-IgG nephropathy)

The common presentation of patients with that type of nephritis is haematuria with minimal associated proteinuria (Berger, 1969; Berger et al, 1971; Lowance et al, 1973; Sinniah et al, 1978). Davies et al (1973) reported upper respiratory tract infection preceding the attack of haematuria which may suggest an infectious aetiology. Hypertension may accompany the adult form (Kapoor et al, 1980). This disease is more common in males than females in their teens and twenties (Sinniah et al, 1981). A past history of fever, pain in the loin, sore throat,dysuria and transient oedema have been reported (Sinniah et al, 1981). Some patients present with the feature of nephrotic syndrome.

Pathology

Light microscopy

The glomeruli appear normal on routine histology, where the lesion is minor. Focal and/or segmental mesangial cell proliferation with increased mesangial matrix is seen when the lesion is more advanced. The glomerular basement membrane is usually normal. Hyalinosis and sclerotic lesions are seen in some adult patients. Generalised diffuse proliferation may be seen, and segmental crescents with or without capsular adhesions have been reported (Sinniah et al, 1981).

Immunofluorescence and immunoperoxidase

The mesangium contains IgA, usually accompanied by C3; IgG or IgM may also be present. IgA is not found in extra-glomerular renal vessels, but IgM has been detected in a few patients (Davies et al, 1973).

Electron microscopy

Electron dense depostis are present in the mesangium. Rarely, small subendothelial and subepithelial deposits can also be seen (Sinniah et al, 1981).

Serological findings

Serum IgA level is slightly elevated, while serum levels of IgG, IgM, C3 and C1q and C4 are normal. Circulating immune complexes may be detected (Glassock, 1979).

1.3.6. Idiopathic membranous glomerulonephritis

The antigen responsible for precipitating this type of disease is not known in most cases. A variety of antigens can be involved, derived

from autologous material, e.g. DNA, thyroglobulin, components of tubular brush border, or heterologous materials, e.g. bacterial, viral or parasitic antigens. In a few instances the antigens have been identified as in lupus nephritis where DNA is apparently the major antigen. In the majority of cases there is no clue as to the nature of the antigen, and the disease is termed idiopathic membranous nephropathy (Heptinstall, 1974).

The term membranous glomerulonephritis was given as the most significant changes involved in GBM. Later studies with the aid of thin sections and special staining demonstrated that the thickening of glomerular capillary walls is produced by:

1. Deposition of proteinous material, presumably containing antigen-antibody complexes between the basement membrane and the epithelial cells.
2. Formation of projections of basement membrane material between deposits (Beregi and Varga, 1974).

It appears probable that the glomerular lesion result from in situ formation of immune complexes in the capillary wall of the glomeruli (Couser and Salant, 1980). The role, if any, of the C3b receptors in the glomeruli in membranous GN is unclear (Cameron, 1979; Gelfand et al, 1975).

Clinical picture

Proteinuria with the nephrotic syndrome is the common presentation, but non-nephrotic proteinuria has been recorded in some patients. Mild

microscopic haematuria has been noted and hypertension may be found in adults (Habib et al, 1973).

Pathology

Light microscopy

The light microscopic observations have been established that usually the changes of glomerular capillary walls evolve in a well-defined manner which has been divided into four stages.

In the first stage, only rarely observed, there are small deposits localised between the GBM and the epithelial filtration slits (Andres et al, 1978).

In the second stage, the deposits are more numerous and tend to form a nearly continuous subepithelial layer interrupted by basement membrane (spikes). There is disappearance of foot processes in addition to other morphological evidence of epithelial cell damage (Hepinstall, 1974).

In the third stage, the deposits show a less dense matrix. The deposits are progressively surrounded by the basement membrane. The spikes of GBM extend and surround the deposits. New layers of basement membrane material appear to be produced. As a consequence of these lesions the basement membrane becomes irregular (Burkholder , 1974; Andres et al, 1978).

In the end stage, the deposits are progressively incorporated into the GBM which is irregular and thickened and which contains dense droplets,

probably lipoproteins. In many glomeruli mesangial proliferation, capsular adhesions and sclerosis are present and the lesions may become indistinguishable from those of other chronic glomerular disease (Heptinstall, 1974).

Immunofluorescence and immunoperoxidase

Diffuse granular staining for IgG with or without C3 is always seen along the peripheral glomerular capillary walls. In the first stage, when only small amounts of immune deposits are present on the GMB, the pattern may appear linear. When the subepithelial deposits are diffuse or in advanced stages, a more patchy or ribbon-like pattern may be seen (Burkholder, 1974).

Electron microscopy

Subepithelial electron-dense deposits are the most striking feature. Germuth and Rodriguez (1979) divided the electron microscopic findings into four stages:

Stage I: Characterised by generalised fusion of the foot processes and small rare electron dense deposits in the subepithelial region.

Stage II: The electron dense deposits are larger in size, more numerous and form a continuous sub-epithelial layer interrupted by newly-formed spikes of basement membrane (Germuth and Rodriguez, 1979; Andres et al, 1978).

Stage III: At this stage the capillary loop is more thickened and the membranous transformation is increased; the dense deposits are less dense (Germuth and Rodriguez, 1979; Andres et al, 1978).

Stage IV: This stage represents the terminal stage of the disease. Large masses of electron dense deposits are present and some sclerotic glomeruli can be seen (Germuth and Rodriguez, 1979; Andres et al, 1978).

Serological findings

Circulating immune complexes may be found (Abrass et al, 1980). The C3 level is usually normal.

1.3.7. Minimal change glomerulonephritis (Lipoid nephrosis)

The term lipoid nephrosis was introduced to designate a condition characterised clinically by the nephrotic syndrome and pathologically by abnormalities that appeared to affect the tubules rather than the glomeruli. The aetiology and pathogenesis of this disease are unknown. The failure to demonstrate immunoglobulins and complement and the lack of any glomerular lesion give the impression of normal histology (Cameron et al, 1974). The disease commonly follows a benign course

Gubler et al (1979 reported that focal and segmental glomerulosclerosis may be a variant or a complication of minimal change disease. Rosen et al (1981) agreed that this was likely due to the following findings:
1. The clinical presentation at the onset of minimal change disease often cannot be distinguished from that of focal and segmental glomerulosclerosis.

2. Focal and segmental glomerulosclerosis is found in patients with relapse of the nephrotic syndrome in whom a previous biopsy has shown minimal change; therefore focal and segmental glomerulosclerosis is not 'per se' responsible for proteinuria.

3. Ultra structural findings in the non-sclerotic glomeruli are identical both in minimal change disease and in focal and segmental glomerulosclerosis.

Clinical picture

The commonest presentation is massive proteinuria, hypoalbuminaemia and generalised oedema, usually accompanied by a puffy face (Cameron et al, 1974). Sometimes there is a past history of urticaria (Thomson et al, 1976).

Pathology

Light microscopy

The absence of any obvious abnormality in the light microscopy is that the most important feature in the diagnosis of the lesion (Heptinstall, 1974; Cameron et al, 1974). If the disease persists for a long time, mild thickening of the capillary wall may be seen (Heptinstall, 1974; Andres, 1978). Tubules and interstitial tissue are usually normal despite the presence of fatty and hyaline droplets in the proximal convoluted tubules (Heptinstall, 1974; Burkholder, 1974).

Immunofluorescence and immunoperoxidase

Immunoglobulins and complement are absent from the glomeruli.

Electron microscopy

The only striking feature seen is fusion of the foot processes of the epithelial cells (Burkholder, 1974; Heptinstall, 1974), so that a layer of cytoplasm replaces the foot processes. These changes return to normal in remission and are thought to be caused by proteinuria

(Leaf and Cotran, 1980). There are usually no electron dense deposits, fluid droplets and vacuoles can be seen in the endothelial cytoplasm (Burkholder, 1974; Heptinstall, 1974).

Serological findings

Elevated levels of circulating immune complexes have been detected (Abrass et al, 1980) but serum complement levels are usually normal (Leaf and Cotran, 1980).

1.3.8. Systemic lupus erythematosus and the kidney (SLE)

Systemic lupus erythematosus is one of the immune complex diseases. It occurs ten times more often in females than in males (Cameron, 1979).

The aetiology of the disease is uknown, but many people consider that it has an infective aetiology. Evans et al (1971) detected raised antibody titres to Epstein-Barr virus in 62 sera out of 100 cases of SLE. Panem et al (1978) found C type virus particles in renal biopsies of patients with SLE and they could detect immune complexes containing HEL-12 virus antigens in the kidney in 43 out of 44 patients with lupus nephritis. The renal involvement in SLE ranges from minimal change to diffuse severe forms of glomerulonephritis (Cameron, 1979). The histological changes may be found in two forms:

1. Diffuse proliferative lupus nephritis

In this group, patients usually present with a nephrotic syndrome. Hypertension is common (Leaf and Cotran, 1980). Recurrent haematuria and proteinuria are usual (Baldwin et al, 1977).

Pathology

Light microscopy

Usually all glomeruli are affected. Diffuse mesangial and endothelial cell proliferation can be seen which may be accompanied by exudative changes. There may be focal regions of greatly thickened capillary wall with fibrinoid necrosis (wire loop lesions); epithelial crescents are always present (Burkholder, 1974; Baldwin et al, 1977).

Immunofluorescence and immunoperoxidase

Granular deposits of IgG and C3 are usually present with mesangial predominance. IgM and IgA may be present (Baldwin et al, 1977; Andres et al, 1978).

Electron microscopy

Mesangial dense depostis are the main feature of this type of nephritis (Andres et al, 1978).

2. Membranous lupus nephritis

The common presentation of this type is with severe proteinuria and may be accompanied by the nephrotic syndrome (Baldwin and McCluskey, 1968; Baldwin et al, 1977).

The histological, immunofluorescent and electron-microscopic pictures are identical with the idiopathic membranous glomerulonephrotic changes described above.

1.3.9. Glomerulonephritis associated with infected atrioventricular shunt (Shunt nephritis)

The association of nephritis with an infected atrioventricular shunt in hydrocephalic patients was reported by Black et al (1965). They described two children with atrioventricular shunts, who presented with the nephrotic syndrome and bacteraemia due to a coagulase negative staphylococcus. Infection of atrioventricular shunt with pneumococcus and enterococcus have been reported (Albini et al, 1979). Stickler et al (1968) reported six cases with diffuse glomerulonephritis and associated infected atrioventricular shunts. The organism was a coagulase negative staphylococcus in all patients.

A proliferative glomerulonephritis may be associated with infected atrioventricular shunts. Albini et al (1979) reported the presence of staphylococcus aureus antigens in glomerular deposits together with IgG and C3. The basis of glomerulonephritis occurring during acute pneumococcal otitis infection, sepsis or staphylococcal otitis appears to be of immune complex pathogenesis.

Proliferative and crescented glomerulonephritis were found in patients with visceral abscess. In some of these patients the blood cultures were negative, in some others they were positive for staphylococcus aureus. Circulating antigen-antibody complexes were found in some patients.

Clinical picture

The presentation might be nephrotic with gross haematuria (Stickler et al, 1968). This presentation may not be the main complaint, but

one of the staphylococcal or pneumococcal infection. The evolution of the disease closely paralleled the course of the infection with complete recovery when a rapid cure of the infection was achieved.

Pathology

Light microscopy

Renal biopsy shows a diffuse glomerulonephritis with proliferation of the endothelial and mesangial cells with glomerular lobulation. Polymorph leucocyte infiltration can be seen and thickening of the basement membrane is usually observed (Sato et al, 1979; Dobrin et al, 1975).

Electron microscopy

Granular dense deposits can be seen in the sub-epithelial region (Dobrin et al, 1975).

Immunofluorescence and immunoperoxidase

Granular deposits of IgG, IgM and C3 are usually seen along the glomerular basement membrane and in the mesangial area (Dobrin et al, 1975). Staphylococcal antigens have been detected in the kidney tissue in some patients (Sato et al, 1979).

Serological findings

The serological findings of this disorder are similar to those of infective endocarditis. Serum complement component levels are often low. Circulating immune complexes can be detected (Dobrin et al, 1975; Sato et al, 1979).

1.4. Localisation and Detection of Antigen

1.4.1. 'Immunocytochemistry'

The concept of using fluorescein-labelled antibodies to localise antigens was pioneered by Coons et al (1941; 1950). This was the basis for the development of immunofluorescent microscopy, which has been modifed and expanded to permit localisation of antigens at the ultrastructural level. That was followed by the immuno-peroxidase techniques pioneered by Nakane, Avrameas and Sternberger (Taylor, 1978). Enzymes which produce permanent reaction products of a characteristic colour have been coupled to antibodies and these conjugates used to localise antigens to be visualised at the light microscopic level. The transition from the use of fluorescent tags to that of stable enzyme conjugates was necessary, as the immuno-fluorescent method has several disadvantages. The requirement for fresh tissues and specialised microscopy, together with the impermanence of the stain and poor morphology of the preparation, limited its use in routine diagnostic histopathology. In contrast, the ease with which various cellular substances can be demonstrated on formalin-fixed, paraffin-embedded sections, the good morphological pictures, made the immunoperoxidase technique preferable for pathological investigations. Various enzymes have been used for this purpose, horseradish peroxidase, glucose oxidase, acid and alkaline phosphatase and cytochrome C, but always horse radish peroxidase was the enzyme of choice. Its high enzymatic activity, the availability of suitable electron donors and the ease with which the reaction product can be demonstrated with light or electron microscopy have made it most widely used for immunohisto-chemical studies (Farr and Nakane, 1981). The enzyme peroxidase is

haemoprotein consisting of a carbohydrate shell surrounding a protein core. It has been isolated from various sources such as fish, horse radish, yeast, sweet potato, broad beans, and Japanese radish (Paul, 1960).

Endogenous peroxidase enzyme present in the granules of neutrophils and eosinophils, red cells, and in liver, and has been demonstrated and purified. Since then blocking reagents for the endogenous peroxidase enzyme have been used to avoid the false positive staining (Heyderman and Neville, 1977).

Diaminobenzidine tetrachloride is the most widely used substrate. It forms an insoluble brown polymer in the presence of peroxidase and hydrogen peroxide., which is deposited at the site of the antigen-antibody reaction.

1.4.2. Immunoperoxidase techniques

This technique can be performed in two main ways, namely peroxidase conjugation and non-conjugation techniques:

1. Peroxidase conjugation

In this method, peroxidase-labelled antibody is used either directly or indirectly. In the direct method the antibody conjugate has specificity directed against the antigen under study. In the indirect method a primary antiserum having specificity against the antigen under study is applied initially, followed by a perixodase-labelled antibody from a second species, directed against the immunoglobulin components of the primary antiserum. This indirect method has certain

advantages over the direct method. It is more sensitive and more versatile by substiution of primary antisera against a wide variety of antigens (Fig. 1.1).

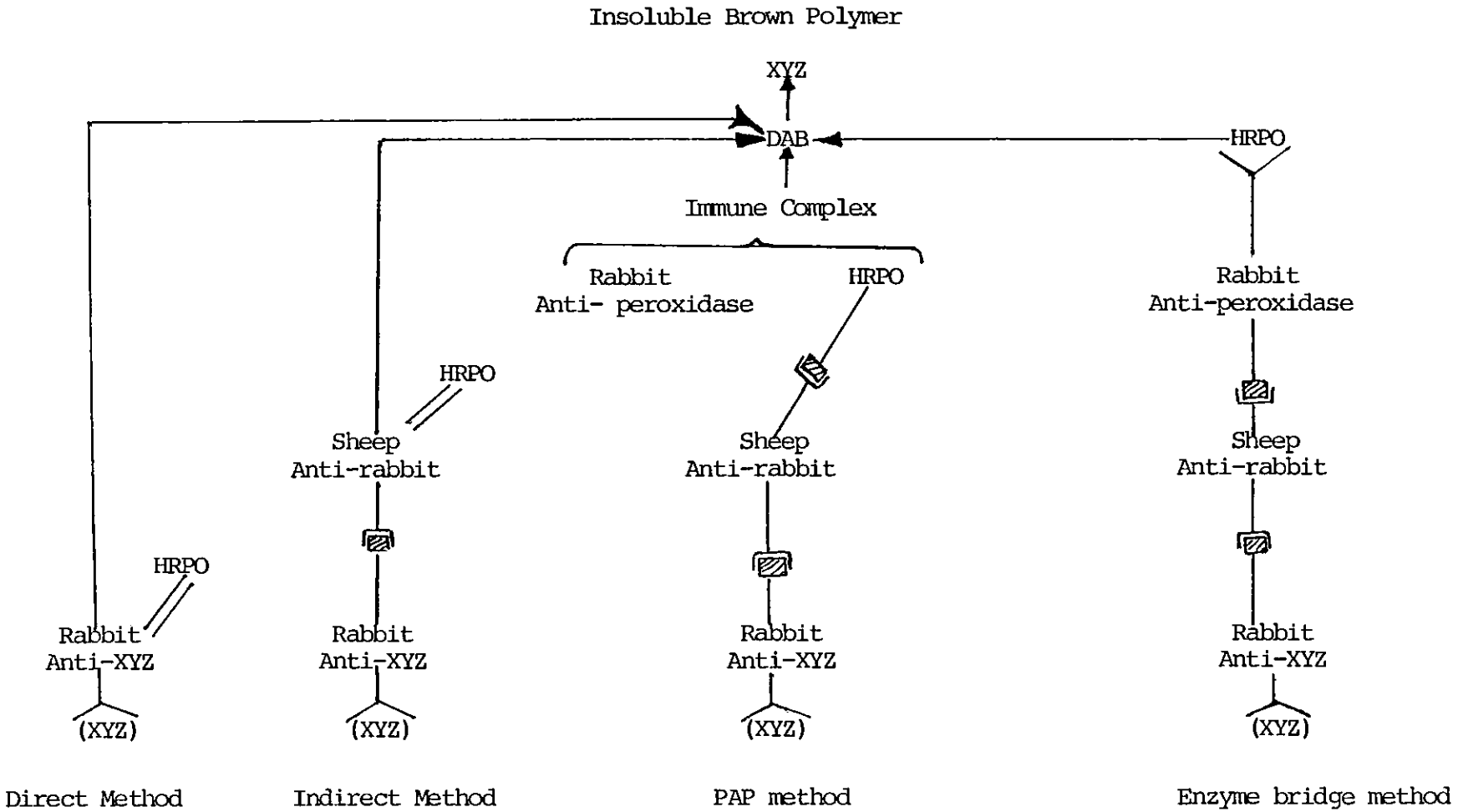
1. Non-conjugated method

This procedure was developed to avoid some of the disadvantages met in conjugated procedures, such as denaturation of antibody, aggregation of unlabelled or partially labelled antibody, inactivation of enzyme and the presence of free enzyme or free unlabelled antibody (Taylor, 1978).

The most commonly used unconjugated method is the peroxidase anti-peroxidase (PAP) technique (Sternberger et al, 1970). In this method both the first and the second antibodies are unlabelled and the third step used an immune complex peroxidase anti-peroxidase raised in the same species in which the first antibody is raised. This method has several advantages. First, there is only the need for one enzyme conjugate per species. Second, it allows the technique to be subject to various specificity controls. Third, since several of the conjugates can bind onto a single first antibody, the colour of the enzyme substrate reaction becomes much stronger. The procedures for the direct, indirect and PAP techniques are outlined in figure 1.1.

There is little technical difficulty in staining sections using the immunoperoxidase technique. The problems are the production of good antisera and the validation of the results achieved with them.

Figure 1.1.



Immunoperoxidase techniques: Symbols: XYZ = antigen under test in tissue on microscopic slide
HRPO = horseradish peroxidase
▨ = conjugate

(After Heyderman, 1979).

Monoclonal antibodies will have the advantage of being well characterised and knowing that the reagents are recognising the same antigenic determinants so that laboratories can compare results.

Controls

1. Normal application

The primary antiserum and the antigen under test = positive staining.

2. Absorption control

This is performed to test the specificity of the positive staining obtained in normal application (1)

a. The primary antiserum + the antigen under test = antibody free serum + antibody-antigen complex.

b. The antibody-free serum + the antigen under test = no staining (Avramease, 1969).

3. Inappropriate antigen control

This is performed to test the specificity of loss of staining in (2). The primary antiserum + unrelated, non-cross-reacting antibody = no loss of activity in the primary serum.

4. Inappropriate antibody absorption control

This is also performed to prove the specificity of the reaction (2). Unrelated non-cross-reacting antibodies + the antigen under test = no loss of activity in the unrelated, non-cross-reacting antiserum.

These are the most reliable controls (Heyderman et al,1979). Other controls such as use of substrate alone, omission of the specific antiserum and substitution with buffer or non-immune serum, comparison with other antisera or with radioimmunoassay, blocking with antibodies from another species, immunodiffusion, are unsatisfactory for the reasons stated below:

1. The use of substrate alone

This is only satisfactory when one is testing an antiserum against a new antigen, to determine that the antigen under test is insensitive to the endogenous enzyme blocking reagent.

2. Omission of the specific antiserum and substitution with buffer or non-immune serum

This control should only be used in very preliminary experiments. The negative staining obtained when the primary antiserum is omitted only indicates that the previous positive staining was due to the primary antiserum; it does not demonstrate the specificity of this antiserum.

3. Comparison with other antisera

Difference in distribution of staining with two different antisera are evidence of differing specificity, but similarity alone cannot be used as evidence of identity. Cells secrete and store a variety of substances which can be localised in only a very small range of sites. Thus antisera to a number of membrane antigens may show similar localisation (Heyderman and Monaghan, 1979).

4. Radioimmunoassay and immunodiffusion data

In both radioimmunoassay and immunodiffusion procedures, using pure antigen label and no immunological cross-reaction is present, the presence of impure antibodies in the antiserum may not interfere with the reaction, while the same impure antibody may be demonstrated in the immunocytochemical staining (Heyderman et al, 1979).

5. Blocking with a serum from another species

This is a negative control using antibodies raised in different species against the same antigen. The idea is that, since both antisera are raised against the same antigen, antibodies from one antiserum can bind onto all the available sites of the antigen under test. If then the other antiserum is applied, there will be no immunological reaction. This reaction may not be reliable, as polyclonal antiserum will probably contain a different mixture of antibodies with different specificity and sub-specificity.

1.5. The Role of Renal Antigens in Immunological Renal Disease

Lesions produced by antibodies to renal antigens have been described early in experimental animals by Lindman, followed by Masugi (Albini et al, 1979). In this experimental model guinea pigs were immunised with homogenates of non-perfused whole rabbit kidney. When the antisera from these guinea pigs were injected into rabbits they developed haemolytic anaemia, proteinuria and died in uraemia. Later considerable evidence has been gained to show that the antigens in this form of nephritis are components of the glomerular basement membrane.

Recent studies (Rudofsky, 1981) showed that non-pathogenic amounts of experimentally induced anti-glomerular basement membrane autoantibodies may cause a modification of the nature of the glomerulus which leads to rapid accumulation of immune complexes and glomerulonephritis in young female mice. Lerner et al (1967) have shown that some forms of nephritis result from autoantibodies directed against constituents of the renal basement membranes.

The basic pathologic mechanism responsible for this form of nephritis is mainly due to the reaction of autoantibodies with constituents of the renal basement membrane.

I will now discuss the renal lesions produced by autoantibodies to glomerular basement membrane antigen, tubular basement membrane antigen, renal tubular brush border antigen, and Tamm-Horsfall glycoprotein.

1.5.1. Anti-glomerular basement membrane glomerulonephritis

This type of glomerulonephritis is usually characterised by extensive formation of epithelial crescents, and by rapid impairment of renal function. Most of the cases are associated with antibody to glomerular basement membrane. The hallmark of anti-GBM antibodies is linear deposits of immunoglobulins, usually IgG, along the GBM (Wilson and Dixon, 1973; Sisson et al, 1974). Linear glomerular basement membrane deposits of immunoglobulins have also been found in SLE (Koffler et al, 1969), kidneys of diabetics, and infrequently in kidneys after perfusion in preparation for transplantation (Wilson and Dixon, 1973).

Detection of circulating or deposited anti-GBM antibodies or both are necessary to support the diagnosis of anti-GBM nephritis. The aetiology of this type of glomerulitis is uncertain. Two hypotheses have been produced:

1. Endogenous glomerular basement membrane (GMB) antigens act as the immunogen after their abnormal release or alteration.

2. Exogenous antigens containing determinants that cross-react with GBM initiate the antibody formation. Toxic exposure (Beirne and Brennan, 1972) and viral infection (Wilson and Smith, 1972) have been implicated.

Because of antigenic similarities in glomerular basement membrane and the alveolar basement membrane of the lung, anti-GBM glomerulonephritis is frequently associated with haemorrhagic penumonitis. The lung shows deposition of anti-GBM antibodies on the basement membrane of alveolar capillaries. This condition is defined as Goodpasture's disease or anti-GBM glomerulonephritis with pulmonary haemorrhage.

Goodpasture's syndrome was first recorded by Goodpasture (1919) in a study of the pulmonary lesions of influenza. While recovering from an influenza attack the patient had haemoptesis, anaemia and proteinuria. The autopsy of this patient showed marked pulmonary alveolar haemorrhage and proliferative glomerulonephritis. Since then a large number of cases with identical findings have been recorded (Benoit et al, 1964).

