THE ROLE OF VARIATIONS OF INHERITED PROPERTIES OF THE GLOMERULUS IN THE PATHOGENESIS OF GLOMERULONEPHRITIS

© LATA MARGRITH CHANDRACHUD (BSc Hons)
UNIVERSITY DEPARTMENT OF MEDICINE
ROYAL INFIRMARY
GLASGOW

THESIS SUBMITTED TO THE UNIVERSITY OF GLASGOW
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

M A Y 1986

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

TABLE OF CONTENTS PAGE NO

PAGE NO

INDEX OF TABLES

INDEX OF FIGURES

26 Binding of alcian blue (250ug/ml) to red cells of various glomerulopathies.

27 Induction of Heymann nephropathy.

28 Electronmicrograph of Lewis rat glomerulus 7 months after injection with tubular antigen (FX1A).

29 Electronmicrograph of DA rat glomerulus 7 months after injection of tubular antigen (FX1A).

30 Glomerular immunofluorescent pattern of control Lewis rat kidney.

31 Glomerular immunofluorescent pattern of Lewis rat kidney after injection with cationic HSA.

32 Glomerular immunofluorescent pattern of control DA rat kidney.

33 Glomerular immunofluorescent pattern of DA rat kidney after injection of cationic HSA.

34 Proteinuria caused by intravenous injection of Hexadimethrine in Lewis and DA rats (HDM).

35 Proteinuria caused by intraperitoneal injection of Puromycin aminonucleoside in Lewis and DA rats (PA).

36 Prostacyclin (PGI_2) release from rat aortic rings.

37 PGI_2 release from rat aortic rings.

38 Renal uptake of cationic IgG in rats fed fatty acid enriched diets.

39(a) Glomerular immunofluorescence pattern after staining with fluoresceinated antibody to human IgG (FITC anti human IgG) in the Lewis strain fed an enriched safflower oil diet.

(b) Glomerular immunofluorescence pattern after staining with FITC anti human IgG in the DA strain fed a safflower oil diet.

40(a) Glomerular immunfluorescence pattern after staining with FITC anti human IgG in the Lewis strain fed an enriched evening primrose oil diet.

(b) Glomerular immunofluorescence pattern after staining with FITC anti human IgG in the DA strain fed an enriched evening primrose oil diet.

41(a) Glomerular immunofluorescence pattern after staining with FITC anti human IgG in the Lewis strain fed a normal diet.

(b) Glomerular immunofluorescence pattern after staining with FITC anti human IgG in the DA strain fed a normal diet.

ACKNOWLEDGEMENTS

I would like to thank the following for their invaluable help during the compilation of this thesis.

Professor A C Kennedy for his supervision and for his guidance during the preparation of this thesis, also for the use of laboratory facilities in the Department of Medicine, Glasgow Royal Infirmary.

Dr J M Boulton Jones for his supervision and for his guidance and encouragement throughout the experimental work and during preparation of this thesis.

Dr M E M Allison for her help with the animal techniques and for the use of her office while preparing this thesis.

Dr Anna Mosely for her expert advice and assistance with the immunofluorescent studies.

Dr A McLay for his help with electronmicroscopy studies.

Dr Jill Belch for her collaboration and advice with the prostaglandin studies.

Graham McWilliams (Senior Honours Student, Department of Physiology, Glasgow University) for his assistance with the red blood cell studies.

Fraser Speirs, Stephen Palmer and the rest of Medical Illustration Staff for their help with the figures.

Dougie Bell and Staff for their help in the Animal House.

My family for their continued encouragement and support.

Patricia Campbell for her help in proof-reading this thesis.

Last but not least Katy Rankin for typing this thesis.

DECLARATION

The work described in this thesis was performed in the University Department of Medicine, Glasgow Royal Infirmary, from August 1982 till January 1986. The work described herein was entirely the author's own unless otherwise indicated.

Some of the work described in this thesis has already been published.

(1) Boulton Jones, J M, Chandrachud, L.
Inherited variations in glomerular properties.
Proceedings of the European Dialysis and Transplant Association, 22, 767-772.

(2) Chandrachud, L, Boulton Jones, J M (1986).
Inherited variations in glomerular properties and some consequences.
Scottish Renal Association Bulletin, 6/2, 2.

The following papers on work in this thesis have been presented by the author:-

(1) Inherited variations in charge of the glomerular basement membrane.
Scottish Society for Experimental Medicine.

JANUARY 1985 - EDINBURGH

(2) Inherited variations in glomerular properties and some consequences.
Scottish Renal Association.

OCTOBER 1985 - DUNDEE

ABBREVIATIONS

HSA	Human serum albumin
HSA^+	Cationized HSA
IgG^+	Cationized IgG
IgG^n	Native (unaltered) IgG
Agg IgG	Aggregated IgG
GBM	Glomerular basement membrane
GCW	Glomerular capillary wall
CL	Capillary loop
PA	Puromycin aminonucleoside
PAN	Puromycin aminonucleoside nephrosis
HDM	Hexadimethrine
rbc	Red blood cell
AB	Alcian blue
IV	Intravenously
IP	Intra peritoneally
IgAN	IgA nephropathy
IMN	Idiopathic membranous nephropathy
MCN	Minimal change nephropathy
MPS	Mononuclear phagocyte system
GAG	Glycoaminoglycan
HN	Heymann nephritis
PHN	Passive Heymann nephritis

SUMMARY

Inherited glomerular properties have been investigated in two strains of rat chosen because one of them, Lewis is susceptible to Heymann nephritis whereas the other DA, is resistant. The glomerular properties examined were the charge on the glomerular basement membrane (GBM) and the mesangial uptake of circulating proteins. The former was studied by measuring the specific renal uptake of cationic IgG which was significantly less in Lewis rats. The deposition of cationic IgG on the GBM was significantly less in Lewis rats. The deposition of cationic IgG on the GBM 15 minutes after its administration, as judged by immunofluorescent studies, confirmed this finding. Interestingly, the specific hepatic and splenic uptake of cationic IgG at 15 minutes and the binding of Alcian blue (a cationic dye) to red cells, were all less in Lewis rats than DA rats. Therefore, the negative charge on all the cell and membrane surfaces examined was less in Lewis rats than DA rats. The second glomerular property, the mesangial uptake of circulating proteins, was studied by measuring the renal uptake of aggregated IgG. This was greater in DA rats than Lewis rats.

The effect of these differences in glomerular properties in the expression of some animal models of glomerular disease was investigated. Lewis rats developed proteinuria following infusion of hexadimethrine, a polycation, into the renal artery whereas DA rats did not. This confirmed the functional significance of the measured difference in GBM charge between the two strains. On the other hand, DA rats developed more proteinuria following puromycin aminonucleoside which may have been due to greater mesangial uptake of the drug. The differences in susceptibility to Heymann nephritis in the Lewis strain

was confirmed. Chronic serum sickness induced by cationic HSA led to capillary loop deposits in Lewis rats whereas DA rats had mesangial deposits even in control kidneys. Therefore, Lewis rats have lesser membrane and cell surface charge, a lower mesangial uptake of circulating proteins than DA rats and are more liable to develop proteinuria and capillary loop deposits. The clinical relevance of these findings was tested by examining the red cell charge of three groups of patients with idiopathic membranous glomerulopathy (IMN), minimal change nephropathy (MCN) and IgA nephropathy (IgAN) who were in clinical remission or who had proteinuria less than 2.2g/24 hours. MCN and IMN are associated with heavy proteinuria which rarely occurs in IgAN. IMN is associated with capillary loop deposits whereas IgAN is associated with mesangial deposits. The red cell charge was significantly greater in patients with IgAN than MCN or IMN but all three patient groups fell within the normal range. If red cell charge correlates with GBM charge in patients as it does in the two strains of rats, the similarities between the kidneys of patients with IMN and Lewis rats and between the kidneys of patients with IgAN and DA rats are striking.

Prostaglandins and their precursors have been shown to have beneficial effects on human and animal glomerulopathies. Modulation of mesangial uptake by prostaglandins and their precursors was investigated. Release of prostacyclin by the aortas of Lewis rats was greater than the aortas of DA rats. Studies of the effect of prostanoids or mesangial uptake of circulating antigen (cationic IgG) showed that all polyunsaturated fatty acid diets and infusions of prostacyclin (PGI_2) or PGE_1 caused an increase in both strains of rats whatever the effect on the uptake by the whole kidney. No difference between the two strains was observed. This may have been due to increased

glomerular production of angiotensin II which is known to increase mesangial uptake. Prostaglandins and precursors have been shown to increase mesangial uptake of antigen. Modulation of mesangial uptake by prostaglandins may offer a new approach to therapy of these nephropathies.

The results of these studies show that the glomerulus is not simply a passive participant of systemic events but that its properties vary within strains and are important and previously unrecognised factors in the individual response to various forms of glomerular injury.

CHAPTER I

RENAL ANATOMY AND PHYSIOLOGY OF THE KIDNEY

1:1:1 Introduction

The kidneys are paired organs which lie retroperitoneally on the posterior abdominal wall. They are bean shaped with a hilum at the mid point of the medial aspect. Each kidney measures 12 x 7 x 3cm but there is considerable variation from person to person. In the adult male they weigh 150-170g each and slightly less in the female. At the hilum the renal artery and vein and the renal pelvis join the organ. The whole kidney is covered by a thin fibrous capsule which is joined at the hilus to the artery, vein and pelvis (1).

The kidney is made up of cortex and medulla which are sharply demarcated on the cut surface where the pyramids are situated (Fig 1). The pyramid is that portion of the medulla which projects towards the pelvis, the distal part being called the papilla. The cortex extends into the space between the pyramids as the columns of Bertin. Faint striations are visible on the cortex running in the direction of the hilum. These are medullary rays which contain collecting tubules and ascending limbs. The cortex contains glomeruli with proximal and distal convoluted tubules and the medulla contains all the remaining constituents of the nephron.

1:1:2 Structure of the Kidney

The functional unit of the kidney is the nephron, an expression first coined by Braus (2). Each human kidney has been estimated to contain about 1,200,000 nephrons. This contrasts with approximately 30,000-34,000 nephrons in each adult rat kidney (3). The essential

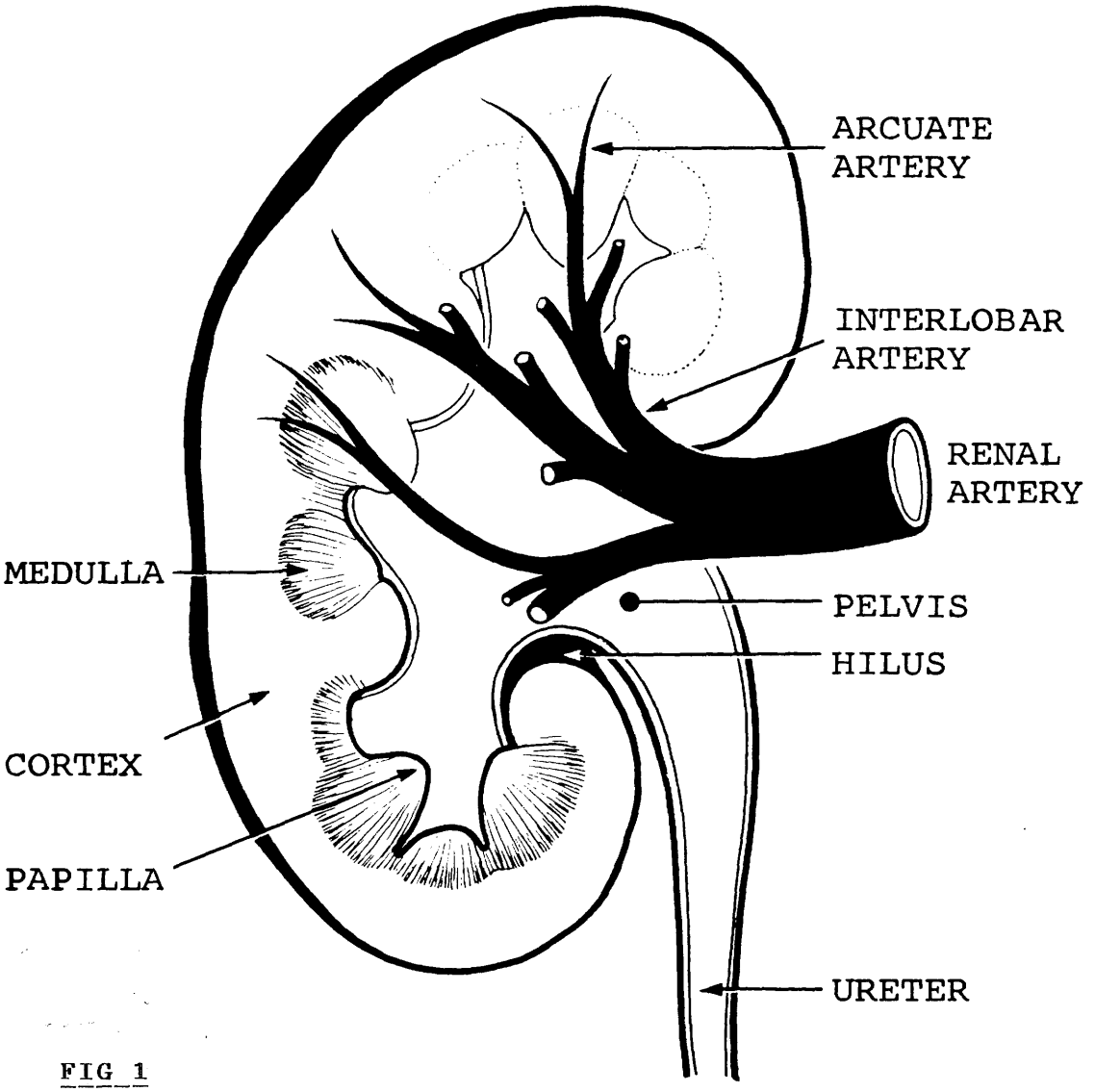


FIG 1

The gross structure of the cut surface of a kidney.

components of the nephron are the renal glomerulus, the proximal tubule, the loop of Henle, the distal tubule and the connecting segment. The nephron is a thin tube approximately 50mm long beginning at the glomerulus and ending where it joins a collecting duct. The upper end lies in the cortex where it is invaginated by a mesh of capillaries - the glomerulus. The next portion of the nephron is a coiled tube called the proximal tubule which passes medially into the medulla towards the hilum. There is a U-shaped bend in the medulla and the nephron then runs towards the cortex. The portion in the medulla is called the loop of Henle. The final portion in the cortex is the distal tube and, like the proximal tubule, it is coiled. At the end of the distal tubule the nephron straightens out, passes medially and joins a collecting duct in the medulla. Several collecting ducts join to form the collecting ducts of Bellini which open directly on the surface of the papillae. A simplified schematic representation of a nephron is shown in Fig 2.

1:1:3 Structure and function of the glomerulus

The glomerular tuft is made up of a coil of specialized capillaries fed by an afferent and drained by an efferent arteriole. The tuft lies in a space the boundary of which is called the glomerular capsule or Bowman's capsule. The capsule is spherical and has an opening directly into the proximal convoluted tubule. The glomerular tuft and glomerular capsule have a continuous lining of epithelial cells which are in direct continuity with those lining the proximal tubules (4). The function of the renal glomerulus is to filter the blood, producing a protein free filtrate of the blood plasma, while retaining the cellular elements of the blood and the plasma proteins in the

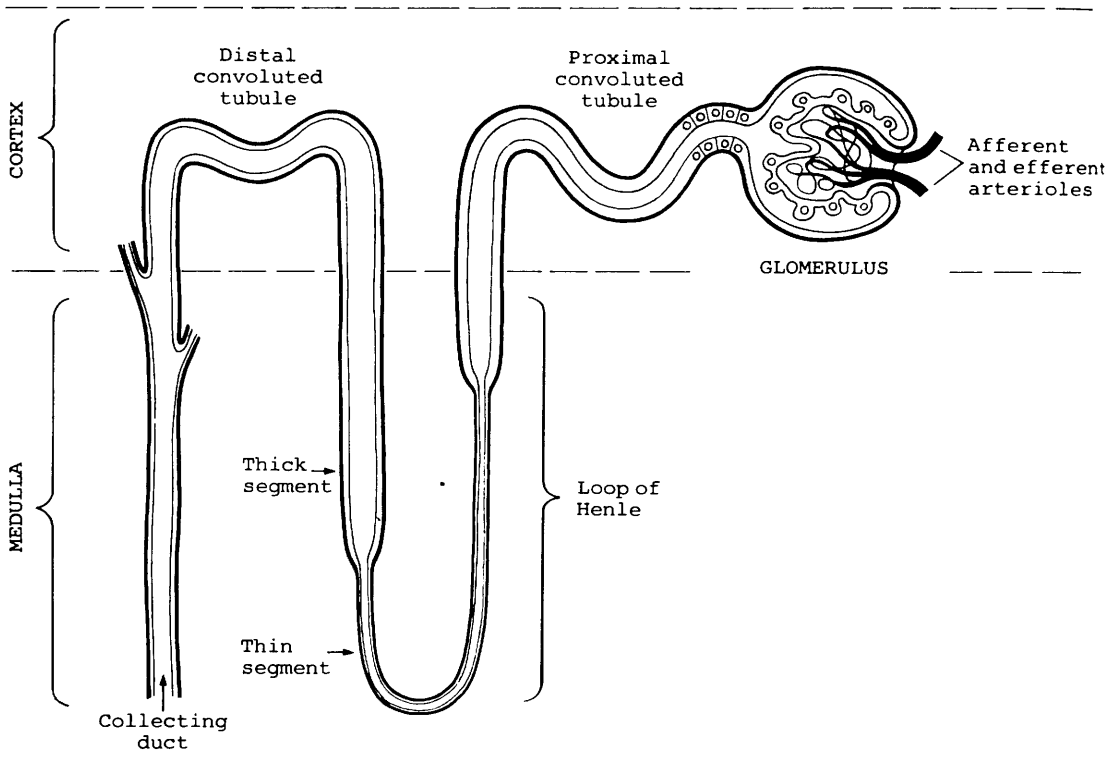


FIG 2 Diagram of a nephron.

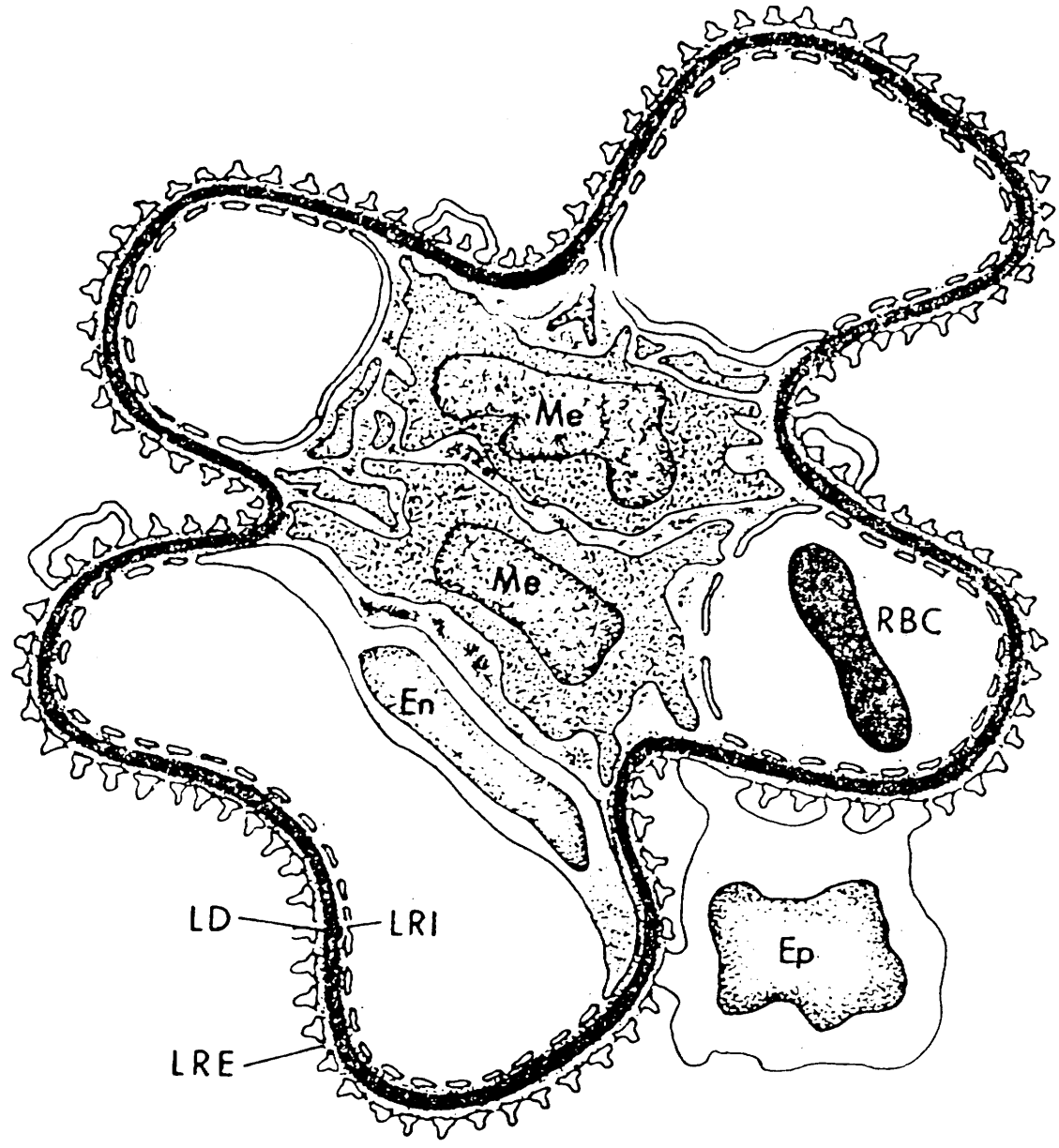
circulation (5). It is at the level of the capillaries that filtration takes place. To carry out this function the structure of the glomerular capillaries are highly specialized. They are divided into peripheral regions which are believed to constitute the main filtration surface and axial regions buried deeper in the glomerular tuft. The glomerular capillary wall is considered to be constructed of four components - three cell types, endothelial, epithelial and mesangial cells and the extracellular basement membrane (Fig 3).

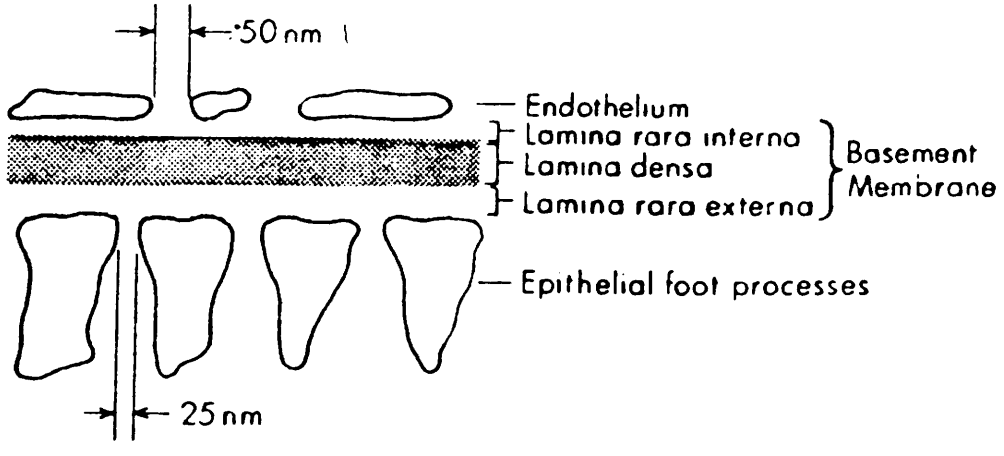
1:1:4 The glomerular basement membrane clearance studies

Renal clearance studies of macromolecules with a wide variety of sizes and charges suggested that charge as well as size played a fundamental role in the transfer of macromolecules across the GBM. Bohrer and Co-workers (1978) showed that there was a decreased fractional clearance of anionic dextran sulphate and an increased clearance of diethylaminoethyl dextran (DEAE) when compared with neutral dextran (8). Rennke and Venkatachalam (1977), using ferritin molecules with variable isoelectric points (pI) showed that as the net negative anionic charge increased penetration into the capillary wall was restricted (9). Conversely, cationic ferritin molecules penetrated regularly through the entire GBM up to the epithelial slit pore. Kelly and Cavallo (1977) showed that as proteinuria occurs and increases in NZB/NZW mice, the localization of native (anionic) ferritin extends across the GBM into the urinary space (10). Kuhn et al (1977) studied the glomerular clearance of endogenous albumin and ferritin in nephrotoxic nephritis and have shown moderate passage of these tracers, especially in areas where loss of epithelial attachment to GBM had occurred (11).

Charge modulates permeability in both healthy and experimentally

FIG 3 - SCHEMATIC DIAGRAM OF GLOMERULAR LOBULE (6)





injured glomeruli but the location of the major charge barrier was uncertain. The epithelial glomerular polyanion was thought to be the primary anionic site, but evidence derived from the ultrastructural studies of cationic probes has led to a reappraisal of this first hypothesis.