The pathogenesis of anti-GBM glomerulonephritis is based on the exposure of the immune system to homologous or heterologous antigen to which it is not tolerant. The resulting antibodies cross-react with the host GBM and perform the function of auto-antibodies (Spielgelberg and Weigle 1965).

Clinical picture

In patients with anti-GBM glomerulonephritis without pulmonary haemorrhage, the common presentation is massive proteinuria, anaemia and impaired renal function. These patients usually present with rapid, progressive glomerulonephritis (Sisson et al, 1974). In patients with associated pulmonary lesions the common presentation is haemoptesis which may be massive and cause death (Benoit et al, 1964).

Pathology

Light microscopy

The renal abnormalities in Goodpasture's disease are quite similar to those of anti-GBM nephritis. In both diseases, when fully developed, diffuse inflammatory and necrotising changes are seen in the glomeruli (Benoit et al, 1964). Early in Goodpasture's disease, the renal changes

may be focal and segmental in the tuft, involving only portions of a capillary loop. Epithelial cell proliferation with formation of crescents in Bowman's space, accumulation of neutrophils, and deposition of fibrinoid material in the crescents are frequent. The proliferation of endothelial and mesangial cells is mild and irregular. Glomerular sclerosis may develop rapidly. Inflammatory cell infiltration of the interstitium is common. The lung pathology characterised by severe alveolar haemorrhage, proliferation of septal cells and accumulation of polymorphnuclear leucocytes and haemosiderin-laden macrophages in the alveoli (Heptinstall and Salmon, 1959).

Immunofluorescence and immunoperoxidase

Smooth linear deposition of IgG is seen in the GBM and sometimes in Bowman's capsule and on tubular basement membrane (TBM). In patients with Goodpasture's syndrome IgG and C3 are localised in a linear manner in alveolar basement membrane of the lung capillaries (Sisson et al, 1974).

Electron microscopy

Electron microscopic studies utilising immunoperoxidase techniques showed that IgG deposits are confined to the lamina densa of the GBM. With the progression of the disease, GBM becomes thickened, fragmented and the IgG deposits lose their sharp, continuous, linear pattern.

Serological findings

Endogenous basement membrane antigens are present in the serum and are excreted into the urine of normal individuals. In patients with glomerulo-

nephritis the amount of basement membrane material is increased in the urine. In Goodpasture's disease rising titres of antibodies to influenza A2 virus have been demonstrated. Anaemia and azotaemia have been recorded in almost all patients (Benoit et al, 1964). The circulating anti-GBM antibodies can be demonstrated by one of these methods:

Immunofluorescence: Normal human kidney tissue is used as a target. This method has been the standard test for anti-GBM antibody determination. It does have the advantage of detecting other reactivities in serum, such as anti-TBM antibodies, anti-renal tubular brush border antibodies, and anti-nuclear antibodies. The indirect immunofluorescence assay is limited by the availability of normal kidney target tissue and the variable degree of reactivity between targets.

Radioimmunoassay: This is to detect anti GBM antibodies in renal eluates and to detect circulating anti-GBM antibodies (Mahieu et al, 1973; Marquardt et al, 1973).

Gel diffusion: This technique is occasionally successful, but it is the least sensitive of the methods available.

1.5.2. Anti-tubular basement membrane nephritis

This type of nephritis is rare in man. In 1953 Hill et al showed that rabbit antisera to rat kidney contained antibodies which bound in vitro to rat GBM as well as TBM. Steblay and Rudofsky (1968) described the first model of auto-immune anti-TBM nephritis. They reported that guinea pigs when injected with crude preparations of rabbit TBM in Freund's

adjuvant developed severe inflammatory reactions characterised by linear deposits of IgG along the TBM, tubular cell damage and interstitial accumulation of mononuclear and giant cells. Since passive transfer of serum induces an accumulation of IgG along the TBM and lesions in the interstitium comparable to those of actively immunised animal, this disease seems to be mediated by auto-antibodies. Lehman et al (1974) showed the range of reactivity of anti-TBM antibodies produced by guinea pigs immunised with bovine TBM. Direct immunofluorescence showed linear deposits of IgG along both proximal and distal tubular basement membrane (TBM). Studies of the IgG eluted from the renal tissue revealed antibodies reacting with both GBM and TBM, although the anti-GBM activity was usually not detectable when low concentrations of eluted IgG were used. Absorption studies of eluates from renal tissue showed that approximately 75% of the IgG reacted with bovine basement membrane preparations; all of the reactive antibodies combined with TBM, whereas only 40% cross-reacted with GBM. These results indicate that TBM has both antigens that are shared with GBM as well as antigens which are distinct from those of GBM (Albini et al, 1979; Wilson and Dixon, 1973; Lehman et al, 1975).

The pathogenesis of anti-TBM nephritis in guinea pigs involves the alternative pathway of complement activation. Nevertheless, tubular and interstitial changes have been described in guinea pigs without detectable accumulation of C3 in the TBM, indicating that complement independent mechanisms also operate. Mononuclear cell infiltrates are usually found in antibody mediated lesion. Rudofsky and Pollara (1976) found that tissue injury induced by passive transfer of anti-TBM

antibodies is prevented if the recipient guinea pigs are depleted of radio-sensitive bone marrow cells before injection of antibody. Tubulo-interstitial nephritis mediated by antibodies to the tubular basement membrane of proximal convoluted tubules have been described in pigs (Steblay and Rudofsky, 1971), rats (Sugisaki et al, 1973; Lehman et al, 1974), and mice (Lehman et al, 1974a). Strain-dependent differences in the appearance of TBM-specific antigen were noted in rats, indicating that this antigen is a tissue restricted alloantigen (Sugisaki et al, 1973; Lehman et al, 1974; Hart and Fabre, 1980). It was shown that transplantation of a homologous kidney from an alloantigen-positive rat to an alloantigen-negative rat generated the formation of antibodies to TBM (Lehman et al, 1974; Hart and Fabre, 1980). A similar observation was made in human kidney transplantation (Wilson et al, 1974); apparently in man, the TBM-specific antigen is also an alloantigen. Present studies by Sugisaki et al (1982) showed that immune response to TBM alloantigen accompanied by deposition of IgG along TBM may be elicited in rats by administration of lymph node cells from TBM antigen-negative donor to a TBM antigen-positive recipient. This alloimmune response was not associated with development of tubulointerstitial nephritis. Hart and Fabre (1980) showed that the appearance of TBM antigen is unrelated to the major histocompatability complex of the rat, and that antigen is inherited as a dominant mendelian trait (Sugisaki et al, 1982). Recent studies by Friedman et al (1982) have demonstrated that tubular immune complexes form in the kidneys of rats passively immunised with heterologous antisera to rat Tamm-Horsfall (T-H) complexes are then rapidly removed once the circulating anti-Tamm-Horsfall antibodies have been cleared from the serum of these rats. Thus, circulating antibodies are required not only for forming, but also for maintaining tubular complexes in this passive model.

1.5.3. Renal lesions produced by brush border antigen

This form of renal lesion is rare in man. It produces a chronic progressive membranous glomerulonephritis (Drummond et al, 1966; Alousi et al, 1967). This form of immunologically mediated renal disease was first described in rats by Heymann et al (1959) as an autoimmune nephrosis. Drummond et al (1966) and Alousi et al (1967) reported that this disease occurs in association with certain forms of human glomerulonephritis. Heymann et al (1965) demonstrated that this nephritogenic antigen is not of glomerular origin but is a renal tubular epithelial antigen. Further studies by Edgington et al (1968) have suggested its origin in the brush border cells of the proximal convoluted tubules of the kidney. Albini et al (1979) reported the possibility of occurrence of cytotoxic tubular lesions by autoantibodies leaking into the urinary space through a damaged glomerular filter. Thyroglobulin-antithyroglobulin immune complexes have been shown in the glomeruli of some patients with thyroiditis and membranous nephropathy. The hallmarks of this type of nephritis are:

1. Granular deposits of immunoglobulin and C3 along the glomerular capillary walls (Dixon et al, 1965; Heymann et al, 1963).

2. The appearance of electron-opaque deposits along the sub-epithelial aspects of the glomerular basement membranes (Feldman, 1963).

Renal tubular brush border immune complex nephritis has been identified in a few patients with sickle cell anaemia and in one patient with renal vein thrombosis. In both of these conditions speculation that alterations in oxygenation of haemodynamics may induce a release of brush border antigen into the circulation and lead to an autoimmune response (Edgington et al, 1968).

1.5.4. Tamm-Horsfall glycoprotein (T-H)

Tamm-Horsfall glycoprotein (T-H) is a renal epithelial protein, originally isolated from human urine (Tamm and Horsfall, 1950). Whilst investigating inhibitors of haemaglutination induced by influenza virus, they isolated the inhibitor which has since become known as Tamm-Horsfall glycoprotein.

Tamm-Horsfall glycoprotien has several unusual physicochemical characteristics that may determine both its normal function in distal tubules (the site of synthesis) and also in pathological conditions (Hoyer and Seiler, 1979). The exact function of Tamm-Horsfall protein is unknown but Hoyer and Seiler (1979) found that this glycoprotein may be a useful antigenic marker for the ascending loop of Henle, although it presumably has an important functional role. Aggregation of Tamm-Horsfall protein leading to increased viscosity and gel formation in vitro is promoted by increasing the concentration of electrolytes within the physiologic ranges. This characteristic of Tamm-Horsfall protein may be responsible for the permeability characteristics of this segment of the nephron (Hoyer and Seiler, 1979).

Organ of origin of Tamm-Horsfall glycoprotein

Immunofluorescent studies of antibody directed against Tamm-Horsfall protein proved that the kidney is the organ of origin of urinary Tamm-Horsfall glycoprotein (Keutel, 1965; McKenzie and McQueen, 1969; Schenk et al, 1971). This study has been confirmed by identification of Tamm-Horsfall glycoprotien in urine produced by the isolated, perfused kidney (Cornelius et al, 1965).

Table 1.1.

Localisation of Tamm-Horsfall glycoprotein in the kidney

Reference	PCT	HL		DCT	MD/DT	CD
		DL	AL			
Pape and Maxfield (1964)	+					
Keutel (1965)	+					
Cornelius et al (1965)	+					
Friedman (1966)		+	+	+		+
Pollak and Arbel (1969)		+	+	+		+
McKenzie and McQueen (1969)			+		+	
Wallace and Nairn (1971)		+	+	+		
Schenk et al (1971)			+		+	
Lewis et al (1972)			+		+	
Hoyer et al (1974)			+	+		
Sikri et al (1979)		+	+			
Hoyer et al (1979)		+	+			
Hoyer and Seiler (1979)		+	+			
Sikri et al (1981)		+	+		+	

PCT = Proximal convoluted tubules
DCT = Distal convoluted tubules
CD = Collected duct
MD = Macula densa
LH = Loop of Henle
DT = Distal tubules
DL = Descending limb
AL = Ascending limb.

Localisation of Tamm-Horsfall glycoprotein within the kidney has been studied by many other authors who have claimed that Tamm-Horsfall glycoprotein is present in the cells of proximal convoluted tubules, distal convoluted tubules, loop of Henle and collecting ducts (Table 1.1).

All authors agree that the glomerulus is devoid of Tamm-Horsfall glycoprotein. The presence of Tamm-Horsfall glycoprotein has been reported in fetal kidneys (Wallace and Nairn, 1971).

Tamm-Horsfall glycoprotein in serum

Since it was first demonstrated by Tamm and Horsfall in 1950, Tamm-Horsfall glycoprotein has been considered a renal-specific glycoprotein found only in urine. Early workers could not detect Tamm-Horsfall glycoprotein in serum using crude immunoprecipitation techniques (Grant and Neuberger, 1973; McQueen, 1962). It was claimed by Hodson et al (1975) that Tamm-Horsfall glycoprotein is not normally present in the circulation. They postulated that only with renal damage could the Tamm-Horsfall glycoprotein enter the interstitium of the kidney and hence the blood. Hanson et al (1976) continued this study and postulated that as Tamm-Horsfall glycoprotein was foreign to the circulation, its presence in the blood could induce antibody production.

A sensitive and specific radioimmunoassay technique capable of detecting Tamm-Horsfall glycoprotein in normal human serum at a level between 5-180 mg/ml has been developed by Avis (1977). Dawnay et al (1980) used the same techniques as well as chromatographic studies and they found that Tamm-Horsfall glycoprotein is mainly present in unaggregated form in the serum with a molecular weight of 73,000

daltons. They also found that serum levels of Tamm-Horsfall glycoprotein are significantly reduced in patients with severe chronic renal disease. Dawnay et al (1980) used the same technique, and they assayed normal human serum and serum from patients with renal disease, bilateral nephrectomy, or renal transplantation for Tamm-Horsfall glycoprotein. They found that Tamm-Horsfall glycoprotein was absent only from the serum of patients with bilateral nephrectomy, and it reappeared in their serum when they were given renal grafts. Dawnay and Cattell (1981) suggested that the level of serum Tamm-Horsfall glycoprotein is strongly correlated with renal function.

Pathology of Tamm-Horsfall

The involvement of Tamm-Horsfall glycoprotein in disease states is discussed below:

1. Rate of excretion of Tamm-Horsfall glycoprotein

The rate of urinary Tamm-Horsfall glycoprotein excretion by normal individuals appears to be fairly constant (McKenzie et al, 1964; Grant et al, 1973). The total urinary excretion of Tamm-Horsfall glycoprotein/24 h is markedly affected in certain pathological conditions as measured by a specific radioimmunoassay (Fasth et al, 1981). McKenzie et al (1964) reported an increase in Tamm-Horsfall glycoprotein excretion rate in patients with the nephrotic syndrome. Transient increase of excretion has been reported in patients with acute renal tubular injury produced by potassium dichromate (Schwartz et al, 1972). Grant et al (1973) reported high rates of excretion per functioning nephron in children with Lignac-Fanconi syndrome. Tamm-Horsfall glycoprotein excretion decreases in proportion to

decrease in glomerular filtration rate in patients with chronic renal failure (McKenzie et al, 1964). Significant decrease in Tamm-Horsfall glycoprotein excretion was observed in patients with renal tubular acidosis (RTA) and extensive staghorn calculi (Schwartz et al, 1972). Total glycoprotein excretion was in the normal range in patients with cadmium nephropathy despite reduced glomerular filtration rate suggesting a significant increase in excretion per functioning nephron (Grant et al, 1973).

2. Cast formation

The main constituent of hyaline casts is Tamm-Horsfall glycoprotein (Fletcher et al, 1970). The factors leading to cast formation are primarily the summation of the factors causing aggregation of Tamm-Horsfall glycoprotein and gel formation in vitro; that is increasing concentrations of hydrogen ions, increase in electrolytes, and increase in Tamm-Horsfall glycoprotein secretion (McQueen and Engle, 1966). Thus, cast formation is observed in renal acidosis (Friedman et al, 1951), in dehydration and in the oliguria related to severe exercise (Schrier et al, 1970). McQueen (1962) found cast formation to be independent of Tamm-Horsfall glycoprotein excretion rate and concentration. It has been suggested that tubular obstruction by casts composed of Tamm-Horsfall glycoprotein plays an important part in the development and maintenance of renal failure.

3. Stone formation

Hallson and Rose (1979) found that Tamm-Horsfall glycoprotein promoted calcium oxalate and calcium phosphate crystal formation and induced clustering of calcium phosphate precipitates. They postulated that

Tamm-Horsfall glycoprotein was involved in the first stage of stone formation. The next step would be mineral deposition on the precipitate, followed finally by attachment of the complex to the renal tubules. To date there is no published evidence to support this hypothesis.

4. Unusual tissue localisation

Osmotic removal of water for extravasated urine greatly facilitates the predictable conversion of soluble urinary Tamm-Horsfall into insoluble extratubular aggregates.

Extratubular Tamm-Horsfall glycoprotein has been demonstrated in medullary cystic disease, obstructive uropathy, chronic pyelonephritis and in some other forms of tubular interstitial nephritis (Resnick et al, 1978). Extratubular Tamm-Horsfall glycoprotein deposits, once formed, may persist for prolonged periods and are usually surrounded by inflammatory cells, and provide an excellent antigenic marker for urinary extravasation (Hoyer and Seiler, 1979). An experimental model of human obstructive uropathy with vesicoureteral reflux (reflux nephropathy) has been produced in pigs by Hodson et al (1975). In this model interstitial deposits of Tamm-Horsfall glycoprotein are present, similar to those seen in human renal tissue. Auto-antibodies to Tamm-Horsfall glycoprotein have been found in some patients and animals suffering from the disease in which extra tubular glycoprotein has been observed (Fasth et al, 1981). Aggregation of Tamm-Horsfall glycoprotein in the extravascular space would also probably prevent prolonged circulation of more than minute quantities of Tamm-Horsfall glycoprotein in plasma. Although Tamm-Horsfall glycoprotein had not been detected previously in serum, extremely small quantities of material that reacted similarly to

Tamm-Horsfall glycoprotein in radioimmunoassay were reported to be present in normal human serum (Avis, 1977).

5. Cystic fibrosis

Cystic fibrosis is a genetic disease, resulting from an inborn error of metabolism that involves all exocrine glands and probably most other tissues and organs (Lobeck, 1972). The disease is diagnosed by an increased electrolyte concentration in the patient's sweat. Dische and co-workers (1959; 1961) suggested that cystic fibrosis was due to systemic changes in the structure of the carbohydrate mainly of mucopolysaccharides throughout the body. They described sugar abnormalities in the glycoprotein fractions of duodenal fluid, sweat, and urine from patients with cystic fibrosis. Maxfield and Wolin (1962) described physical differences in Tamm-Horsfall glycoprotein isolated from the urine of cystic fibrosis patients. Recent studies have observed autoantibodies to Tamm-Horsfall in some patients with cystic fibrosis (Fasth and Kollberg, 1980). Serum levels of IgG and IgA were elevated but IgM was normal.

6. Immunologic responses

It is known that renal antigens can be involved in autoimmune diseases and evidence is accumulating that Tamm-Horsfall glycoprotein is one such antigen. It was originally suggested that continuous liberation of renal antigens during pyelonephritis might trigger autoimmune phenomena. This hypothesis was reported by the work of Losse et al (1975) who experimentally induced pyelonephritis in rabbits. They found autoantibodies in the rabbit serum directed against a specific kidney antigen, or against an antigen present in kidney and liver. Injection of tubular basement membrane

induced renal tubular disease in guinea pigs (Steblay and Rudofsky, 1971) and interstitial nephritis in rats (Lehman et al, 1974). It has been shown that when Tamm-Horsfall glycoprotein in complete Freund's adjuvant is injected into rats, they develop tubulointerstitial nephritis involving the thick ALH, Macula densa and the first portion of the DCT (Hoyer, 1980). Granular and nodular deposition of the rat immunoglobulin G and C3 appeared along the basal portions of the involved tubules followed a few days later by mononuclear cell infiltration around the tubules. Friedman et al (1982) showed that circulating anti-Tamm-Horsfall antibodies precipitate and maintain tubular immune complexes in the kidneys of rats. Hanson et al (1976) found raised levels of IgG antibody directed against Tamm-Horsfall glycoprotein in 10 girls with pyelonephritis. They suggested that measurement of autoantibodies to Tamm-Horsfall glycoprotein might be useful to demonstrate and differentiate upper and lower urinary tract infection. However, Fasth et al (1977) found no increase in Tamm-Horsfall glycoprotein antibody in patients with asymptomatic infection of the upper and lower urinary tract. Hodson et al (1975) reported antibody to Tamm-Horsfall glycoprotein in pig serum after surgically induced reflux. They suggested that this was an evidence that formed urine is driven into the lymphatic or blood stream during reflux, and that antibody to Tamm-Horsfall glycoprotein could be used as a guide to detect vesico-ureteric reflux in humans. Losse et al (1975) suggested that liberation of tubular antigens into the blood stream might cause the formation of antigen-antibody complexes which could be deposited at the glomerular basement membrane, and cause acute glomerulonephritis. This could explain the presence of Tamm-Horsfall glycoprotein in the glomerular capsular space in patients with tubulointerstitial disease (McGiven et al, 1978).

Cochrane and Dixon (1976) found abnormal cell-mediated immune responses to Tamm-Horsfall glycoprotein, using the leucocyte migration test, in patients with autoimmune liver disease and renal tubular acidosis. They found material in the cell membrane of normal liver cells that cross-reacted with antibody raised against Tamm-Horsfall glycoprotein in rabbits (Tsantoulos et al, 1974). They suggested that as a result of liver damage, the Tamm-Horsfall glycoprotein-like material is released into the blood, this stimulates an immune response against Tamm-Horsfall glycoprotein and the kidney tubules are damaged by this causing renal tubular acidosis.

Autoantibodies to Tamm-Horsfall glycoprotein have been observed recently in some patients with cystic fibrosis (Fasth and Kollberg, 1980). The highest values were found among people with liver involvement. Autoantibodies to Tamm-Horsfall glycoprotein have been found in patients with urinary tract infection (pyelonephritis and previous renal infection); only IgA antibodies were significantly elevated (Fasth et al, 1981).

2.1. Isolation and Estimation of Immune Complexes from Human Sera

a. Materials

1. Standard serum: Taken from a pool of more than 20 sera from the antenatal clinic, i.e. normal human serum with respect to IgM, IgG and C1q levels. Individual normal sera were also taken from normal volunteers. All sera were stored at -70°C until used (not more than 3 months).

2. Test sera: Either used fresh or stored at -70°C.

3. Antisera: (Anti-C1q, IgG, IgM and IgA) raised in rabbits by immunisation with purified components, and standardised by comparison with known reference antibody.

4. Barbitone buffered saline (BBS): Stock solution was made up from 85 g sodium chloride (BDH Chemicals Ltd.); 3.75 g sodium barbitone (BDH Chemicals Ltd.); 5.75 g barbitone (diethyl barbituric acid) (BDH Chemicals Ltd) in 2 l distilled water.

5. Working BBS: This was made by dilution of stock solution 1:5 with distilled water, pH 7.5.

6. Ethylene diamine tetra acetic acid (EDTA): 0.5 M was made up from 74.44 gm EDTA disodium salt (Fisons Laboratories reagent) dissolved in 1 l distilled water, pH 7.6 and pH 8.6, adjusted with 10 M NaOH

7. Polyethylene glycol (PEG): MW 6000 daltons (Hopkins and Williams Ltd.). Stock solution 20% w/v, e.g. 20 g PEG in 100 ml working BBS.

8. Working solution of PEG: 12% PEG in working BBS and 60 mM EDTA, pH 7.5; (6 ml w/v PEG in BBS; 3 ml 0.2 M EDTA solution, pH 7.6; 1 ml working BBS).

9. Washing solution of PEG: 2% working PEG in 10 mM EDTA, pH 7.6, and BBS, pH 7.5; (5 ml stock PEG in BBS; 2.5 ml 0.2 M EDTA solution, pH 7.6; 425. ml BBS).

10. Agarose (Indubiose A37) (IBE Ltd.).

b. Method

The idea of this method involved precipitation of soluble immune complexes from serum by the addition of PEG. The amounts of C1q, IgG and IgM in the precipitate were measured by single radial immunodiffusion (SRID), and the presence of IgA detected by double diffusion in agarose gel. Values were compared with those obtained from both pooled and individual normal human sera from healthy individuals (Mancini et al, 1965).

PEG precipitation

0.1 ml working solution of PEG, pH 7.6, was added to 0.5 ml of the test serum. The solutions were mixed thoroughly and left overnight at 4°C. Solutions were mixed again before centrifugation at 1000 g

for 20 min at 4°C. The supernatant was decanted and kept at 4°C. The precipitate was washed once with 2 ml of the washing solution, mixed, and then spun as above . The wash was discarded and the pellet redissolved in 0.5 ml BBS. This was mixed well and left for at least 30 min at room temperature.

Preparation of plates for single radial immunodiffusion (SRID)

For estimation of C1q, IgG and IgM, each Mancini gel plate contained a total volume of 25 ml including the antiserum. The Mancini plate was prepared as follows: (example for 10% anti C1q antibody) 250 mg agarose; 5 ml 0.2 M EDTA, pH 8.6; 17.5 ml working BBS; 2.5 ml anti-C19.

The agarose, EDTA and BBS were gently heated until the agarose dissolved. The solution was then cooled to 50°C and the antibody added. The solution was then injected between two glass plates (250 mm x 250 mm), held 1 mm apart by a rubber mask separator. When it cooled, as shown by clouding of the gel, one plate was slid off, the mask removed, and 4 mm diameter holes were punched in the gel. The gel was then removed by suction. The distance between the centres of the wells in each row and column was 15 mm.

SRID

Ten µl of each sample was put in each well. Ten µl of pooled or single normal human sera (suitably diluted in BBS) were added as standards. The plates were incubated overnight at 37°C and precipitin ring diameter was measured the following day to the nearest 0.1 mm. The amount of antigen was calculated from the standards included in each plate.

Double diffusion

A petri dish containing a 1 mm thick 1% agarose gel was prepared by pouring the heated agarose solution into a horizontally placed dish. When cool, a hexagonal pattern of 4 mm diameter wells was then punched in it. Two µl anti-IgA antiserum was placed in the central well, Ten µl of the test serum in one of the peripheral wells and normal human serum in another well as a control. The plates were incubated at +37°C overnight and examined for the presence or absence of a precipitin the following day.

Calculation of Results

When the antigen diffusion out of the 4 mm diameter well has ceased (at equilibrium), the area within the 'precipitin ring' formed is proportional to the amount of antigen originally present. Measurement of the diameter of this ring gives an expression of the amount of antigen in the sample tested (Mancini et al, 1965).

Example:

Let the mean of the diameter of 3 normal serum standards = S (mm) and unknown diameter = U (mm). Knowing that the diameter of the well = d = 4 (mm); and the dilution factor (D) of the normal serum for each plate was as below:

Plate	Assessment of:	NHS dilution	D
Anti C1q	precipitate	undiluted NHS	1
Anti C1q	supernatant	5/6 dilution	5/6
Anti IgG	precipitate	1/15 dilution	1/50
Anti IgM	precipitate	1/5 dilution	1/5

Then, since area of precipitin = area of outer circle-area of inner circle

Standard area of precipitin = $A = \pi(\frac{S}{2})^2 - \pi(\frac{d}{2})^2$

$$A = \pi(\frac{S^2}{4} - \frac{4^2}{4})$$

Unknown area of precipitin = $X = \pi(\frac{U}{2})^2 - \pi(\frac{d}{2})^2$

$$X = \pi(\frac{U^2}{4} - \frac{4^2}{4})$$

and so the ratio $\frac{X}{A}$ represents an expression for the relative amount of antigen in PEG pellet compared with "standard" normal human serum.

$$\frac{X}{A} = \frac{\pi(\frac{U^2}{4} - \frac{4^2}{4})}{\pi(\frac{S^2}{4} - \frac{4^2}{4^2})}$$

$$\frac{X}{A} = (\frac{U^2 - 4^2}{S^2 - 4^2})$$

since A = 100%

therefore $X = \frac{(U^2 - 4^2)}{(S^2 - 4^2)} \times 100\%$

and allowing for the dilution factor (D)

$$X = \frac{(U^2 - 4^2)}{S^2 - 4^2} \times D \times 100\%$$

A computer program, on a Texas Instruments computer, was used to calculate the above data. Measuring C1q, IgG, and IgM present in the PEG precipitate of 80 normal sera, the mean and standard deviation (SD) of this control group were calculated. An upper limit of normal designated as means + 2 SD. Values above these limits for any component (C1q, IgG and IgM) indicate positive immune complex.

Estimation of C3 and C4

Serum C3 and C4 were measured by single radial immunodiffusion using the technique shown in Table 2.1 with the following modifications:

1. The distance between the centres of the wells in each row and column of the anti-C3 and anti-C4 plates was 20 mm.
2. Five µl of serum samples and standards were used for the anti-C3 plates.
3. In the anti-C4 plate three different normal human sera were used as standards.

The calculation of results was carried out as described previously. The dilution factor (D) is one. The sera that these results represent are a mixture of aliquots of various different pools from antenatal clinics, and various serum samples from laboratory 'normals' - at the most, 3 samples from any one person.

Table 2.1

Some arbitrary values that are used in the laboratory to represent sera from normal volunteers

	Upper limit of normal (mean + 2 SD) (%)		
	Mean	SD	ULN
C1q	22	15	52
IgG	0.39	0.15	0.69
IgM	2.22	1.75	5.72
IgA	Lower limit of sensitivity = 0.18%		

Complement components

	Mean	SD	Mean ± 2 SD
C1q	110	9	92 - 128
C3	101	11	79 - 123
C4	100	10	80 - 120

For these purposes serum levels of components are taken to be as follows:

C1q - 200 µg/ml

IgG - 10 mg/ml

IgM - 2 mg/ml

IgA - - 1.4 - 4.0 mg/ml

2.2. Preparation and labelling of $F(ab)_2$ antibody fragments

2.2.1. Preparation of $F(ab)_2$ antibody fragments

Materials

1. Sera tested: Forty-six serum samples were taken from patients with chronic renal disease, mainly chronic glomerulonephritis. The serum samples were supplied by the Renal Unit of St. Mary's Hospital, London.

2. Standard sera

3. Antisera

4. Polyethylene glycol

5. Barbitone buffer

6. Agarose

7. Ethylene diamine tetra acetic acid

8. Sodium phosphate (0.5 M, pH 8.2) (BDH Ltd.).

Materials (2 - 7) were prepared as described in section 2.1.

9. 3 N acetic acid (BDH Ltd.)

One hundred and seventy-four ml glacial acetic acid was diluted to 1 l with distilled water.

10. 3 N sodium hydroxide (BDH Ltd.)

One hundred and twenty gm in 1 l

11. Pepsin (Sigma Chemical Company)

This was obtained from hog stomach mucous (1:60,000) and stored (desiccated) below 0°C.and prepared as 1 mg/ml in 3 N acetic acid.

Method

The immune precipitate was treated with pepsin by a modification of the method of Lachmann (1971). 0.5 ml of immune precipitate was adjusted to pH 3 using 3 N acetic acid. Pepsin was dissolved in 0.2 ml acetic acid in a concentration of 1 mg/ml. Pepsin was then added to the immune precipitate (ratio 1:50).

Example: If the amount of IgG in a particular sample, estimated by SRID is 5%, and the normal IgG content in serum is 10 mg/ml, then the amount of IgG in this sample will be 5/100 x 10,000 µg/ml = 500 µg/ml. So the amount of pepsin in acetic acid needed to digest the IgG present in 1 ml of immune precipitate will be 10 µl and for 0.5 ml of immune precipitate will be 5 µl (according to the ratio stated above; 1 unit pepsin:50 units protein). This mixture was incubated for 5-6 h at 37°C in a water bath; the pH was raised to 5 using 3 N NaOH and then raised to 8 using sodium phosphate (0.5 M, pH 8.2). The mixture was then transferred to a dialysis sac and dialysed overnight against PBS at 4°C. The contents of the dialysis sac were then centrifuged at 4500 g for 10 min at room temperature. The supernatant was stored in glass tubes at -20°C.

2.2.2. Preparation of glutaraldehyde peroxidase

Materials

1. Peroxidase

Horseradish peroxidase type II (Sigma Chemical Company)

2. Glutaraldehyde

This was made up from 25% stock solution, diluted 1:10 with distilled water. EM grade MW 100. 12. Agar aids.

3. Phosphate buffer (0.1 M, pH 6.8)

280 ml 0.2 M NaH_2PO_4; 720 ml 0.2 M $NaHPO_4$. Make up to 2 l with distilled water, pH adjusted to 6.8 with 0.1 N hydrochloric acid.

4. Ethylene diamine tetra acetic acid saline (EDTA saline)

Ten mM EDTA was made up from 3.72 gm EDTA disodium salt (Fisons Laboratory Reagents) dissolved in 1 l of normal saline (0.9% NaCl)

Method

The glutaraldehyde peroxidase used for labelling purposes throughout this study was prepared at 10 mg/ml. For preparing 5 ml of 10 mg/ml glutaraldehyde peroxidase, 50 mg of horseradish peroxidase was mixed with 2 ml of phosphate buffer (0.1 M, pH 7.2) and 0.5 ml of glutaraldehyde solution diluted 1 in 10. The mixture was left overnight at room temperature for the reaction of the glutaraldehyde with the horseradish peroxidase. The following day the mixture was transferred to a dialysis sac and dialysed against 10 mM EDTA saline overnight at 4°C. Next day the mixture was spun (4500 g for 5 min at room temperature) and the precipitate discarded; the volume of the supernatant was adjusted to 5 ml with EDTA saline and stored in glass tubes at -20°C.

2.2.3. Labelling of $F(ab)_2$ antibody fragments

Materials

1. $F(ab)_2$ antibody fragment

Prepared from patients' sera as described in section 2.2.1.

2. Glutaraldehyde peroxidase (10 mg/ml)

Prepared as described in section 2.2.2.

3. Carbonate/bicarbonate buffer (0.5 M, pH 9.5)

0.5 M sodium carbonate (BDH Chemicals Ltd.): 0.5 M sodium bicarbonate (Hopkin and Williams Ltd.).

Equal volumes of each were mixed together and the pH adjusted to 9.5.

4. Ethanolamine (0.1 M) (BDH Chemicals Ltd.)

5. Phosphate buffered saline (PBS) (0.2 M, pH 7.2)

40 ml 0.2 M phosphate buffer, pH 7.2; 17 gm sodium chloride.

Make up to 2 l with distilled water.

Method

The amount of $F(ab)_2$ fragments in the sera was determined by Biuret reaction (see 2.2.4). It was assumed to be half of the amount of IgG rather than 2/3, to make allowance for the recovery we normally achieved. As the ratio of glutaraldehyde peroxidase to IgG is 2:1, for $F(ab)_2$ antibody fragment, the glutaraldehyde peroxidase was added in the ratio 1:1

Example

If the IgG content of the serum is 5% (0.5 mg/ml) so the amount of $F(ab)_2$ in this particular sample is assumed to be 0.25 mg/ml and 0.125 in 0.5 ml of serum. Therefore the amount of glutaraldehyde peroxidase needed for labelling 0.5 ml of this sample will be 0.125 mg which is present in 1.25 ml as the glutaraldehyde is 10 mg/ml; 0.6 ml of 0.5 M carbonate/bicarbonate buffer, pH 9.5, was added and the mixture incubated at room temperature for 18 h. Next day 0.1 ml of 0.1 M ethanolamine was added, the mixture was then incubated in a water bath at 37°C for 30 min. The mixture was dialysed overnight against PBS and stored in glass tubes at -20°C until used.

A partial purification of peroxidase labelled $F(ab)_2$ was performed by precipitation with Na_2SO_4 in presence of carrier of normal serum or antiserum. The specificity of labelled $F(ab)_2$ antibody was demonstrated by immunoelectrophoresis.

Purification of peroxidase labelled $F(ab)_2$

The labelled mixture was added to normal human serum or rabbit antiserum was used as a carrier to allow precipitation of $F(ab)_2$ with 20% Na_2SO_4 in a water bath at 37°C for 30 min. This partial purification of peroxidase labelled $F(ab)_2$ does not remove undigested soluble complexes, Fc or some smaller products completely; all these contaminants are labelled. The preparation was dialysed against saline overnight at 4°C. This preparation was used for immunoelectrophoresis. Immunoelectrophoresis was performed as described in Section 2.5).

2.2.4. Biuret method for estimation of protein

Materials

Stock Biuret reagent; working Biuret reagent; 0.1 M sodium carbonate solution (as diluent).

Method

One ml of appropriate diluted sample was mixed with 1 ml of working Biuret reagent, and incubated for 15 min at 37°C. One ml of 0.1 M sodium carbonate was used as a blank without any protein. The optical densities of the solutions were read at 540 nM in a Guildford 300-N microsampler spectrophotometer. The protein concentrations of the samples were read from a standard graph of optical density at 540 nM against the amount of protein added.

It was found that the amount of protein in $F(ab)_2$ samples was about half the amount of protein present in the original IgG samples.

2.3. Prepration of peroxidase labelled globulin

2.3.1. Preparation of globulin

Materials

1. Sera tested

One hundred serum samples were taken from patients undergoing renal biopsy in the Renal Unit of St, Mary's Hospital. Biopsy was usually for renal disease, mainly chronic glomerulonephritis. These sera were stored at -70°C until used.

2. Sodium sulphate (26.7%) (Fisons Chemicals Ltd)

This was prepared by dissolving 26.7 gm of sodium sulphate in 100 ml distilled water.

3. Sodium sulphate (20%)

Twenty gm of sodium sulphate was dissolved in 100 ml of distilled water.

4. Acetate buffer (0.02 M, pH 5.5)

Solution A was made up from 0.02 M acetic acid; 1.15 ml in 1 l distilled water.

Solution B was made up from 0.02 M Na acetate; 1.64 gm in 1 l distilled water.

14.8 ml of solution A and 35.2 ml solution B were made to 100 ml with distilled water.

The pH was adjusted to 5.5.

Method

Serum was dialysed against 0.1 M phosphate buffer, pH 7.8. 0.1 ml of dialysed serum was added to 0.3 ml of 26.7% sodium sulphate solution (i.e. final concentration is 20%). The mixture was thoroughly mixed and incubated in a water bath at 37° for 30 min. The mixture was then centrifuged (4500 g for 10 min at room temperature). The supernatant was then decanted and the precipitate washed twice with 1 ml of 20% sodium sulphate solution. The precipitate was dissolved in 0.1 ml phosphate buffer (0.1 M, pH 7.8) and dialysed against running tap water overnight. The following day the dialysis sac was transferred to 0.02 M acetate buffer, pH 5.5, and left overnight at 4°C. This step was done to precipitate macroglobulins and acid euoglobulin (IgM). Next day the contents of the dialysis sac were centrifuged at 4500 g for 5 min at room temperature and the supernatant saved.

The amount of IgG was measured by single radial immunodiffusion.

2.3.2. Labelling the globulin

I have used the standard method of Avrameus and Ternynk (1971).

Materials

Globulin

This was prepared from the sera of renal patients as described in section 2.3.1.

2. Glutaraldehyde peroxidase (10 mg/ml)

This was prepared as described in section 2.2.2.

3. Carbonate bicarbonate buffer (0.5 M, pH 9.5)

0.5 M sodium carbonate (BDH Chemicals Ltd.); 0.5 M sodium bicarbonate (Hopkins and Williams Ltd.).

Equal volumes of each were mixed together and the pH adjusted to 9.5

4. Phosphate buffered saline (PBS) (0.2 M, pH 7.2)

Forty ml of 0.2 M phosphate buffer, pH 7.2; 17 gm sodium chloride. Made up to 1 l with distilled water.

5. Ethanolamine (BDH Chemicals Ltd.)

0.1 M soultion was prepared.

Method

The pH of the globulin (IgG) was adjusted to 7.2 using 0.5 M carbonate/ bicarbonate buffer, pH 9.5. 0.1 ml of globulin was added to 0.2 ml of glutaraldehyde peroxidase (10 mg/ml) and 0.3 ml of 0.5 M carbonate/ bicarbonate buffer, pH 9.5. This mixture was left overnight at room temperature. Next day 0.1 ml of 0.1 M ethanolamine was added to destroy the aldehyde groups of any residual glutaraldehyde and was left in a water bath at 37°C for 30 min. The mixture was dialysed overnight against 0.2 M phosphate buffered saline (PBS), pH 7.2, at 4°C. The following day the contents of the dialysis sac were centrifuged at 4500 g for 10 min and the supernatant stored at 4°C in a glass tube.

2.4. Preparation of peroxidase-labelled Tamm-Horsfall antibody

2.4.1. Isolation of Tamm-Horsfall glycoprotein

Tamm-Horsfall antigen was isolated and purified from normal human urine using the original method of Tamm and Horsfall (1950) and adopted by Goodall and Marshall (1978). This antigen was a gift kindly donated by Mrs. A.A. Davies. Urine was collected in 2 litre measuring cylinders containing 2 mg of sodium azide as preservative. Urine from healthy men was pooled and the pH adjusted to 6 using N HCl. Tamm-Horsfall glycoprotein was precipitated by adding 0.58 M NaCl and left overnight at 4°C. A fluffy precipitate formed which formed a pellet on centrifugation (6000 g for 10 min at 4°C). The pellet was resuspended in 100 ml of 0.58 M NaCl and centrifuged as before. The pellet was resuspended in 10 ml of water and freeze-dried.

2.4.2. Raising antiserum to Tamm-Horsfall glycoprotein

The first batch of Tamm-Horsfall antiserum was supplied by Mrs. A.A. Davies. She raised Tamm-Horsfall in rabbit and the rabbit IgG was isolated by chromatography on Whatman DE23 cellulose. The next batch of Tamm-Horsfall antiserum was raised by the author, mixing 0.5 ml of Tamm-Horsfall antigen solution (1 mg/ml) and 0.5 ml of complete Freund's adjuvant to form an emulsion. This was achieved by forcing the mixture backwards and forwards through a fine needle between two syringes (Berlin and McKinney, 1958). 0.5 ml of the emulsion was injected intramuscularly (0.1 ml injected into 5 dorsal sites) into a New Zealand white rabbit. Two weeks later, 0.5 ml of the emulsion (1 ml/ml) was injected intravenously into the marginal ear vein. After a further 10 days, the rabbit was bled from an ear vein. The

blood was collected and allowed to clot. Serum was separated by centrifugation 2000 g for 15 min at room temperature), and the serum was stored in 0.5 ml aliquots at -20°C.

The specificity of Tamm-Horsfall antibody was tested against reference Tamm-Horsfall antigen and whole human serum using immuno-electrophoresis and double diffusion method. The test antiserum was shown to be monospecific Tamm-Horsfall antibody.

2.4.3. Preparation of Tamm-Horsfall antibody

The precipitation of Tamm-Horsfall antibody (IgG) from the antiserum was the same method as described before for the preparation of globulin from serum (Section 2.3.1).

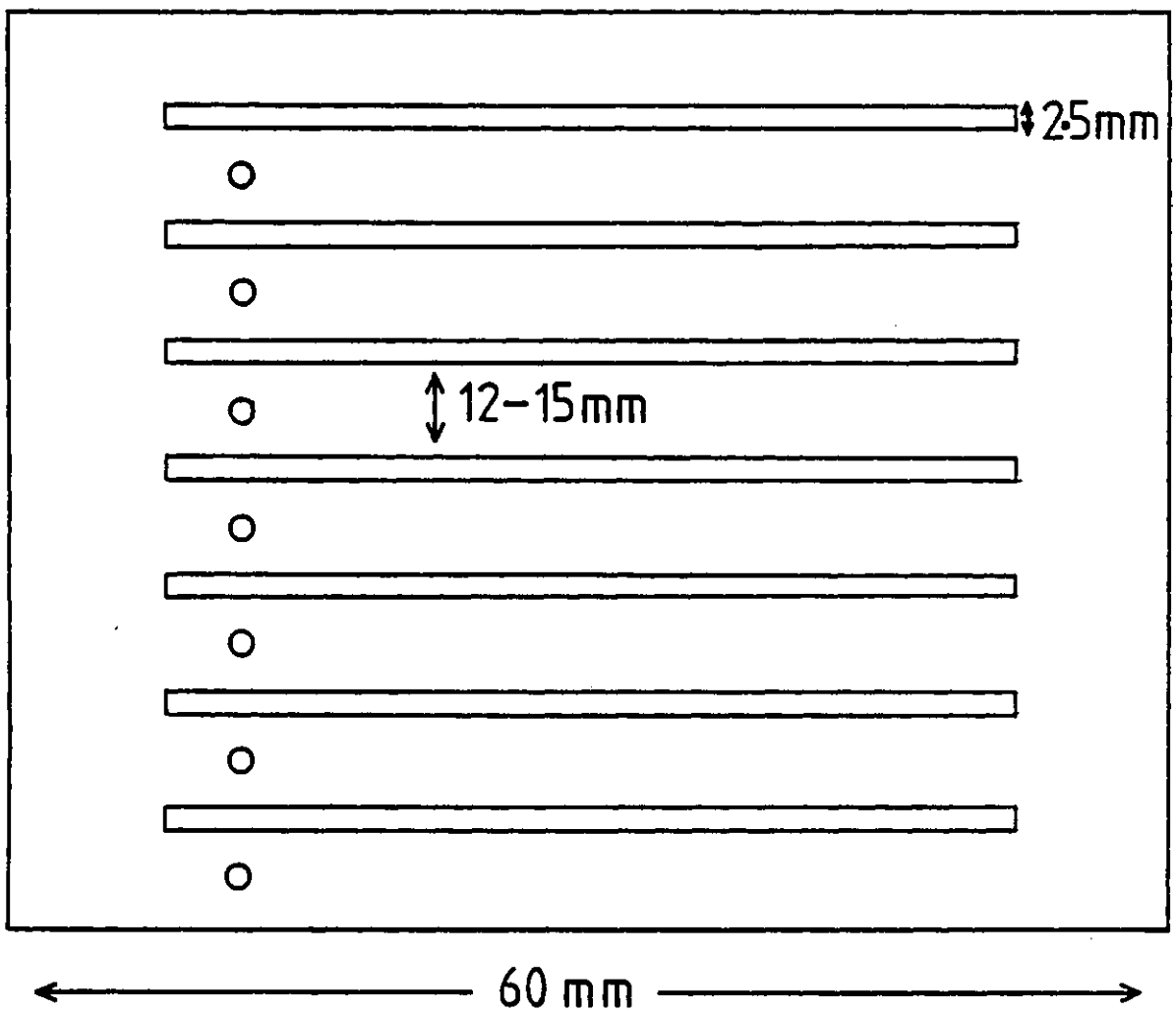
2.4.4. Labelling Tamm-Horsfall antibody

The materials and method used for labelling Tamm-Horsfall antibody was that of Avrameus and Ternynck (1971) which was discussed in Section 2.3.2.

2.5. Immunoelectrophoresis

Agarose (0.5 g) was heated in 50 ml EDTA (25 mM, pH 8.6) until it dissolved. The hot solution was poured into a mould, for a slab gel, made from two pieces of glass clamped together with a 1 mm gasket between them. When the gel had set, the gasket and upper sheet of glass were removed. The gel was left on the lower sheet of glass and 4 mm diameter wells were cut into it with 2.5 mm troughs between the wells. Antigen samples (10 µl) were placed in the wells. Bromophenyl blue was added to a well containing a control serum, and a voltage was applied across the gel until the dye front reached the end of the gel (e.g. 90 min at 80 mA and 90 volts). The buffer chambers contained 50 mM EDTA, pH 8.6. The gel was removed from the buffer and the desired antisera or antibody were put into the troughs. The gel was placed in a damp, sealed box and left overnight at room temperature (figure 2.1).

Figure 2.1.



Arrangement used for immunoelectrophoresis

2.6. Immunoperoxidase techniques

I have used the direct immunoperoxidase method of Avrameus and Ternynck (1969) for materials treated by Tamm-Horsfall antibody. I have developed another method for treating the renal biopsy tissues with labelled globulin and found that the longer the labelled antibody stayed on the tissue the stronger the staining obtained, but not with paraffin sections which I have used for Tamm-Horsfall glycoprotein.

2.6.1. Direct immunoperoxidase technique

Materials

1. Renal biopsy tissues

Renal biopsies were obtained from patients with chronic renal disease, mostly chronic glomerulonephritis. The tissues supplied were mainly from the renal cortex.

2. Liquid nitrogen

3. Tissue Tek II (OCT)

This is compound embedding media for frozen tissue specimens to mould it into blocks.

4. Phosphate bufferd saline (PBS) (0.1 M, pH 7.2)

Prepared as previously

5. Labelled antibody

These include labelled globulin, labelled $F(ab')_2$, and Tamm-Horsfall antibody, prepared and labelled as described earlier.

6. Substrate solution (Graham and Karnovsky, 1966)

Three mg of 3,3 diaminobenzidine tetrachloride (DAB) was dissolved in 4 ml of phosphate buffer (0.1 M, pH 7.2); 50 µl of 10 volume per cent hydrogen peroxide was added immediately before use. Substrate solutions were freshly prepared for each batch of slides.

7. Humid chamber

Sealed damp box containing a wet sponge was used to keep the slides from drying.

Method

Each renal biopsy specimen was processed into several pieces for frozen and paraffin sections.

a. Frozen section technique

Two or more blocks, about 4 x 3 x 1 mm of renal biopsy tissue were moulded in OCT embedding medium on a strip of aluminium foil and frozen in liquid nitrogen for a few seconds. Frozen blocks with their aluminium foil shell were kept in pre-cooled bijou bottles at -70°C until used.

The method used for staining with $F(ab')_2$ antibody fragments was that of Avrameus and Ternynck (1969).

The frozen tissue blocks were mounted on a frozen chuck of a Lipshaw cryostat with a few drops of OCT and left for a few minutes to cool to -20°C, or were dipped in liquid nitrogen. Four micron cryostat sections were picked up on PTFE multispot slides (12 spots per slide)

and washed in 0.1 M phosphate buffered saline, pH 7.2, for 30 min. The slides were then dried at room temperature for 15 min; sections were incubated with peroxidase labelled conjugates diluted 1/4 with PBS. The slides were then incubated in a humid chamber at 4°C overnight. The labelled conjugates used were:

1. $F(ab')_2$ antibody fragments.
2. Anti IgG as a control.

At the end of incubation, slides were washed in phosphate buffer and then allowed to dry at room temperature. Sections were stained as described by Graham and Karnovsky (1966). The slides were then dehydrated in ascending alcohols, cleaned in xylol and mounted in DPX.

b. Paraffin section technique

The tissue was fixed in 10% formol saline overnight at room temperature and then embedded in paraffin wax at 58°C overnight. Four micron paraffin sections were cut with a light rotary microtome (Leitz-Weizlar) and kept at room temperature.