Ultrastructural tracer studies

The nature and site of the glomerular permeability barrier still is an area of controversy. The earliest studies using electron dense tracers suggested that the barrier was primarily at the level of the GBM. In 1961 Farquhar and colleagues showed that ferritin readily traversed the endothelial fenestrae but did not penetrate to any extent beyond the lamina densa (12). They concluded that the GBM , particularly the lamina densa was the main filtration barrier, and that the epithelial cells played a monitoring role by controlling the entry into the urinary space of those molecules which had escaped the barrier. Using enzyme tracers many groups of workers concluded that the final barrier for macromolecules was at the level of the filtration slit, and that its' diaphragm was important in these regards (13).

On the basis of the then available evidence, Karnovsky and colleagues proposed the double barrier or filter hypothesis that:

(1) The GBM restricts the passage of protein over a wide range of molecular size.
(2) The slit pore, or slit diaphragm, functions as an additional barrier for relatively smaller molecules that were incompletely restricted by the GBM (14,15).

This hypothesis, however, was based solely on size discrimination when this was considered to be the main determinant of glomerular

filtration.

Venkatachalam noted the behaviour of two relatively large molecules, myleperoxidase and catalase. Myleoperoxidase rapidly traversed the GBM whereas catalase (in contrast to myeloperoxidase) crossed slowly. He postulated that these differences might be ascribed to variations in molecular charge, the isolectric point of myleoperoxidase being greater than 10 compared to 5.7 for catalase (16).

Studies by Caulfield and Farquhar using neutral dextrans showed that restriction of the tracer molecule was not at the slit pore but at the level of the GBM and concluded once again that the GBM represents the primary barrier to the filtration of macromolecules (17). Chang et al and Rennke et al reported the physiologic studies detailing the importance of the effect of molecular charge on glomerular permeability to macromolecules. While anionic ferritin was completely excluded from the GBM cationic ferritin penetrated the GBM in greatly increased amount (18,19).

Nature and location of anionic sites in glomeruli

After determining by clearance studies that the glomerulus has both size and charge selective properties attention shifted to investigating which glomerular component(s) was responsible for establishing the charge barrier function of the glomerulus. This was a matter of considerable importance because loss of the charge barrier function has been found in several experimental diseases characterized by proteinuria, which are used as models for human diseases (20).

Cationic stains such as colloidal iron and alcian blue have been used to demonstrate the anionic sites. Using these stains, negatively

charged groups were shown to be present in all the layers of the capillary wall (ie) in the GBM and on the surfaces of endothelial and epithelial cells. The thick sialic rich, cell surface coat of the epithelium, captured the attention of a number of workers (21). This membrane, however, is on the wrong side of the GBM to produce its charge selective properties (22). However, it has been shown to be essential for the maintenance of the normal arrangement of foot processes and their slit pores. Neutralization of the coat by perfusion with polycations disrupts the arrangement of the foot processes and reperfusion with polyanion restores them to their normal state (23).

Various other probes were used as stains to detect anionic sites in the GBM and to determine where the greatest concentration of negatively charged groups occured. Lyzozyme (MW 14,000) because of its high isoelectric point (pI 11.0) bound to anionic sites in the GBM and in effect acted as a stain for anionic groups. Using this method Caulfield et al found anionic sites in all layers of the basement membrane (24). Further studies showed that a network of negative sites were concentrated in the laminae rarae. Cationized ferritin bound to sites within the laminae rarae but not the lamina densa or epithelial cell surface, thereby demonstrating that the sites with the highest density charge or concentration of negatively charged group in the glomerulus correspond to the sites where the heparan sulphate proteoglycans are located in the laminae rarae (25).

Role of glycosaminoglycans in the permeability of the GBM to macromolecules

Glycosaminoglycans are polysaccharides that are found in tissues of vertebrates usually in covalent assocation with protein. Seven types

are commonly recognised, six of these are structurally related, with a carbohydrate back-bone consisting of alternating uronic acid (L iduronic acid and/or D - glucuronic acid) and hexosamine (D - glucosamine or D - galactosamine) residues. All except one, hyaluronate, are sulphated. The glycoaminoglycans are widely distributed within the animal kingdom and are commonly distinguished in vertebrate tissues. They are all polyanions that have acidic sulphate and/or carboxyl groups (26).

As the glycoaminoglycan variety of proteoglycans are among the most highly negatively charged molecules known, it was assumed that GAG might be one of the GBM components responsible for endowing the GBM with its properties as a charge selective filter. Direct evidence for this assumption was provided by the following experiments. Perfusion of the whole kidney by GAG degrading enzymes in situ, led to marked changes in the permeability of the GBM with increasing clearance of Bovine serum albumin (BSA MW 65,000) and anionic ferritin (MW 480,000) (28,29). Removal of all GAG (including heparan sulphate) by digestion with heparinase, led to increased leakage of both ferritin (MW 480,000) and BSA (MW 65,000) into the urinary spaces. Removal of hyaluronic acid alone by digestion with streptomyces hyaluronidase or removal of most GAG's but not heparan sulphate with chrondroitinase led to increased clearance of BSA but not ferritin (27,28). These findings suggested that both heparan sulphate and hyaluronic acid may play a role in maintaining the charge selective filter properties of the GBM.

However, it is still not clear if alterations in GAG are responsible for the loss of charge selectivity found in some glomerular diseases. Previous workers have reported reduced binding of cationic probes in anionic sites in the laminae rarae in experimental animals

with glomerular diseases associated with proteinuria (29). The proposed structure for the GBM can be seen in Fig 5 (30).

1:1:5 THE MESANGIUM

Structure

The glomerular mesangial cells reside in an intercapillary position in the centre of several capillary loops (31) Fig 4. Zimmerman utilizing light microscope techniques provided an excellent description of the mesangial region as early as 1933 (32). Decisive proof for the existence of a third glomerular cell, distinctive from the endothelium and epithelium, resulted from electron microscope observations of the kidney. The studies by Farquhar et al and Latta et al clearly demonstrated that these cells actively phagocytosed electron dense tracers and were functionally distinct from glomerular endothelial cells (33,34,35).

Mesangial cells are quite irregular in shape, contain an indented nucleus and have numerous elongated cytoplasmic processes extending from the main body of the cell (36). The nucleus is much denser than that of adjacent endothelial cells, a feature that permits identification of mesangial cells even at the light microscopic level. There is evidence from recent studies that at least some of the phagocytic cells in the mesangium of the rat kidney constitute a functionally distinct population of mesangial cells bearing I-region-associated antigens (Ia)(37,38). However, the predominant cell in the mesangium is a non-phagocytic, Ia negative cell that resembles a smooth muscle cell which contracts when stimulated by

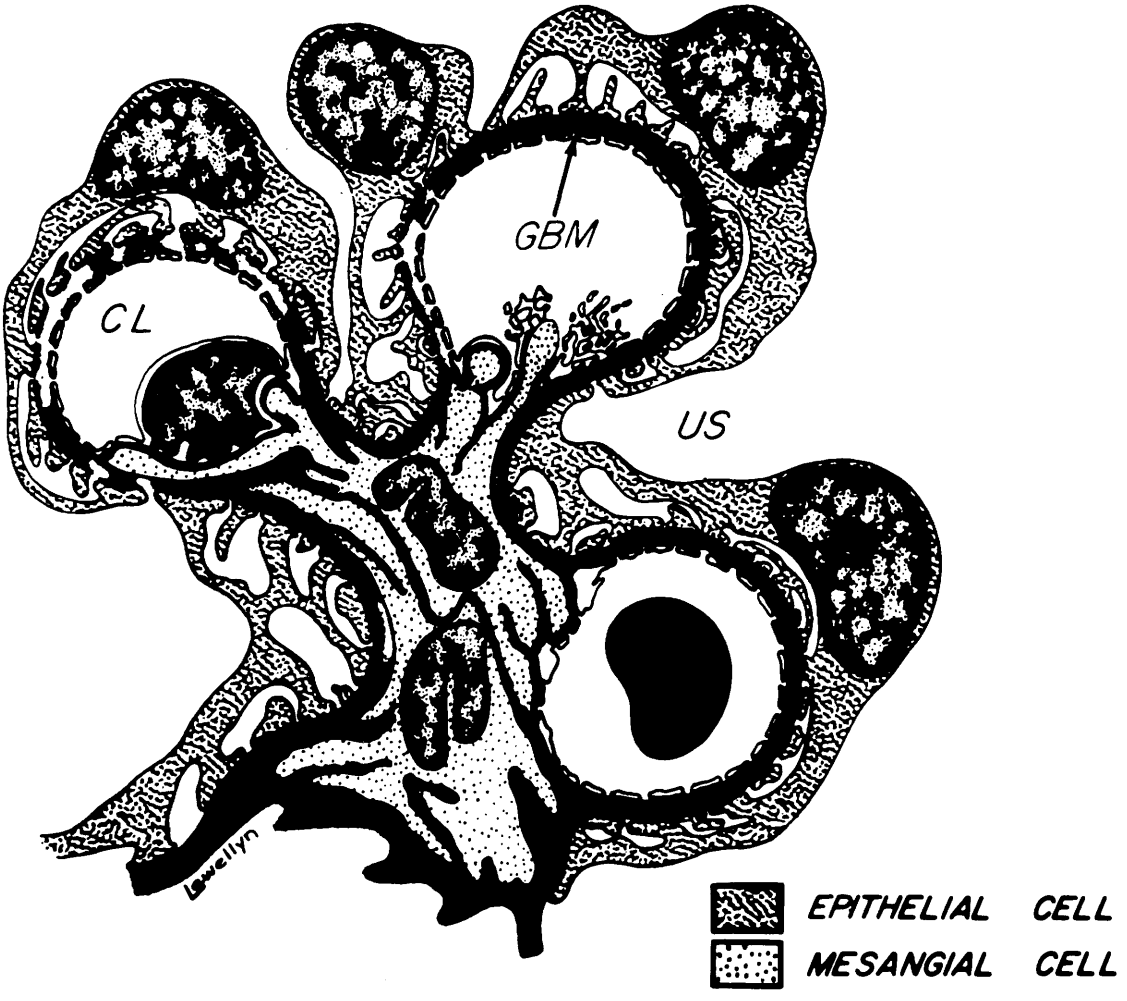


FIG 4 – A CROSS SECTION OF A GLOMERULAR LOBULE DEMONSTRATING THE RELATIONSHIP BETWEEN THE ENDOTHELIAL, EPITHELIAL AND MESANGIAL CELLS (7)

CL = CAPILLARY LUMEN
US = URINARY SPACE

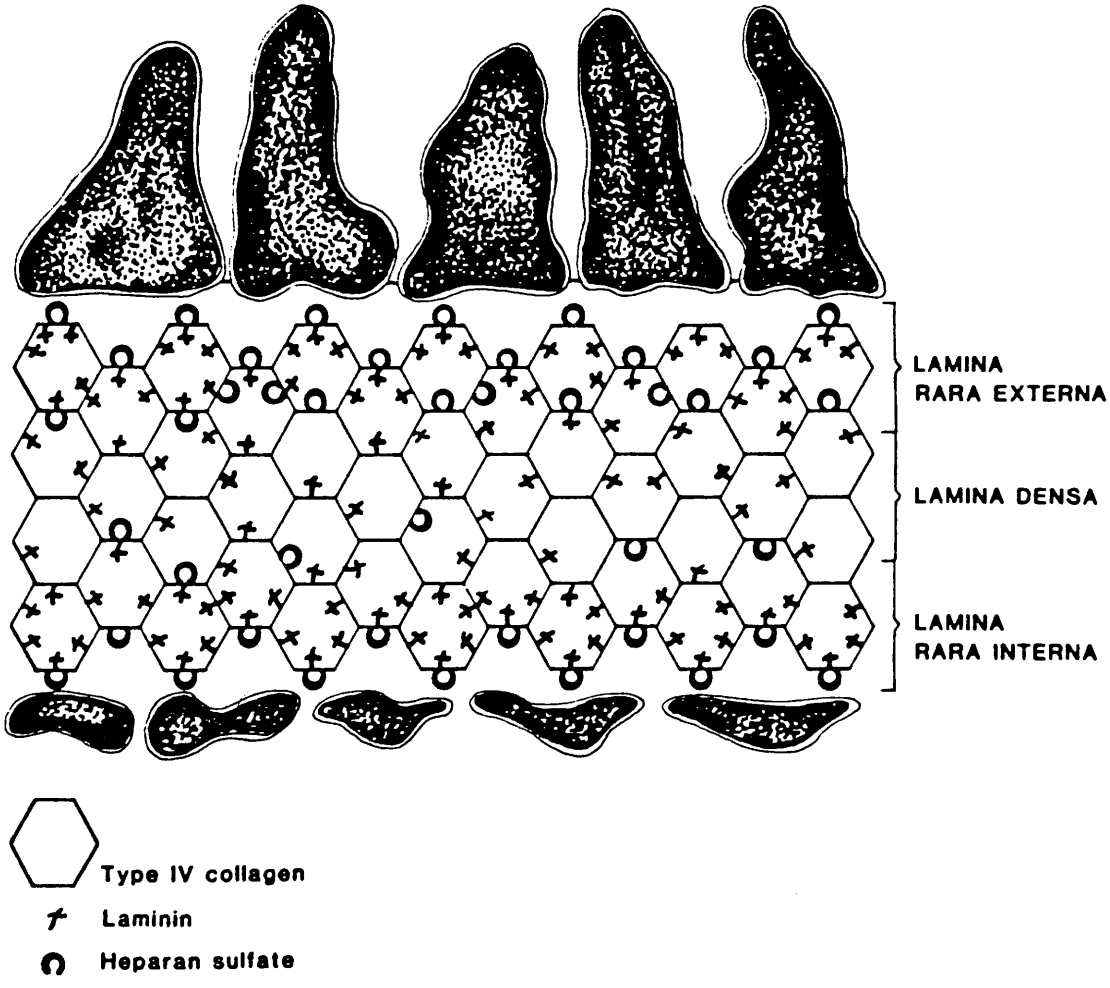


FIG 5 - SCHEMATIC REPRESENTATION OF THE PROPOSED SUPRAMOLECULAR ORGANISATION OF THE GLOMERULAR BASEMENT MEMBRANE (30)

angiotensin II and arginine vasopressin (39). The cell is capable of synthesising collagen types I,II and IV in vitro and lacks Fc or C3 receptors. The function of this cell is probably to regulate flow through the glomerular capillaries under the influence of hormones particularly angiotension II produced locally within the glomerulus. The intracapillary position of the mesangium has made it relatively inaccessible to study either in the intact animal or in vitro. Harper et al have devised a technique in which isolation of pure, homogeneous cultures of rat glomerular cells is possible (40). One cell type was polygonal, forms domes in culture, has cilia and stains predominantly and diffusely with the anti-platelet myosin antibody. The other cell type was spindle-shaped, had numerous filaments and stained along its filaments with anti myosin antibody from both muscle and non muscle sources. Since the first set of characteristics was common to epithelial cells and the latter to smooth muscle cells, isolation of glomerular epithelial and mesangial cells was thought to have been achieved. The glomerular capillary loop is almost encircled by the GBM except in an area overlying the mesangium, where only the fenestrated endothelial cell processes separate the mesangial matrix from the blood in the capillary. All circulating molecules with no restriction due to size, enter the mesangium and are thought to flow towards the hilum where they disappear (41).

Function

The exact functions of the mesangium are poorly defined but there is evidence to suggest that it has an important role in keeping the glomerular basement membrane free of macromolecular debris (42,43). Their probable role as supportive elements of the mesangium of the glomerular tuft has been suggested.

Mesangial cells possess contractile as well as phagocytic capabilities (44). Experimental studies involving the intravenous injection of ferritin (35) thorotrast (33,44), colloidal carbon (44,45) and aggregated proteins (46,47) have demonstrated the uptake of the tracer material by the mesangium. Factors such as blood level, physiochemical characteristics and haemodynamic factors influence mesangial uptake (48). Once in the mesangial area, complexes can either pass back into the blood, be phagocytosed and broken down by resident phagocytes or pass down the mesangial channel towards the glomerular stalk (49). The precise route by which these complexes leave the glomerulus is unknown although they appear to pass close to the juxtaglomerular apparatus.

Many substances accumulate within the mesangial region; some persist while others are relatively rapidly eliminated. An example of the latter is heat aggregated IgG (49) which disappears from the mesangium within days whereas carbon particles (45,51) or ferritin protein conjugates (50) can persist for months. Evidence suggests that molecular size and charge influence the degree of uptake and persistence of macromolecules with the mesangium (51,52). Batsford et al using ferritin isomers showed that molecular size was found to be a major determinant of the extent and rapidity of antigen uptake, but had only minor influence on persistence. The rapidity of uptake and antigen persistence was charge dependent, but to a limited extent. Biologic (toxic) activity of macromolecules appears to be a very important determinant of mesangial handling since glutaraldehyde-treated ferritin was taken up much more rapidly and to a greater extent than native ferritin and could persist for very prolonged periods (53). The fate of the antigenic material within the glomerulus has pathogenic relevance since not only would its mere

presence interfere with the clearing function but it could act as planted target antigen for circulating antibody leading to in situ immune complex formation.

The mesangium in human diseases

That injury to the glomerulus might impair macromolecular traffic through the mesangium is suggested by studies on rats with long standing nephrotic syndrome and focal sclerosis induced by repeated injections of puromycin aminonucleoside (54,55). Aggregated IgG administered to these animals persisted for prolonged periods of time within the glomeruli, which suggested that the mesangial clearing mechanisms were impaired.

There are numerous diseases in man in which pathological changes are limited or predominantly involve the mesangium in which the above process might be involved. Diseases such as anaphylactoid purpura, mesangial IgA, mesangiocapillary glomerulonephritis, mesangial lupus and diabetic nephritis have many common features including focal or diffuse mesangial cell proliferation, deposition of IgA along with other immunoglobulin and complement components in the mesangium (56). However, the exact pathogenesis of these lesions remains obscure.

1:1:6 Renal prostaglandins

Prostaglandins are synthetised from 20 carbon polyunsaturated acids containing three, four or five double bonds (57). These fatty acids are contained in the phospholipids of cell membranes phospholipids of all tissues. In man the main precursor of prostaglandins is arachidonic acid (AA) which in response to many different stimuli (58), is released from the phospholipids of cell membranes and rapidly metabolised into oxygenated products including prostaglandins and thromboxanes of the 2-series.

Prostaglandins and thromboxane are ubiquitous in mammalion tissue. They subserve a host of actions involving regulation of smooth muscle tone, the blood coagulation cascade, hormone secretion, the inflammatory response, central nervous system function and fluid and electrolyte transport (59).

Interest in renal prostaglandins began with the description by Lee et al of a vascoactive acidic lipid in the rabbit renal medulla (60). Subsequent studies showed that the kidney contains all enzymes necessary for prostanoid and thromboxane synthesis, and that the generation of each of these products can be demonstrated in renal tissues (Table 1)(61).

Recent research has led to significant advances in the understanding of renal prostaglandin biochemistry and physiology. The physiological role of prostaglandins in modifying various renal functions has become increasingly apparent over the last years. At present there is convincing evidence that prostaglandins influence:-

SITE	PROSTAGLANDINS SYNTHESISED [a]	REFERENCES
(Medulla)		
Interstitial cells	$PGE_2 > PGF_{2\alpha}$	(62)
Collecting tubule	$PGE_2 > PGI_2 > PGD_2$	(63)
Thick ascending limb	PGE_2 $PGF_{2\alpha}$	(64)
(Cortex)		
Glomerular epithelium	PGI_2 PGE_1	(65)
Mesangial cells	$PGE_2 > TXB_2 > PGF_{2\alpha} >$ 6-keto $PGF_{1\alpha}$	(66)
Arterioles	$PGI_2 > PGF_2 > PGE_2$	(67)
Glomeruli	6-keto $PGF_{1\alpha}$ TXB_2 PGE_2 $PGF_{2\alpha}$	(68)

TABLE 1 SITES OF PROSTAGLANDIN SYNTHESIS WITHIN THE KIDNEY

a DATA ARE BASED ON MICRODISSECTION AND CELL CULTURE STUDIES OF RAT AND RABBIT GLOMERULAR CELLS.

(1) Renal blood flow particularly following injury.

(2) Release of renin

(3) The urinary concentrating mechanism (61).

SECTION 2

1:2:1 Classfication of human glomerulonephritis

Disorders of glomerular structure and function constitute one of the major problems encountered in the practice of clinical nephrology. Beginning with Richard Bright in the 1830's, the study of glomerular diseases has focused on the relationship of clinical findings to morbid anatomy (69). Samuel Wilks (1853) produced the first valid attempt to classify Bright's disease according to its clinical and pathological features (70). His special contribution was to recognise that the contracted granular kidney could arise as part of a general decay in which the systemic arterial system as a whole was intimately concerned. In 1914, Volhard and Fahr published their splendid illustrated monograph on Bright's disease illustrating all forms of the disease with a wealth of clinical, anatomical and histological detail. Their classification remains the foundation on which our present clinical and pathological conception of Bright's disease is built (71). However, this classification did not emphasise the two ways in which Bright's disease commonly presents; the acute form with haematuria, and the insidious form with oedema. As a result, Ellis (1942) proposed a new classification of nephritis which was essentially clinical in nature. To acute nephritis he gave the name Type I nephritis. To those cases of gradual onset and which presented with oedema he called them Type II nephritis (72).

Until comparatively recently these studies were largely based upon information derived from post mortem examination of the kidney. Thus, only a portion of the spectrum of glomerulopathic processes were uncovered, and a vast area of natural history remained uncharted. This situation has improved considerably because of four developments.

First, the increasing use of percutaneous kidney biopsy in diagnosis and in the management of patients (73). Second, improved histologic techniques and the application of electron microscopy revealed a number of previously unrecognised structural features. Third, the use of immunofluorescence made possible the identification of immunoglobulins and complement in various patterns within renal tissue. Lastly, laboratory experimentation served to develop models of immunologically mediated kidney disease.

A classification of glomerulonephritis is shown in Table 2. Glomerulonephritis of the first group are presumed, or known to be caused, by immunological mechanisms because immunoglobulins and complement can be demonstrated in glomerular structures. Glomerular diseases that are caused by antigen antibody complexes formed in the circulation and/or in situ are listed in subgroup 1A.

Subgroup 1B includes glomerular diseases caused by autoantibodies that react with native glomerular basement membrane (GBM) antigens. Subgroup 1C lists membrano-proliferative glomerulonephritis Type II or dense deposit disease, in which there is a striking accumulation of C3, without significant amounts of immunoglobulins in glomerular structures. Subgroup 1D, renal allograft rejection is a condition in which sensitised cells, transplantation antibodies and transplantation antigen/antibody complexes are instrumental in tissue damage ie, the host mounts an immunological assault on the transplanted kidney.

Lastly, group II consists of glomerular diseases in which there is no impressive evidence of pathogenetic immunological mechanisms. This assumption is based on the absence of immunoglobulins and complement in renal structures. However, this conclusion may be occasionally

TABLE 2

Classification of human glomerulonephritis

I. Diseases presumed or known to result from immunologic mechanisms

A. Immune complex glomerulonephritis

1. Diseases of known etiology
   a) Poststreptococcal glomerulonephritis
   b) Glomerulonephritis associated with other bacterial infections; staphylococcal; pneumococcal; bacterial endocarditis; atrioventricular shunt infections; visceral abscesses; secondary syphilis; leprosy.
   c) Glomerulonephritis associated with viral infections: hepatitis B infection; dengue fever; mumps; varicella; subacute sclerosing panencephalitis; infectious mononucleosis.
   d) Glomerulonephritis associated with protozoal infections: malaria; schistosomiasis; toxoplasmosis; kala-azar; filariasis.

2. Renal diseases associated with systemic diseases of unknown etiology
   a) Lupus nephritis
   b) Nephritis with anaphylactoid purpura
   c) Nephritis in polyarteritis nodosa
   d) Nephritis in idiopathic "mixed" cryoglobulinemia

3. Primary renal diseases of unknown etiology
   a) Membranous glomerulonephritis
   b) Membranoproliferative glomerulonephritis Type I
   c) IgA-IgG glomerulonephritis; IgM glomerulonephritis
   d) Some cases of rapidly progressive glomerulonephritis
   e) Some cases classified as focal glomerulonephritis
   f) Some cases classified as chronic sclerosing glomerulonephritis
   g) Unclassified glomerulonephritis

B. Anti-glomerular basement membrane glomerulonephritis: some cases of rapidly progressive glomerulonephritis and Goodpasture's syndrome

C. Diseases associated with local deposition of complement: Membranoproliferative glomerulonephritis Type II or basement membrane dense deposit disease?

D. Renal allograft rejection (multiple immunologic mechanisms)

II. Diseases without impressive evidence of immunologic mechanisms

A. Most cases of chronic sclerosing glomerulonephritis
B. Some cases of focal glomerulonephritis
C. Nephrotic syndrome with minimal glomerular lesions (Lipoid nephrosis)*
D. Hemolytic-uremic syndrome and other coagulopathies
E. Diabetic nephropathy
F. Amyloidosis and dysproteinemias
G. Unclassified glomerular diseases

* Although not generally classified as a form of glomerulonephritis, it is included here for convenience.

erroneous especially in acute immune complex disease in which immune deposits may be scanty or may disappear before resolution of the lesions.