For Tamm-Horsfall antibody staining I used 4 micron paraffin sections as Tamm-Horsfall staining appears to be more clear and obvious in paraffin sections than in frozen sections. The paraffin sections were dewaxed in xylene, hydrated in descending alcohols, rinsed in water and washed in PBS for 30 min. The labelled Tamm-Horsfall antibody was applied to the slides in 1/4 dilution with the aid of a Pasteur pipette and they were incubated in a humid chamber at 4°C overnight. Next day the slides were washed for 30 min in PBS and allowed to dry at room temperature. Sections were then stained as described previously.

With every batch of slides used there was a control section taken from normal human kidney.

2.6.2. Delayed immunoperoxidase technique

I have developed a new technique in the laboratory for the reaction between antigen deposited in the renal tissue and the labelled globulin. During my work on frozen sections of renal biopsies with labelled globulin I noted that some of the specimens which did not give specific staining could give better results if they were incubated with the labelled globulin for a longer time at the same dilution. This was not as good a staining as I had hoped, because although there was specific staining of the deposits, it was weak and the deposits were less localised and the granularity was blurred. I looked for the reason which might have caused this weak staining and thought that some of the complexes in the tissue might dissolve in the drop of buffer on the section and be lost when the section was washed. I tried 3% PEG in 10 mM EDTA as a buffer for diluting the globulin 1 in 4 (i.e. the final concentration of the PEG was 2.3%) to keep the complexes precipitated. I think this system overcame the problem and I did get good staining using it.

The idea of delayed technique

The reaction between antigen and antibody is a reversible mass action equilibrium. There is continuous dissociation and re-association between the antigen and the antibody within the immune complex molecule resulting in the formation of free antigen and free antibody:

$$AgAb \rightleftharpoons Ag + Ab$$

So if we apply the labelled globulin on the tissue and allow sufficient time for the reaction, a new equilibrium will occur between the free antigen sites in the complex and the labelled globulin. Of course, that will occur only if the antibody complexed in the tissue is the same as the labelled one which we have applied, so that they can both identify the antigen complexed in the renal biopsy, otherwise the reaction will not take place:

$$AgAb \rightleftharpoons Ag + Ab \quad + \ *Ab \rightleftharpoons Ag*Ab + Ab$$

Preliminary experiments

Experiment 1

Five slides were prepared, each with six frozen sections of renal biopsy tissue which was known to show granular deposits, presumably of immune complex when stained with labelled IgG antibody.

Labelled IgG was applied in the following dilution using 0.2 M PBS, pH 7.2.

neat 1:2 1:4 1:8 1:16 1:32

on sections 1, 2, 3, 4, 5, and 6.

Slide No. 1. washed and stained after 24 h

Slide No. 2. " " " " 48 h

Slide No. 3. " " " " 72 h

Slide No. 4. " " " " 96 h

Slide No. 5. " " " " 120 h

The score of each section is shown in Table 2.2 and examples of these sections are shown in figures 2.2 to 2.7.

Table 2.2.

THE EFFECT OF TIME ON FROZEN SECTIONS STAINED BY LABELLED IgG

Slide No. 1.

Dilution	Section 1	Section 2	Section 3	Section 4	Section 5	Section 6
Neat	High background					
1 in 2		High background				
1 in 4			Weak granular deposit			
1 in 8				Weak granular deposit		
1 in 16					Very weak granular deposit	
1 in 32						Very weak granular deposit

Washed after 24 hours

Table 2.2. (continued) - THE EFFECT OF TIME ON FROZEN SECTIONS STAINED BY LABELLED IgG

Slide No. 2.

Dilution	Section 1	Section 2	Section 3	Section 4	Section 5	Section 6
Neat	High background					
1 in 2		High background				
1 in 4			Brighter granular deposit			
1 in 8				Brighter granular deposit		
1 in 16					Weak background	
1 in 32						Weak background

Washed after 48 hours

Table 2.2. (continued) - THE EFFECT OF TIME ON FROZEN SECTIONS STAINED BY LABELLED IgG

Slide No. 3.

Dilution	Section 1	Section 2	Section 3	Section 4	Section 5	Section 6
Neat	High background					
1 in 2		High background				
1 in 4			Good specific granular deposit			
1 in 8				Good specific granular deposit		
1 in 16					Good specific staining	
1 in 32						Good staining weak background

Washed after 72 hours

Table 2.2. (continued) - THE EFFECT OF TIME ON FROZEN SECTIONS STAINED BY LABELLED IgG

Slide No. 4.

Dilution	Section 1	Section 2	Section 3	Section 4	Section 5	Section 6
Neat	Very high background					
1 in 2		High background				
1 in 4			Very good specific granular deposit			
1 in 8				Very good specific granular deposit		
1 in 16					Very good specific granular deposit	
1 in 32						Very good staining Weak background

Washed after 96 hours

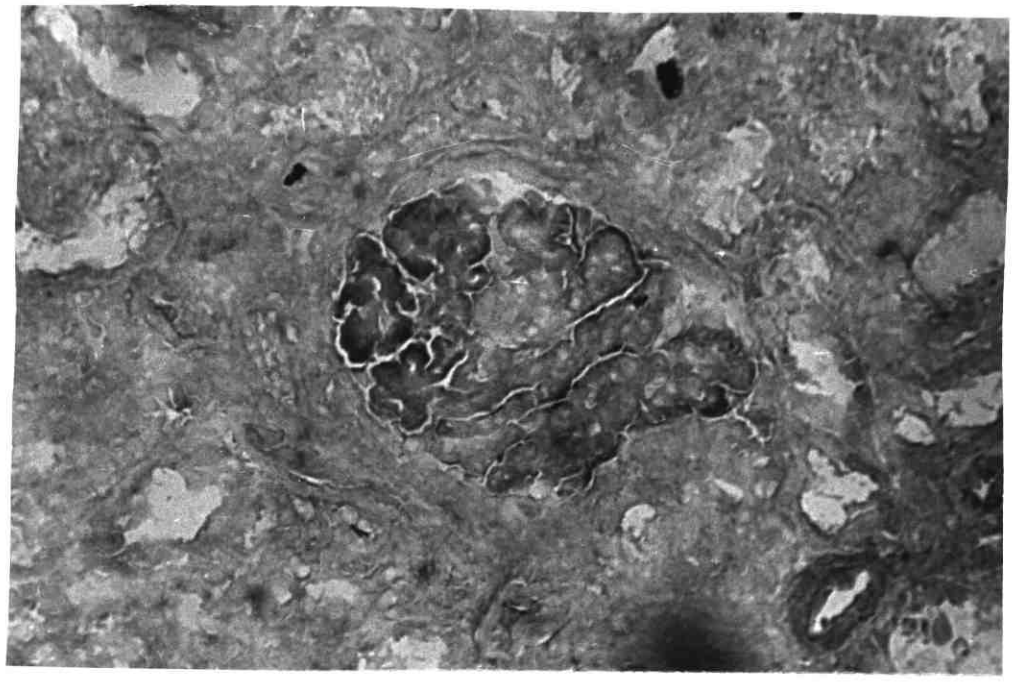
Table 2.2. (continued) - THE EFFECT OF TIME ON FROZEN SECTIONS STAINED BY LABELLED IgG

Slide No. 5.

Dilution	Section 1	Section 2	Section 3	Section 4	Section 5	Section 6
Neat	Very high background					
1 in 2		Very high background				
1 in 4			Very good specific granular deposit			
1 in 8				Very good specific granular deposit		
1 in 16					Very good specific granular deposit	
1 in 32						Very good staining Weak background

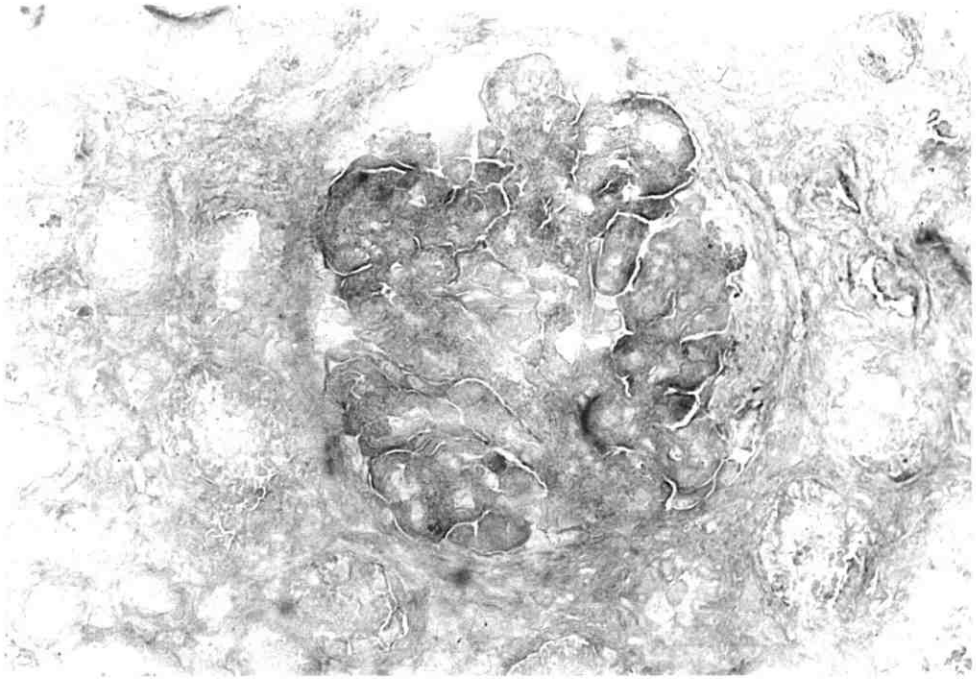
Washed after 120 hours

Figure 2.2.



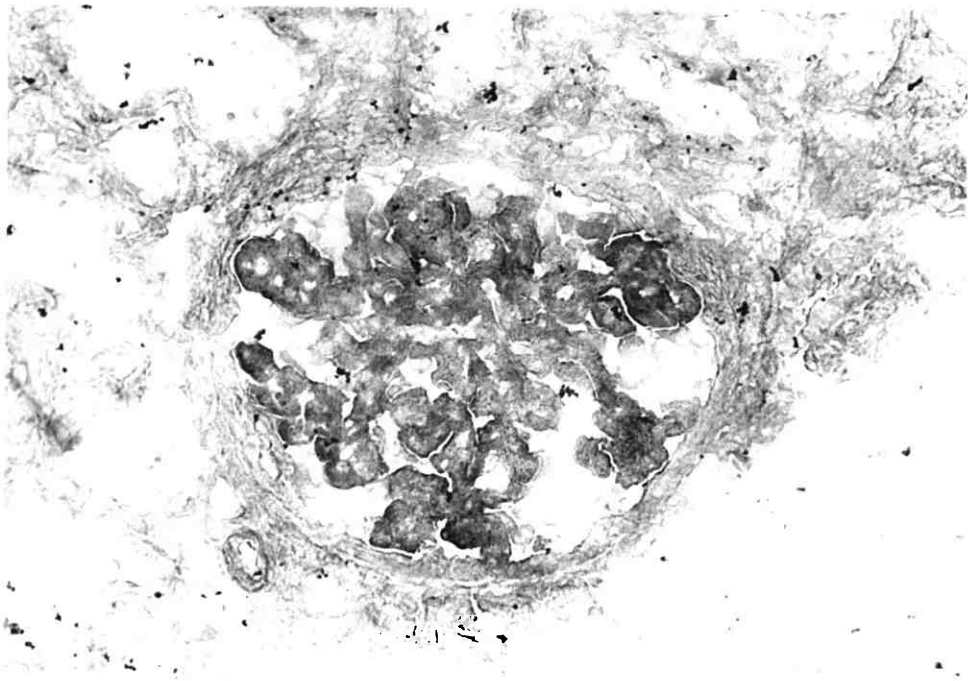
Direct immunoperoxidase staining in a patient with mesangial proliferative glomerulonephritis showing granular deposits of immune complex in the mesangium with high background. Tissue incubated with peroxidase-labelled IgG diluted 1 in 2 for 24 hr. (x 132).

Figure 2.3.



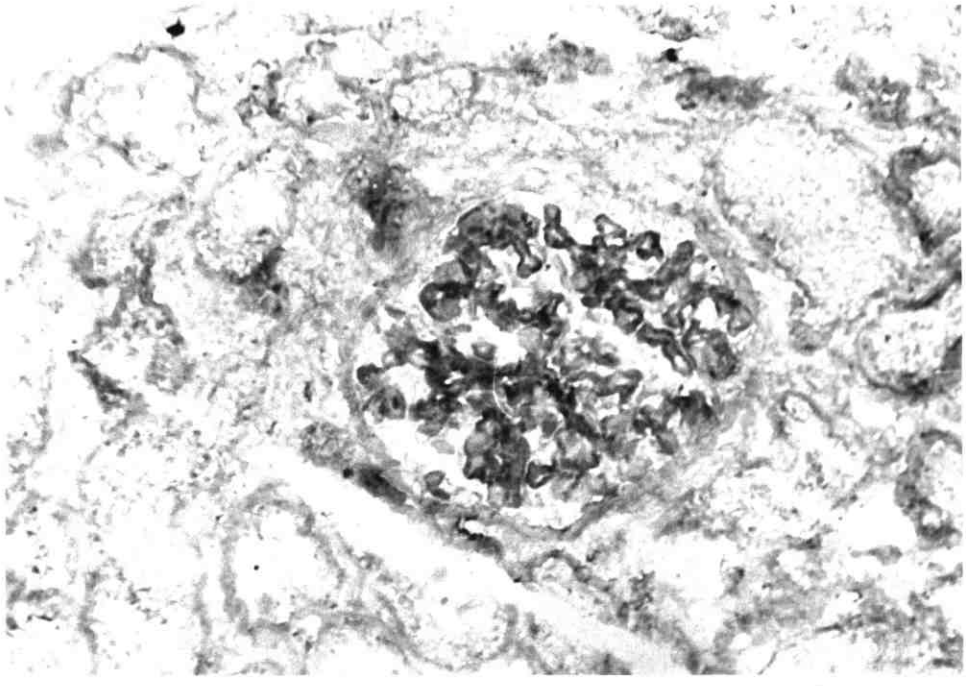
Direct immunoperoxidase staining in a patient with mesangial proliferative glomerulonephritis, showing very weak or nearly absence of deposits. Tissue incubated with peroxidase-labelled IgG diluted 1 in 32 for 24 hr. (x 132).

Figure 2.4.



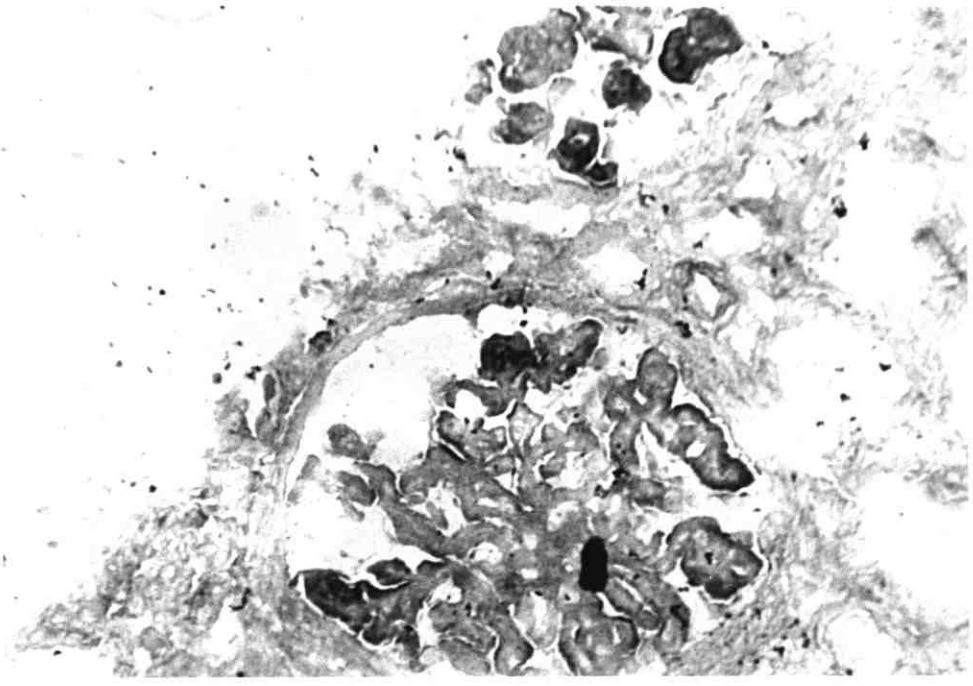
Delayed immunoperoxidase staining in a patient with mesangial proliferative glomerulonephritis showing presence of mesangial granular deposits of immune complex. Tissue incubated with peroxidase-labelled IgG diluted 1 in 4 for 48 hr (x 132).

Fig. 2.5.



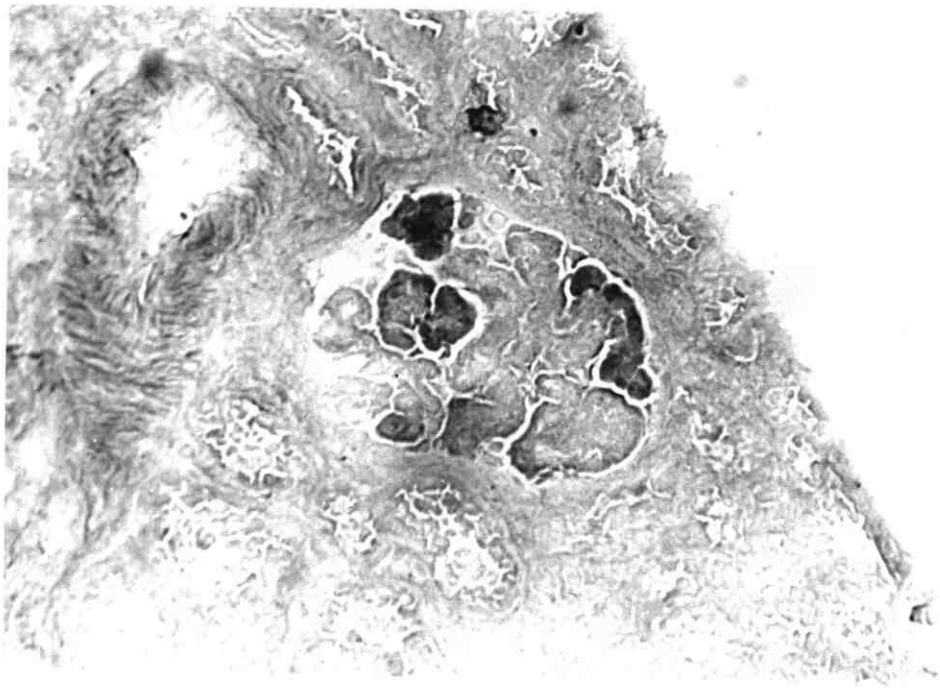
Delayed immunoperoxidase staining in a patient with mesangial proliferative glomerulonephritis showing mesangial granular deposits of immune complexes with improved clarity. Tissue incubated with peroxidase labelled IgG diluted 1 in 4 for 72 hr. (x 132).

Figure 2.6.



Delayed immunoperoxidase staining in a patient with mesangial proliferative glomerulonephritis showing mesangial granular deposits of immune complexes with excellent clarity. Tissue incubated with peroxidase-labelled IgG diluted 1 in 4 for 96 hr. (x 132).

Figure 2.7.



Delayed immunoperoxidase staining in a patient with mesangial proliferative glomerulonephritis showing mesangial granular deposits of immune complexes with good clarity. Tissue incubated with peroxidase-labelled IgG diluted 1 in 4 for 120 hr. (x 132).

Experiment 2

Renal biopsy tissue which is known to show granular deposits of immune complexes when stained for IgG was chosen. Frozen sections were cut and picked up on three slides.

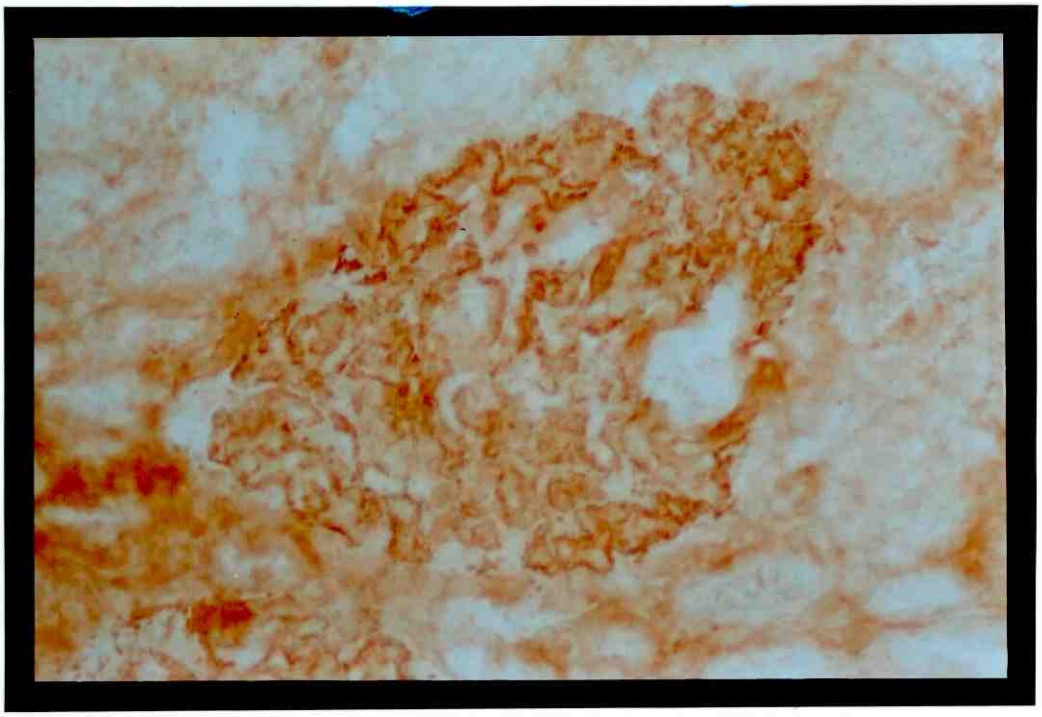
On slide No. 1, labelled globulin, diluted 1 in 4 with 0.1 M PBS, pH 7.2, was applied for 24 h.

On slide No. 2, labelled globulin, diluted 1 in 4 with 0.1 M PBS, pH 7.2, was applied for 96 h.

On slide No. 3, labelled globulin, diluted 1 in 4 with 3% PEG in 10 mM EDTA in PBS, i.e. the final concentration of PEG was 2.3% for 96 h.

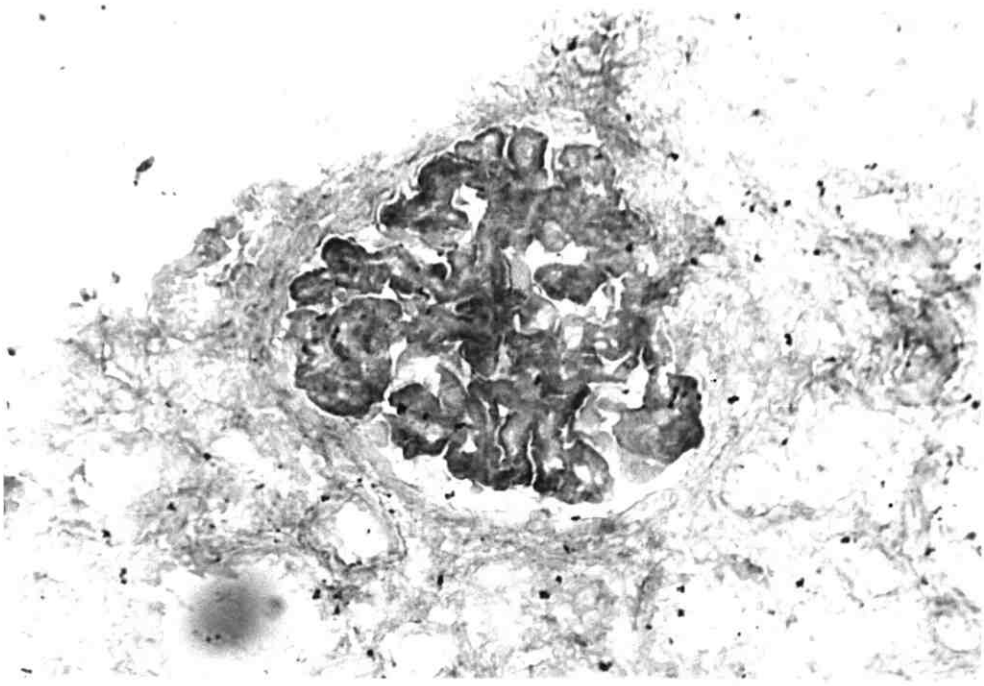
Figures 2.8, 2.9 and 2.10 show slides Nos. 1, 2, and 3 respectively.

Figure 2.8.