SECTION 2

IMMUNE COMPLEX DISEASE

1:2:2 Circulating immune complexes

The importance of immune complex nephritis as a major immunopathogenetic mechanism in renal disease has been recognized for many years. The interaction of antigen and antibody sets in motion immunopathological processes that ultimately caused renal disease, particularly glomerulonephritis (74). The glomerular capillary, a sophisticated ultrafilter, seemed to be a prime target for immunologic injury. The understanding of this process has been greatly facilitated by immunofluorescence studies in which granular or irregular deposits of immunoglobulin and complement were found in renal structures in patterns that resemble those found in experimental models of immune complex nephritis.

In the glomeruli, three main types of immune complex nephritis can be distinguished in humans and experimental animal models. The deposits may be present predominantly on the epithelial side of the GBM (membranous glomerulonephritis): predominantly in the mesangium (IgA -IgG glomerulonephritis) or in the mesangium and between the endothelium and GBM (mesangiocapillary glomerulonephritis Type I).

The mechanism responsible for the variation in the deposition of immune complexes were not fully known. In the late fifties, Germuth et al and Dixon et al published their classical studies on experimental serum sickness in the rabbit. They studied two different models, acute serum sickness and chronic serum sickness (75,76).

Acute immune complex disease

Intravenous injection of radiolabelled bovine serum albumin into rabbits was used to study acute serum sickness (75). Intravenous injection of the radiolabelled foreign protein yielded a predictable non-immune half disappearance time ($T\frac{1}{2}$) in the recipient rabbit thus allowing determination of the commencement of disease. After injection, radiolabelled BSA was eliminated from the circulation in three phases as can be seen from Fig 6.

(1) Phase I in which BSA equilibrates between intra and extra vascular spaces.

(2) A slower phase in which BSA is catabolized at about the rate of the rabbits own albumin.

(3) A more rapid phase which was thought to be caused by an immune elimination of the antigen. After antigen has been completely removed from the circulation, free antibody appears.

However, of greatest importance was the observation that during the phase of rapid elimination of the antigen, complexes of antibody and antigen were detected in the circulation and lesions of acute immune complex disease developed in arteries, glomeruli, joints and hearts. Glomerulonephritis was marked by endothelial swelling with some lifting of endothelial cells from the underlying basement membrane. Proteinuria was marked with little or no haematuria. BSA antigen, host and globulin and C3 were detectable in a granular pattern along the GBM. This granular appearance became the hallmark of immune complex disease. With the electron microscope, little is seen beyond the swelling of endothelial cells. Therefore, acute one shot serum sickness resulted in transient renal damage which occurred without the participation of complement or polymorphonuclear leukocytes and caused no significant permanent structural glomerular damage.

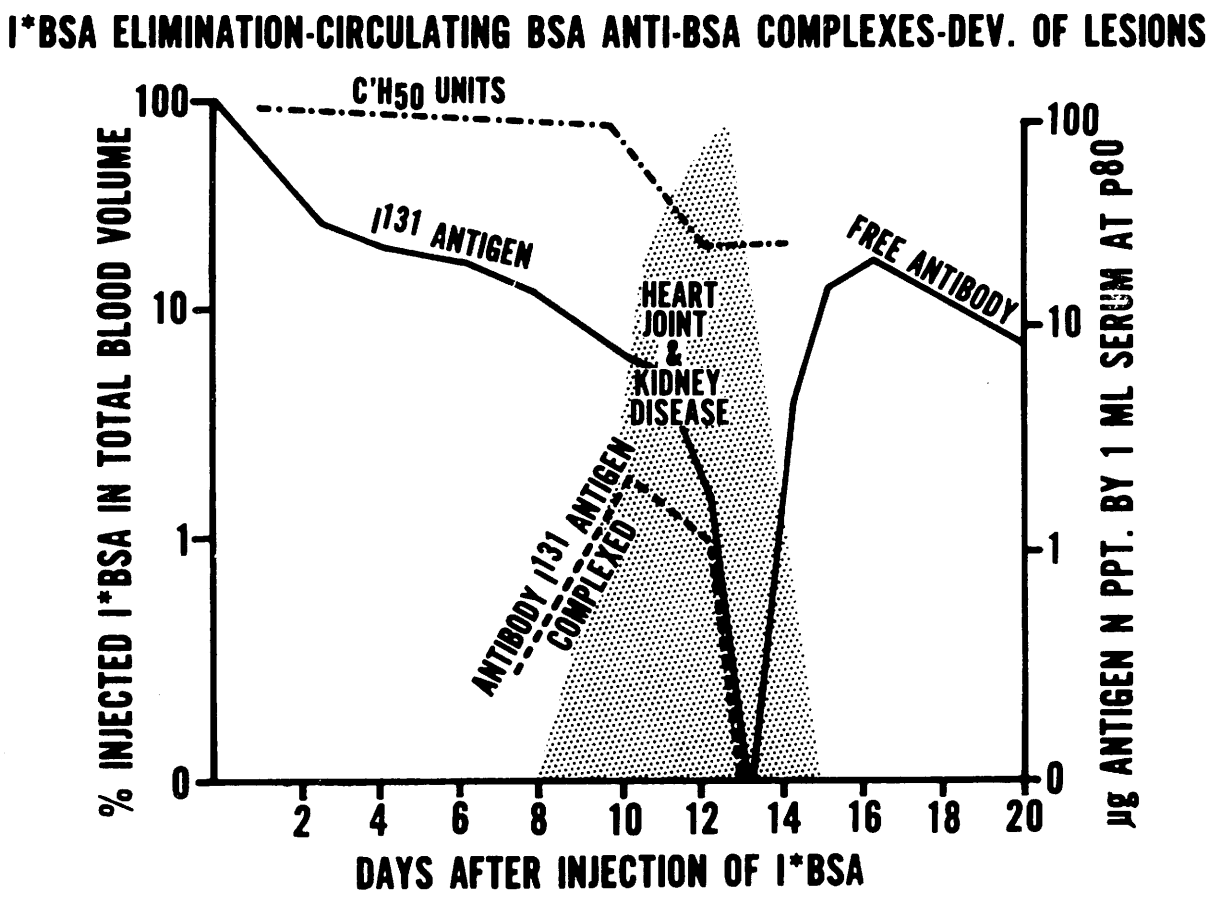


FIG 6 – EXPERIMENTAL ONE SHOT SERUM SICKNESS (75)

Chronic immune complex disease

Experimental chronic immune complex disease has been used extensively as a model in understanding human glomerulonephritis (76). Intravenous daily injections of 12.5mg of BSA into rabbits produced four responses (Table 3). Some rabbits failed to produce antibody and did not develop any glomerular lesion. A second group produced low levels of antibody so that circulating antigen-antibody complexes were formed in antigen excess. These small complexes penetrated the glomerular loop and produced diffuse glomerulonephritis. A third group produced intermediate levels of antibody. In these animals daily injections of antigen caused the formation of intermediate-sized, poorly soluble circulating complexes which localized within the subendothelial mesangial system. Lastly, the fourth group produced high levels of antibody which resulted in the formation of particulate complexes. These particulate complexes were removed by the reticulo-endothelial system and the glomerulus remained unaltered. Thus this one model (chronic serum sickness) could cause almost all histological changes seen in human GN (ie focal, diffuse, membranous). Complexes were identified by discrete deposits of antibody and/or complement.

Therefore, in studying the pathogenesis of experimental serum sickness, the possibility was considered that immune complexes which were found in the tissues were either deposited there from the circulation, or formed locally through an Arthus type reaction. Since the investigators were able to demonstrate immune complexes in the circulation at the time of the development of glomerular deposits, they proposed the first possibility as the pathogenic mechanism for the glomerulonephritis associated with experimental serum sickness. Therefore, the contention was that glomerulonephritis resulted from

TABLE 3

IMMUNE RESPONSE IN RABBITS AFTER DAILY INJECTION OF BSA

GROUP	ANTIGEN/ANTIBODY RESPONSE	SIZE OF COMPLEXES	NATURE OF DEPOSITS
1	Large antigen excess	$< 5.7 \times 10^5$	No deposits
2	Small antigen excess	5.7×10^5	Small capillary loop deposits
3	Small antibody excess	1.6×10^6	Large mesangial deposits
4	Large antibody excess	$> 1.6 \times 10^6$	No deposits

passive trapping of circulating immune complexes. Factors regulating the glomerular deposition of circulating complexes have been extensively studied and defined. They included systemic factors, glomerular factors and properties of the immune complexes themselves. Systemic factors included renal blood flow, MPS function and erythrocyte C3b receptors which collectively determined the plasma level, disappearance kinetics and renal delivery of immune complexes

Glomerular factors included hydrostatic pressure and filtration fraction which determined the driving forces by which immune complexes entered the capillary wall or mesangium from the circulation (78). Other glomerular factors included charge and permeability characteristics of the glomerulus itself and mesangial clearing function (79). Subtle forms of immune deficiency were suggested as an enabling factor leading to an inadequate immune response and failure to clear the antigen (80). Some evidence for this hypothesis has been found. Inherited complement deficiencies have been found more commonly than expected in patients with immune complex disease (81). Mice with an inherited pre-deposition to this immune complex nephritis disorder have been shown to produce antibodies of low avidity (82). Patients with membranous nephropathy who are DR3 (HLA Type) positive have a deficiency in splenic Fc receptors (83). Another factor thought to regulate glomerular deposition of immune complex is the size of the complex. Germuth suggested that large complexes localized in the mesangium whereas smaller immune complexes deposited along the GBM. The size of such an immune complex and thereby its site of deposition was governed largely by the antigen antibody ratio and the size of antigen as demonstrated by the studies of Germuth and Dixon (75,76).

1:2:3 'In Situ' immune complex formation

Recent studies provide strong evidence for a second mechanism of immune complex formation in glomeruli, the reaction of free circulating antibody with antigens already present or fixed in the glomerulus by mechanisms that reflect properties of the glomerulus itself and result in in situ deposit formation (84).

Firstly, the issue of whether preformed immune complexes can be passively trapped in a subepithelial site remains controversial (85). Secondly, it does not accord with the poor correlation between circulating immune complex measurements and glomerular immunopathology in some glomerular diseases (86). Thirdly, intravenous injection of preformed complexes very rarely causes any pathogenic lesion in experimental animal models (87). As a result the hypothesis that circulating immune complexes are the only, or even the main cause of immune complex glomerular disease is in dispute.

It is interesting to note that the idea that immune complexes may be formed locally is more than 75 years old. In 1903, Arthus demonstrated that a necrotising vasculitis developed at the site where immune complexes were formed in the vessel wall through binding of circulating antibody to an antigen which was injected locally (88). A few years later Schick put forward the idea that such a pathogenesis was operating in glomerulonephritis following a streptococcal infection. He supposed that during the infection streptococcal products bound to the GBM and later on reacted with antibody which originated from the immune reaction of the host (89).

Experimental in situ immune complex formation

Male Lewis rats immunised with an antigen derived from the luminal brush border of proximal tubular epithelial cells (FX1A), developed proteinuria and subepithelial immune deposits indistinguishable from those in membranous nephropathy in man (90,91). This animal model is known as Heymann nephritis after the man who pioneered the studies in the late sixties. It was suggested that autologous immune complex nephritis (AICN) involved loss of tolerance to tubular antigen which was thought to normally circulate in nanogram quantities which led to (92,93), antibody formation and deposition of small circulating immune complexes. Tubular antigen was identified in circulating immune complexes in autologous immune complex nephritis (AICN) rats and was present in glomerular deposits in AICN (94). The immunological characteristics of the AICN Model were difficult to reconcile with a CIC pathogenesis. In AICN subepithelial deposits developed in marked antibody excess when antigen was virtually absent from the circulation (95). Unless the number of antigenic determinants involved were very limited, this situation would have resulted in formation of large immune complexes that would be localized in the mesangium rather than exclusively in the subepithelial space observed (90).

Evidence for an in situ mechanism for the formation of subepithelial deposits were derived largely from studies of the passive Heymann nephritis (PHN) model: in which a lesion identical to AICN is produced in hours by the administration of heterologous antibody to FX1A in an isolated kidney (96,97). This showed that the deposits found in Heymann nephritis, previously believed to be the result of trapping of circulating immune complexes, containing brush border antigens, could be produced by antibody binding to an intrinsic glomerular antigen.

Couser et al also investigated the mechanism of subepithelial deposit formation in PHN in vitro using the isolated perfused rat kidney system. A single by-pass system was used which allowed the renal venous effluent to run to waste. Granular deposits of IgG were demonstrated along capillary walls within 10 minutes by immunofluorescence and a membranous pattern of small deposits were detected by electronmicroscopy at two hours (98). Van Damme et al exposed anti FX1A antibody in vitro to enzyme or acid-treated normal rat kidney sections and demonstrated glomerular antibody binding in a granular pattern. Moreover they showed that when anti FX1A IgG was perfused into a bloodless rat kidney and localized by an immunoperoxidase EM technique, antibody binding throughout the glomerular capillary wall, including the subepithelial space and slit pores, could be demonstrated (99).

These earlier studies provided the first conclusive evidence for the in situ formation of glomerular immune aggregates. Subsequent studies have extensively re-evaluated the nature of glomerular immune complex deposition at mesangial, subendothelial and subepithelial sites as discussed below.

SUBEPITHELIAL GLOMERULAR IMMUNE COMPLEX FORMATION

Fixed glomerular antigens

Subepithelial immune complex deposits are now believed to form primarily on a local or in situ basis rather from circulating immune complex trapping. This mechanism however may involve insoluble fixed glomerular antigens or exogenous soluble antigens. The best studied model of an in situ mechanism involving a fixed glomerular antigen is the Heymann nephritis model as discussed earlier (91). The antigen responsible for this lesion is now believed to be a glycoprotein with a molecular weight of 660,000 daltons (100). The antigen is distributed along the glomerular epithelial cell membrane, where it is associated with endoplasmic reticulum and coated pits at the cell surface. The antigen is apparently cross-reactive with the proximal tubular brush border (101).

Neale and Wilson reported in situ immune deposit formation and proteinuria due to an endogenous capillary wall antigen (102). They showed that a spontaneous nephropathy in NZW rabbits resulted from antibody to another non-glomerular basement membrane antigen, which is localised along the epithelial cell foot processes and slit pores. Except for anti GBM nephritis, no human renal disease has been shown to result from antibody reactivity with an endogenous glomerular antigen. However, this mechanism has certainly not been excluded particularly in membranous nephropathy, in which the lesion closely resembles that induced by the Heymann antigen experimentally.

Planted non glomerular antigens

Subepithelial deposits can also occur in nephritis induced by exogenous antigens. Multiple mechanisms have been identified that can lead to such planted antigen deposits. Several involve charge-dependant mechanisms between the negative charge group in the glomerular basement membrane and the cationic antigen. In 1982, Border et al showed by inducing chronic serum sickness, using cationic bovine serum albumin (BSA) rabbits developed predominantly subepithelial deposits; independent of the immune response or of the quantity and size of the circulating immune complexes (103). Native BSA that had not been modified gave rise to mesangial deposits. Moreover, they demonstrated the initial binding of cationic antigens to the capillary loop using immunofluorescence and radiolabelling techniques. However, they showed that the antigen does not persist and the disease does not develop unless the antigen is bound to antibody and produces immune complexes (104,105).

The mechanism of cationic antigen-induced immune complex nephritis has been applied to studies of post-infectious nephritis in humans. Vogt et al have isolated several anionic and cationic antigens in glomerular immune deposits in 8 of 18 patients with early post streptococcal GN (106). Evidence has also been shown for the participation of a streptococcal cytoplasmic endostreptosin, in immune deposits in patients with early post streptococcal GN (107).

A second mechanism of in situ subepithelial immune complex formation related to charge, involves large cationic antigens such as ferritin, which may first localise along the subendothelial surface of capillary walls. Secondary fixation of antibody leads to formation of large

latticed subendothelial deposits, which can subsequently dissolve and individual reactants can cross the capillary wall to reform as large immune complex aggregates in the subepithelial space (108). This mechanism may be operative in diseases in which subepithelial and subendothelial deposits co-exist as in patients with severe lupus nephritis.

Although most attention has been directed at glomerular localisation of cationic antigens, anionic antigens such as native BSA and DNA are important in producing experimental and clinical immune complex nephritis (109,110). Fleuren et al have demonstrated in situ subepithelial immune complex formation with native BSA with a pI of only 4.6 (111). DNA has also been demonstrated in subepithelial immune deposits in patients with membranous lupus nephropathy and has been shown to localise in the glomeruli in free form, although it, too, is anionic (112,113). Two additional charge-related mechanisms may account for the local formation of immune complex deposits containing anionic antigens. Anionic antigens may bind to specific cationic antibodies already localised in the glomeruli due to their charge. This mechanism may account for the finding of Fleuren, Grond and Hoedemaeker with native BSA (111).

Alternatively, anionic antigens may bind electrostatically to non-immune cationic proteins that bind to glomerular anionic sites prior to immune deposit formation. Evidence for this mechanism comes from the work of Chan, Boyd and Fritzler who produced typical subepithelial BSA immune complex deposits in mice by first administering cationized non antibody IgG folowed by anionic BSA and then anti BSA antibody (114).

Evidence that some mechanism involving exposure of the glomeruli to cationic molecules, or other processes leading to loss of anionic sites, before immune deposit formation begins, has been provided in the NZB/W model of murine SLE, in which loss of glomerular anionic sites and increased glomerular permeability appear to precede the development of glomerular IC deposits (115). A variety of proteins involved in immune reactions are cationic and may be involved in such a mechanism (111,117).

MESANGIAL IMMUNE COMPLEX DEPOSITS

Fixed mesangial antigens

No substantial clinical or experimental evidence has been reported of mesangial injury or glomerulonephritis caused by antibody reacting directly with such intrinsic mesangial structures. However, the mesangium exhibits a variety of antigenic determinants that are quite accessible to circulating antibodies, including actin, myosin, glycoaminoglycan, fibronectin, laminin, type V collagen, procollagen type IV and other less well defined antigenic structures (118,119,120).

Planted mesangial antigens

The capacity of the mesangium to localise antigens and the nephritogenicity of in situ immune complex formation within the mesangium, are well illustrated by the studies of Mauer and co-workers. Aggregated human IgG was administered intravenously into rabbits; it localised without causing injury in the glomerular mesangium. Rabbit kidneys containing mesangial human-aggregated IgG deposits, were then transplanted into normal recipients (rabbits) who

were passively administered antibody to human IgG. The recipients developed an acute focal proliferative glomerulonephritis much like that associated with mesangial deposits in diseases such as IgA nephropathy or lupus nephritis in humans (121). Similar degrees of histological and functional glomerular injury have not been produced by preformed IC infusion (122). Charge has also been shown to play a part in mesangial trapping of potential antigens. Cationic antigens have been shown to localize and persist longer at this site (123,124).

The fact that severe glomerulonephritis does not occur by infusion of preformed IC, but does occur acutely when similar quantities of IC's form in situ in the mesangium, suggests that in situ formation may be a more likely mechanism for causing acute inflammatory lesions in diseases such as lupus nephritis, IgA nephropathy and Henoch Schonlein purpura.

Non-glomerular antigens

Non-glomerular antigens may cause glomerular injury by two mechanisms. Large molecules such as ferritin, which cannot readily penetrate the GBM, may localise by charge interaction with anionic sites on the inner surface and produce subendothelial deposits (108).

The affinity of certain plant lectins such as concanavalin A (Con A), for glucose and mannose residues in capillary-wall glycoproteins can result in glomerular localisation of antigen. The studies of Golbus and Wilson showed that when anti-con A antibody was injected, a linear pattern of IC deposits along the inner surface of the capillary wall was produced accompanied by an acute proliferative glomerulonephritis (125). Viruses and other material are known to contain such

lectin-like components and may, therefore, have the potential for producing deposits by this mechanism in humans (126). Additional evidence has come from the work of Batsford who showed that by altering the net negative charge of the GBM by infusion of cationized ferritin, a nephrotic syndrome resulted 5-7 days later in the apparent absence of persistent ferritin or subsequent IC formation in the glomeruli (108).

SUBENDOTHELIAL IMMUNE DEPOSIT FORMATION

Fixed glomerular antigens

There is currently little clinical evidence of a fixed antigen mechanism in the development of glomerulonephritis at this site. However, nephrotoxic serum (NTS) glomerulonephritis has been reported to have been induced in the rabbit by anti endothelial antibodies (127). Antibodies to endothlelial cell surface and Ia antigens have been reported in lupus nephritis and would contribute to subendothelial deposit formation by this mechanism (128,129).

1:2:4 Prostaglandins and renal disease

Effects of arachidonate oxidation products are diverse and can induce most of the major pathophysiological events that characterise the inflammatory process. Acting as local hormones, these metabolites, therefore, have a profound effect on vascular musculature, platelet aggregability and the immunological system. Alterations in the balance of the components of this cascade may affect kidney function and structure.

Effect on proteinuria

Indirect evidence that the arachidonate pathways may be activated in glomerulonephritis come from clinical and experimental observations. Long term cyclooxygenase inhibition with oral indomethacin decreases urinary protein excretion in some patients with the nephrotic syndrome (130,131). A single oral dose (75mg) of this non-steroidal, anti-inflammatory agent to patients with proteinuria >3g/24 hours (due to various forms of immunologically mediated glomerulopathies decreases urinary protein excretion within 45-60 minutes, particularly when given under conditions of volume depletion (132). The decrement in proteinuria exceeds that of glomerular filtration rate. Studies by Tiggler, Hulme and Wijdeveld have demonstrated that in patients with the nephrotic syndrome, indomethacin decreases proteinuria and the glomerular permeability to macromolecules such as polyvinylpyrrolidone, by influencing intrarenal and glomerular hemodynamics presumably as a result of PG synthesis inhibition (133).

Effect on glomerulonephritis

Certain prostaglandins, particularly of the E series, appear to have a beneficial effect on the progression of certain forms of glomerulonephritis. Pharmacological doses of PGE_1 alter the course of lupus glomerulonephritis both in NZW/W and in MRL/1 mice (134,135). A pronounced increase in mouse survival and striking reductions in the amounts of immune deposits in renal glomeruli were noted. In addition, PGE_1 treatment reduced the levels of circulating immune complexes in both strains (136).

Recently, several reports have shown the effects of dietary polyunsaturated fatty acids (PUFA) on immune complex forms of glomerulonephritis and on the progression of animal models of chronic renal disease. Dietary enrichment with the polyunsaturated fatty acid eicosapentanoic acid, prevented proteinuria and prolonged survival in NZB/NZW mice (137). A high linoleic acid diet had a protective effect on the development of murine apoferritin-induced immune complex nephritis (138).

Role in renal failure

PUFA's are precursors of the prostaglandin (PG) thromboxane (Tx) family and of the leukotrienes. This may provide a possible explanation for the beneficial effects on the kidney of altering the supply of PUFA's in the diet. The PG - Tx metabolites are formed through the cyclooxygenase and the leukotriene metabolites through the lipooxygenase pathways. Acting as local hormones, these metabolites have profound effects on vascular musculature, platelet aggregability and the immunological system. Alteration in the balance of the components of this cascade may affect the kidney function and structure. That these polyunsaturated fatty acids are important factors in the maintenance of renal function is suggested by the findings of Kimberly and Plotz (139) in patients with systemic lupus erythematosus and those of Ciabattoni et al in patients with glomerulonephritis (140). Both groups reported that cyclooxygenase blockade had deleterious effects on renal function in these two groups of patients with impaired renal function. The increased prostaglandin found in patients with chronic renal disease may suggest a compensatory mechanism when renal function is decreased (141). Injections of pharmacological doses of prostaglandin or substances that block synthesis of thromboxane, have been shown to have beneficial effects both in various models of renal disease and in

humans with diminished renal function due to chronic glomerulonephritis (142).

SECTION 1:3:1

Aims of the study

The glomerulopathies are a group of disorders in which the main abnormality occurs in the glomeruli or filtering units of the kidney. They are the cause of renal failure in one third of the patients on dialysis in Europe. The two commonest glomerulopathies, which together account for nearly one half, are membranous and IgA nephropathies (23% each in Glasgow Royal Infirmary). Both types of glomerulopathy are mediated by immune complexes but in membranous nephropathy, the complexes form on the basement of the capillary wall whereas they are found in the mesangium in IgA nephropathy.

No adequate explanation has yet been found to account for individual susceptibility or resistance to a particular nephropathy. Immune complexes are thought to be common mediators of glomerular disease, but are frequently detected in the sera of patients whose renal function and structure is normal and conversely are often not detected in patients with glomerulonephritis. More recently the charge of the antigen has been shown to be an important factor in its site of immune complex deposition within the kidney.

In this thesis, the postulate that the role of the glomerulus itself may play an important part in determining the response of the glomerulus to injury was investigated. The specific aims of this thesis are as follows:

(1) To examine two glomerular properties to see if they influence renal handling of antigen.

   a Charge on the glomerular basement membrane
   b Mesangial uptake of proteins

These properties were examined in two strains of rat; one of which is susceptible to Heymann nephritis and the other of which is resistant.

(2) To study the consequences of different glomerular properties on various animal models of glomerular injury.

(3) To investigate the effect of prostaglandins and their precursors in modulating glomerular uptake of antigens.