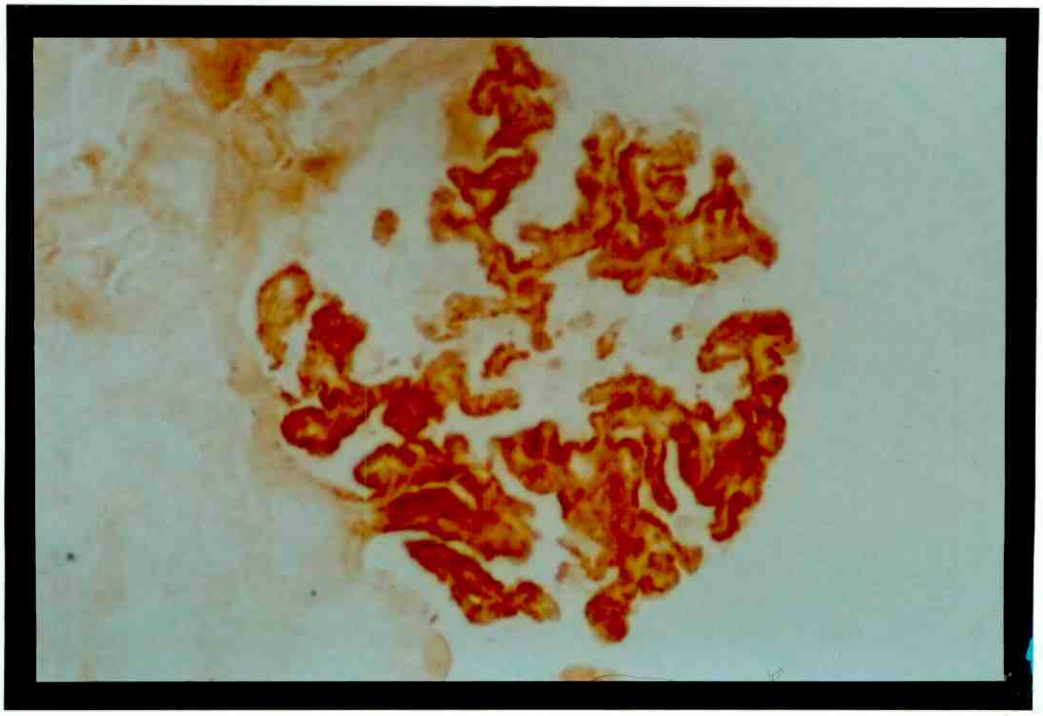
Delayed immunoperoxidase staining in a patient with mesangiocapillary glomerulonephritis showing weak and diffuse staining pattern of the immune complexes deposited alongside the glomerular capillary walls. Tissue incubated with peroxidase-labelled globulin prepared from the patient's own serum diluted 1 in 4 with PBS for 24 hr. (x 320).

Figure 2.9.



Delayed immunoperoxidase staining in a patient with mesangiocapillary glomerulonephritis showing better staining pattern of immune complexes. Tissue incubated with peroxidase-labelled globulin diluted 1 in 4 with PBS for 96 hr. (x 132).

Figure 2.10.



Delayed immunoperoxidase staining in a patient with mesangiocapillary glomerulonephritis showing excellent granular deposits of immune complexes alongside the glomerular capillary walls. Tissue incubated with labelled globulin prepared from patient's own serum diluted 1 in 4 with 3% PEG in 10 mM EDTA for 96 hr. (x 320).

2.7 Reaction between labelled globulins and tissue biopsies using the delayed technique

Materials

1. Renal biopsy

Renal biopsy specimens were obtained from patients with chronic glomerulonephritis.

2. Patients' sera

Sera used for preparing the globulins were from the same patients as those subjected to renal biopsy.

3. 3% Polyethylene glycol

This solution was made up from a stock solution of 30% PEG in 10 mM EDTA. One volume of this solution was added to 9 volumes of 0.1 M PBS, pH 7.2. just before using the labelled globulin. The labelled globulin was diluted 1 in 4 with this solution, i.e. the final concentration of PEG on the tissue was 2.3%.

Method

The tissue sections were washed in 10 mM EDTA in PBS, pH 7.2, for 30 min. The sections were left to dry at room temperature for about 15 min. The diluted globulin were added to the sections with Pasteur pipettes. The slides were then incubated in the humid chamber for 4 days at 4°C. They were washed in 0.1 M PBS, pH 7.2, for 30 min, stained for peroxidase and examined.

2.8. The effect of acidity on immunoglobulins

The idea of testing the effect of acid conditions on immunoglobulins arose after the unsatisfactory results I got from testing the labelled $F(ab)_2$ on renal biopsies. The possibility of destruction of immunoglobulins by the acid conditions needed for pepsin digestion led me to this experiment.

Materials

1. Test serum

Serum from patients with rheumatoid arthritis and a hyperviscosity syndrome, known to contain very large amounts of IgG, IgM and rheumatoid factor (SCAT titre 200,000,000, IgG 75 g/l, IgM 30 g/l).

0.2 M glycine, pH 3 (BDH Ltd.).

7.5 gm glycine dissolved in 1 l distilled water, the pH was adjusted to 3 using N Hcl.

0.2 M EDTA, pH 7.6

As discussed previously.

0.3% sheep red cells sensitised with optimal amounts of rabbit anti-sheep red cell antibody

Dithiothreitol 1 mg/ml (BDH Ltd.).

Method

One ml of 0.2 M EDTA, pH 7.6 was added to each of 6 polystyrene tubes. 0.1 ml of serum was added to 0.4 ml of 0.2 M glycine, pH 3. 0.1 ml of

serum was added to each tube after 2 min, 5 min, 10 min, 20 min and 25 min, to be neutralised. IgM and IgG in the serum were then measured using single radial immunodiffusion. Several dilutions of untreated serum were used as standards.

A curve has been drawn for changes in Mancini estimate of IgG and IgM in the serum acidified for different periods (2 min - 25 min).

The agglutination titre of 0.3% sheep red cells sensitised with rabbit antibody was determined on the heated samples. This was carried out in barbitone buffered saline as diluent, and also in buffered saline containing dithiothreitol (1 mg/ml). Disulphide bonds holding the IgM subunits together were reduced to prevent agglutination by IgM, and only reduces IgG. Antibody titres were carried out by two doubling dilutions. The first attempts are shown in Table 3.20.

The results were difficult to interpret because the large amount of IgG antibody present in this sample. Thus 0, 5, 15 and 25 min incubation samples were subjected to gel filtration chromatography on G-200 Sephadex in BBS by Professor Mowbray. After this there was still some IgG present, presumably because of IgG-IgM rheumatoid factor aggregates, but the amount was very small so that the effect of acidification on the IgM antibody could be specifically selected (Table 3.21).

The serum fractions were subjected to gel filtration on G-200 Sephadex, and the void fraction reconstituted to the original volume before assay. Results are expressed at $\log_2$ titres, using doubling dilutions of antibody (0.2 ml volumes) and addition of 0.2 ml of 0.3% sensitised sheep red cells.

A comparison was made between the composition of the immune complexes found in the sera and in the renal biopsies of patients with renal disease, mainly glomerulonephritis.

Renal biopsies and sera from 200 patients in the Renal Unit at St. Mary's Hospital were studied for circulating and deposited complexed immunoglobulins and complement.

Forty-six renal biopsies were stained with peroxidase labelled $F(ab')_2$ antibodies obtained from circulating immune complexes.

One hundred renal biopsies and patients' sera were studied to determine whether the patients' own labelled circulating IgG reacted with sections of the renal tissue.

One hundred and forty-four renal biopsies were stained with Tamm-Horsfall antibody.

3.1. Composition of Circulating and Deposited Immune Complexes in Renal Disease

Table 3.1.

FORMS OF GLOMERULONEPHRITIS IN 200 CASES USED IN THE STUDY

Histological Diagnosis	No. of patients
Diffuse mesangial proliferative GN (MPGN)	33
Systemic lupus erythematosus (SLE)	15
IgA nephropathy	15
Membranous glomerulonephritis	19
Diabetic glomerulosclerosis	16
Focal and segmental proliferative GN	21
Minimal change glomerulonephritis	13
Diffuse mesangiocapillary glomerulonephritis Type I (MCGN)	16
Miscellaneous group	52
Total	200

The miscellaneous group includes interstitial nephritis, primary hypertensive nephrosclerosis, acute tubular necrosis, diffuse endocapillary proliferative glomerulonephritis, polyarteritis nodosa, diffuse sclerosing GN, amyloid, haemolytic uraemic syndrome, multiple myeloma idiopathic interstitial fibrosis of the lung, infective endocarditis, progressive systemic sclerosis and unclassifiable group. The number of patients in any of these groups was too small for them to be considered separately.

I have compared the composition of the immune complexes in the circulation with the composition of those present in the glomeruli in all patients studied (Table 3.2). Also I have compared the frequency with

which the individual immunoglobulins and C1q were found in the circulating immune complexes with the frequency of their presence in the deposited complexes in each of the various major forms of renal disease encountered (Tables 3.3 - 3.6).

The correlations between circulating and deposited immune complexes in respect to different classes of immunoglobulins and complement (C1q) are highly significant for IgA (χ^2 = 28, $P < 0.0005$) and C1q (χ^2 = 7, $P < 0.01$, Table 3.2). In studying every individual complexed immunoglobulin and complement (C1q) with respect to different forms of renal disease, I found that there is a highly significant correlation between circulating and deposited IgA complexes with mesangial proliferative glomerulonephritis, membranous glomerulonephritis and focal/segmental glomerulonephritis (χ^2 = 5.38, 6.11, 11.75; $P < 0.05$, 0.05, 0.001 respectively, Table 3.5). However, with every class of complexed immunoglobulins and complement (C1q) (Table 3.2), and with every form of renal disease studied (Tables 3.3 - 3.6), there are some patients who only have immunoglobulins and/or complement (C1q) in their kidneys and do not have any circulating immune complexes detectable by the method used and vice versa. In another group, I could not detect either circulating or deposited complexes. A possible explanation for these differences will be considered in Chapter 4. The presence of deposited C1q in complexes was shown to occur only when IgG or IgM complexes were present without IgA (χ^2 = 26.7, $P < 0.0005$, Table 3.7), or all three immunoglobulins together (χ^2 = 5.6, $P < 0.05$). Deposited IgA complexes alone do not show any

significant association. Thus, deposited IgA complexes show a significant association with C1q, only because of the accompanying deposits of IgG and IgM

Deposited C_3 in complexes shows a highly significant correlation with all immunoglobulin classes and deposited C1q (Table 3.8).

In studying the deposited immunoglobulins with respect to different forms of renal disease I found that, in all forms, about 50% of the cases show all three immunoglobulins are deposited in the tissue. Only in Berger's disease does IgA alone (12/15) tend to deposit more than the other immunoglobulins (Table 3.9).

Table 3.2.

COMPOSITION OF CIRCULATING AND DEPOSITED IMMUNE COMPLEXES IN 200 CASES OF RENAL DISEASE

Immunoglobulins and complement present in circulating complexes

Immunoglobulins and complement found in renal glomeruli		IgG −	IgG +	IgM −	IgM +	IgA −	IgA +	C1q −	C1q +
IgG	−	45 (23%)	34 (17%)						
	+	72 (36%)	49 (25%)						
IgM	−			33 (17%)	25 (13%)				
	+			82 (41%)	60 (30%)				
IgA	−					74 (37%)	61 (31%)		
	+					10 (5%)	55 (28%)		
C1q	−							74 (37%)	24 (12%)
	+							59 (30%)	43 (22%)
Total		200		200		200		200	
X^2		0.13		0.01		28.00		7.00	
Probability		NS		NS		<0.0005		<0.01	

NS = not significant

Table 3.3.

COMPARISON BETWEEN PRESENCE OF IgG IN CIRCULATING IMMUNE COMPLEXES AND IN GLOMERULI IN DIFFERENT FORMS OF RENAL DISEASE

Deposited IgG Complexes		Circulating IgG Complexes								
		MPGN	SLE	IgA nepropathy	membranous GN	Focal and seg. GN	Diabetic GN	Misc.	MCGN	Minimal change GN
		− +	− +	− +	− +	− +	− +	− +	− +	− +
MPGN	−	15(45%) 3(9%)								
	+	10(30%) 5(15%)								
SLE	−		1(7%) 2(13%)							
	+		5(33%) 7(47%)							
IgA nepr.	−			2(13%) 5(33%)						
	+			6(40%) 2(13%)						
Memb. GN	−				1(5%) 1(5%)					
	+				10(53%) 7(37%)					
Focal and seg. GN	−					6(29%) 3(14%)				
	+					8(38%) 4(19%)				
Diab. GN	−						3(19%) 1(6%)			
	+						7(44%) 5(31%)			
Misc.	−							4(8%) 18(35%)		
	+							15(29%) 15(29%)		
MCGN	−								3(19%) 0(0%)	
	+								10(63%) 3(19%)	
Minimal change GN	−									10(77%) 1(8%)
	+									1(8%) 1(8%)
Total		33	15	15	19	21	16	52	16	13
X^2		1.24	0.07	3.23	/	0.00	0.36	5.54	/	/
P		NS	NS	NS	/	NS	NS	< 0.05	/	/

P = probability
NS = not significant

Table 3.4.

COMPARISON BETWEEN PRESENCE OF IgM IN CIRCULATING IMMUNE COMPLEXES AND IN GLOMERULI IN DIFFERENT FORMS OF RENAL DISEASE

Deposited IgM Complexes		Circulating IgM Complexes																			
		MPGN		SLE		IgA nepropathy		Membranous GN		Focal and seg. prolif.GN		Diabetic GN		Misc.		MCGN		Minimal change GN	
		−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+
MPGN	−	5(15%)	3(9%)																
	+	13(39%)	12(36%)																
SLE	−			1(7%)	1(7%)														
	+			7(47%)	6(40%)														
IgA nepr.	−					3(20%)	3(20%)												
	+					7(47%)	2(13%)												
Memb. GN	−							1(5%)	1(5%)										
	+							9(47%)	8(42%)										
Focal and seg. GN	−									7(33%)	0(0%)								
	+									9(43%)	5(24%)								
Diab. GN	−											8(50%)	2(13%)						
	+											3(19%)	3(19%)						
Misc.	−													1(2%)	9(17%)				
	+													27(52%)	15(29%)				
MCGN	−															0(0%)	3(19%)		
	+															4(25%)	9(56%)		
Minimal change GN	−																	7(54%)	3(23%)
	+																	3(23%)	0(0%)
Total		33		15		15		19		21		16		52		16		13	
X^2		0.27		/		1.25		/		3.28		1.57		9.58		/		/	
P		NS		/		NS		/		NS		NS		< 0.005		/		/	

P = probability

NS = not significant

Table 3.5.

COMPARISON BETWEEN PRESENCE OF IgA IN CIRCULATING IMMUNE COMPLEXES AND GLOMERULI IN DIFFERENT FORMS OF RENAL DISEASE

Deposited IgA complexes		Circulating IgA Complexes: MPGN −	MPGN +	SLE −	SLE +	IgA nepropathy −	IgA nepropathy +	Membranous GN −	Membranous GN +	Focal and seg. GN −	Focal and seg. GN +	Diabetic GN −	Diabetic GN +	Misc. −	Misc. +	MCGN −	MCGN +	Minimal change GN −	Minimal change GN +
MPGN	−	9(27%)	14(42%)																
	+	0(0%)	10(30%)																
SLE	−			5(33%)	5(33%)														
	+			0(0%)	5(33%)														
IgA nepr.	−					0(0%)	0(0%)												
	+					10(67%)	5(33%)												
Memb. GN	−							9(47%)	5(26%)										
	+							0(0%)	5(26%)										
Focal and seg. GN	−									8(38%)	3(14%)								
	+									0(0%)	10(48%)								
Diab. GN	−											12(75%)	2(13%)						
	+											0(0%)	2(13%)						
Misc.	−													10(19%)	26(50%)				
	+													0(0%)	16(31%)				
MCGN	−															11(69%)	3(19%)		
	+															0(0%)	2(13%)		
Minimal change GN	−																	10(77%)	3(23%)
	+																	0(0%)	0(0%)
Total		33		15		15		19		21		16		52		16		13	
X^2		5.38		3.75		/		6.11		11.75		/		5.50		/		/	
P		<0.05		NS		/		< 0.05		< 0.001		/		<0.05		/		/	

P = probability

Table 3.6.

COMPARISON BETWEEN PRESENCE OF C1q IN CIRCULATING IMMUNE COMPLEXES AND IN GLOMERULI IN DIFFERENT FORMS OF RENAL DISEASE

Deposited C1q Complexes		Circulating C1q Complexes								
		MPGN	SLE	IgA nepropathy	membranous GN	Focal and seg. GN	Diabetic GN	Misc.	MCGN	Minimal change GN
		− +	− +	− +	− +	− +	− +	− +	− +	− +
MPGN	−	16(49%) 5(15%)								
	+	10(30%) 2(6%)								
SLE	−		5(33%) 2(13%)							
	+		6(40%) 2(13%)							
IgA neph.	−			9(60%) 3(20%)						
	+			2(13%) 1(7%)						
Memb. GN	−				5(26%) 2(11%)					
	+				9(47%) 3(16%)					
Focal and seg. GN	−					12(57%) 3(14%)				
	+					3(14%) 3(14%)				
Diab.	−						3(19%) 1(6%)			
	+						10(63%) 2(13%)			
Misc.	−							7(13%) 7(13%)		
	+							9(17%) 29(56%)		
MCGN	−								6(38%) 0(0%)	
	+								10(63%) 0(0%)	
Minimal change GN	−									11(85%) 1(8%)
	+									0(0%) 1(8%)
Total		33	15	15	19	21	16	52	16	13
X^2		0.23	0.02	/	0.03	1.89	/	3.33	/	/
P		NS	NS	/	NS	NS	/	NS	/	/

P = probability

Table 3.7.

ASSOCIATION OF DEPOSITED C1q WITH DEPOSITED IMMUNOGLOBULINS IN 200 RENAL BIOPSIES

Deposited C1q complexes	Deposited Immunoglobulins: IgG with M & A		IgM with G & A		IgA with G & M		IgG or IgM		IgA without G or M	
	−	+	−	+	−	+	−	+	−	+
−	45 (23%)	53 (27%)								
+	34 (17%)	68 (34%)								
−			33 (17%)	65 (33%)						
+			25 (13%)	77 (39%)						
−					74 (37%)	24 (12%)				
+					61 (31%)	41 (21%)				
−							45 (23%)	53 (27%)		
+							13 (7%)	89 (45%)		
−									84 (42%)	14 (7%)
+									101 (51%)	1 (1%)
Total	200		200		200		200		200	
X^2	3.31		2.04		5.62		26.71		/	
P	NS		NS		<0.05		<0.0005		/	

P = probability

NS = not significant

Table 3.8

ASSOCIATION OF DEPOSITED C_3 WITH DEPOSITED IMMUNOGLOBULINS AND C1q

IN 200 RENAL BIOPSIES

C_3 Deposited Complexes	Deposited Immunoglobulins											
	IgG		IgM		IgA		IgG or IgM		IgA without IgM IgG		C1q	
	−	+	−	+	−	+	−	+	−	+	−	+
−	45 (23%)	13 (7%)										
+	34 (17%)	108 (54%)										
−			33 (17%)	14 (7%)								
+			25 (13%)	128 (64%)								
−					74 (37%)	15 (8%)						
+					61 (31%)	50 (25%)						
−							45 (23%)	48 (24%)				
+							13 (7%)	94 (47%)				
−									74 (37%)	5 (3%)		
+									111 (56%)	10 (5%)		
−											74 (37%)	40 (20%)
+											24 (12%)	62 (31%)
Total	200		200		200		200		200		200	
X^2	49.59		50.68		13.45		31.73		/		26.86	
P	< 0.0005		< 0.0005		< 0.0005		< 0.0005		/		< 0.0005	

P = probability

Table 3.9 DEPOSITED IMMUNOGLOBULINS WITH RESPECT TO DIFFERENT FORMS OF RENAL DISEASE

Form of renal disease	G & M no A	G & A no M	M & A no G	G & M and A	G alone	M alone	A alone	Total
Diffuse mesangial proliferative GN	5	2	3	20	2	1	0	33
Systemic lupus erythematosus	5	1	1	7	1	0	0	15
IgA nephropathy	0	1	0	2	0	0	12	15
Membranous GN	5	2	2	10	0	0	0	19
Diabetic glomerulosclerosis	5	2	1	5	0	3	0	16
Focal and segmental GN	5	3	2	7	2	1	1	21
Minimal change glomerulonephritis	1	0	0	3	6	3	0	13
Diffuse mesangiocapillary GN Type I	4	2	2	5	2	1	0	16
Miscellaneous group	12	7	1	30	1	1	0	52
Total group	42	20	12	89	14	10	13	200
Percent	21	10	6	45	7	5	7	100

3.2.Reaction of Peroxidase-Labelled $F(ab')_2$ with Renal Biopsies

I have compared the staining of renal biopsies with labelled $F(ab')_2$ from patients with circulating complexes, another patient's $F(ab')_2$ and normal human IgG as a positive control.

Table 3.10.

NUMBER OF RENAL BIOPSIES THAT STAINED POSITIVELY WITH PEROXIDASE-LABELLED $F(ab')_2$

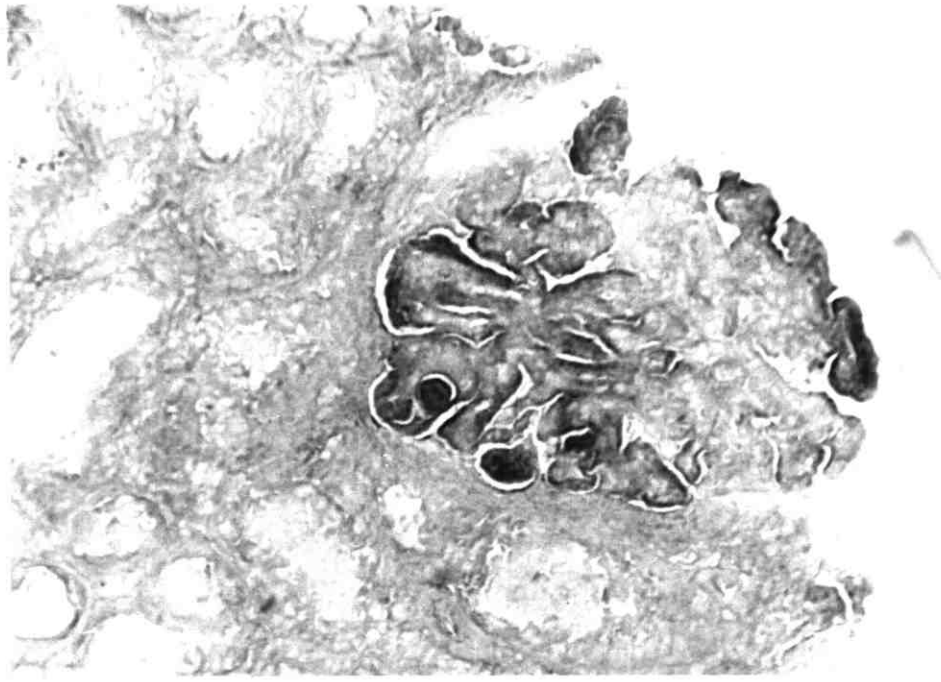
Labelled material used	No. positive	No. negative	Total
Patient's own $F(ab')_2$	26 (57%)	20 (44%)	46
Another patient's $F(ab')_2$	28 (61%)	18 (39%)	46
Normal human IgG	40 (87%)	6 (13%)	46

From the above table it can be seen that there is no difference between frequency of positive staining obtained with the patient's own $F(ab')_2$ and another patient's $F(ab')_2$ in most of the biopsies studied. This might be due either to insensitivity of the method used, or to a non-specific binding of the labelled $F(ab')_2$ to the deposits. There was no evidence of any antibody specificity in this experiment.

Figures 3.1 and 3.2 show the staining of one of the positive renal biopsies with patient's own $F(ab')_2$ and another patient's $F(ab')_2$.

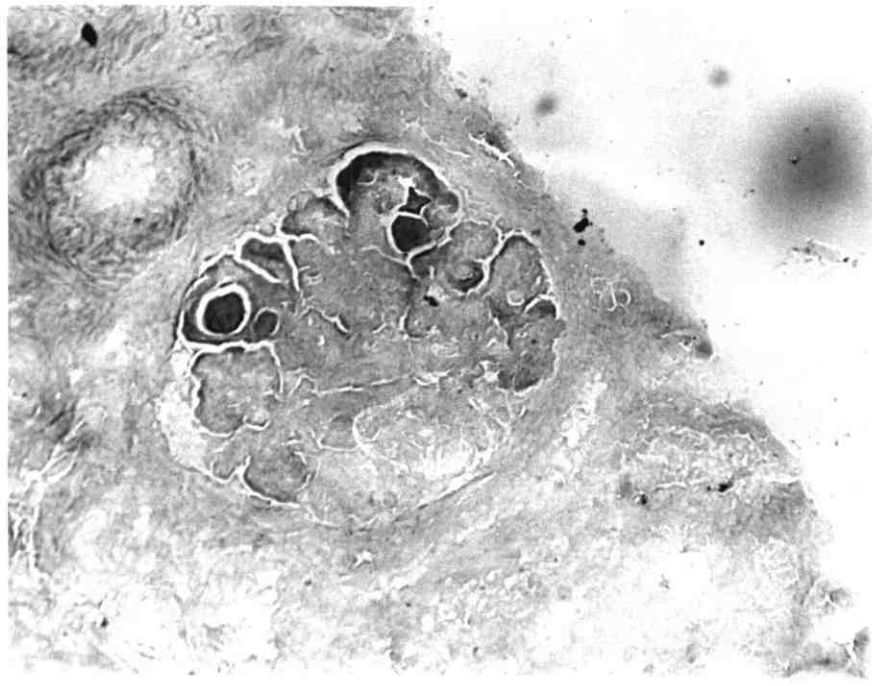
On studying the composition of the circulating complexes used for preparing $F(ab')_2$, I found that half of these patients (23/46) have circulating IgM or IgA complexes but not IgG complexes (Tables 3.11, 3.12).