CHAPTER TWO

MATERIALS AND METHODS

SECTION 2:1:1

The materials and suppliers used for the following experiments are as follows:

BDH CHEMICALS LTD	Sodium chloride (NaCl)
	Potassium dihydrogen orthophosphate (KH_2PO_4)
	di-potassium hydrogen orthophosphate (K_2HPO_4)
SIGMA CHEMICALS LTD	Puromycin Aminonucleoside (PA)
	Hexadimethrine (HDM)
	Human IgG
	Human serum albumin (HSA)
	1-ethyl 3 (3 dimethylaminopropyl) carbodiimide hydrochloride (EDC)
	N, N dimethyl 1-3 propanediamine (DMPA)
	FITC conjugated antihuman IgG
	FITC conjugated anti rat IgG
DIFCO LABORATORIES	Freunds complete adjuvant.
AMERSHAM RADIO-CHEMICAL CENTRE LTD	I-125, I-131
CLAY ADAMS	Polyethylene tubing PE10, PE50, PE90
MAY & BAKER	Sodium pentobarbitone (60mg/100ml)
OLAK	Inbred DA and Lewis rats.
SPECIAL DIET SERVICES	Fat-free diet Rat and mouse No1 expanded diet.

KODAK LTD	High speed ektachrome film
MACFARLAN SMITH	Anaesthetic ether
BRITISH PETROLEUM CHEMICALS	74OP Methylated spirits
WELLCOME REAGENTS LTD	Mouse liver powder
MILES LABORATORIES INCORPORATED	Tissue Tek II OTC compound
PARK DAVIS & CO	Gelatin capsules, size 00
BRITISH OXYGEN CO	Liquid nitrogen

METHODS

SECTION TWO

2:2:1 Modifications of Proteins

Cationization of proteins was performed according to the method of Danon et al (143), using 1 ethyl 3 (3 dimethylaminopropyl) carbodiimide hydrochloride (EDC) as activator and N,N dimethyl 1,3 propanediamine (DMPA) as a nucleophile to replace carboxyl groups. The reaction is based on the activation of the carboxyl groups of the protein to be modified with a water soluble carbodiimide (EDC) and the subsequent reaction of the activated carboxyl with a nucleophile of the general type (+RNH), such as DMPA, to obtain the tertiary amine type derivatives. The following procedure was used:-

(1) The required amount of protein (16mg) was measured out and dissolved in 0.5ml of buffer A pH 5.5 (see appendix for buffers).

(2) 20ul of DMPA was added to 250ul of buffer A.

(3) 30mg of EDC was dissolved in 250ul of buffer A.

The three reactants were quickly mixed together and allowed to stand overnight at room temperature. The reaction mixture was then dialysed three times in large volumes of working buffer pH8.6. The degree of cationization was estimated by isoelectric focusing. The reaction conditions produced cationized human IgG with a pI exceeding 9.5 and human serum albumin with a pI exceeding 8.0.

2:2:2 Determination of isoelectric point

The isoelectric range of the modified proteins was determined by isoelectric focusing in thin layers of polyacrylamide gels, pH range 3-10, using a Pharmacia flat bed electrophoresis unit (FBE 3000) with accompanying power supply (ECPS 3000/150). The following procedure was carried out:-

1 Preparation of agarose solution

0.3g isoelectric focusing agarose , 3.6g sorbitol was dissolved in a conical flask by heating in a boiling water bath.

2 Mould preparation

While the agarose was being dissolved the mould was set up.

(a) It was ensured that the levelling table was horizontal using a spirit level.

(b) 2ml of distilled water was poured into the levelling table.

(c) A sheet of gel bond with the hydrophilic surface up was placed onto the levelling table. Agarose will adhere only to the hydrophilic side.

(d) The film was rolled so that it was flat against the levelling table and excess moisture and air bubbles were removed from the gel.

(e) The gel casting frame was placed in position over the plastic film and the edges fastened down with six spring clips.

(f) The levelling table and plastic was prewarmed.

3 Moulding

(a) The agarose gel solution was allowed to cool to around 75°C. The Pharmalyte (pH 3-10) was added, mixed and then poured into

the mould. The flask was moved around so that the solution flowed to all corners of the mould. Air bubbles were avoided.

(b) Once the gel had set (10-15 minutes) a scalpel was run around the edge of the gel casting frame and the gel. The plastic film was removed from the levelling table by levelling with a scalpel from one end. The gel was allowed to harden fully before use (1 hour at 4°C).

Running the experiment

1 The gel was placed on the flat bed cooling unit. The electrode strips were soaked in the appropriate electrode solutions

Cathode IM NaOH
Anode 0.05M H_2SO_4

The strips were blotted on filter paper for about one minute to remove excess liquid. The gel was prefocused at 500 volt hours.

2 The samples and a pI calibration kit pH 3-10 was applied using the paper sample applicators. Up to 25ul can be applied on the filter paper applicators.

3 The power supply was set to deliver a maximum of 15W and 1500V with unlimited current and was run for 90 minutes.

4 The sample applicators were removed after 45 minutes.

Fixing and staining

See appendix for fixing and staining solutions.

1 The gel was fixed immediately after the run was completed in the fixing solution for 30 minutes.

2 The gel was washed in two lots of destaining solutions, each for 15 minutes.

3 The gel was dried before staining. Three layers of filter

paper were placed carefully on top of the gel followed by a glass plate and weight of about 1kg. After 15 minutes, all this was removed and the gel was dried with a hair dryer.

4 The film was placed in staining solution for 5 to 10 minutes.

5 The gel was destained until the background was clear (15-20 minutes).

6 The film was dried with a hair dryer.

Determination of pI using standard curve

1 A piece of graph paper with 1cm markings and 0.1cm sub-divisions was used. The gel was positioned over the grid so that the origin of the grid was lined up with the cathode and of the gel.

2 Using the grid, the distance from the cathode of each protein pI marker to the nearest 0.05cm was determined.

3 The known pI value of each pI marker versus its distance from the cathode was plotted. Fig 7

4 The distance from the cathode of the proteins of interest to the nearest 0.05cm using the grid was determined.

5 From the pI calibration curve and known distances of the protein(s) of interest from the cathode the pI of the protein(s) was determined (Fig 8).

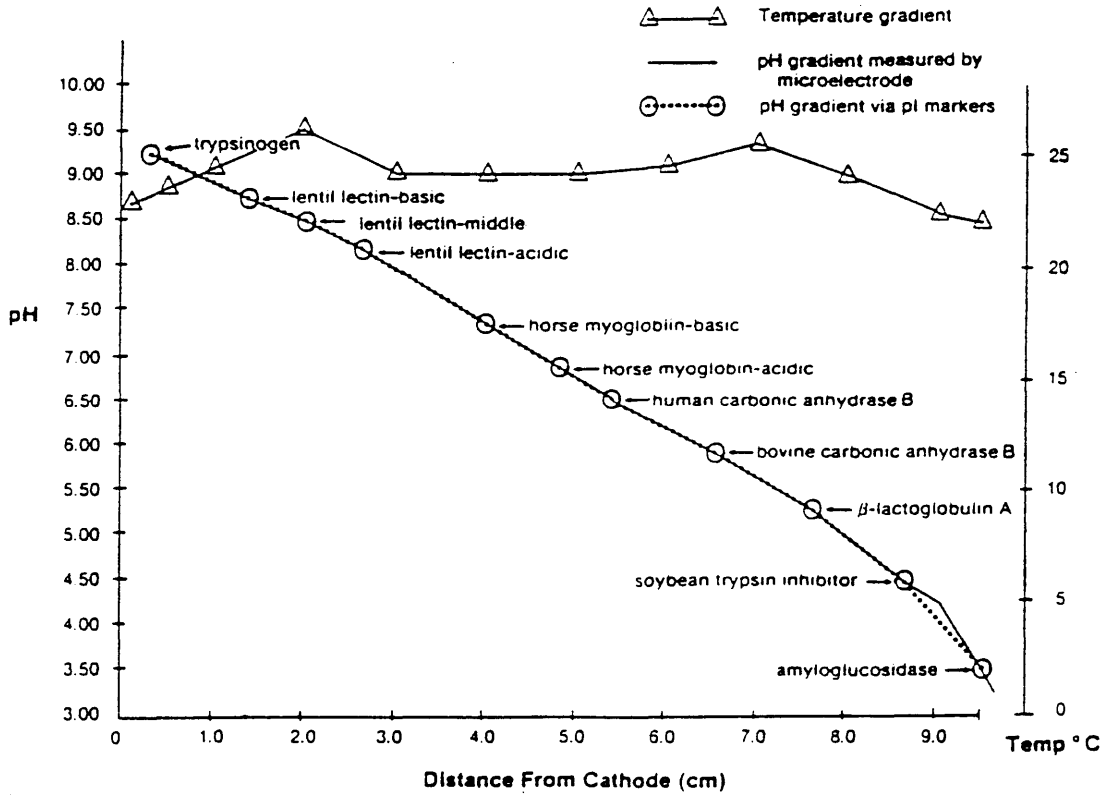
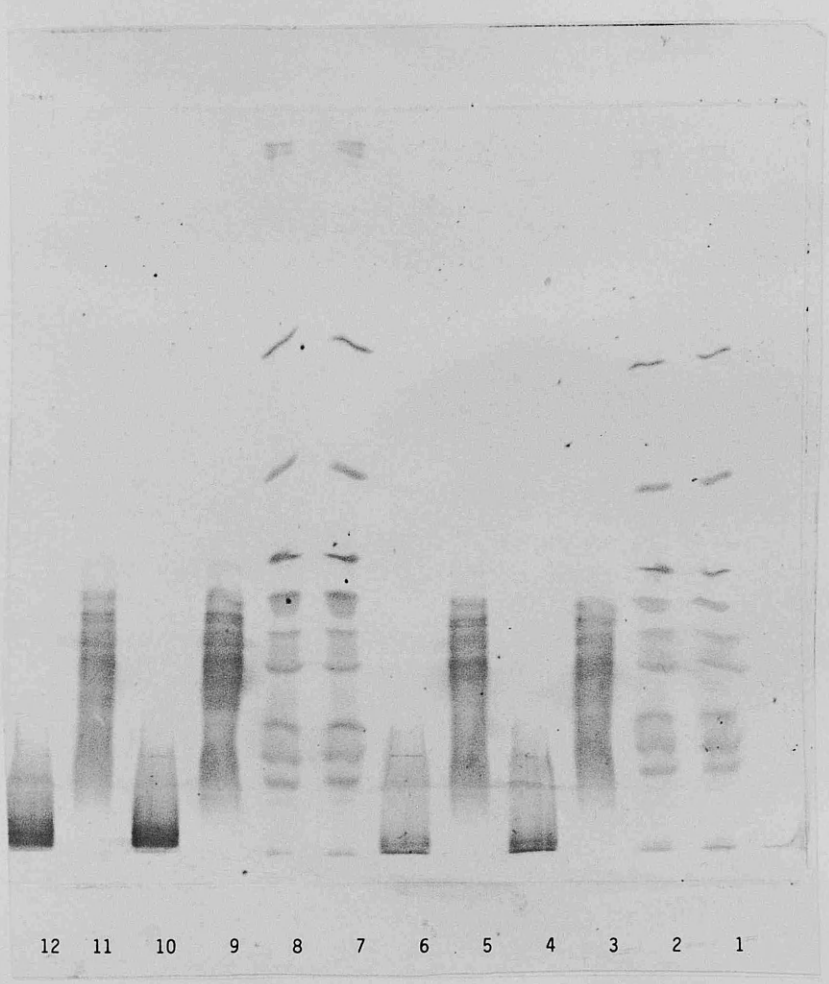


FIG 7 Determination of pH Gradient Profile using the Broad pI Calibration Kit on a 5% PAA Gel containing Pharmalyte 3-10.

ISOELECTRIC FOCUSING GEL **(Fig 8)**



+ ve

amyloglucosidase (pI-3.50)

soybean trypsin inhibitor (pI-4.55)

B-lactoglobulin A (pI-5.20)

bovine carbonic anhydrase B (pI-5.85)
human carbonic anhydrase B (pI-6.55)
myoglobin-acidic (pI-6.85)
myoglobin-basic (pI-7.35)

lentil lectin acidic (pI-8.15)
lentil lectin middle (pI-8.45)
lentil lectin basic (pI-8.65)

trysinogen (pI-9.30)

Well No 1 2 7 8 contain standard samples

4 6 10 12 contain IgG+

3 5 9 11 contain IgG^N

2:2:3 Alkali aggregation of human IgG

IgG was aggregated using the method described by Whaley et al (144). Equal volumes of human IgG (20mg/ml) and sodium hydroxide (200mmol/l) were mixed at room temperature and then dialysed extensively against phosphate buffered saline. This procedure allows the pH to fall gradually to 7.2 and retains the aggregates in a soluble form. The degree of aggregation was checked using SDS electrophoresis.

2:2:4 Radiolabelling of proteins

Human IgG and HSA were iodinated with I-125 and I-131 respectively by reaction with iodine monochloride (145). This procedure introduced an atom of iodine into the phenolic ring structures of tyrosine residues. The following methods and experimental procedure was used.

Materials

(1) Iodine monochloride (ICl) (25nmol/ul)

(2) 1M Glycine buffer pH 10.0.
7.15g glycine was made up to 100ml with deionized water and adjusted to pH 10.0.

Method

(1) The protein was assayed using the Lowry method (146).

(2) The concentration of ICl: protein being 2.5:1.

(3) The volume of glycine buffer to be used was estimated; 250ul buffer/1ml of protein.

(4) The volume of isotope used was either 1mCi I-125 1mCi I-131 in 10ul of dilute sodium hydroxide.

Procedure

(1) 1ml of protein was added to a LP3 tube. 250ul of glycine buffer was added.

(2) To this mixture 10ul of either I-125 or I-131 was added.

(3) The appropriate volume of ICl to be used was put in the LP3 cap, the cap was placed on the tube and mixed quickly by inversion.

(4) Free iodine was removed by dialysis using large amounts of working buffer. The buffer was changed 4 or 5 times until only minimal radioactive counts could be detected in the buffer.

Preparation of injectable doses of protein

After radiolabelling the protein concentration was reassayed using the Lowry method (146). The concentration was then adjusted so that the dose administered to the rats was in a volume of 0.1ml. The protein solution was drawn up into 1ml syringes. The syringes were weighed before and after injection.

SECTION 2:3:1

IMMUNOFLUORESCENT TECHNIQUES

Absorption of antisera

Conjugation of FITC to protein causes an increase in the net negative charge of some serum proteins and these may bind to tissue sections resulting in excessive background staining. These highly negative charged proteins can be removed either by absorption with tissue powders or by ion exchange chromatography. The former method was found to be effective and convenient and was, therefore, used for the absorption for all FITC conjugated antisera before being used in the immunofluorescence test. Mouse liver powder was washed twice in PBS, centrifuging at 1000g for five minutes and then incubated with the antiserum for one hour at room temperature on a Matburn mixer. This mixture was centrifuged at 1000g for five minutes and the procedure repeated. The supernatant resulting from the second absorption was centrifuged a second time to remove any trace of liver powder and was aliquotted and stored at -20°C.

RENAL IMMUNOFLUORESCENT METHODS

Freezing and storing rat kidney material

The rat kidneys were cut in half longitudinally and cortical samples taken from both poles. The section was immersed immediately in Tissue-Tek II contained within a gelatin capsule. The piece of cortex was aligned horizontally within the upright capsule, frozen in a mixture of dry ice and methylated spirit and stored in a nunc tube at -70°C till required.

Immunofluorescence test

Using a Slee cryostat, 4μ sections were cut from the embedded renal rat cortex and air dried for 30 minutes. Sections were washed twice for a total of 20 minutes in PBS while shaking on a Griffen flask shaker. These sections were then fixed for 10 minutes in ether alcohol at 4°C followed by 90% alcohol for 20 minutes at 4°C and washed 3 times in PBS for 15 minutes. The glass slides were dried leaving the sections moist and the sections were incubated with one drop of the relevant antiserum in a humid chamber for 30 minutes at room temperature. This was followed by 3 washes in PBS for 20 minutes, sections were then mounted in citifluor. Control sections from each kidney were tested each time.

(a) One section was incubated with PBS rather than antisera during each incubation.

(b) Normal rat kidneys of the same strains were incubated either with PBS or the appropriate antisera during each incubation.

Microscopy and photography

The microscope used was a Leitz Ortholux II with a high pressure mercury vapour lamp, HBO 200 light source, a BG38 heat filter, a 4mm BG12 interference filter, an inbuilt barrier filter at setting 3 and a supplementary barrier filter, K510. This system is designed to allow only light below a wavelength of 490nm to reach the tissue and only light above 520nm to reach the eye pieces since the peak of FITC absorption is at 490nm and the peak emission is at 520nm.

The specimens were examined within 24 hours of being stained. Sections were usually viewed using x10 wide-field eye pieces and a x40 objective lens. Only where localization of staining was difficult was an oil

immersion x100 objective lens used.

All sections were assessed for intensity of stain on a scoring system of zero to three, zero = negative, one = weak, two = moderate, three = intense.

The sections were also described according to the pattern of staining, CL = capillary loop and M = mesangial.

All photography was done using a Zeitz Orthomat camera attachment.

The observer did not know the source of the sections examined.

SUMMARY OF IMMUNOFLUORESCENCE METHODS

1 Embed rat cortex in Tissue-Tek in a gelatin capsule and snap freeze immediately.

2 Store at -70°C in a sealed container.

3 Cut 4μ frozen sections.

4 Air dry for 30 minutes.

5 Wash in PBS, 2 x 10 minutes.

6 Fix in ether alcohol at 4°C for 10 minutes.

7 Continue fixation in 95% alcohol at 4°C for 20 minutes.

8 Wash in PBS, 3 x 5 minutes.

9 Incubate with antisera in humid container, 30 minutes.

10 Wash in PBS 3 x 7 minutes.

11 Mount in citifluor.

12 Examine sections and photograph.

13 Store at 4°C in the dark.

SECTION 4

ANIMAL STUDIES

Two strains of rat were used in this study. The Lewis strain (Fig 9) and the DA strain (Fig 10). Rats of 200-250g were used in all the experiments apart from the diet feeding experiments when 100g rats were used.

Healthy male rats were used throughout the study unless indicated.

2:4:1 RENAL UPTAKE OF PROTEINS

Changes in renal uptake with time

The rats were placed into a jar containing ether. When the rats were slightly anaesthetised they were removed from the jar. The rats were placed on a thermoregulated electric blanket; the blanket was connected to a homeothermic control at 37°C which maintained body temperature at 37.0 ± 0.5 C.

An incision was made in the neck and the jugular vein exposed by blunt dissection. Intravenous injections of proteins were given into the jugular vein under ether narcosis. Cationized IgG, native IgG and aggregated IgG were given at various times and in doses of 0.25mg, 1mg or 5mg. The animals were sacrificed and the kidneys, liver and spleen were removed. The uptake of the various proteins was measured by counting one whole kidney, liver and spleen. Sections of the other kidney were snap frozen in liquid nitrogen and the site of deposition

FIG 9 - LEWIS RAT

FIG 10 - DA RAT

Fig 9

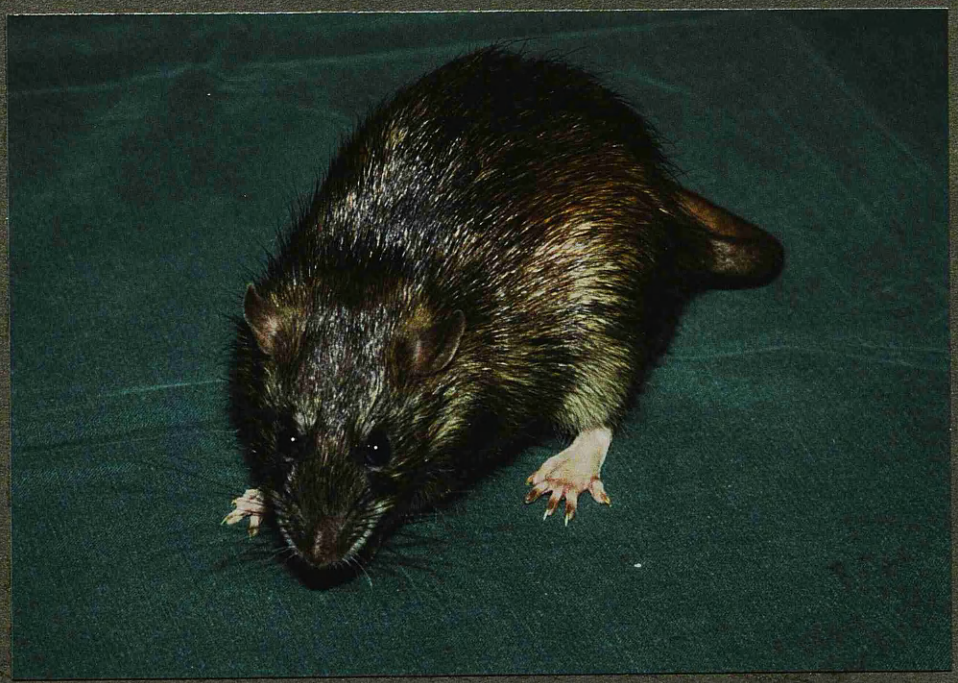


Fig 10

of IgG determined by standard immunofluorescent techniques using FITC anti human IgG. The uptake of cationic IgG by female Lewis and DA rats was studied at 1 hour and 3 hours using the same technique.

2:4:2 Renal artery perfusion studies

The procedure used was that described by Hoyer (147). Rats were anaesthetised with ether and the abdominal cavity exposed through a mid-line incision. The aorta and inferior vena cava were exposed by blunt dissection. The left spermatic and adrenal arteries were ligated and divided. Clamps were then placed across the aorta and inferior vena cava above and below the left renal artery. The origin of the right renal artery was outwith the clamps. A small incision was made in the left renal vein. 1ml of saline was infused over 20 seconds into the left renal artery through a 26 gauge needle placed in the aorta between the clamps. Varying quantities of IgG (5ug, 50ug and 200ug) were then infused in a volume of 0.1ml followed by 1ml of saline. The perfusate which leaked through the incision in the renal veins was removed by gauze swabs. The incisions in vein and artery were repaired with 7-0 silk sutures. The clamps were removed following total ischaemic time of less than 15 minutes. The abdomen was closed in 2 layers with 4-0 sutures. The animals were sacrificed 15 minutes after infusion of IgG, the kidneys removed and the uptake of IgG in both kidneys measured.

2:4:3 Changes in glomerular permeability caused by IgG

This was assessed in two ways:-

(1) 5mg of I-125 IgG^{+} was injected IV via the jugular vein at time zero. Fifteen minutes later 5mg of I-131 HSA was given IV. The rats were housed in metabolic cages and all urine

collected over the next 24 hours. The urine was dialysed against a large volume of buffered saline and the urinary excretion of HSA measured.

Control rats were given I-131 HSA only and housed in metabolic cages for 24 hours. There were 6 rats in each group.

(2) The second method of assessing glomerular permeability was to measure the increased renal uptake of HSA resulting from a 1mg bolus of IgG^+. One group of rats had only 1mg of HSA injected with no prior IgG given, the rats were sacrificed at 15 minutes and the renal uptake measured (HSA_s). A second group of rats were given 1mg of IgG^+ IV at various times followed by 1mg HSA 15 minutes before sacrifice and the renal uptake measured (HSA_c). The baseline value was then substracted from the renal uptake of HSA given 15 minutes before sacrifice following IV injections of IgG^+, ie, $HSA_c - HSA_s$.

2:4:4 Charge on red cells in DA and Lewis rats

The method used was that described by Levin (148). The rats were bled out through the abdominal aorta. Nine volumes of blood were mixed with one volume of 3.8% trisodium citrate. The red blood cells were separately centrifuged and washed three times in phosphate buffered saline, and resuspended in the same buffer in a final concentration of 1.2×10^6ml.

Anionic sites on the red blood cells were quantified by the binding of the cationic dye alcian blue 8 GX (AB). The optical density of AB at 650mm is linearily related to its concentration (Fig 11). Suspensions of rbc's from the two strains of rat were incubated with solutions of AB at varying concentrations and the amount of dye bound to the cells was calculated from the difference in optical density, between the original solution and the supernatant after removal of the cells by centrifugation.

Effect of [AB] on alcian blue binding

1ml of a suspension of rbc's (1.2×10^6/ml) was incubated with 1ml of a solution of AB varying in concentration from 10 to 2000μg/ml and incubated at 37 ^{o}C for 30 minutes. After removal of the cells by centrifugation, the optical density of the supernatant was measured. The amount of AB bound per 10^6 cells was plotted against the residual (free) AB concentration. This showed a sigmoid curve indicating saturation of binding sites at AB concentration above 600μg/ml (Fig 12).

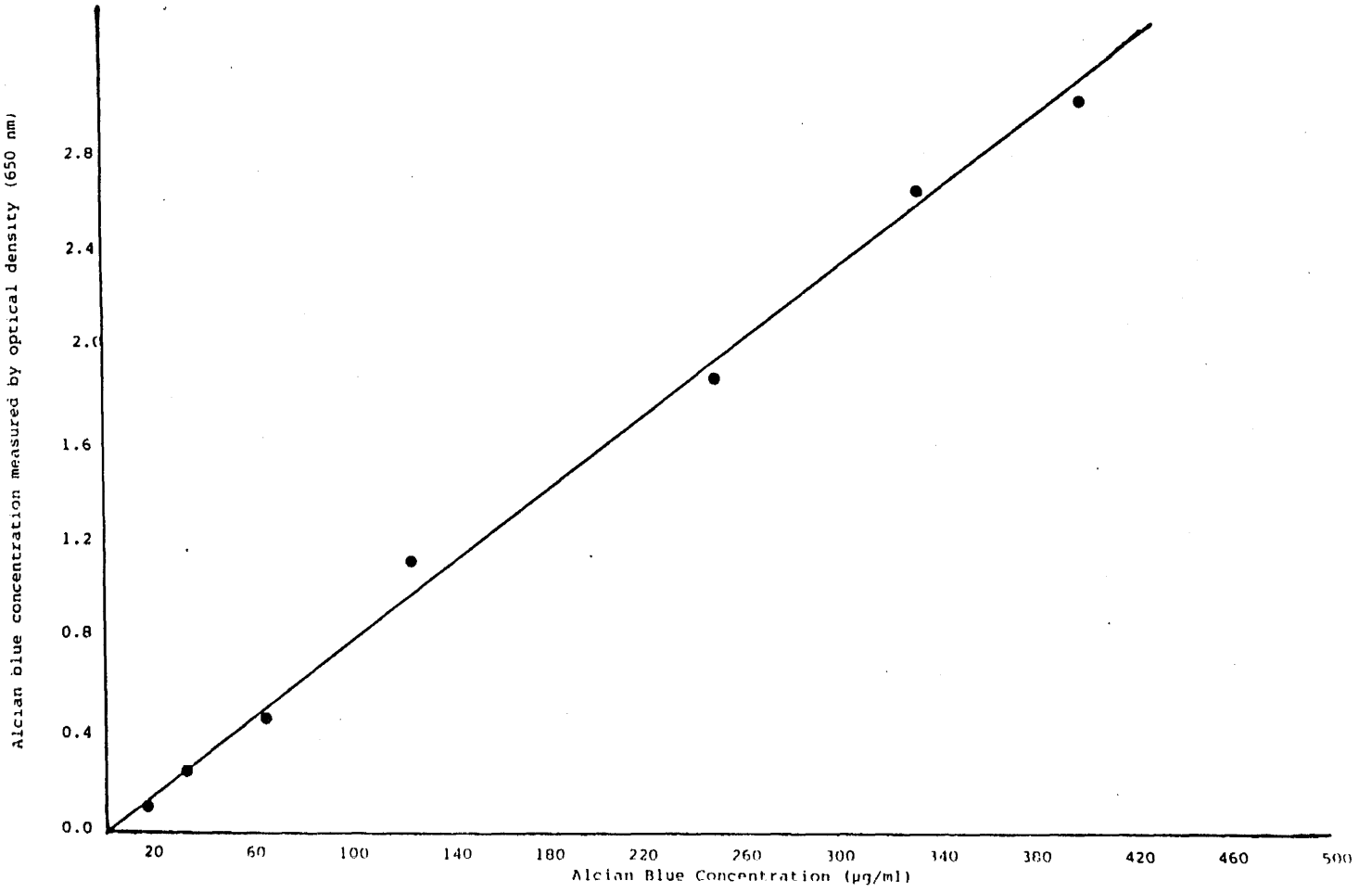


FIG 11 Standard Optical Density Curve for Alcian Blue.

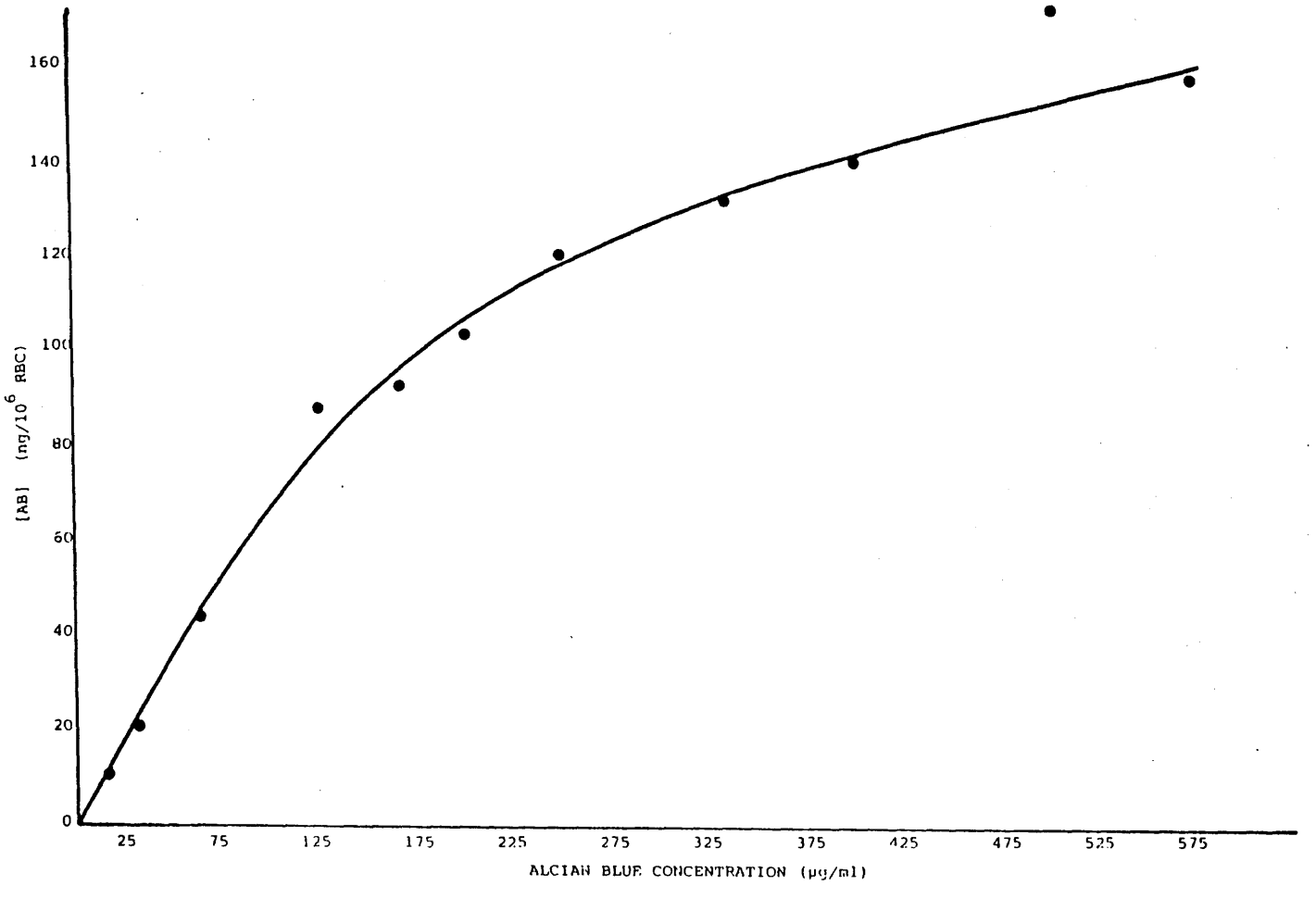


FIG 12 Alcian Blue Binding to Red Cells

Increasing concentrations of Alcian blue were added to a fixed number of red blood cells. The Alcian blue bound increased with increasing concentrations until a plateau was reached above 600µg/ml.

2:4:5 Charge on red cells in patients with different glomerulopathies

The charge on the red blood cells was examined in three groups of patients all of whom were in complete or partial remission. The three groups of patients were defined by the appearance of their renal biopsy and comprised patients with IgA (IgAN) membranous (IMN) and minimal change (MCN) nephropathies. A group of controls made up of hospital staff was also studied. Thirteen patients had IgAN (9 men and 4 women). Their mean age was 34.8 (range 17-55). Ten still had haematuria, one was receiving atenolol for hypertension, one was receiving glyceryl trinitrate, nifedipine and a diuretic, and one had steroid and salbutamol inhalers for asthma.

Ten patients had had one or more episodes of nephrotic syndrome due to minimal change nephropathy. Their mean age was 45.4 years (range 24-73), five of which were male. None was receiving prednisolone at the time of study.

Twelve patients with IMN had presented with nephrotic syndrome but were in complete or partial remission at the time of biopsy. Three had taken part in the MRC study and previously may have had steroids. None was receiving steroids at the time of study. There were three women and nine men with a mean age of 48.4 years (range 25-66). 24-hour urines were measured for proteinuria. Details of quantitative proteinuria and creatinine clearance can be seen in table 4 for all groups of patients studied.

TABLE 4

PATIENT	NEPHROPATHY	QUANTITATIVE PROTEINURIA g/24 hrs	MEAN PROTEINURIA g/24 hrs	CREATININE CLEARANCE ml/min	CREATININE CLEARANCE MEAN $\pm$ SD
1		0.47		143	
2		0.01		114	
3		0.63		77	
4		0.05		123	
5	IgA	0.21	0.56	ND	100.8 $\pm$ 30.7
6		0.40		65	
7		0.05		120	
8		0.23		130	
9		2.3		50	
10		0.24		125	
11		0.23		84	
12		0.82		122	
13		1.70		57	
1		0.24		144	
2		1.95		78	
3		0.05		107	
4		0.07		148	
5		0.06		56	
6	IMN	0.57	0.33	69	93.9 $\pm$ 26.4
7		0.11		116	
8		0.80		86	
9		0.12		82	
10		0.04		98	
11		0.04		57	
12		0.06		116	
1		1.59		164	
2		0.05		101	
3		0.07		104	
4		0.05		162	
5		0.03		103	
6	MCN	0.01	0.25	143	124 $\pm$ 25.4
7		0.03		103	
8		0.63		111	
9		0.09		104	
10		0.05		150	

Measurement of charge

This was carried out using the same protocol as described previously. Two different concentrations of alcian blue 125μg/ml and 250μg/ml were used. Patients were venesected in the morning and the test carried out soon after. Each test was performed in triplicate.

Competitive inhibition of alcian blue binding by polycations

Increasing concentrations of protamine sulphate or hexadimethrine were added to produce a dose dependant inhibition of alcian blue binding to normal red blood cells.

SECTION 5

Experimental animal models of glomerulonephritis

Four different animal models of glomerulonephritis were investigated in the two strains of rats and the effects of their different glomerular properties on their response to these models were examined.

2:5:1 Heymann nephritis (autologous immune complex nephropathy)

Male Lewis and DA rats (200g) were used. There were 4 animals in each group.

Preparation of crude kidney fraction

This was carried out following the method of Edgington (149). The proximal tubule brush border named FX1A was lyophilised and stored at -20 ^{o}C. (This antigen was kindly donated by Dr M E M Allison).

Induction of Heymann nephritis with FX1A

12.5mg of FX1A was mixed with 0.25ml of 0.9% saline which was then emulsified with the same volume of Freunds complete adjuvant. 0.5ml of the mixture was then injected intradermally at several sites into the rat's back. Control rats were injected with Freunds complete adjuvant only. 24 hour urines were collected at fortnightly intervals for 20 weeks.

Estimation of proteinuria

24 hour urine collections were made fortnightly. Protein content was determined by using the Rice method (150). The mean proteinuria of non-immunised rats was < 10mg/day. Proteinuria of 10mg or greater per day was considered abnormal and was accepted as presumptive evidence of disease. Section of kidney cortex were fixed in 2% gluteraldehyde phosphate buffer and prepared for electron microscopy studies. EM studies were kindly done by Dr A McLay (Pathology Department, Glasgow Royal Infirmary).

2:5:2 Chronic serum sickness model

This model was first described by Border in rats and demonstrated that by injecting cationized HSA intravenously on alternate days, mesangial deposits developed (124). Interestingly, when the experiment was repeated in rabbits membranous deposits evolved (103).

Experimental protocol

The method used was a modification of the procedure by Border (124). Male Lewis and DA rats (200g) were immunised with an intravenous injection containing 1mg of cationic HSA and 10ug endotoxin, as adjuvant under ether narcosis. One week later, intravenous injections of 1mg of cationic HSA were given until day 21 when the rats were sacrificed and renal tissue taken for immunofluorescence studies.

Prior to immunisation and during the course of the experiment, animals were housed in metabolic cages and 24 hour urine collections were obtained for protein estimations. Urinary protein was quantified by the Rice method (150).

2:5:3 Infusion of hexadimethrine

Hexadimethrine is a polycation and was used to test the hypothesis that neutralization of polyanion of the glomerular basement membrane will lead to loss of charge-dependant glomerular permselectivity.

Experimental protocol

The experimental procedure used was that described by Hunsicker et al (117). Male Lewis and DA rats (200g) were used in this study. There were six rats in each group. Each rat was pretreated with an intraperitoneal injection of diphenhydramine (25mg/kg) to reduce HDM toxicity related to histamine release from mast cells. The rats were anaesthetised with sodium pentobarbitone 60mg/kg body weight intraperitoneally. While under anaesthesia, an IV polythene catheter (PE50) was introduced into the jugular vein and the rats volume expanded over a period of 10 minutes with a quantity of saline equal to 5% of the body weight. The bladder was catheterised with PE90 tubing. Each animal was then permitted to equilibriate for 30 minutes on a saline infusion at a rate of 0.24ml/min. After equilibration an infusion of HDM (0.33mg/ml in 0.9% saline) was begun at the same rate of 0.24ml/min for 60 minutes and then saline was infused again at the same rate for a further 40 minutes. Sequential 10 minute urine collections were obtained throughout the study into pre-weighed vials containing paraffin oil. Volumes were determined by weight assuming a specific gravity of 1.0. Protein content was estimated by the Lowry method.

2:5:4 Puromycin aminonucloeoside nephrosis (PAN)

Puromycin aminonucleoside causes proteinuria five days after a single intra peritoneal injection in rats. The only histological change is epithelial foot process fusion therefore, it is suggested that PAN is associated with minimal change nephropathy. However, experimental animal models have produced conflicting views on the pathogenesis of PAN nephropathy and, therefore, the above view may be erroneous. Thus, the effect of puromycin aminonucleoside (PA) on the two strains of rat was studied.

Experimental Protocol

Male Lewis and DA rats (200g) were used in this study. There were eight rats in each group. All animals used were housed in individual metabolic cages with free access to standard rat chow and water. Initial urine collections were obtained twice during three days before PA administration. All rats had less than 10mg/24 hours of protein in their urine which was considered normal.

The rats received one 16mg dose of PA intraperitoneally. 24 hour urine collections were measured on alternate days over a period of three weeks. Urinary protein content was estimated by the Rice method.

2:6:1 Measurement of PGI_2 in aortic rings

Aortas were removed from male Lewis and DA rats and PGI_2 levels measured using the method of McLaren et al (151). Aortas were placed immediately into Hartmann's solution after removal from the body (six aortas taken from six separate Lewis or DA rats were used). The aortas were cleaned, freed from any fatty tissue and then chopped into pieces about 10mm long. They were then blotted once on a piece of filter paper and weighed. Pieces of similar weight were used for the experiments and incised longitudinally to allow maximum exposure of the endothelium. They were then incubated in Hartmann's solution at 37 ^{o}C and the buffer changed at various intervals as shown below.

SAMPLING TIME	VOL BUFFER
15 minutes	10ml
30 minutes	10ml
45 minutes	10ml
60 minutes	10ml
90 minutes	5ml
2 hours	5ml
$2\frac{1}{2}$ hours	5ml
3 hours	5ml
4 hours	5ml
5 hours	5ml
6 hours	5ml
7 hours	5ml
8 hours	5ml
9 hours	5ml

PGI_2 levels in the sequential supernatants were measured. The amounts of PGI_2 released per minute was calculated (ng/min).

SECTION 6

2:6:2 Modulation of glomerular properties by prostaglandins and their precursors

Quite recently there has been considerable interest in the role of prostanoids in the kidney. Experimental reports have shown beneficial effects of some prostanoids in modulating the course of some animal models of glomerulonephritis.

The effect of different fatty acid diets, fed over a six week period to the two strains of rat, on the glomerular uptake of proteins was examined.

Experimental protocol

A fat free diet was prepared by Special Diet Services (Essex). 100g male Lewis and DA rats were used in the study. The rats were divided into five groups as illustrated below. The oils were kindly donated by Efamol (Guilford). The composition of oils is illustrated in table 5.

			OIL	ACTIVE INGREDIENT	PG PRODUCTS
GP1	FAT FREE DIET	+	SAFFLOWER OIL	ARACHIDONIC ACID	2-SERIES PG
GP2	FAT FREE DIET	+	FISH OIL	EICOSAPENTAENOIC ACID	3-SERIES PG
GP3	FAT FREE DIET	+	EVENING PRIMROSE OIL	GAMMA LINOLEIC ACID	1-SERIES PG
GP4	FAT FREE DIET	+	OLIVE OIL	OLEIC ACID (Inert)	-
GP5	NORMAL DIET		-	-	-

TABLE 5

	OIL COMPOSITIONS			
	SAFFLOWER OIL	OLIVE OIL	EVENING PRIMROSE OIL	FISH OIL
14:0				14%
16:0	Palmitic	9–16%	6%	16%
18:0			1%	4%
18:1	Oleic 7–42%	65–85%	8–12%	16%
18:2	Linoleic 55–81%	4–12%	70–75%	1%
18:3	γ Linoleic	-	7.5– 9%	$<$ 1% (α)
20:4				
20:5	EPA			19%
22:6				12%
18:4 or 21:0				5%
16:1				5%

The basic recipe used was as follows:-

43ml oil
500g fat free diet
2.7g butylated hydroxy tolulene
150ml diethyl ether

The oil, ether and tolulene were mixed together. This mixture was added to the fat free diet and thoroughly mixed. It was then put in the drying oven overnight to allow the ether to evaporate.

The rats were weighed before and twice weekly during the six weeks to make sure they were feeding on the diet.

Glomerular uptake of cationized IgG

At the end of the 6 weeks the rats were injected intravenously with I-125 cationized IgG^{+} (1mg) as previously described. The rats were sacrificed one hour after the injection of IgG^{+} and one kidney counted in a Beckmann Gamma counter and the other kidney taken for immunofluorescence studies.

Kidney sections were stained with FITC anti human IgG and FITC anti rat IgG by standard immunofluorescent techniques.

2:6:3 Renal infusions of PGE_1, PGI_2

Operative procedure

The rats were anaesthetized with sodium pentobarbitone (0.1ml/100g) and placed on a thermoregulated blanket as before. Two PE50 cannulae, one containing saline (sodium chloride solution, 154nmol/l) and the other containing heparinised saline (30iu/ml) were placed in the right jugular vein and right carotid artery respectively. Blood pressure was continually recorded from the carotid artery with a blood pressure transducer (Bell & Howell) and a MX4 4 channel recorder (Devices). The recording system was calibrated with a mercury monometer from zero to 140mm Hg by step wise (20mm Hg) increases in pressure. The blood pressure was measured to within $\pm$ 2mmHg.

Experimental protocol

PGI_2 was supplied by the Wellcome foundation Ltd, London in a vial containing 0.5mg. This was diluted in 50ml of glycine buffer pH 10.5. 1ml of this solution was then further diluted 1/100 with more glycine buffer giving a final concentration of 100ng/ml. PGE_1 was supplied by UpJohn, West Sussex, in a concentration of 500μg/ml. This was diluted with saline to give a final concentration of 500ng/ml. The biological activity of these very dilute solutions of PGI_2 and PGE_1 was checked by measuring their ability to inhibit platelet aggregation. Biological activity was found to be present. PGE_1 and PGI_2 or glycine buffer were infused via the jugular vein at a rate of 0.05ml/min using a Harvard infusion pump and continued for 3 hours. Blood pressure was monitored continually. Two hours after the start of the infusion, 1mg of radiolabelled cationic IgG was given via the jugular cannula. The uptake of IgG^+ was measured in one kidney in a Gamma counter. The other kidney was taken for immunofluorescent studies.

CHAPTER 3

SECTION I

RENAL UPTAKE OF PROTEINS

3:1:1 Changes in renal uptake with time

The renal uptake of IgG^+ (cationized IgG) and IgG^n (native IgG) are shown in Figs 13 and 14 respectively. There was significantly less uptake in the kidneys of male Lewis rats at all times compared with DA rats. The specific uptake of IgG due to charge ($IgG^+ - IgG^n$) is shown in Fig 15 and was also greater in DA rats at all times except 24 hours when there was no specific uptake.

3:1:2 Effect of dose of IgG^+ on renal uptake

The effect of dose on IgG^+ on renal uptake was examined in the 2 strains of rat one hour following 0.25mg, 1mg or 5mg of IgG given intravenously (table 6). The renal uptake in Lewis rats was 50.0% that of DA rats at the lowest dose but rose to 70.8% following the highest dose. Therefore, the greatest relative difference followed the smallest dose of IgG but the difference in absolute amounts was greatest after 5mg.

3:1:3 Renal artery perfusion studies

The uptake of IgG^+ (in μg/g of kidney) by the kidney of the 2 strains of rat 15 minutes following injections of 5,50 and 200μg of IgG into the left renal artery was as shown in Table 7. The amount deposited in the left kidney was again greater in DA rats and the ratio of Lewis/DA was least after the smallest dose. The amount found in the non perfused right kidney was minimal indicating that there was no systemic spillover of IgG^+.

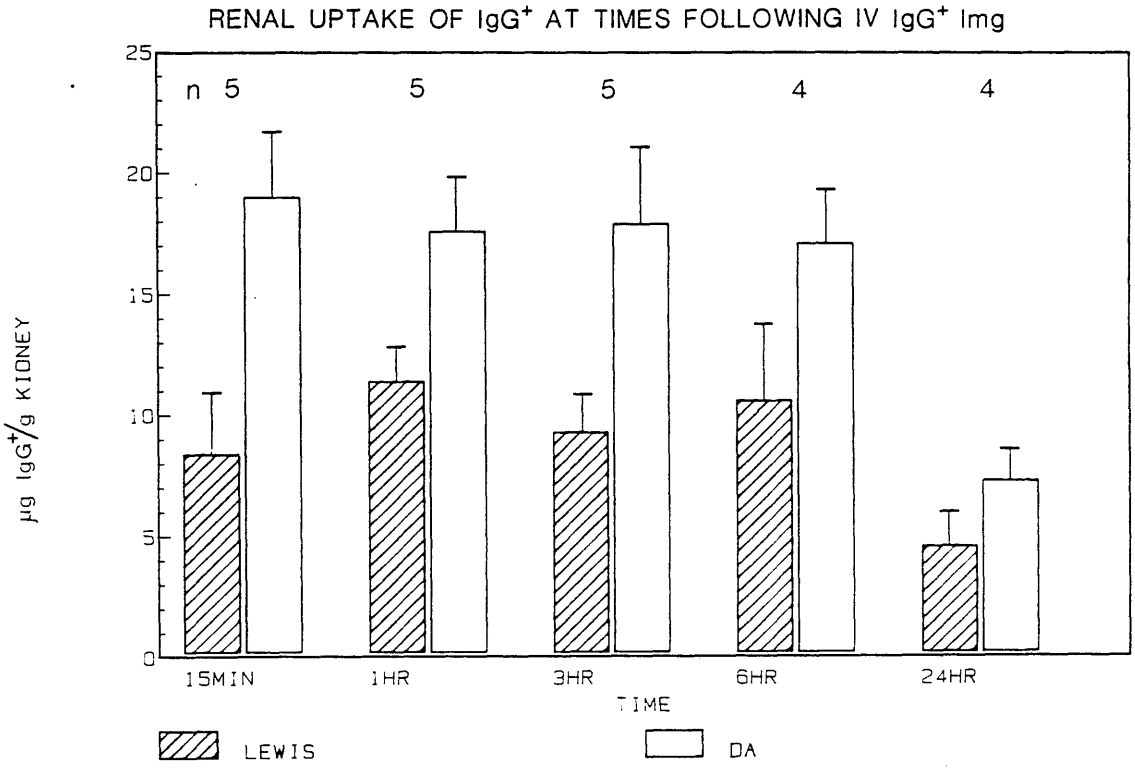


FIG 13 IgG^+ content (in $\mu g/g$ of kidney ± SD) of kidneys of Lewis and DA rats at various times following IV bolus of 1mg IgG^+. n = number of experiments at each time. The differences between the strains were significant $p < 0.025$ at all times using a student t-test.