Figure 3.1.



Direct immunoperoxidase staining in a patient with focal and segmental proliferative glomerulonephritis showing focal deposits of immune complexes in the mesangium. Tissue incubated with peroxidase-labelled $F(ab')_2$ prepared from patient's own serum diluted 1 in 4 with PBS. (x 132).

Figure 3.2.



Direct immunoperoxidase staining of the glomerulus in a patient with focal and segmental proliferative glomerulonephritis showing focal mesangial deposits of immune complexes. Tissue incubated with peroxidase-labelled $F(ab')_2$ prepared from another patient's serum diluted 1 in 4 with PBS (x 132).

Table 3.11.

NUMBER OF RENAL BIOPSIES STAINED POSITIVELY WITH THE PATIENT'S OWN AND ANOTHER PATIENT'S $F(ab')_2$ IN 23 PATIENTS WITH CIRCULATING IgG, IgM AND IgA IMMUNE COMPLEXES

Diagnosis	Number of Positives	Positive staining: Patient's own $F(ab')_2$	Positive staining: Another Patient's $F(ab')_2$	Total
Diffuse mesangial proliferative (MPGN)	8	5	3	15
Systemic lupus erythematosus (SLE)	5	3	2	10
Diffuse messangiocapillary glomerulonephritis (MCGN)	6	2	4	10
IgA nephropathy	1	1	1	7
Miscellaneous group	3	4	3	4
Total	23	15	13	46

Table 3.12.

NUMBER OF RENAL BIOPSIES STAINED POSITIVELY WITH THE PATIENT'S OWN AND ANOTHER PATIENT'S $F(ab')_2$ IN 23 PATIENTS WITH CIRCULATING IgM AND IgA IMMUNE COMPLEXES BUT NO IgG COMPLEXES

Diagnosis	Number of Positives	Patient's own $F(ab')_2$	Another patient's $F(ab')_2$	Total
Diffuse mesangial proliferative glomerulonephritis (MPGN)	7	3	4	15
Systemic lupus erhthematosus (SLE)	5	2	4	10
Diffuse mesangiocapillary glomerulonephritis (MCGN)	4	2	2	10
IgA nephropathy	6	3	4	7
Miscellaneous group	1	1	1	4
Total	23	11	15	46

I have shown experimentally that IgM antibody is acid labile (see Section 3.5) and it is known that yields of $F(ab')_2$ from IgA and possibly IgM are very low (Johnston and Thorpe, 1982), so destruction by the acid condition needed for pepsin digestion of IgM and IgA complexes in half of the sera studied may be a cause of the lack of specific staining found. In the other patients who do have circulating IgG complexes, the cause of negative or non-specific staining may be due to too short a time of incubation of labelled $F(ab')_2$ on the tissue section. In experiments I found that it needs four days to obtain positive staining. For this reason I studied other biopsies by the delayed technique (see Section 3.3).

These two main possibilities may be the case of the unsatisfactory results in this section. I did not try the slow method with $F(ab')_2$ as I got good results with free antibody in the serum this way.

3.3. The Delayed Technique

3.3.1. Reaction between renal biopsies and peroxidase labelled globulin using the Delayed Technique

In 63 cases the reaction between labelled serum IgG and the complexes deposited in the renal biopsies was studied, using serum from the patients themselves, and from other renal biopsy patients. Normal human IgG was used as a control, and was reacted with each biopsy.

Table 3.13.

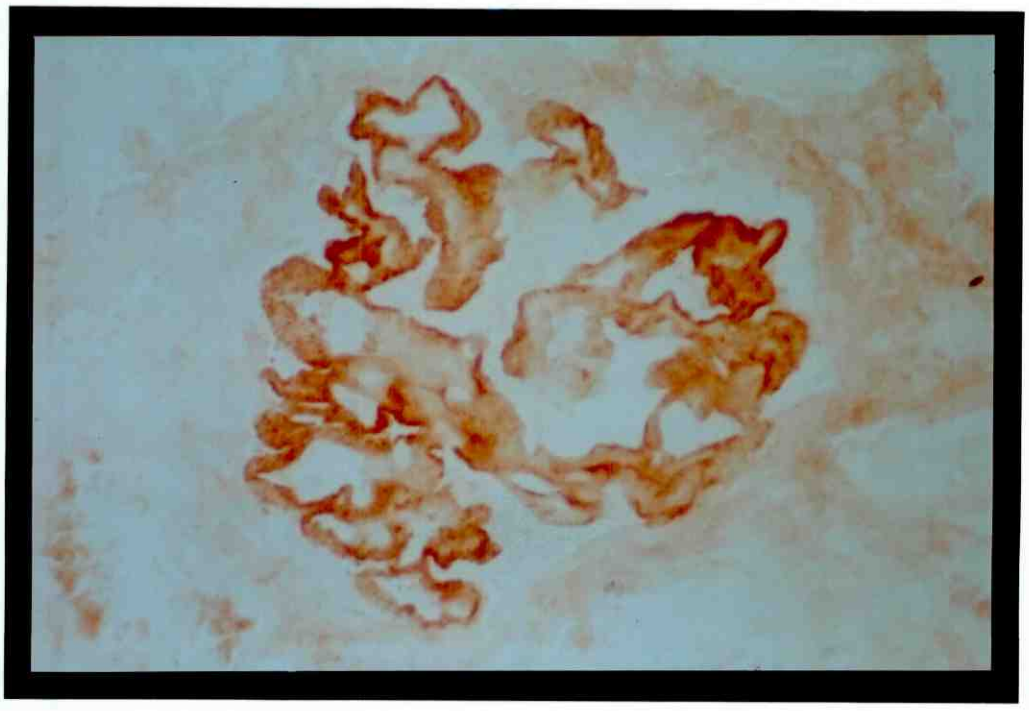
COMPARISON OF STAINING OF BIOPSIES WITH THEIR OWN AND OTHER PATIENTS' GLOBULIN

staining with other globulin	Staining with own globulin	
	-	+
-	5 (8%)	52 (83%)
+	2 (3%)	4 (6%)

From Table 3.13 we can see that 52 patients (83%) showed positive staining with their own labelled globulins, but not with another patient's globulin.

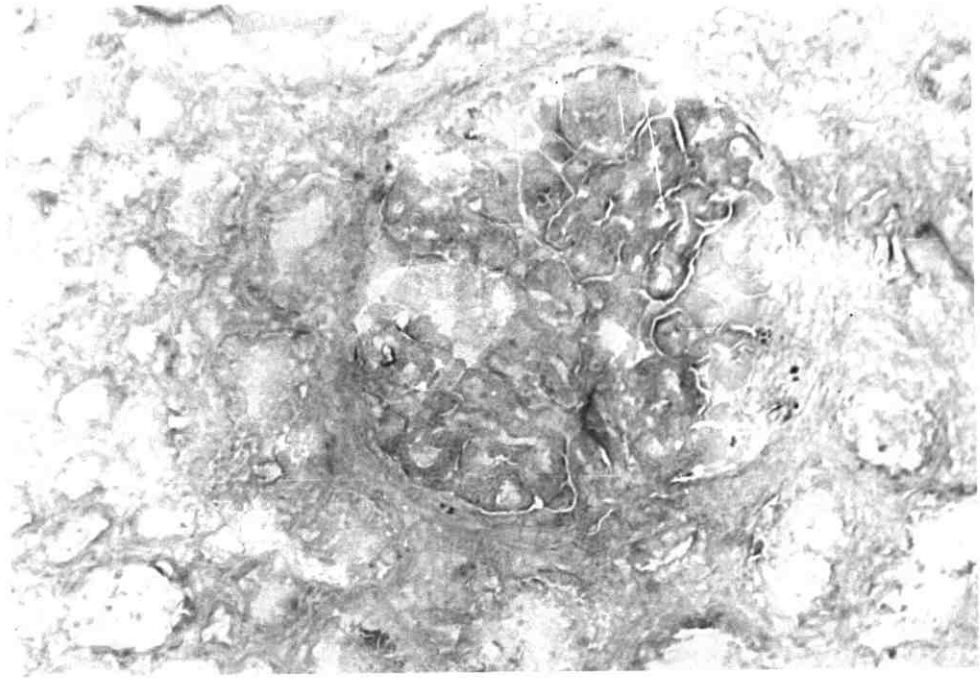
Only four patients (6%) showed staining with both labelled globulins, and only two patients showed staining with another patient's globulin but not with their own. This shows that in most cases of glomerulonephritis there is an excess of free circulating antibodies to antigens in the deposited

Figure 3.3.



'Delayed' immunoperoxidase staining of glomerulus in a patient with mesangiocapillary glomerulonephritis showing granular deposits of the immune complexes in the mesangium and alongise the capillary walls. Tissue incubated with peroxidase-labelled globulin prepared from patient's own serum diluted 1 in 4 with 3% PEG in 10 mM EDTA for 96 hr. (x 320).

Figure 3.4.



Delayed immunoperoxidase staining of glomerulus showing the absence of immune complexes in a patient with mesangiocapillary glomerulonephritis. Tissue incubated with peroxidase-labelled globulin prepared from another patient's serum diluted 1 in 4 with 3% PEG in 10 mM EDTA for 96 hr. (x 132).

complex. Although labelled globulin reacted with the patient's own deposited antigen, this antibody did not react with that of another patient's biopsy. There were two patients who showed positive staining with the other patient's globulin but not with their own. They may represent technical failures to detect the reaction with their own globulin, or have been in antigen excess. The implication will be considered in a later chapter.

Thus, in this experiment it can be seen that most patients do not share a common antigen in their immune complex deposits, and that different antigens may be involved in different patients, even though they have the same form of glomerulonephritis.

Figures 3.3 and 3.4 show positive and negative staining of renal biopsies with patient's own and another patient's globulin.

3.3.2. Study of the reaction between immune complexes in renal biopsies and 24 labelled globulins in patients with glomerulonephritis

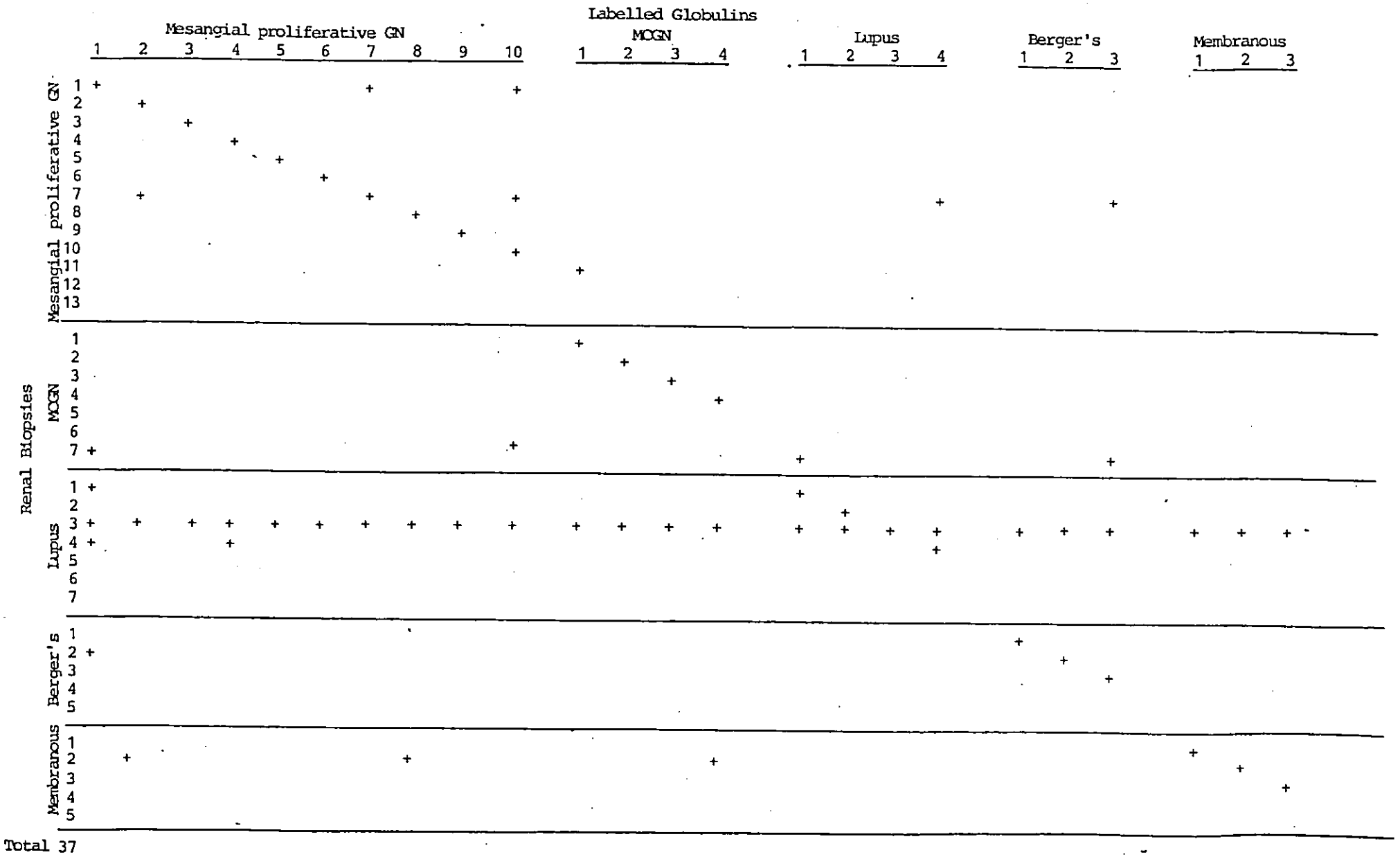
The results of the previous experiment (3.3.1) led me to do a further experiment using renal tissue from more than one other patient, i.e. the labelled globulin from any one patient was reacted not only with his/her own renal biopsy, but also with biopsies and kidneys from 36 other patients, using the delayed method.

This experiment confirmed the previous one (3.3.1). As shown in Table 3.14 the biopsies were reacted with 23 different globulins. In all cases, I got positive staining in tissue reacted with its own globulin, but not with another globulin. In only one patient with SLE

the biopsy showed positive results with all labelled globulins which must be assumed to be non-specific. It is thus very rare to find positive staining with different globulins. This experiment shows that the deposited antigens are rarely the same in different patients, so that the labelled antibodies could detect their own complexes but not those of the other patients.

Table 3.14.

FRACTION OF POSITIVE REACTION BETWEEN LABELLED GLOBULIN AND 37 RENAL BIOPSIES USING THE DELAYED METHOD



3.4. Reaction of Tamm-Horsfall Antibody with Renal Biopsies

I have studied the reaction of Tamm-Horsfall antibody with renal tissue as an example of an immune reaction involving an autologous antigen present naturally in the renal tissue. Renal glomerular basement membrane antigen, renal tubular basement antigen and renal brush border antigen are known to be autologous antigens present in the renal tissues and they can precipitate renal immune complex disease if they combine with their corresponding antibodies. Tamm-Horsfall glycoprotein is a mucoprotein secreted from the renal tubules. Under certain circumstances the synthesis and secretion of Tamm-Horsfall glycoprotein is diminished or ceases completely. I have studied the conditions under which Tamm-Horsfall glycoprotein secretion is altered. I found a correlation between the presence of Tamm-Horsfall glycoprotein and renal function.

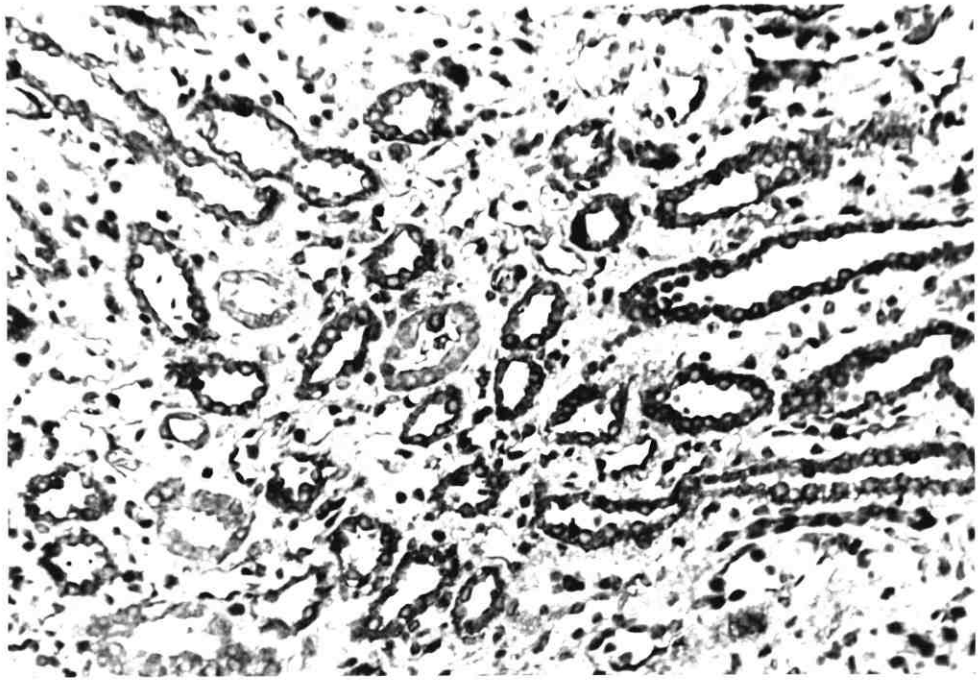
The Tamm-Horsfall staining in the renal tubules was scored from 0 to 4+ according to the intensity and the distribution of the staining obtained by peroxidase labelled Tamm-Horsfall antibody (Table 3.15).

Table 3.15

DISTRIBUTION OF TAMM-HORSFALL GLYCOPROTEIN IN RENAL BIOPSIES SHOWN BY IMMUNOPEROXIDASE STAINING

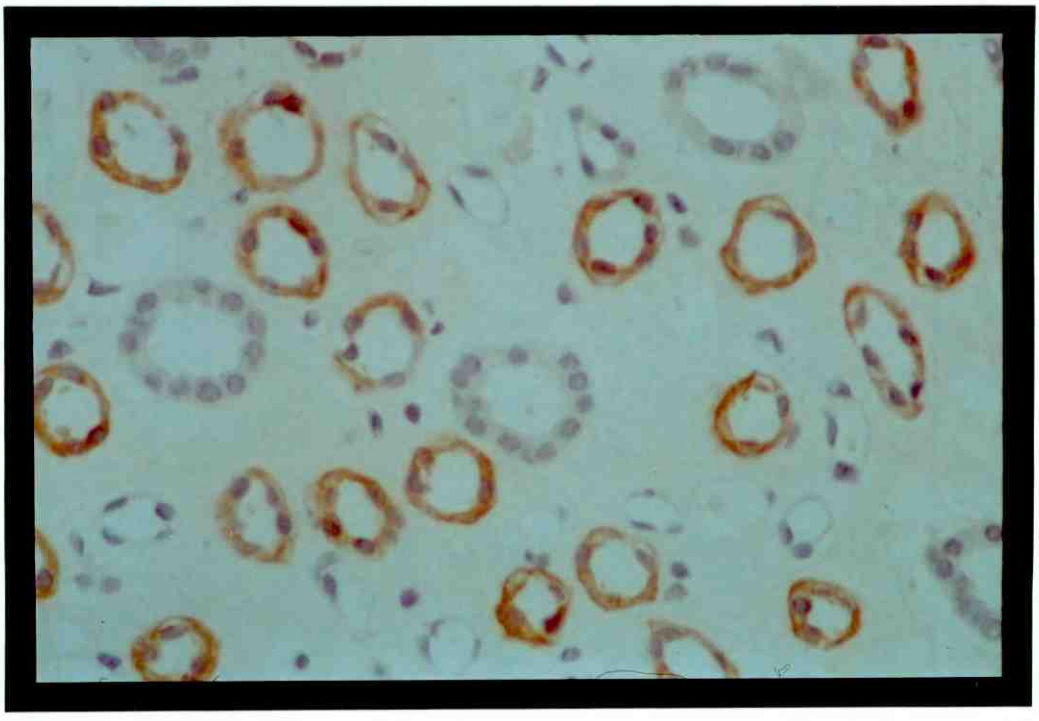
No. of renal biopsies	0	1+	2+	3+	4+
144	46 (32%)	25 (17%)	26 (18%)	27 (19%)	20 (14%)
17 normal kidney tissue (control)	0	0	0	5 (29%)	12 (71%)

Figure 3.5.



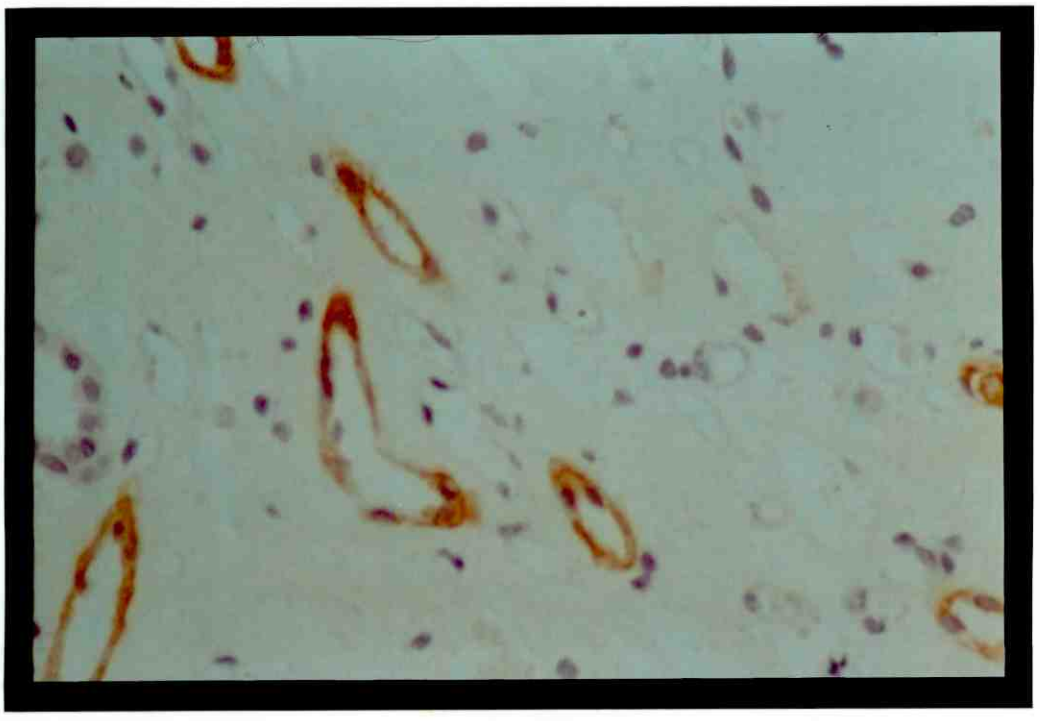
Direct immunoperoxidase staining of renal tubules in a patient with mesangial proliferative glomerulonephritis showing grade 4+. Tissue incubated with peroxidase-labelled Tamm-Horsfall antibody diluted 1 in 4 with PBS. (x 132).

Figure 3.6.



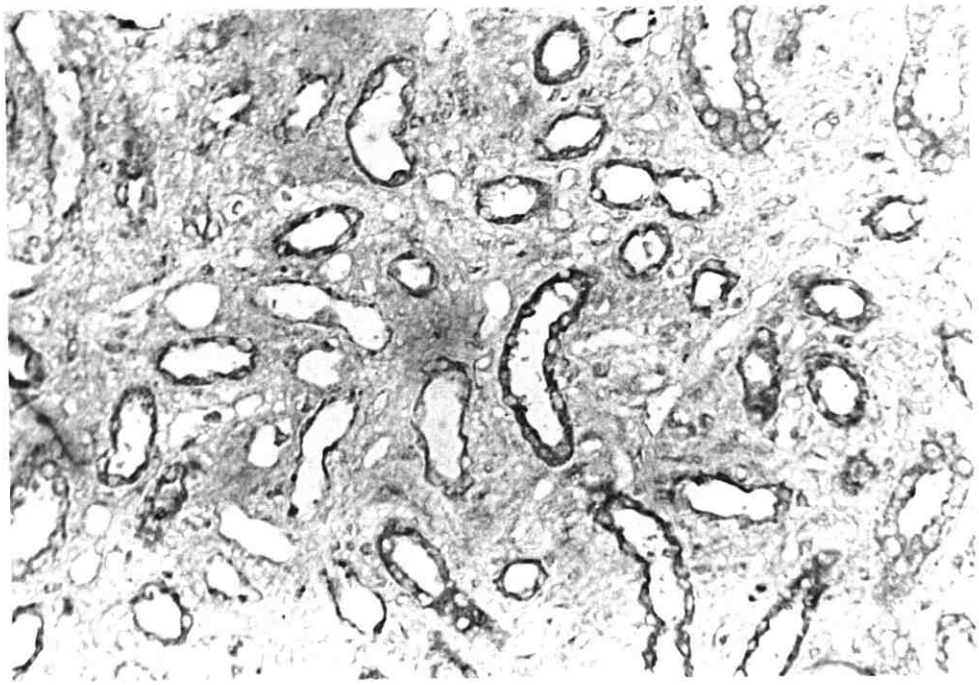
Direct immunoperoxidase staining of renal tubules in a patient with mesangial proliferative glomerulonephritis showing grade 3+. Tissue incubated with peroxidase-labelled Tamm-Horsfall antibody diluted 1 in 4 with PBS. (x 320).

Figure 3.7.



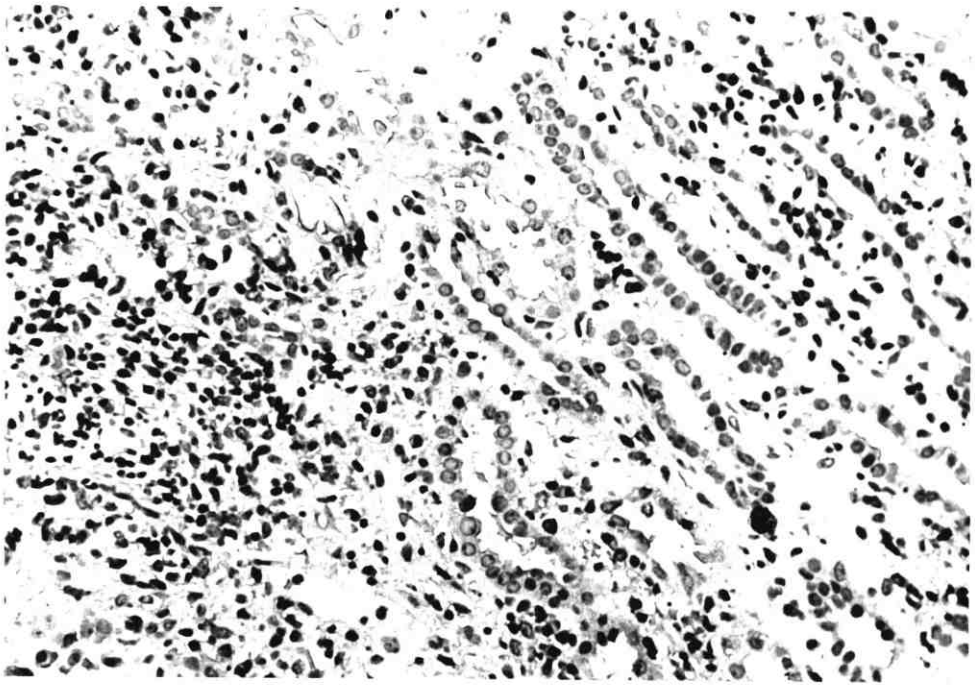
Direct immunoperoxidase staining in a patient with focal and segmental proliferative glomerulonephritis showing grade 2+. Tissue incubated with peroxidase-labelled Tamm-Horsfall diluted 1 in 4 with PBS. (x 320).

Figure 3.8.



Direct immunoperoxidase staining in a patient with SLE showing grade 1+. Tissue incubated with peroxidase-labelled Tamm-Horsfall diluted 1 in 4 with PBS. (x 132).

Figure 3.9.



Direct immunoperoxidase staining in a patient with mesangial proliferative glomerulonephritis showing grade 0. Tissue incubated with peroxidase-labelled Tamm-Horsfall diluted 1 in 4 with PBS. (x 132).

There is a difference in the staining of renal biopsies and normal kidneys for Tamm-Horsfall glycoprotein. The normal control taken from normal human kidneys obtained at autopsy (17) showed staining which lay between 3+ and 4+. The renal biopsies showed, in general, a greatly reduced intensity and distribution of staining. As most of them lay between 0 and 2+ staining (97/144; 67%), I have studied the factors that might affect Tamm-Horsfall secretion in the renal tubules and found an association between renal functions as indicated by creatinine clearance, and Tamm-Horsfall staining. A division was made into patients with creatinine clearance above and below 60 ml/min. (see Table 3.16).

Table 3.16.

NUMBER OF CASES OF RENAL DISEASE WITH AND WITHOUT RENAL FAILURE STAINED BY LABELLED TAMM-HORSFALL ANTIBODY SCORED FROM 0 to 4+

Diagnosis	0	1+	2+	3+	4+	Total
Renal disease with Ccr $\leq$ 60 ml/min.	10 (23%)	10 (23%)	16 (36%)	4 (9%)	4 (9%)	44
Renal disease with Ccr $>$ 60 ml/min	10 (10%)	15 (15%)	10 (10%)	38 (38%)	27 (27%)	100
Normal controls	-	-	-	5 (29%)	12 (71%)	17

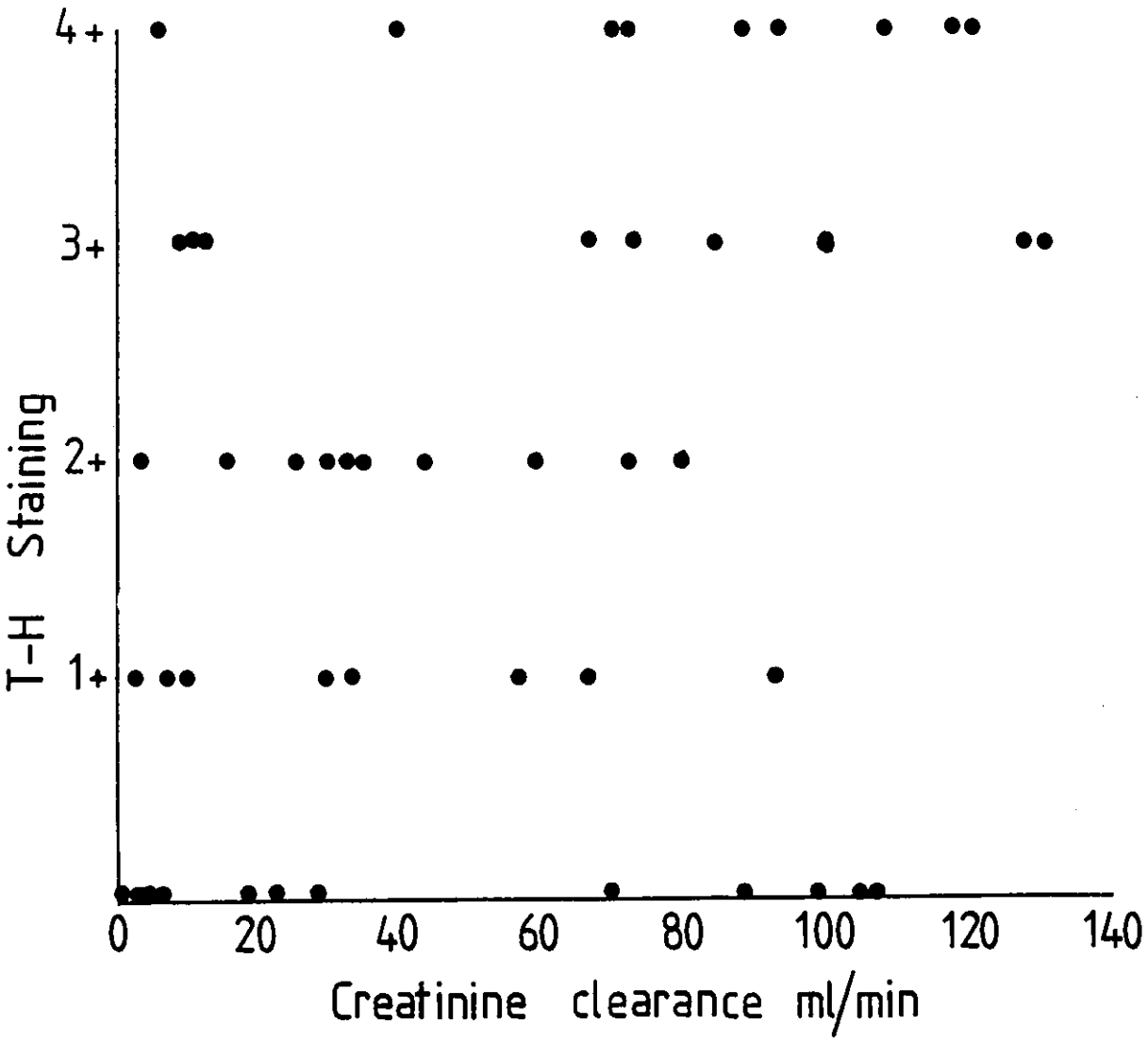
In studying Table 3.16 we notice that in renal disease with failure of the kidney most cases lie between 0 to 2+ (36 cases; 82%); while only eight cases lie between 3+ and 4+ (18%). On the other hand, in renal disease without failure, most cases lie between 3+ and 4+ (65 cases; 65%)

while the rest (35 cases; 35%) show 0 to 2+ staining. The normal controls show 3+ or 4+ staining.

This shows that there is an association between the presence of Tamm-Horsfall glycoprotein in the renal tubules and the renal function. I have calculated the regression line between Tamm-Horsfall staining and renal failure. This line shows a slope which tells us that there is a positive association between these two factors, although a poor correlation ($r = 0.31$).

I have calculated the regression line for Tamm-Horsfall staining on age and on sex for these patients, and found no correlation between them ($r = -0.117$; $r = -0.13$ respectively). I have also studied the intensity and distribution of Tamm-Horsfall staining in different forms of renal lesions and found no correlation between the different forms of renal lesions and found no correlation between the different forms of renal disease and Tamm-Horsfall staining.

Figure 3.10.



The regression line of Tamm-Horsfall staining on renal failure ($r = 0.31$).

Table 3.17

NUMBER OF RENAL BIOPSIES STAINED WITH TAMM-HORSFALL ANTIBODY IN RESPECT TO DIFFERENT FORMS OF FENAL DISEASE SCORED FROM 0 TO 4+

Diagnosis	0	1+	2+	3+	4+	Total
Diffuse mesangial proliferative GN (MPGN)	10 (30%)	5 (15%)	4 (12%)	8 (24%)	6 (16%)	33
Diffuse mesangio-capillary GN Type 1 (MCGN)	3 (19%)	2 (13%)	2 (13%)	4 (25%)	5 (31%)	16
IgA nephropathy	2 (13%)	3 (20%)	3 (20%)	5 (33%)	2 (13%)	15
Systemic lupus erythematosus (SLE)	5 (33%)	3 (20%)	1 (7%)	3 (20%)	3 (20%)	15
Membranous GN	8 (42%)	0 (0%)	5 (26%)	2 (11%)	4 (21%)	19
Focal and seg. GN	9 (43%)	5 (24%)	6 (28%)	1 (5%)	0 (0%)	21
Miscellaneous	9 (36%)	7 (28%)	5 (20%)	4 (16%)	0 (0%)	25
Total	46 (32%)	25 (17%)	26 (18%)	27 (19%)	20 (14%)	144

I also tried to correlate Tamm-Horsfall staining with the presence of proteinuria but there was no correlation between them.

Table 3.18

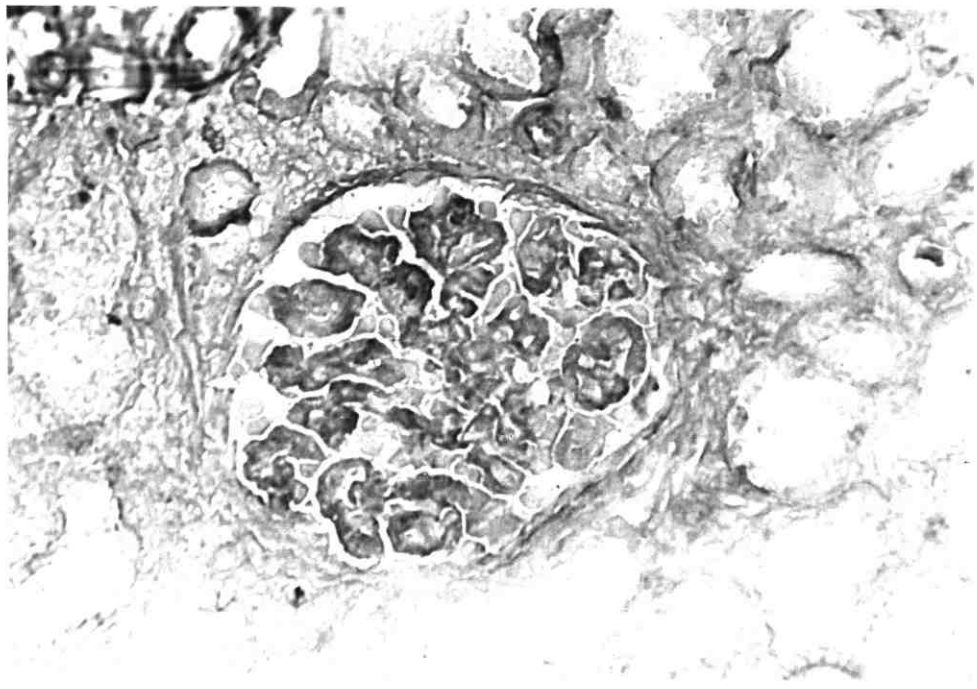
NUMBER OF CASES OF RENAL DISEASE WITH AND WITHOUT PROTEINURIA STAINED WITH LABELLED TAMM-HORSFALL ANTIBODY

Diagnosis	0	1+	2+	3+	4+	Total
Renal disease with proteinuria	10 (22%)	8 (18%)	8 (18%)	10 (22%)	9 (20%)	45
Renal disease without proteinuria	36 (36%)	17 (17%)	19 (19%)	15 (15%)	12 (12%)	99
Normal control	-	-	-	5 (19%)	12 (71%)	17

As we can see from Table 3.18, the distribution of Tamm-Horsfall glycoprotein in renal tubules is nearly the same in both groups. This shows that the presence of proteinuria does not affect the presence or absence of Tamm-Horsfall glycoprotein.

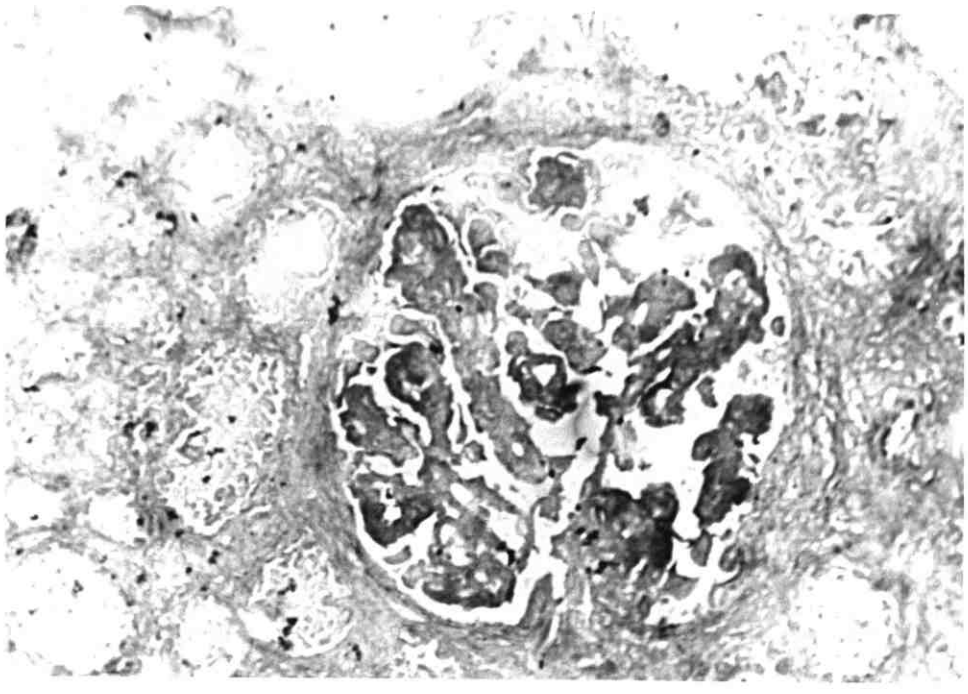
I have found two patients in whom the glomerular deposits stained with Tamm-Horsfall antibody; one of these patients had a uterine carcinoma associated with membranous glomerulonephritis. The other patient had lupus nephritis (Figs. 3.11 and 3.12). The possibility and explanation of the presence of Tamm-Horsfall glycoprotein in such an ectopic place will be discussed in a later chapter.

Figure 3.11.



Direct immunoperoxidase staining in a patient with SLE showing mesangial deposits of immune complexes. Tissue incubated with peroxidase-labelled Tamm-Horsfall antibody diluted 1 in 4 with PBS. (x 132).

Figure 3.12.



Direct immunoperoxidase staining in a patient with membranous glomerulonephritis showing diffuse mesangial deposits of immune complexes. Tissue incubated with peroxidase-labelled Tamm-Horsfall antibody diluted 1 in 4 with PBS. (x 132).

3.5. The Effect of Acidity on Immunoglobulins

I did this experiment to see if the method used to prepare $F(ab')_2$ antibody was damaging to IgM present in complexes so that it was useless in detecting antigen in deposited complexes. I found that the antigenicity of IgM is completely destroyed by the acid conditions, but IgG is not materially affected by the acid.

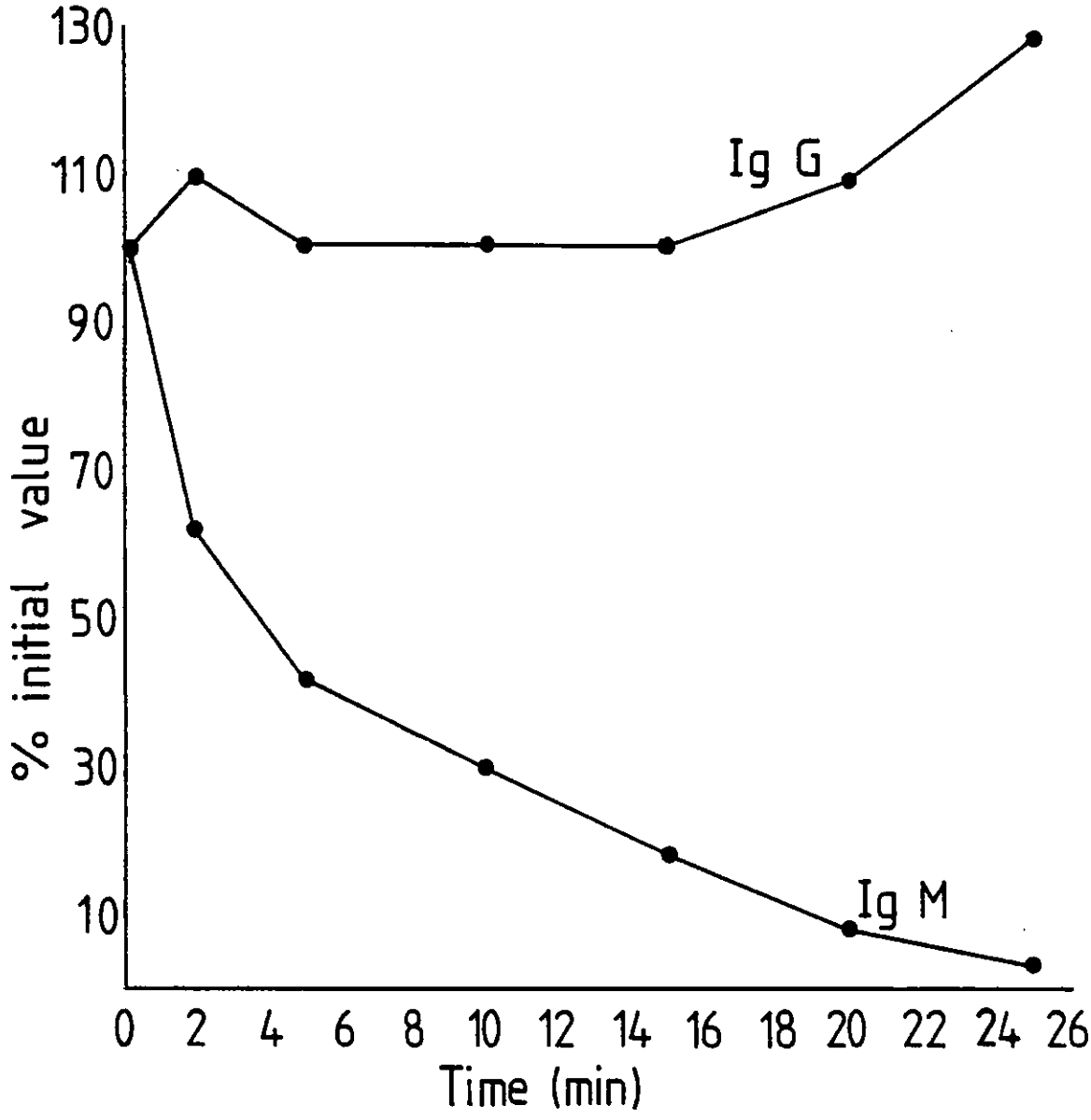
Table 3.19

MANCINI ASSAYS OF IgG AND IgM AFTER INCUBATING IN ACID (pH 3) FOR VARYING TIMES (2 min - 25 min)

Time	IgG		IgM	
	Ring diameter mm	Per cent time 0	Ring diameter mm	Per cent time 0
Standard value	12.5	100	8	100
2 minutes	13	109	6.5	62
5 minutes	12.5	100	6	41
10 minutes	12.5	100	5.5	30
15 minutes	12.5	100	5	18
20 minutes	13	109	4.5	8
25 minutes	14	128	4.2	3

A curve was drawn for IgG and IgM comparing the amount of each after incubation in acid for times between 2 and 25 mins.

Figure 3.13.



Change in Mancini estimate of IgG and IgM in serum acidified for periods of 2 min to 25 min expressed as percentage initial value.

Figure 3.13 shows that the reaction of IgM with its antibody diminishes progressively with time when it is incubated in acid conditions. After 25 min there was hardly any antigenic material left. IgG showed no consistent decrease in antigenicity with time in acid conditions.

This experiment confirmed my thoughts about the effect of acidity on some immunoglobulins, which was one of the causes of insensitivity of the $F(ab')_2$ method used. Of course this did not by itself show that because the Fc region of the IgM failed to react with anti-IgM that the binding sites for antigens were damaged. The second part of the experiment studied the decrease in binding of IgM and IgG rheumatoid factor to antibody coated red cells after exposure to acid. The results of this are shown in Tables 3.20 and 3.21.

Table 3.20

EFFECT OF ACIDIFICATION OF SERUM SAMPLE STUDIED INCUBATED FOR DIFFERENT TIMES (2-25 min) IN ACID CONDITIONS ON THE AGGLUTINATION TITRES AND OF SENSITISED SHEEP RED CELLS WITH AND WITHOUT DITHIOTHREITOL

Time of incubation in acid per min	Agglutination Titre	
	With dithiothreitol	Without dithiothreitol
0	18	11
2	17	11
5	17	12
10	16	12
15	14	13
20	12	11
25	12	12

The results of this experiment were difficult to interpret because of the large amount of IgG antibody present in the serum sample. This serum sample was subjected to gel filtration chromatography on G-200 Sephadex in BBS by Professor Mowbray, and the agglutination titres of sensitised sheep red cells were studied (Table 3.21).

Table 3.21.

EFFECT OF ACIDIFICATION OF SERUM SAMPLE AND INCUBATION FOR DIFFERENT TIMES, ON THE AGGLUTINATION TITRES OF SENSITISED SHEEP RED CELLS WITH AND WITHOUT DITHIOTHREITOL

Time of incubation in acid per min	Agglutination Titre	
	With dithiothreitol	Without dithiothreital
0	8	2
5	7	2
15	4	1
25	2	1

The results shown in Table 3.21 demonstrate the loss of IgM agglutinating activity after acidification at nearly the same rate as the loss of reactivity to anti-IgM antibody, Since the rheumatoid factor activity is located on the V region of the $F(ab')_2$ fragments, which are presumably the same as the variable region of the IgG antibody in this serum. Surprisingly the IgG binding sites are not affected by acidification. However, the implication of this will be considered in the Discussion.

DISCUSSION

From the analysis of circulating and deposited immune complexes in renal disease in general I could differentiate four groups of patients. In one group there were circulating as well as deposited immune complexes; in a second group I found neither circulating nor deposited immune complexes. The third group of patients had circulating but not deposited complexes; and in the last group there were no circulating but only deposited complexes. However, there was only a statistically significant correlation between complexed circulating and deposited IgA and C1q. The differences in the composition and in the fate of immune complexes in patients with renal disease, mainly glomerulo-nephritis, led me to think that there may be differences in clearance of circulating immune complexes from the circulation, dependent on the class of immunoglobulins, and the complement components present in the complex. Thus, some complexes may be rather selectively eliminated from the circulation, and may be preferentially deposited in the glomeruli. Other mechanisms may be operating for the presence or absence of circulating or deposited complexes. I will discuss below the possible mechanismsoperating in each group of patients studied.