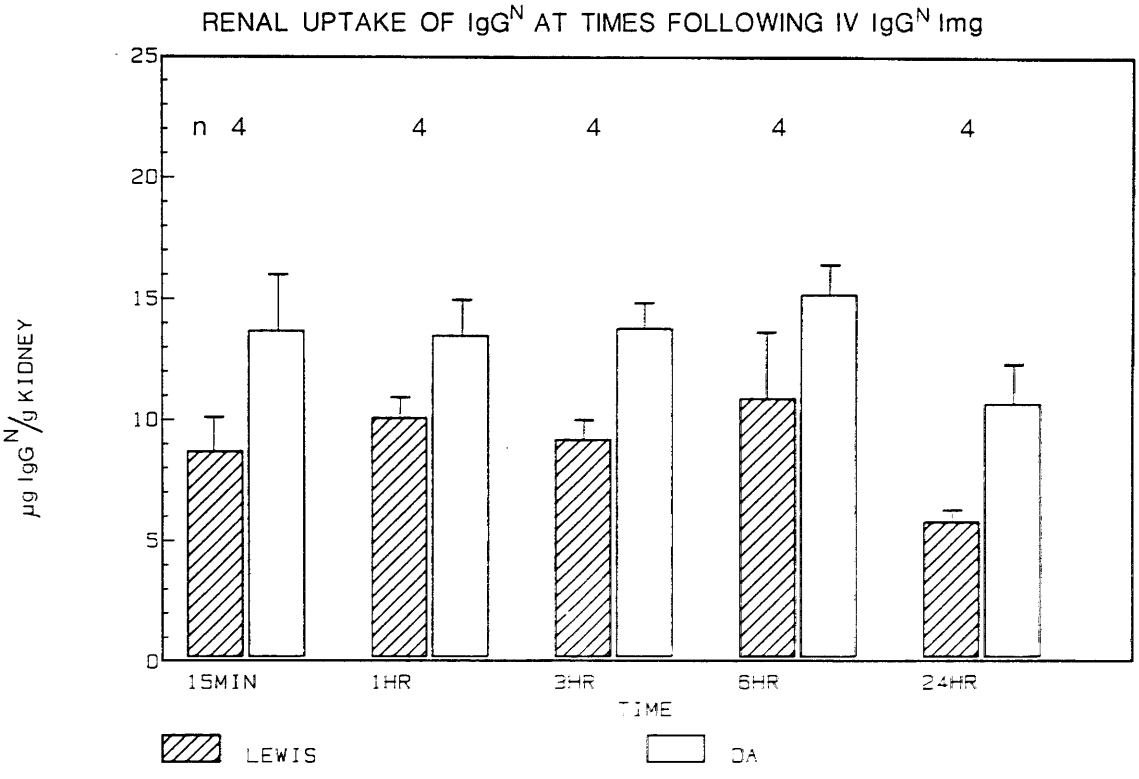


FIG 14 Uptake of IgG^N (in μg/g of kidney ± SD) by the kidneys of Lewis and DA rats at various times following an IV bolus of IgG^N 1mg. Each value is the mean of 4 experiments. The differences were significant at each time point $p<0.025$ using a student t-test.

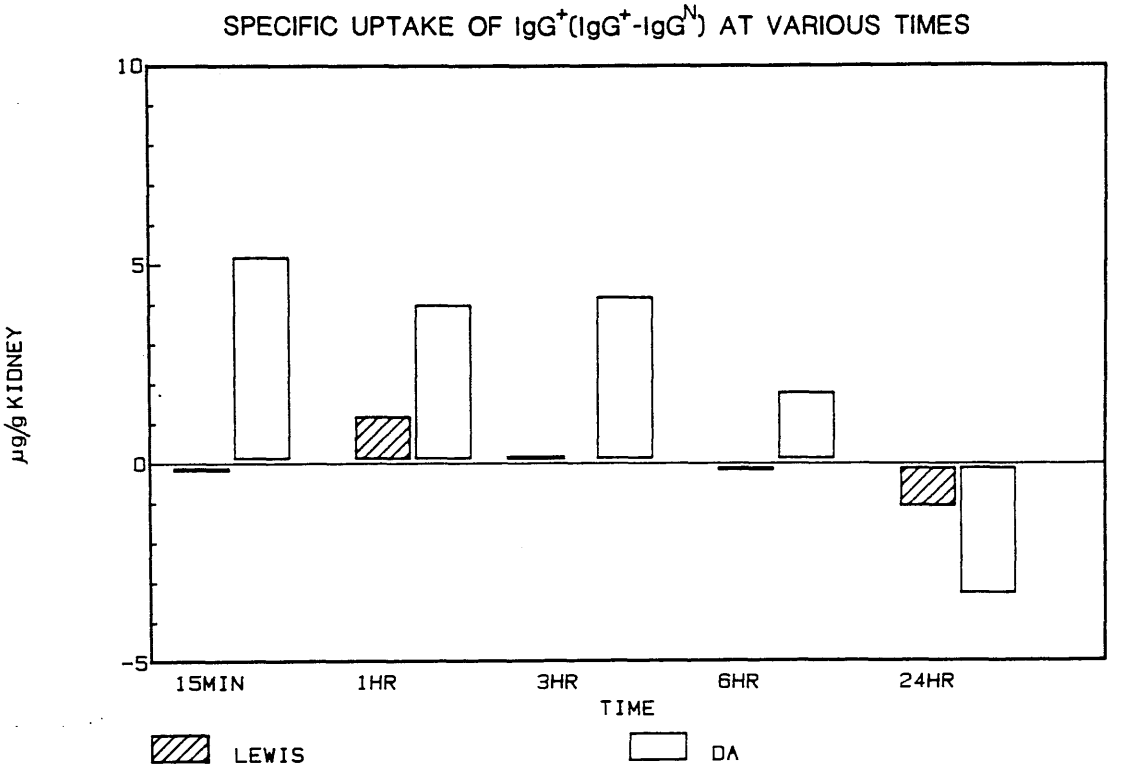


FIG 15 The specific uptake of IgG^+ due to its charge ie IgG^+-IgG^N was greater in DA rats at all times up to 6 but not including 24 hours. Results expressed as $\mu g/g$ of renal tissue.

T A B L E 6

EFFECT OF DOSE ON RENAL UPTAKE

DOSE OF IgG^+	NO	LEWIS	DA	LEWIS/DA %
0.25mg	3	1.9	3.8	50.0
1.0 mg	5	11.6	17.8	64.9
5.0 mg	4	43.5	61.4	70.8

Effect of dose on renal uptake of IgG^+ one hour after an IV bolus. Results expressed as $\mu g/g$ of renal tissue. (mean value of number of experiments indicated.)

TABLE 7

RENAL ARTERY PERFUSION OF IgG^+

IgG^+ injected	No	LEWIS		DA		LEWIS/DA % (L kidneys)
		L	R	L	R	
5 μg	3	0.05	0.01	0.16	0.02	31.2
50 μg	3	2.9	0.06	8.2	0.06	35.4
200 μg	3	24.1	0.06	47.2	0.06	51.0

FIG Renal uptake of IgG^+ following perfusion of left kidney. Results expresed as μg IgG^+ /g of kidney are the mean of the number of experiments indicated.

3:1:4 Renal uptake of IgG^+ in female rats

The difference in uptake of IgG^+ between sexes of the 2 strains of rat was studied. The renal uptake of IgG^+ one hour and 3 hours after an IV bolus of 1mg IgG^+ is shown in Fig 16. The uptake was least in male Lewis rats. The differences between the sexes of DA rats or between females of the 2 strains were comparatively small.

3:1:5 Uptake of IgG^+ by other organs

The uptake of IgG^+ and IgG^n in liver, spleen and kidney 15 minutes after an IV bolus of 1mg of the protein is shown in Table 8. This demonstrates the specific uptake of IgG ($IgG^+ - IgG^n$) is greater in DA rats in all tissues studied and that it is relatively less in the kidney compared to the liver or spleen.

3:1:6 Renal uptake of aggregated IgG

The MW of aggregated IgG by SDS electrophoresis was greater than 1×10^6 daltons. The renal uptake of aggregated IgG following boluses of 1mg and 5mg is shown in Figs 17 and 18 respectively. The renal uptake by Lewis rats was 65-70% of that of DA rats at all times but the rate of removal from the kidneys was similar in both strains.

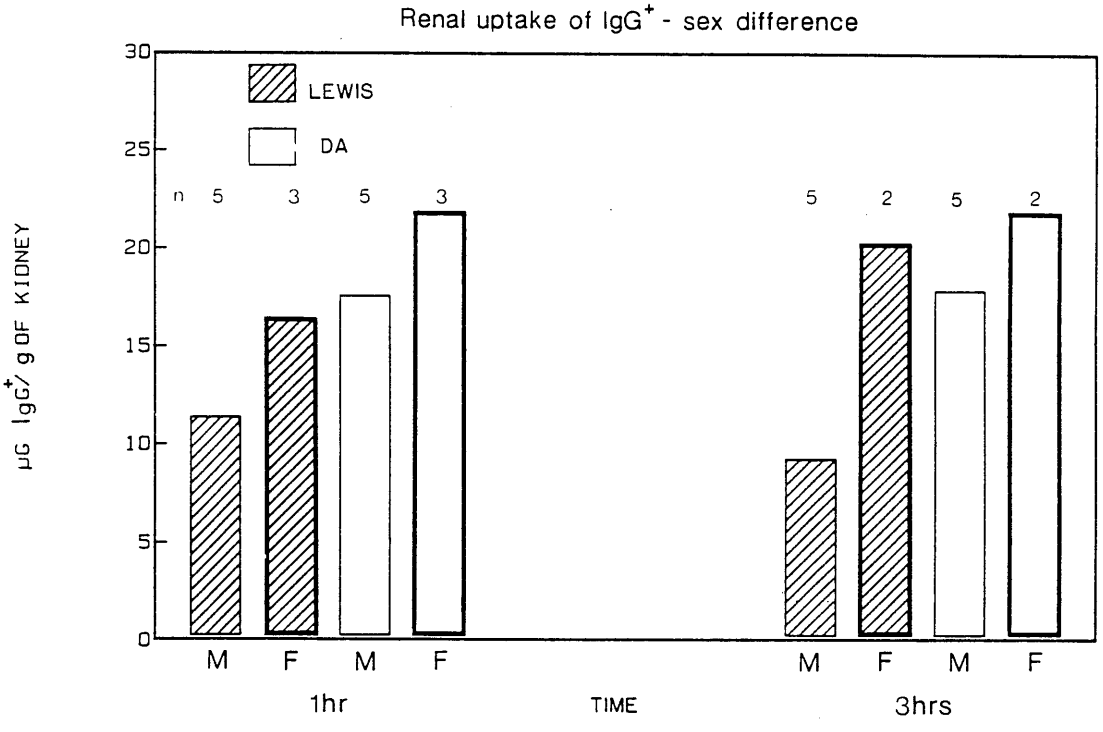


FIGURE 16 Renal uptake of IgG^+ at 1hr and 3hrs following an IV bolus of IgG^+ 1mg by the kidneys of male and female Lewis and DA rats. The values (in μg IgG^+/g of kidney) are the mean of the number of experiments indicated.

T A B L E 8

UPTAKE OF IgG^+ BY OTHER ORGANS

	L E W I S			D A		
	IgG^+	IgG^N	DIFF	IgG^+	IgG^N	DIFF
SPLEEN	21.4	18.0	3.4	32.0	22.4	9.6
LIVER	33.3	15.6	17.7	52.8	19.9	32.9
KIDNEY	8.6	8.8	-0.2	19.2	13.9	5.3

Uptake of IgG^+ and IgG^N in μg/g of organ 15 minutes after IV bolus of IgG^+ 1mg or IgG^N 1mg. Each value of IgG^+ is a mean of 5 experiments and each value of IgG^N is a mean of 4 experiments.

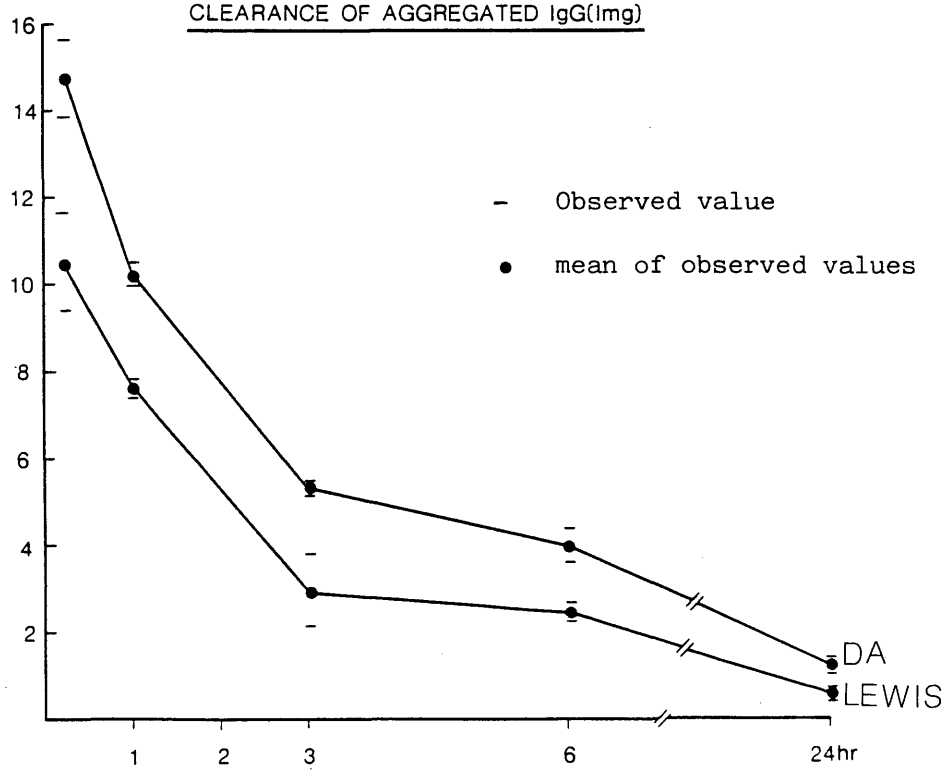


FIG 17 The renal uptake of IgG (in μg/g of kidney) at various times following an IV bolus of 1mg agg IgG. Each value is the mean of 2 experiments as indicated.

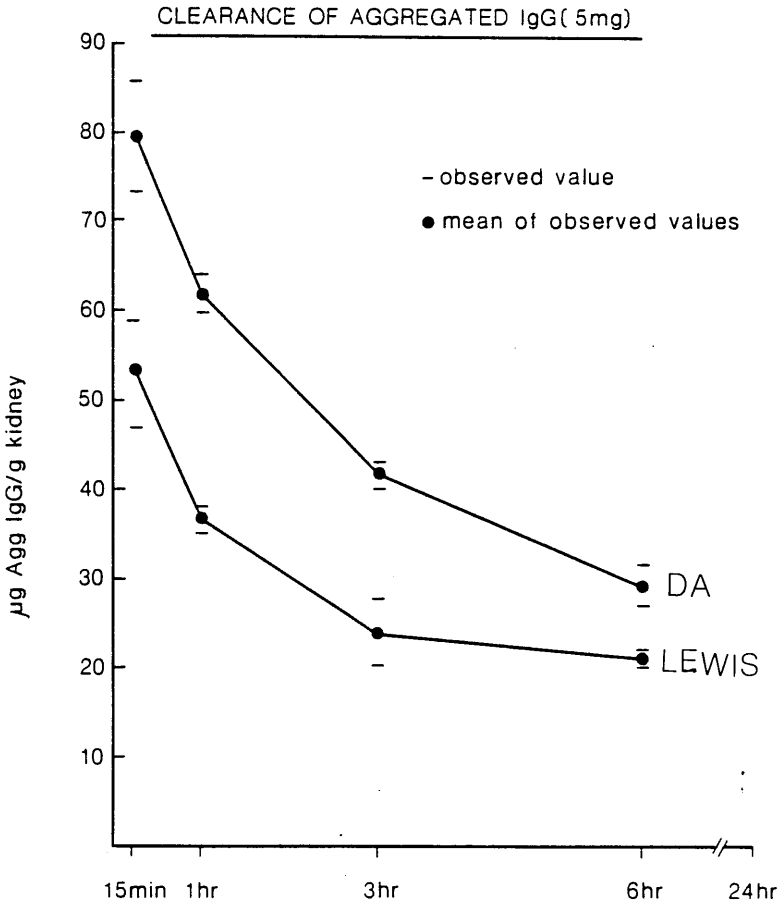


FIGURE 18 The renal uptake of agg IgG in (μg/g of kidney) by Lewis and DA rats at varying times. Each point is the mean of 2 experiments.

SECTION 2

3:2:1 Glomerular localisation of antigens by immunofluorescence

(a) IgG IF studies showed that at 15 minutes IgG^+ could be detected along the GBM. As expected, it was present in greater amounts in DA rats. At 1 hour, IgG^+ was detected both on the capillary loops and in the mesangium. By 3 hours it could be detected only weakly in the mesangium of DA rats and not at all in the Lewis rats. No extra glomerular IgG^+ was detected. Figs 19 and 20 show the staining of FITC anti human IgG at 15 minutes. Table 9 shows a summary of glomerular localisation following IgG^+.

(b) IgG^n - IgG^n could not be detected by routine IF techniques although whole kidney counts showed it to be present. The procedure was repeated but without washing the slides prior to fixing and adding anti human IgG. Thereafter, weak mesangial staining of IgG^n was found in both strains (Fig 22). It was concluded that IgG^n was present in the mesangium but was easily removed because it was unbound.

(c) Aggregated IgG - Deposits of aggregated IgG were detected in the capillary loop and mesangium at 15 and thereafter only mesangium at 1 hour (Fig 21).

TABLE 9

GLOMERULAR LOCALISATION OF IgG^+ FOLLOWING IV BOLUS IgG^+ 1mg.

TIME	LEWIS	DA
15 minutes	++ cap loop	+++ cap loop
1 hour	+ cap loop and mesangial	++ cap loop and mesangial
3 hours	-	+ mesangial

FIG 19 - GLOMERULAR IMMUNOFLUORESCENCE PATTERN OF CATIONIC IgG AT 15 MINUTES IN THE LEWIS STRAIN.

(x350)

FIG 20 - GLOMERULAR IMMUNOFLUORESCENCE PATTERN OF CATIONIC IgG AT 15 MINUTES IN THE DA STRAIN.

(x350)

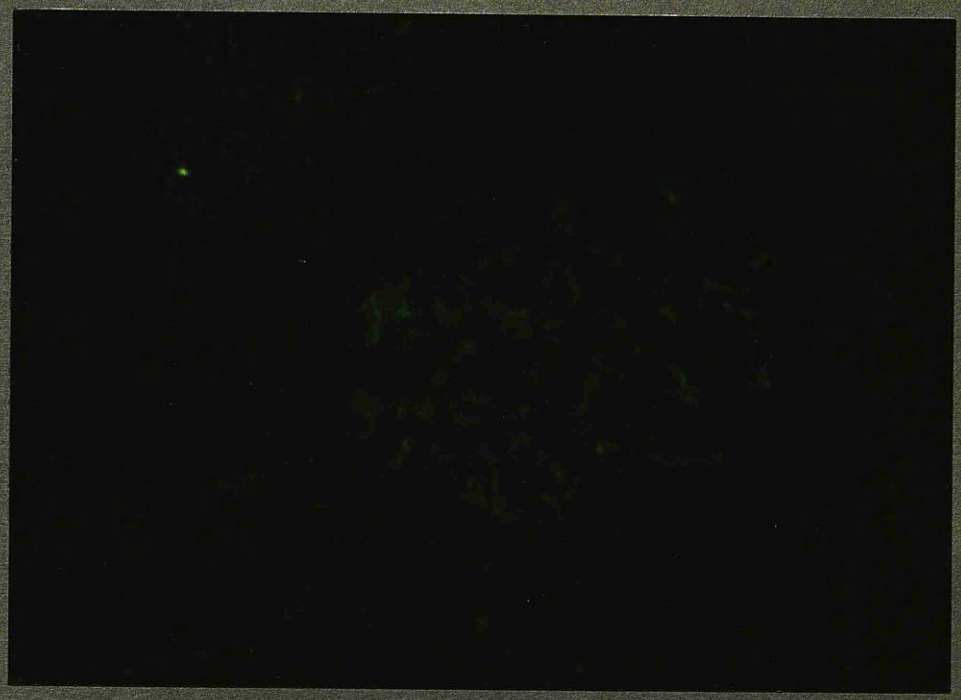


Fig 19

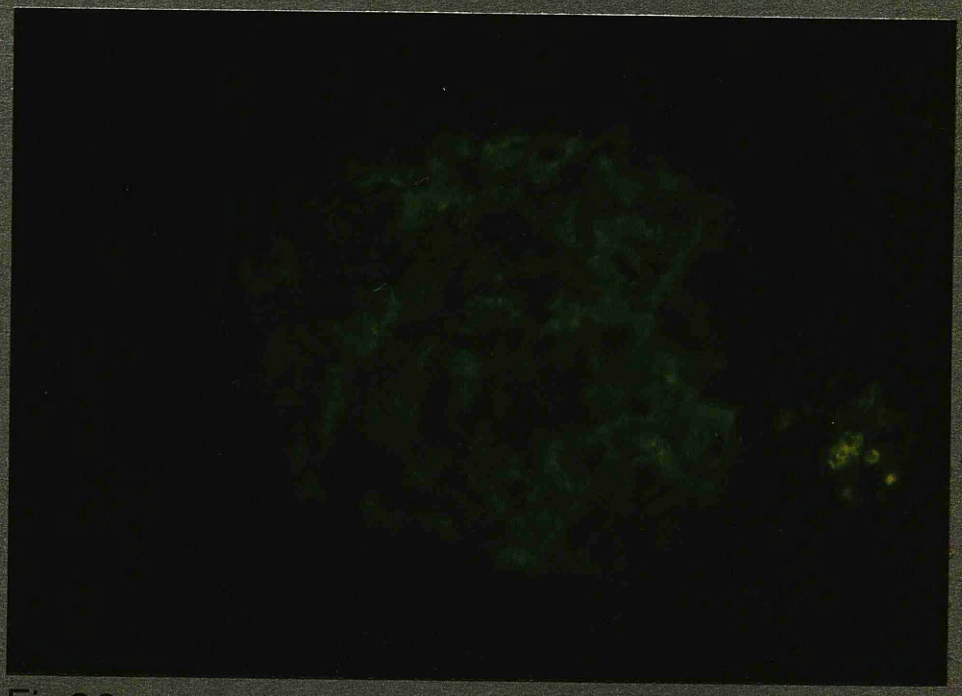


Fig 20

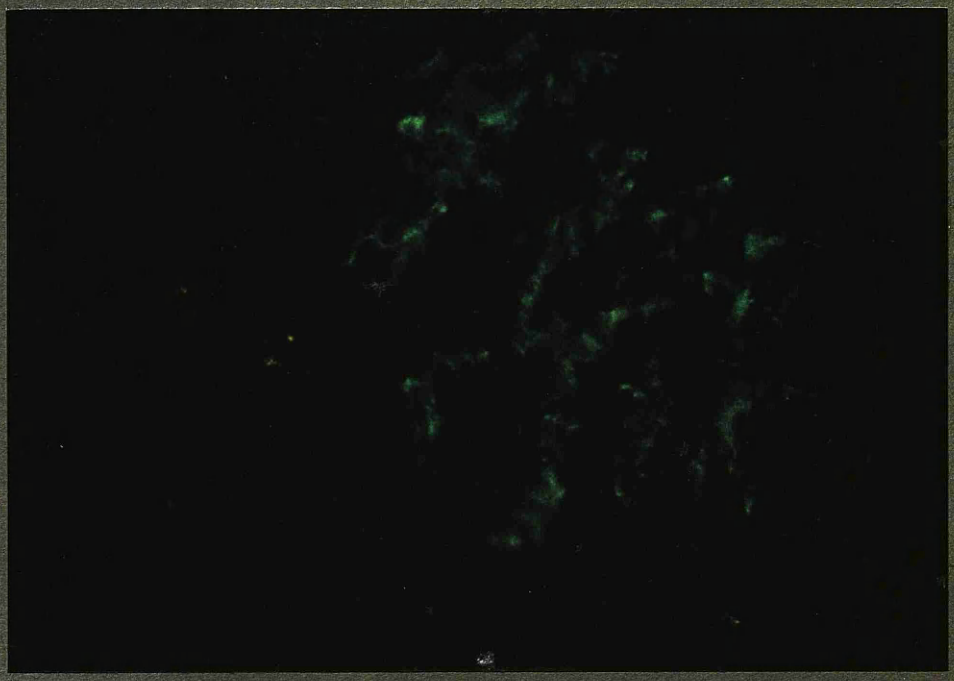


Fig 21

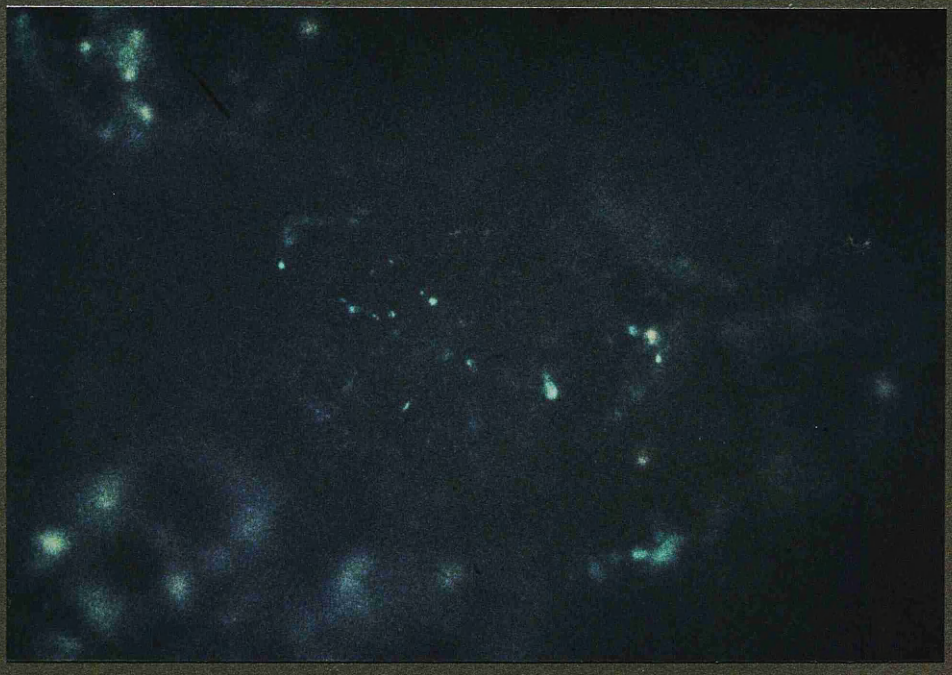


Fig 22

FIG 21 - GLOMERULAR IMMUNOFLUORESCENCE PATTERN OF AGGREGATED IgG IN THE LEWIS STRAIN AT 1 HOUR.

(x350)

FIG 22 - GLOMERULAR IMMUNOFLUORESCENCE PATTERN OF NATIVE IgG AT 15 MINUTES IN THE LEWIS STRAIN.

(x350)

SECTION 3

3:3:1 Changes in glomerular permeability

The urinary excretion of HSA^N after a bolus of 5mg of IgG^+ can be seen in Table 10. The mean urinary loss of HSA^n in Lewis rats was 60.2μg whereas in DA rats it was 16.2μg. The urine excretion of HSA^n only (ie. without IgG^+) can be seen in Table 11. The mean urinary loss in Lewis and DA rats was 9.65 and 5.48 μg/24 hours respectively.

The second way of assessing glomerular permeability was to measure increased renal uptake of HSA resulting from a bolus of IgG^+. Table 12 shows that although the specific uptake of IgG^+ ($IgG^+ - IgG^n$) was less in Lewis rats, the increase in uptake of HSA ($HSA_c - HSA_s$) was greater at all times from 1 hour. No increase could be detected in either strain at 15 minutes and the increase in DA rats was only marginal at all times.

CHANGES IN GLOMERULAR PERMEABILITY

T A B L E 10

STRAIN	NO	E X C R E T I O N I^{131} -HSA		
		MEAN (μg/24 hrs)	RANGE (μg/24 hrs)	SEM (μg/24 hrs)
LEWIS	6	60.0	28 - 104	12.7
DA	6	16.2	10.4 - 20.4	2.0

Urine excretion (μg/24 hrs) of I^{131}- HSA following IV boluses of 5mg I^{131}- HSA and 5mg IgG^{+} in DA and Lewis rats. Difference significant ($p<0.025$) student t-test.

T A B L E 11

	NO	I^{131} HSA	
		MEAN μg/24 hrs	RANGE μg/24 hrs
LEWIS	3	9.65	3.56 -13.8
DA	3	5.48	1.83 - 8.81

Urine excretion (μg/24 hrs) of I^{131} HSA only in DA and Lewis rats.

TABLE 12

EFFECT OF IgG^+ (1mg) ON RENAL UPTAKE OF HSA

TIME	LEWIS				DA			
	IgG^+-IgG^N	HSA_c	HSA_s	DIFF	IgG^+-IgG^N	HSA_c	HSA_s	DIFF
15 mins	-0.26	4.5	3.5	1.0	5.3	11.7	11.9	-0.2
1 hr	1.3	10.2	3.5	6.7	4.0	12.5	11.9	0.6
3 hrs	0.1	8.8	3.5	5.3	4.1	13.2	11.9	1.3
6 hrs	-0.3	11.2	3.5	7.7	1.9	14.4	11.9	2.5
24 hrs	-1.2	10.7	3.5	7.2	-3.4	13.5	11.9	1.6

HSA_c = Renal uptake of HSA given 15 min before death but after IgG^+ 1mg given at times indicated.

HSA_s = Renal uptake of HSA given 15 min before death but with no prior IgG^+.

DIFF = $HSA_c - HSA_s$

SECTION 4

3:4:1 Binding of alcian blue to red cells of DA and Lewis rats

The alcian blue binding by red cells of Lewis and DA rats is shown in Fig 23. There was significantly less binding to the red cells of Lewis rats at concentrations of alcian blue of 125μg/ml and 250μg/ml ($p < 0.05$ using student t-test) but not 62.5μg/ml.

3:4:2 Competitive inhibition of alcian blue binding by polycations

The addition of the polycations hexdimethrine or protamine sulphate produced a dose dependent inhibition of AB binding (Fig 24). The concentration of alcian blue used in these experiments was 125μg/ml. There was little displacement of AB binding below this concentration for either HDM or protamine sulphate.

3:4:3 Alcian blue binding to red cells of various glomerulopathies

Studies of AB binding to the red cells (ng AB per 10^6 RBC) of patients with three different nephropathies and a control group is shown in Figs 25 and 26. All values for the patients fell within the range of controls. Patients with IgAN had significantly greater binding than did patients with MCN or IMN whether the final concentration of AB was 125μg/ml or 250μg/ml. There was no difference between the groups when the concentration of AB was 62.5μg/ml. The statistical values of these differences is shown in Table 13.

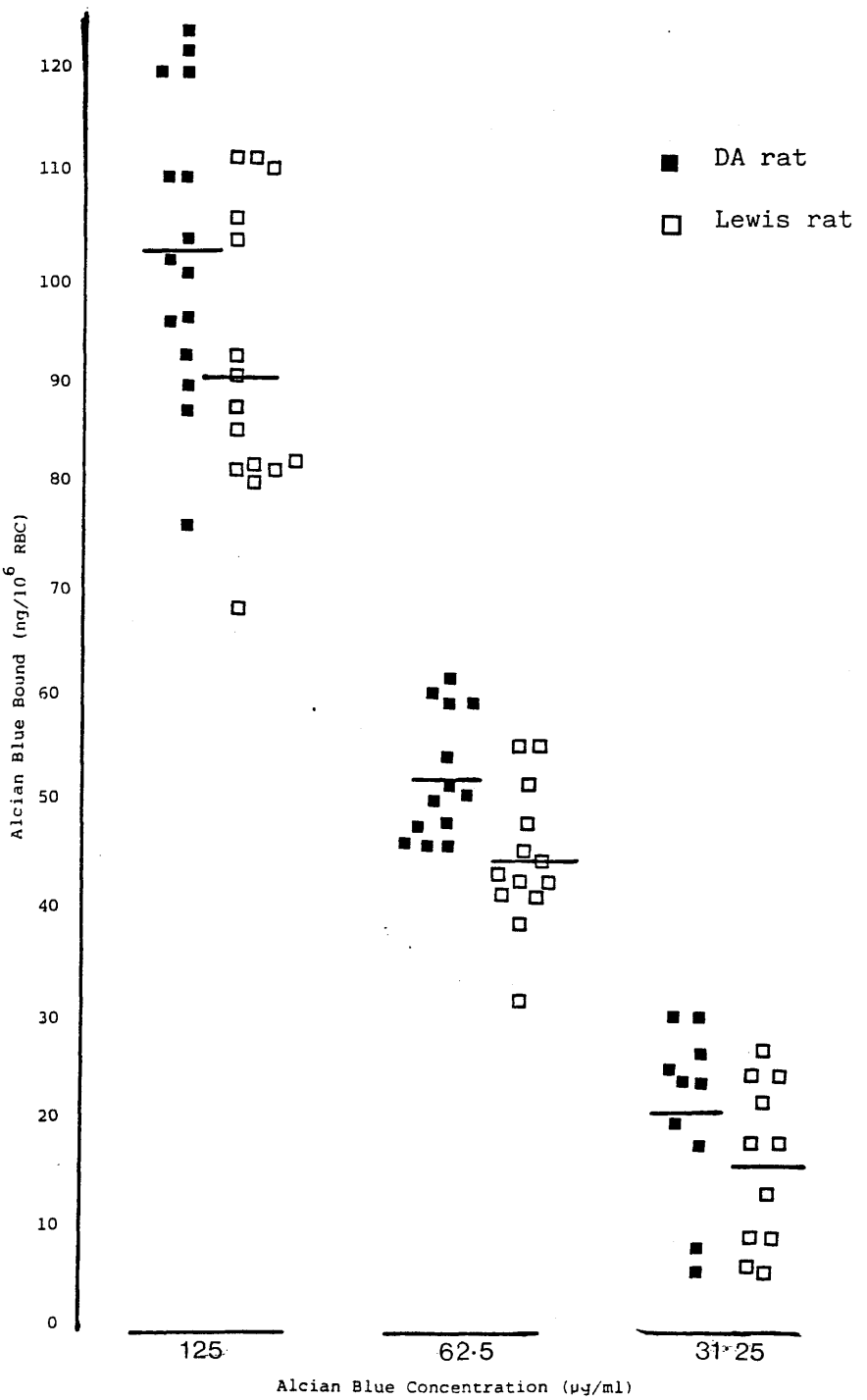


FIG 23 Binding of Alcian blue to red cells of Lewis or DA rats. Means are indicated by horizontal lines.

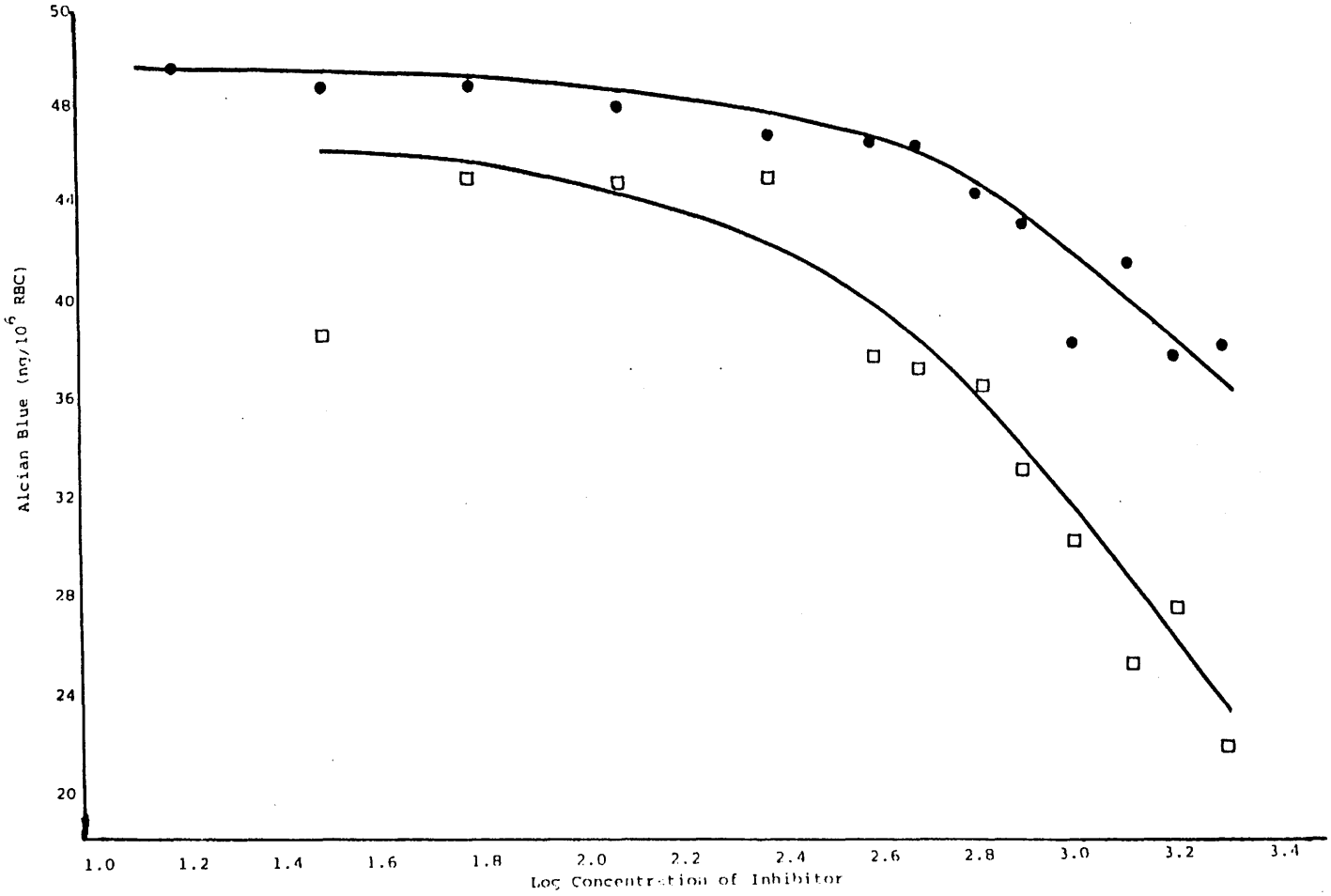


FIG 24 Competitive inhibition of Alcian blue binding by polycations.

Increasing concentrations of protamine sulphate (□) or hexadimethrine (●) progressively inhibited the binding of Alcian blue to normal red blood cells.

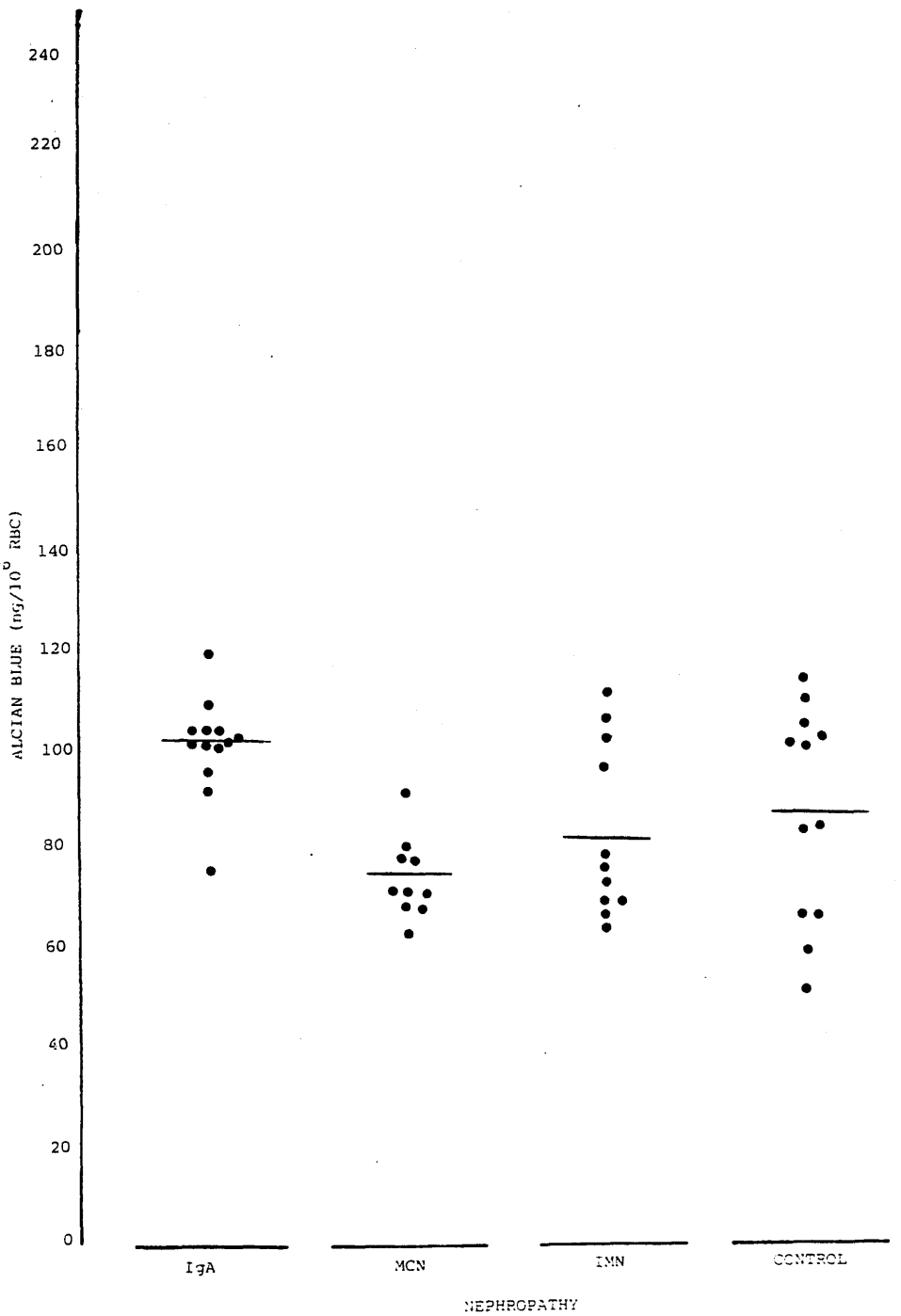


FIG 25 Binding of Alcian blue (125μg) to red cells of normal controls or patients with either mesangial IgA, minimal change (MCN) or membranous nephropathy (IMN). Means are indicated by horizontal lines.

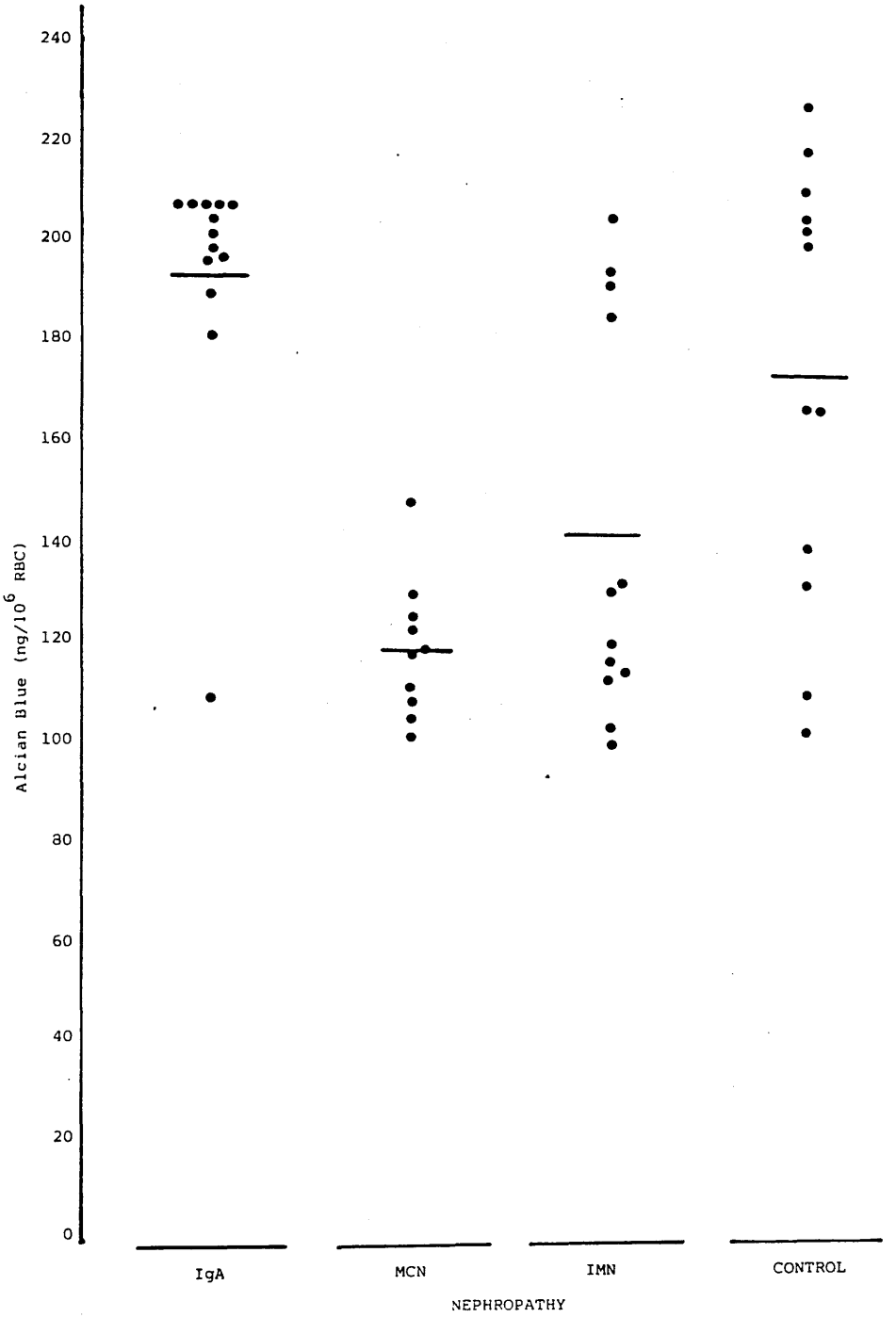


FIG 26 Binding of Alcian blue (250μg) to red cells of normal controls or patients with either mesangial IgA, minimal change (MCN) or membranous nephropathy (IMN). Means are indicated by horizontal lines.

TABLE 13

RELATION / AB CONCENT.	IgA:MCN	IgA:IMN	MCN:IMN	IgA:CONTROL	MCN:CONTROL	IMN:CONTROL
250µg/ml	$P<0.001$	$P<0.001$	$P>0.05$	$P>0.05$	$P<0.01$	$P>0.05$
125µg/ml	$P<0.001$	$P<0.01$	$P>0.05$	$P<0.05$	$P>0.05$	$P>0.05$
62.5µg/ml	$P>0.05$	$P<0.02$	$P>0.05$	$P>0.05$	$P>0.05$	$P>0.05$

Statistical analysis between groups of patients or controls using the student t-test

CHAPTER 4

SECTION I

SOME CONSEQUENCES OF VARIATION IN GLOMERULAR PROPERTIES INVESTIGATED BY ANIMAL MODELS OF GLOMERULAR INJURY

4:1:1 Heymann nephritis model (autologus immune complex nephropathy)

Proteinuria was detected in Lewis rats from 12 weeks onward but not DA rats (Fig 27). Control rats from both strains did not develop proteinuria.

Electron micrographs of the sections of the glomeruli of Lewis and DA rats are shown in Figs 28 and 29 respectively. Subepithelial deposits characteristic of Heymann nephritis were detected in Lewis but not DA rats.

4:1:2 Chronic serum sickness

Alternate day injections of cationic HSA produced no proteinuria in either of the 2 strains of rat over a 21 day period. Immunofluorescent studies of sections of kidney cortex showed deposits of rat IgG in both strains of rat. The deposits were less dense in Lewis rats and were found mainly on the capillary loop (Fig 31). No deposits were found in Lewis control kidneys (Fig 30) DA rats on the other hand, had dense mesangial deposits of rat IgG in both control and experimental kidneys (Figs 32 and 33). HSA could not be detected in kidneys of either strain by immunofluorescent techniques.

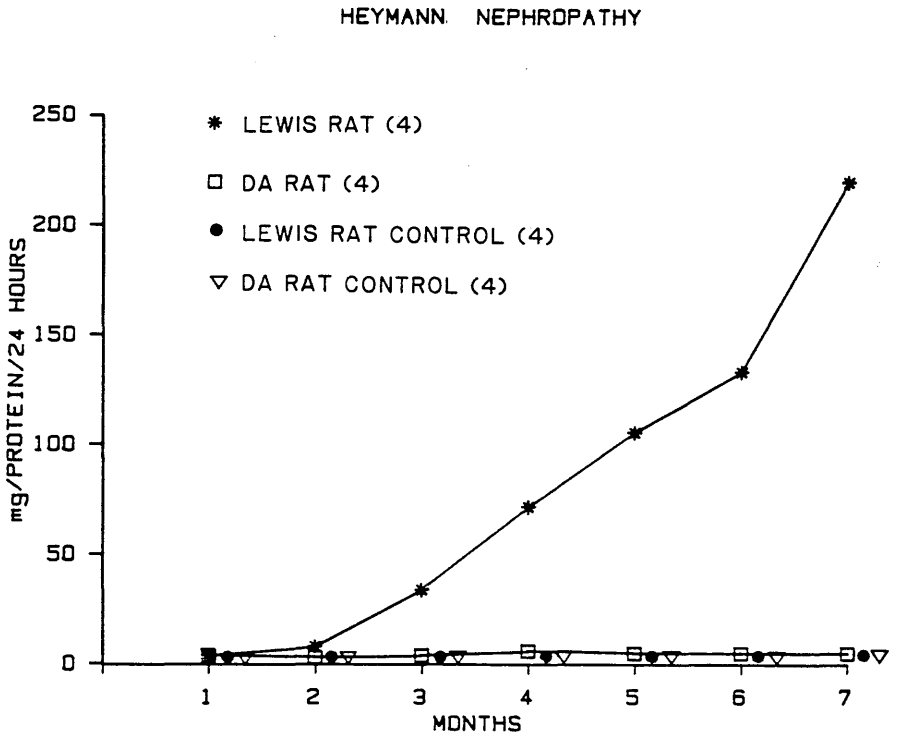


FIG 27 Urine protein excretion (mg/24 hours) in Lewis and DA rats after intradermal injection of 12.5mg tubular antigen. Each point is the mean of 4 experiments.

FIG 28 - ELECTRONMICROGRAPH OF LEWIS RAT GLOMERULUS 7 MONTHS AFTER INJECTION OF TUBULAR ANTIGEN (FX1A).