1. Glomerular deposition of circulating immune complexes

In the first group of patients studied in whom I could detect immune complexes both in the circulation and in their tissues, the renal lesion may be due to deposition of some of the circulating complexes. The pathogenesis and mechanism of most glomerular disease is thought to involve the formation of circulating immune complexes and their deposition in the filtering structures of the kidney (Wilson and Dixon, 1974). The factors influencing the deposition of circulating immune

complexes in the kidney glomeruli are various, and depend on the host and the immune complexes themselves. Host factors involving the presence of C_3b and Fc receptors on the glomeruli may facilitate the arrest of immune complexes (Gelfand et al, 1975; Mizoguchi et al, 1978). The presence of a high pressure, and an effective filtration barrier in glomeruli favour the arrest of circulating immune complexes in the walls of glomerular capillaries (McCluskey and Bhan, 1981). Host factors may thus play a role in deposition of circulating immune complexes. Circulating immune complexes may occur in other individuals, but lacking one or more of the factors which favour their arrest, may not deposit in the glomeruli. The intrinsic properties of the circulating complexes themselves may also play a role in their deposition on the tissue. Some circulating complexes will never deposit in the tissue and may be either eliminated by the phagocytic system or excreted in urine. In seropositive rheumatoid arthritis, with vasculitis due to immune complex, deposition, serum immune complexes are present which tend to enhance the uptake of aggregated IgG in the radiobioassay test of Onyewotu et al (1975). A similar enhancement has been seen with seropositive sera for infective endocarditis and post-streptococcal glomerulonephritis (Mohammed et al, 1977).

Other circulating complexes tend to deposit once formed, dependent on the size of circulating complexes. Certain strains of group A streptococcal, particularly M type 12, may be nephritogenic because they share antigenic determinants with the kidneys (Johnson and Stollerman, 1969). In patients with sub-acute infective endocarditis, despite high levels of circulating immune complexes, very few get

glomerulonephritis. The glomerular involvement in such cases occurs as a result of specific mechanisms. It is either due to specific antibody combined with streptococcal antigens to form complexes, or is due to auto-antibodies (that is anti-globulins or immunoconglutinins) to circulating antigen. Both mechanisms might operate in the same patient. The nature of the circulating complex molecule itself and the way it forms may determine its fate.

2. Passage of circulating immune complexes in urine without significant renal deposits

In the second group of patients studied in whom glomerulonephritis was clinically and histologically diagnosed, I could not detect either circulating or deposited complexes. Circulating or glomerular passage of these complexes in urine without significant deposition may result in tissue injury (Stachura et al, 1981). Data obtained from the studies of experimental models of glomerulonephritis imply that not only deposited but also soluble immune complexes can produce glomerular damage. My experiments have shown that deposited complexes are soluble and can be washed away. Germuth et al (1979) produced fatal immune complex glomerulonephritis without any evidence of immune deposits in the glomeruli.

The absence of glomerular immune deposits was noted in another group of patients studied (third group). This finding, as well as the reported absence of circulating immune complexes in another group of patients (fourth group) without glomerular deposits, may suggest the possibility that the pathogenesis of some forms of glomerulonephritis does not involve immunological mechanisms. Stachura et al (1981)

could detect circulating immune complexes and significant alterations of serum immunoglobulins and complement in the majority of cases of idiopathic crescentic glomerulonephritis without immune deposits. In addition, urinary immune complexes with an electrophoretic pattern corresponding with those of the serum immune complexes were detected in several of their patients. Thus, passage of ICS into urine may account for the lack of glomerular deposits in some cases of glomerulonephritis.

IgA nephropathy characterised by mesangial deposits of IgA has been considered an immunologically mediated disease, in spite of the fact that circulating complexes have not usually been demonstrated in the circulation of these patients using solid phase C1q and Raji cell radioimmunoassay for detection of immune complexes in glomerulonephritis patients (Tung et al, 1978). This is possibly because the circulating immune complexes are transient, but if they are composed of IgA as the antibody, these techniques, unlike the PEG precipitation technique which does detect them,will not react with IgA complexes. I found circulating IgA complexes in 6 out of 15 Berger's patients. However, Stachura et al (1981) found circulating immune complexes in 8 out of 11 patients with IgA nephropathy.

There may be factors leading to the formation of immune complex deposits as important as those responsible for their removal. Within the glomerulus the rate and mechanisms of removal of complexes depends on their size. Mesangial deposits generally disappear quickly, either through phagocytes and degradation, or by traversing the glomerulus through mesangial channels (Michael et al, 1979). Apparently, insoluble

complexes anywhere in the glomerulus can be solubilised by excess free antibody. I have shown experimentally that mesangial deposits have disappeared by addition of excess antibody on the renal biopsy sections and incubating them for a long time (see Delayed Method).

3. In situ formation of immune complexes

This comprises another mechanism of glomerulonephritis; the reaction of free circulating antibody with antigens already present or fixed in the glomerulus. Trapping of antigen by the glomerulus is largely dependent on properties of the glomerulus itself. In other words, not every glomerulus has the ability to fix antigen and consequently attract the circulating antibody. The glomerular properties responsible for fixation of antigen includes mesangial uptake, biochemical, antigenic and charge characteristics of the glomerular capillary wall, and possibly filtration properties. In my survey I found a group of patients in which immune complexes had been found only in tissue with no minimal detection of any circulating complexes, but in whom free circulating antibodies were present. In this group of patients the in situ mechanisms of glomerular deposition of immune complexes may be the cause of glomerulonephritis.

From the observations obtained in the present study of differences in the circulating and deposited complexes, I could say that in glomerulonephritis in general, several mechanisms and more than one factor specific to the patients and to the causative antigen are needed for glomerulonephritis to occur.

The present study demonstrates a highly significant association between deposited complement components (C1q and C_3) and the presence of IgM or IgG in

complexes. These data are in line with the knowledge that immune complex formation with IgG or IgM activates the complement system (Müller-Eberhard, 1975). The immunoglobulin class which determines the antibody's valancy for specific antigen seems to play an important role in activating complement. It has been reported that IgG or IgM complexes activate primarily the classical pathway, whereas heat aggregated IgA activates the alternative pathway. The pathway of complement activation by various immunoglobulin isotypes has been reviewed (Götz and Müller-Eberhard, 1976). My results support this as I found both C1q and C3 in complexes occurring in association with IgG and IgM but not with IgA alone. This suggests that activation of complement system by either pathway may occur with different immunoglobulin in complexes. Nevertheless, it would seem likely that tissue damage is largely a result of complement activation by locally deposited complexes, whether this occurs by the classical or the alternative pathway.

The Effect of Acidity on Immunoglobulins

It has been stated above that IgM antibody loses complement fixing reactivity on exposure to pH less than 3, even when the protein concentration is high. Little or no inactivation of IgG antibody occurs even at pH 2 (Stollar et al, 1976). The pepsin digestion method used for preparing the $F(ab')_2$ antibody fragments from immune precipitates has been described as a vigorous procedure for digesting proteins and only exceptional protein antigens resist this digestion (Lachmann, 1971). It is known that the yields of $F(ab')_2$ from IgA and probably IgM are low (Johnston and Thorpe, 1982).

I have added to these observations and investigated the effect of the acid conditions which I used for pepsin digestion on the stability of IgG and IgM antibodies. The results showed that complete destruction of IgM antibody occurred, but IgG did not show significant reduction in activity. Thus, in those patients with IgM complexes, the yields of $F(ab')_2$ antibody fragments from digestion of the complexes with pepsin would be near to zero, and this might explain some of the negative results obtained with labelled antibody made from complexes using this technique. It could not be the only explanation, as complexes with only IgM or IgA, but without IgG, were only found in 50% of the patients studied. It is probable that biopsies with complexes formed in antibody excess did not react with the labelled $F(ab')_2$ antibody during the short exposure used in this group of experiments. I feel that this is the likely cause of the majority of the negative results using this approach.

This view is supported by the observation that using the delayed technique, free,labelled IgG antibody of the patients react with their own complexes in the great majority of patients, if given sufficient time.

Thus, failure of the $F(ab')_2$ prepared from the complexes to react is probably because the complexes were in antibody excess. The ability to precipitate and label the free antibody in the serum and react it with the deposited complexes demonstrates that the complexes were in antibody excess. Therefore, $F(ab')_2$ antibody prepared from the circulating complexes could only react slowly with the deposited complexes by re-equilibration. In the $F(ab')_2$ experiments insufficient time was used for this reaction to occur. Thus, this was the reason for the negative results obtained with $F(ab')_2$ from the complexes when compared with the patients' results using the delayed method.

The Delayed Method for the Identification of Antigens Complexed in Human Renal Biopsies

The delayed method used for identification of complexed antigens deposited in human renal biopsies is a reproducible reaction between the patient's circulating IgG antibody and the immune complexes deposited in their own kidneys. In contrast, this circulating antibody rarely showed a reaction with biopsies of other patients. This means that although the circulating antibody which I have been labelling has the same specificity as the antibody deposited in the patient's own kidneys, in other patients one must assume that different antigens are complexed. This implies that there are quite a few antigens present in deposited complexes in different patients, even in those patients with the same form of glomerulonephritis.

1. Idiotypic differences in immunoglobulins

Idiotypic differences are those related to individual variable region sequences produced by each clone of antibody forming cells. There is a gene coding for the variable region of the antibody, and it occurs on the chromosome carrying the genes for the constant region. This leads to the belief that we inherit genes which enable us to make particular antibodies of particular sub-classes and that the capacity to produce an antibody response is limited by the store of specificities encoded by the genes on this chromosome. Idiotypic differences are identified by specific antisera. If the antibody raised by a rabbit

to a bacterium is used to coat organisms which are injected into another rabbit of identical allotypes, the second rabbit may make antibodies against the coating antibodies. These second antibodies will recognise the variable regions of some of the injected antibody, and the antiserum will react with only a restricted population of immunoglobulins in the serum of the first rabbit. It has been found that the antigen-binding site of the antibody is part of an important idiotypic determinant. Binding of hapten induces a conformational change that alters the idiotypic determinants that are not located in the antigen-binding site (Brient and Nisonoff, 1970). If that is the case in my experiments, the circulating antibody (idiotype) of a particular patient, produced by a particular antigen will react specifically with antigen complexed in their own tissue to an anti-idiotype, but not with another patient's tissue containing different anti-idiotypes, even if the inciting antigen is the same. Therefore, individual variation of the variable region of the antibody may account for this difference. Idiotypic determinant, which is found close to or in the antibody combining site, may be shared by different antibody classes (with the same V gene and different C genes), but they are very rarely found to be the same among different individuals.

Idiotypic cross-reaction in outbred animals is found less frequently than in inbred animals. Oudin and Bordenave (1971) studied the

the cross-reaction of anti-idiotypic antisera prepared by injection of rabbit antibodies specific for Salmonella abortus-equi into another rabbit. They showed that the frequency of these cross-reactions was about 3%.Oudin and Michel (1973) elicited antibodies in rabbits by injecting Salmonella typhi. They mixed the resulting antiserum with Salmonella typhi to bind the antibodies to the intact bacteria and then injected the washed agglutinate into a recipient rabbit. Serum from the recipient reacted with donor serum containing anti-Salmone lla typhi antibodies but not with pre-incul ation serum from the same rabbit, or with serum taken after anti-Salmonella typhi antibodies had disappeared. These authors also demonstrated that the antiserum specific for the anti-Salmonella typhi antibodies from one rabbit did not react with the anti-Salmonella typhi serum taken from any of 17 other rabbits. In my experiments, antibodies from the patient's serum showed specific reaction with their own complexes in the tissue but not with the other 23 renal biopsies, which is in agreement with the findings discussed above.

These observations show that identical idiotypes appeared to be present only on antibodies of a particular specificity. Antibodies with different specificities either in the same individual or in different individuals do not bear the same idiotypes. Antibody from different individuals with the same specificity may have different idiotypes.

2. Presence of different antigens in different patients

It seems that different antigens can precipitate the same form of glomerulonephritis. The antibodies produced will be different from one patient to another and these antibodies will only react with their specific antigens but not with those of other patients. Some patients can change their antigenic structure during infection (Goodman et al, 1979). Antigenic variation in influenza virus is common and responsible for new influenza pandemics. It is possible that multiple antigens with different immunogenecities may induce continuous production of more than one antibody. Thus failure of circulating antibody in one patient to detect the complexed antigen in another patient's tissue could be explained by either of these mechanisms.

3. Antibody diversity

It has been proposed that to deal with the vast range of antigens present in the environment requires a large repertoire of specific antibody molecules. To do this requires a generator of diversity (Lennox and Cohn, 1967). There are restrictions on the total number of antibody combining sites, which are made of the hypervariable region of heavy and light chains. If the hypervariable regions comprise locations which can be occupied by 10-15 amino acids from the 20 possible amino acids, then there can be at least 10^{20}-10^{30} different antibody molecules, if both heavy and light chains contribute equally to the combining site. Among this vast number of possible antibodies it is very unlikely for the immune response to involve the same antibody in two different individuals. So that the labelled antibody prepared from patients' serum could not react with

other patients' complexes deposited in the renal tissue. Most likely it is different from the antibody in the complexes. Even with long incubation to allow dissociation and reassociation of the deposited complex; the labelled antibody could not replace the original antibody.

Besides the previous evaluation of the delayed method for the identification of antigen complexed in renal biopsies, the delayed method has revealed the immune complexes of glomerulonephritis occur in antibody excess. Otherwise I would not have been able to label free circulating antibody in the serum of these patients. The question of whether immune complex glomerulonephritis occurs in antigen or antibody excess has been a point of argument for a long time. Hawn and Janeway (1947), McClean et al (1951), Germuth (1953) believed that soluble complexes formed in the circulation deposits in tissue. The basis of this belief came from studies in animals of the effects of a single large dose of serum, followed by purified protein. In the early phase during induction of the clinical manifestation of serum sickness, the antibody-antigen complex is in antigen excess. Thereafter, free antibody appears, but only after the point at which inflammation, accompanied by appearance of immune reactants in the kidney and proteinuria become apparent. Thus, in this model, the appearance of circulating immune complexes and proteinuria coincide with a period of antigen excess. However, there is also good evidence that immune complexes in antibody excess can cause injury. Clark et al (1981) reported an experiment involving injection of ferritin in pre-immunised pigs. A severe form of nephritis with proteinuria and haematuria was induced and with repeated injections a mesangiocapillary glomerulonephritis was obtained.

The persistence of insoluble complexes formed at equivalence or in antibody excess must be limited by the activity of the reticulo-endothelial system (Mannik, 1980). However, the kidney receives 25% of the cardiac output, and formed immune precipitate could enter or form within the kidney, regardless of reticuloendothelial function.

Even so, until recently, most workers believed that soluble circulating immune complexes were the principal pathogenic agent in immune complex disease. Germuth et al (1967, 1977) demonstrated that rabbits given constant daily doses of a fixed antigen responded within a week or two in one of four ways. First, little or no antibody was produced and no renal disease was observed; second, a high antibody titre was observed and no renal disease developed; third a poor antibody titre was reported and mesangial glomerulonephritis was found; and finally, those animals who from the start had, or later developed, a poor immune response in moderate antibody excess developed severe nephritis. Germuth et al (1972) showed that the third group with mesangial deposits had relatively large soluble immune complexes in the circulation, whilst the fourth group had smaller immune complexes. These workers interpreted the glomerulonephritis induced as being the result of soluble immune complexes formed in antigen excess. Germuth and Rodrigues (1973) re-examined the above data and they re-interpreted their results. They calculated that in those rabbits with a relatively poor antibody response, at the time of induction of nephritis, the plasma must have swung each day after each injection from moderate antibody excess to moderate antigen excess. They speculate that the complexes formed in moderate antigen excess might be the cause of mesangiopathic glomerulonephritis. Germuth et al (1977) concluded that mesangiopathic

lesions might result from deposition of complexes formed either at equivalence or in antibody excess.

Thus all these data can be interpreted equally to suggest that poorly soluble or insoluble complexes at or near equivalence were the pathogenic agents in these animals (Cameron and Clark (1982).

My experiments have shown that immune complexes present in the human renal tissue are soluble, since it was found that the staining of the deposits was very bright after the addition of PEG to the buffer used for diluting the labelled antibody. In the absence of PEG, the staining was weak and blurred and the granular deposits became very diffuse. I assume that some of the formed complexes may have dissolved in the drop of buffer over a matter of days and have been to some extent at least, washed away. Complexes formed locally would only be likely to be held locally if they were insoluble, or attached to receptor, say for C_3b or Fc receptors. Trapping by filtration is a mechanism for the localising of soluble complexes produced somewhere else. Thus, since the complexes do dissolve in the drop of buffer and are thus soluble, they are likely not to have been made locally. So I suggest that the glomerular lesions in human kidney occur as a result of soluble immune complexes which form in antibody excess.

Reaction of Tamm-Horsfall antibody with renal biopsies

Tamm-Horsfall glycoprotein is a specific renal epithelial protein which is predominantly localised in the cells of the proximal and distal convoluted tubules of loop of Henle and the collecting duct (Table 1.1). The biological role of Tamm-Horsfall glycoprotein is possibly related to the permeability property of the ascending loop of Henle (Hoyer and Seiler, 1979). Various reports have stated that Tamm-Horsfall glycoprotein might be one of the renal tubular antigens involved in some of auto-immune renal disease (Losse et al, 1975).

These findings prompted me to study Tamm-Horsfall glycoprotein in relation to renal disease. This was performed by examining the Tamm-Horsfall glycoprotein present in renal biopsies using labelled Tamm-Horsfall antibody. My study demonstrates that generally in glomerulonephritis the intensity and distribution of staining of Tamm-Horsfall glycoprotein was greatly reduced in comparison with staining of normal kidney tissue (the control group). However, there was no significant correlation between different forms of glomerulonephritis and the presence of Tamm-Horsfall glycoprotein. This might be so because it seems that the presence of Tamm-Horsfall glycoprotein depends more on renal function than on other factors. So in any form of glomerulonephritis without renal failure, the presence of Tamm-Horsfall glycoprotein seems to be unaffected. It has been shown in studying the correlation between the presence of Tamm-Horsfall glycoprotein and creatinine clearance as an assessment for renal function. This finding shows that Tamm-Horsfall glycoprotein secretion by the renal tubular cells is related to renal function.

The intensity and distribution of Tamm-Horsfall staining was compared with age, sex, and presence of proteinuria. There was no apparent correlation between Tamm-Horsfall staining and any of these factors, so that from these observations it seems that the presence of Tamm-Horsfall glycoprotein is related only to renal function. Dawnay et al (1980) suggested that the serum level of Tamm-Horsfall glycoprotein measured by a sensitive and specific radioimmunoassay is strongly correlated with renal function. It has been demonstrated that Tamm-Horsfall glycoprotein is normally present in human serum (Avis, 1977; Grant et al, 1979; Dawnay et al, 1980), and its level is affected by renal function; so that serum Tamm-Horsfall glycoprotein is strongly linked with urinary Tamm-Horsfall glycoprotein and may be dependent on it as renal tubules are the site of synthesis of this glycoprotein. It seems that both serum and tubular Tamm-Horsfall glycoprotein are affected by the same factors. A low grade of Tamm-Horsfall staining in renal biopsies might be taken as evidence of poor renal tubular function.

Normally Tamm-Horsfall glycoprotein is demonstrated in the cytoplasm of the cells of the ascending limb of the loop of Henle and distal tubules (Table 1.1). I could detect Tamm-Horsfall glycoprotein in the glomerular tuft in two patients; one patient with SLE, and another with membranous glomerulonephritis associated with cervical carcinoma. The explanation of the presence of Tamm-Horsfall glycoprotein in such an ectopic place will be discussed below.

1. Filtration of Tamm-Horsfall glycoprotein from plasma components

The presence of Tamm-Horsfall glycoprotein deposits in the glomerular tuft could result from filtration of a plasma and secretion by glomerular constituents such as mesangial cells, or perhaps from secretion by the parietal epithelial cells lining the capsular space, particularly when tubular epithelium has replaced the normal epithelial lining (McGiven et al, 1978). It appears that Tamm-Horsfall glycoprotein, which under normal conditions is sequestered within tubular cells of the ascending limb of Henle's loop and the distal convoluted tubules, may be released from these sties as a result of tubular damage and penetrated into the renal interstium. Serum Tamm-Horsfall glycoprotein level may be a factor in precipitating such conditions, If the serum level of Tamm-Horsfall glycoprotein increases under certain pathological conditions, there may be excretion of some of this protein through the kidney and there may be arrest of this protein by the filtering system of the kidney.

2. Retrograde passage of Tamm-Horsfall glycoprotein

It is possible that there is a retrograde passage of Tamm-Horsfall glycoprotein from its site of synthesis in the distal tubules, along the loop of Henle and proximal convoluted tubules into the glomerular mesangium.

3. Extra-tubular Tamm-Horsfall glycoprotein

Extra-tubular Tamm-Horsfall glycoprotein has been demonstrated in medullary cystic disease, chronic pylonephritis, obstructive uropathy, and some other forms of tubular interstitial nephritis (Resnick et al, 1978). However, it is more likely that this extra-tubular Tamm-Horsfall glycoprotein may pass to the circulation to reach the glomeruli.

4. Presence of ectopic tubular epithelium cells

The presence of Tamm-Horsfall glycoprotein in the glomeruli might be due to the presence of some ectopic tubular cells in that situation, which are capable of secreting Tamm-Horsfall glycoprotein.

There has been a strong suggestion of the development of immunological reactions against Tamm-Horsfall glycoprotein in renal disease. Experimental studies in pigs (Hodson et al, 1975) suggested that reflux nephropathy led to the formation of Tamm-Horsfall antibody. Autoantibody to Tamm-Horsfall glycoprotein associated with renal damage and urinary tract infection in adults has been found by Fasth et al (1981). Forty-one out of forty-seven of these patients has vesicoureteric reflux.Cell-mediated immunity against Tamm-Horsfall glycoprotein has been reported in patients with autoimmune liver disease associated with renal tubular acidosis (Tsantoulos et al, 1974). Thus, the presence of Tamm-Horsfall glycoprotein complexes in the glomeruli may be due to the immunological reactions against Tamm-Horsfall glycoprotein, especially if the glomeruli are diseased . The presence of Tamm-Horsfall glycoprotein containing deposits within the renal interstitium in patients with medullary cystic disease, obstructive uropathy and vesicoureteral reflux has been reported by Resinick et al (1978).

The addition of Tamm-Horsfall antisera to the study of renal biopsies may represent useful evidence of retrograde tubular passage of urine; possibly associated with tubulo-interstitial damage and intra-renal reflux and undetectable by other methods of investigation.

CONCLUSION

Immunopathological studies in animal and man have established the role of immune complexes in the pathogenesis of immunologically mediated renal disease. The pathogenesis of different forms of renal disease involves more than one mechanism. The present study shows that the difference in clearance of circulating immune complexes from the circulation depends on the class of immunoglobulin and the complement components present in the complexes. Classical pathway activation of complement may occur when IgG or IgM is present in the complexes, but not with IgA alone.

A new method has been developed for studying the complexed antigens. This method depends on the slow equilibration of labelled globulin prepared from the patient's own serum with the complexed antigen in the renal tissue. Two important observations have emerged from this study. First, in general immune complex mediated renal disease occurs in antibody excess. Second, patients with glomerulonephritis rarely share the same antigen complexed in their kidney even if they have shown the same form of disease.

I have studied Tamm-Horsfall glycoprotein as one of the autologous antigens which might be involved in immune complex glomerulonephritis. This study has shown a positive correlation between the presence of Tamm-Horsfall in renal tubules and renal function. Also it has shown that under certain pathological conditions Tamm-Horsfall glycoprotein is present in the glomerular tuft.

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ABBREVIATIONS

GN	Glomerulonephritis
GBM	Glomerular basement membrane
AH	Anti-hyaluronidase
ASO	Anti-streptolysin O
ASK	Anti-streptokinase
ADnase	Anti-deoxyribonuclease
MPGN	Diffuse mesangial proliferative glomerulonephritis
MCGN	Diffuse mesangio-capillary glomerulonephritis
C_3NeF	C_3 nephritic factor
DDGN	Dense deposit glomerulonephritis
PAP	Peroxidase anti-peroxidase
T-H	Tamm-Horsfall glycoprotein
RTA	Renal tubular acidosis
DT	Distal convoluted tubules
PCT	Proximal convoluted tubules
CD	Collecting tubules
MD	Macula densa
LH	Loop of Henle
DL	Descending limb
AL	Ascending limb
Ccr	Creatinine clearance
ICS	Immune complexes

g	gram
mg	milligram
µg	microgram
l	litre
µl	microlitre
cm	centimetre
mm	millimetre
M	Molar
mM	millimolar
°C	Temperature in degrees Centigrade
w/v	weight/volume
%	Percentage
IgG	Immunoglobulin G
IgA	Immunoglobulin A
IgM	Immunoglobulin M
C1q	Complement one q
C_3	Complement three
C_4	Complement four
Ag	Antigen
Ab	Antibody
min	Minute
PBS	Phosphate buffered saline
EDTA	Ethylene diamine tetra-acetic acid
PEG	Polyethylene glycol
$F(ab')_2$	Fragment antibody binding
Fc	Fragment constant