(x15500)

FIG 29 - ELECTRONMICROGRAPH OF DA RAT GLOMERULUS 7 MONTHS AFTER INJECTION OF TUBULAR ANTIGEN (FX1A).

(x15500)

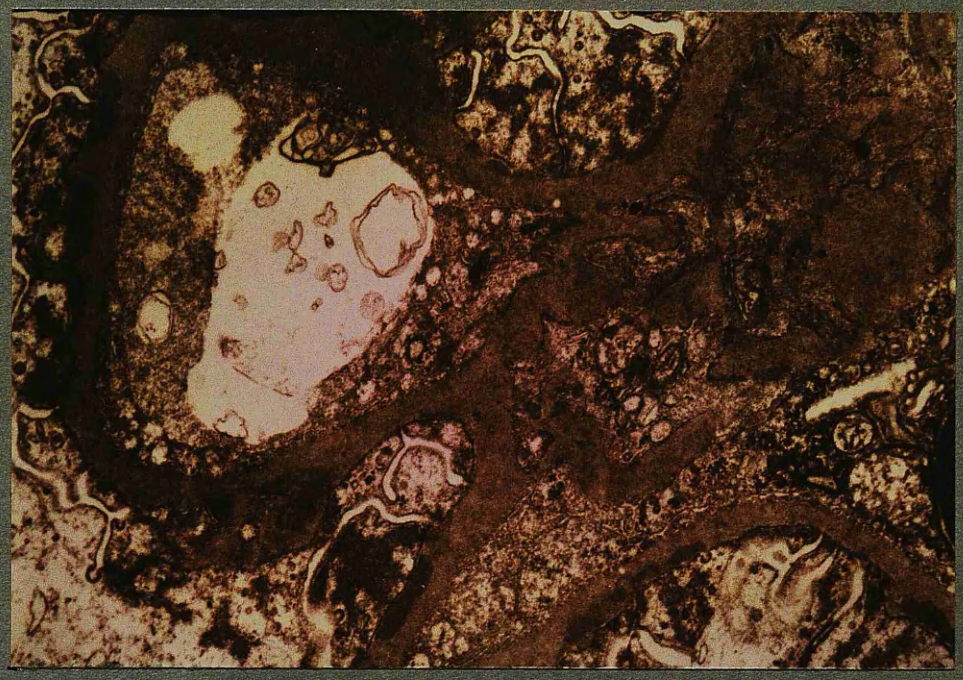


Fig 28

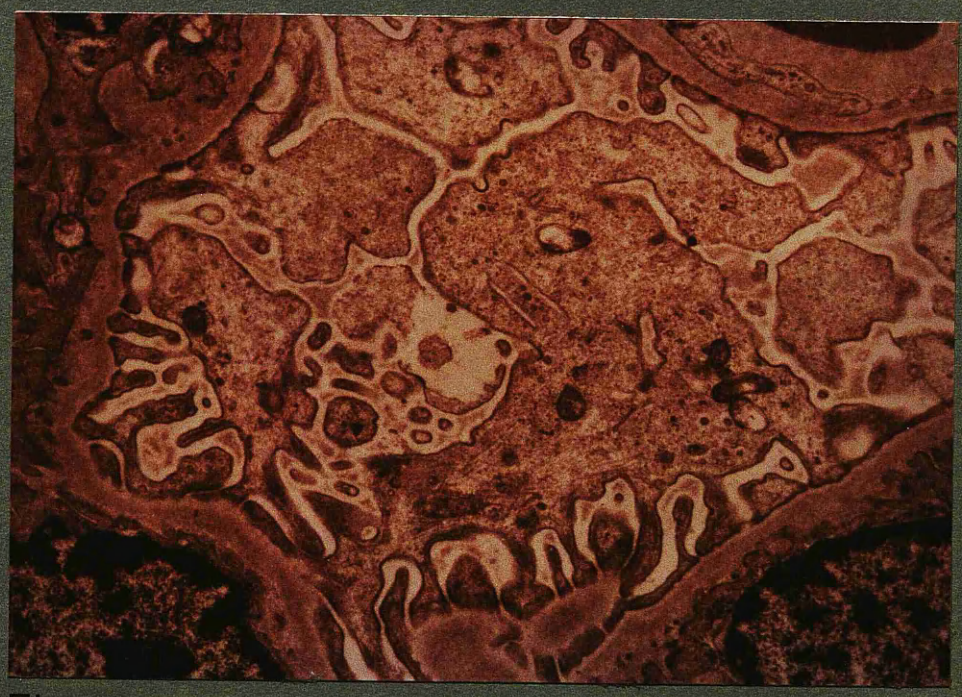


Fig 29

FIG 30 - GLOMERULAR IMMUNOFLUORESCENT PATTERN OF CONTROL LEWIS RAT KIDNEY. (x350)

FIG 31 - GLOMERULAR IMMUNOFLUORESCENT PATTERN OF LEWIS RAT KIDNEY AFTER INJECTION OF CATIONIC HSA. (x350)

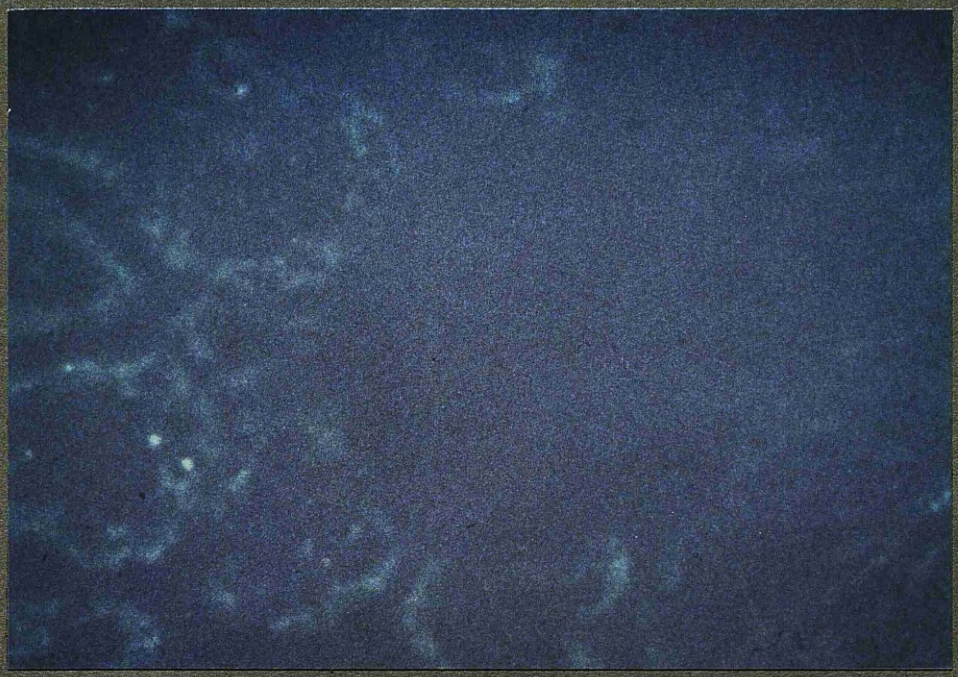


Fig30

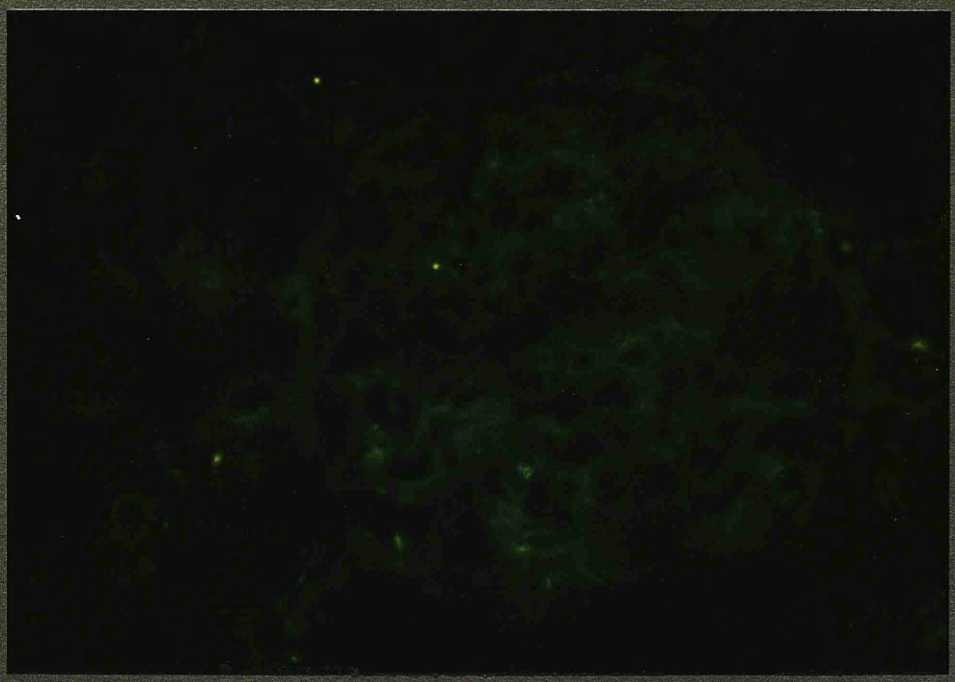


Fig31

4:1:3 Infusion of hexadimethrine (HDM)

Urine protein excretion in both strains was similar during the period of saline infusion. It remained unchanged in DA rats. Forty minutes after the start of the infusion of HDM, the urine protein loss of Lewis rats increased sharply and continued to increase until the end of the infusion when it quickly returned to basal values (Fig 34). Both strains of rat started to become oliguric towards the end of the saline infusion causing urine volumes to tail off.

4:1:4 Intravenous injection of puromycin aminonucleoside

A single intra peritoneal injection of 16mg of Puromycin aminonucleoside produced proteinuria in both strains of rat by day 5. Fig 35 shows the mean protein excretion of the rats. This was measured on alternate days for 3 days before the intraperitoneal injection and every alternate day thereafter for 21 days. Proteinuria was twice as much in DA rats compared to that of Lewis rats.

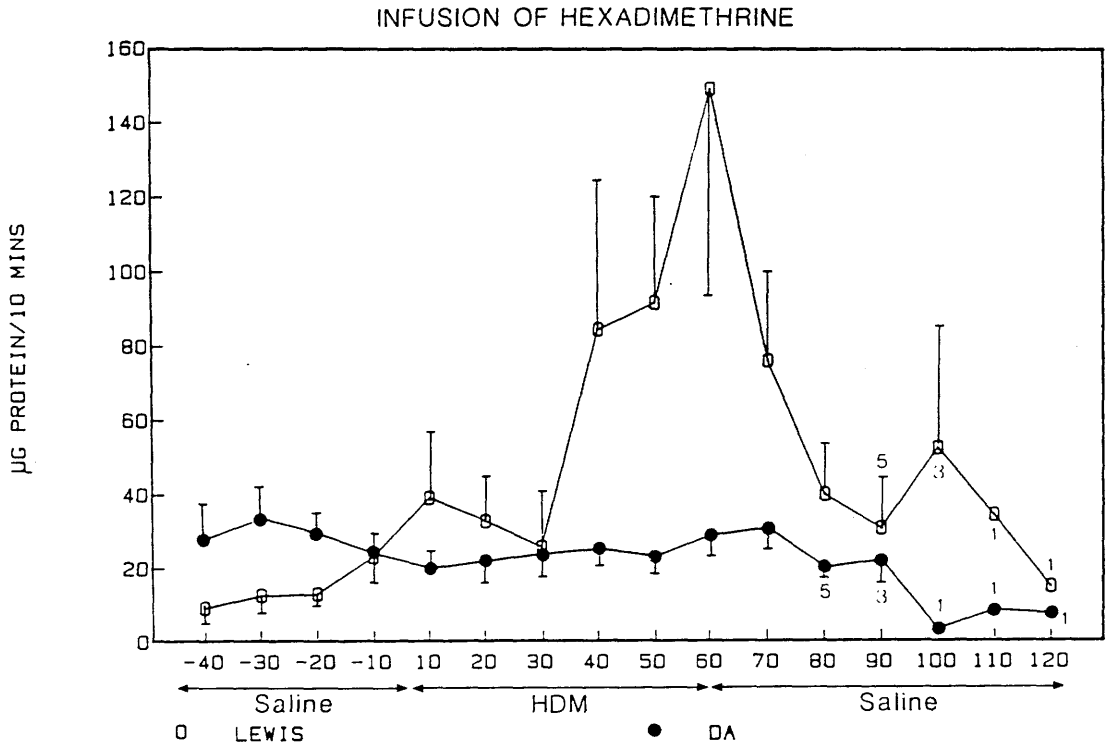


FIG 34 Urine protein excretion (μg/10 minutes) of DA and Lewis rats before, during and after an infusion of hexadimethrine. Each point is the mean and standard error of the mean of 6 experiments unless indicated.

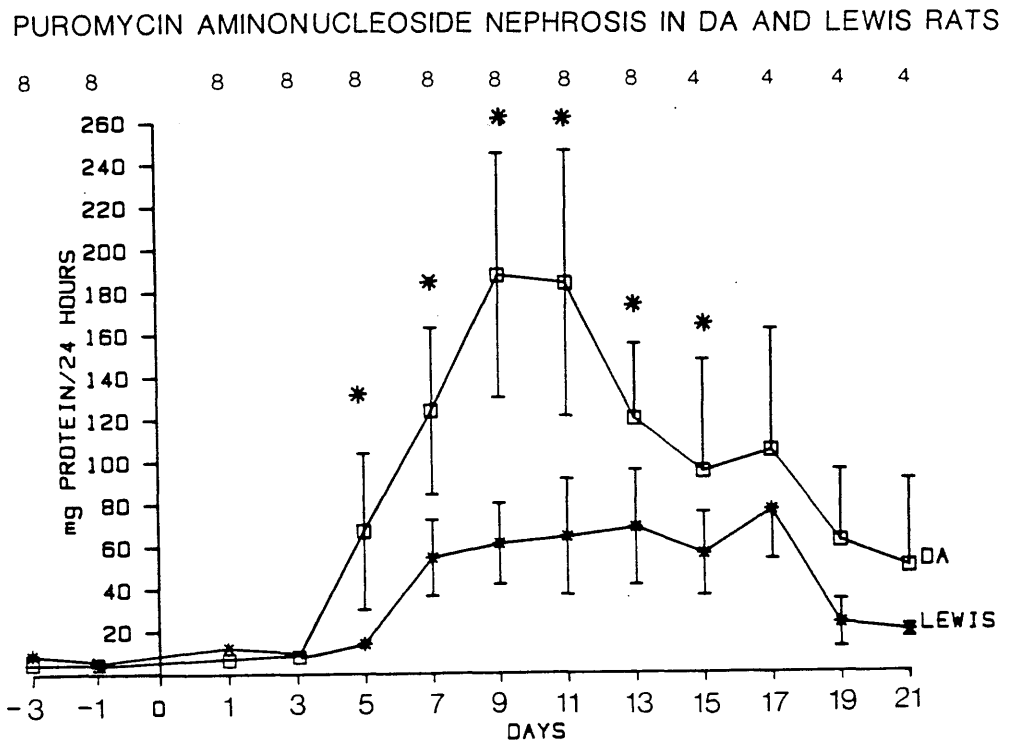


FIG 35 Urine protein excretion (mg/24 hours) of DA and Lewis rats after an intraperitoneal injection of PA. The number in each group is indicated along the top of the graph. Each point is the mean ± SD.* $p<0.05$ using student t-test.

CHAPTER 4

SECTION 2

MODULATION OF THE GLOMERULAR DEPOSITION OF ANTIGEN BY FATTY ACIDS AND PROSTANOIDS

4:2:1 Release of PGI_2 from rat aortas

The release of PGI_2 from two sets of aortas, three rats in each group in each experiment can be seen in Figs 36 and 37. Both experiments showed that there was a greater release from Lewis aortic rings compared to DA aortic rings. Exhaustion times are different in the two sets of experiments. This was probably due to fluctuations in room or buffer temperatures therefore causing a faster rate of exhaustion.

4:2:2 Renal uptake of cationic IgG by Lewis and DA rats on a fatty acid diet

Lewis and DA rats were fed on one of five different diets for six weeks as described in the methods section 6. The renal uptake of cationic IgG was measured and the results shown in Fig 38. The shaded areas represent the uptake of cationic IgG while on a normal laboratory diet mean$\pm$2SD as established earlier (Fig 13).

Arachidonic acid enriched diets, caused a slight increase in renal uptake of cationic IgG in Lewis rats and a marked decrease in DA rats. Evening primrose oil caused an increase in uptake in Lewis rats but no change in DA rats.

The renal deposition of cationic IgG was determined by immunofluorescence and changes in deposition of rat IgG was also examined (table 14). The FITC anti human IgG was diluted 1:4 with

distilled water. At this concentration no deposits of cationic IgG could be detected in the kidneys of either DA or Lewis rats fed on a normal diet. However using a straight dilution of the antisera mesangial deposits were detected (Figs 41a and 41b). Quite dense deposits of cationic IgG were found in the glomeruli of rats fed on the arachidonic acid enriched diet (Figs 39a and 39b). They were more apparent in DA than Lewis rats. Similar, but less marked deposits, were found in the kidneys of rats fed on other diets (Figs 40a and 40b) and were least in the rats fed on eicosapentanoic acid enriched diets.

Not only was the glomerular deposition of human cationized IgG increased, despite the changes found in the whole kidney, but increased deposits of rat IgG were also found (table 14). There was close symmetry in the pattern of deposition in the two strains which were mostly mesangial with some capillary loop.

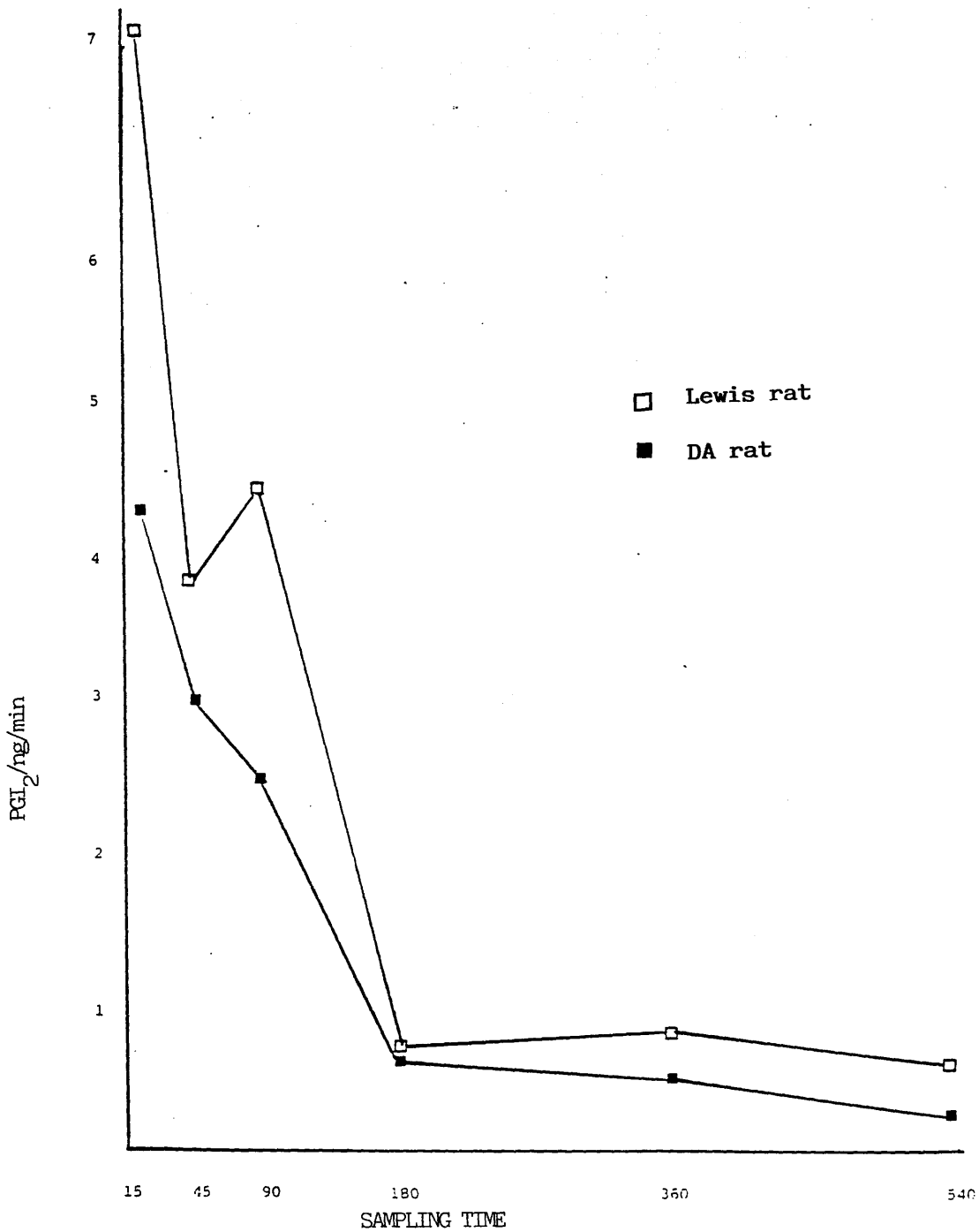


FIG 36 PGI_2 release from aortic rings. Each point is a mean of 3 experiments.

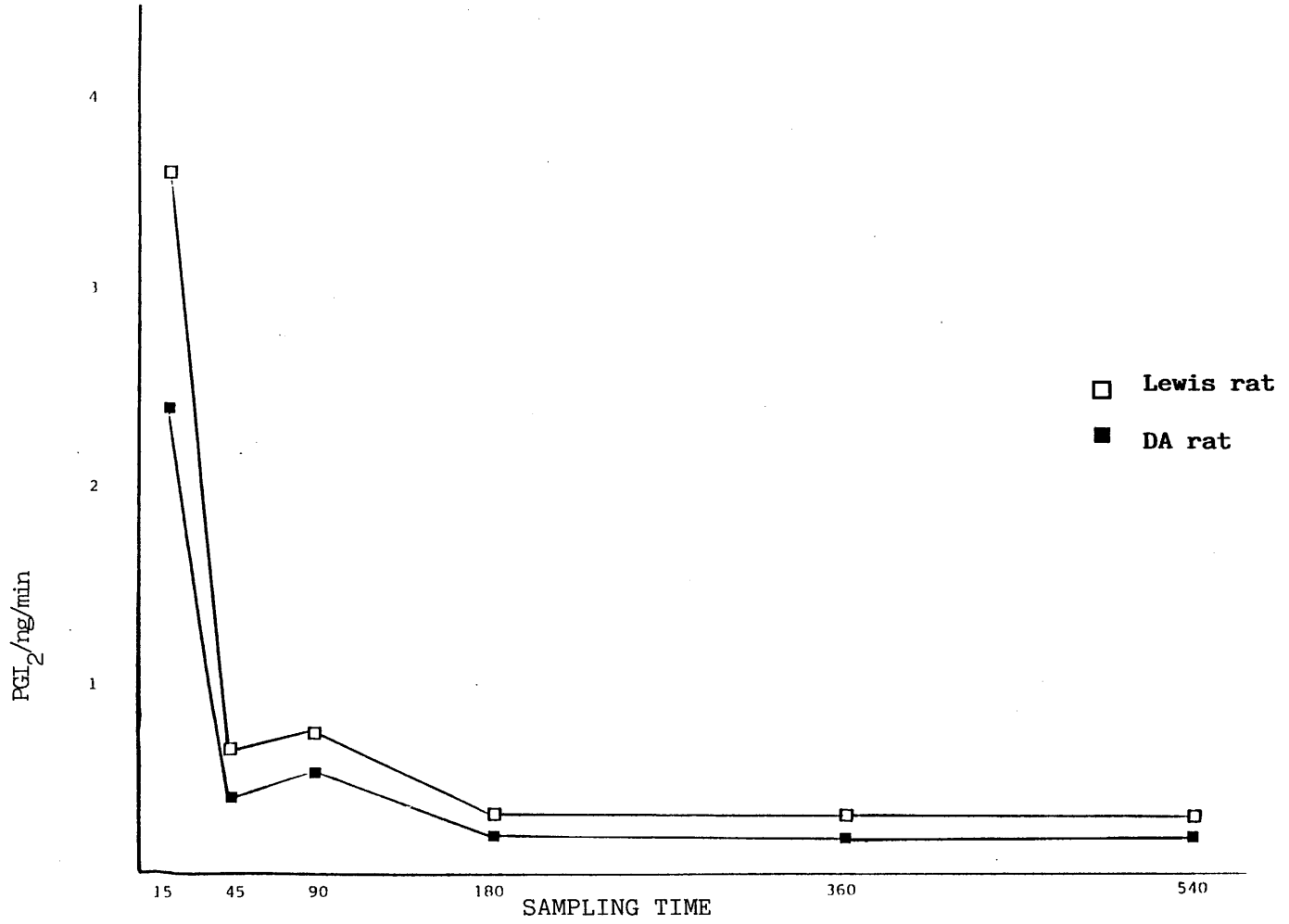


FIG 37 PGI_2 release from aortic rings. Each point is a mean of 3 experiments.

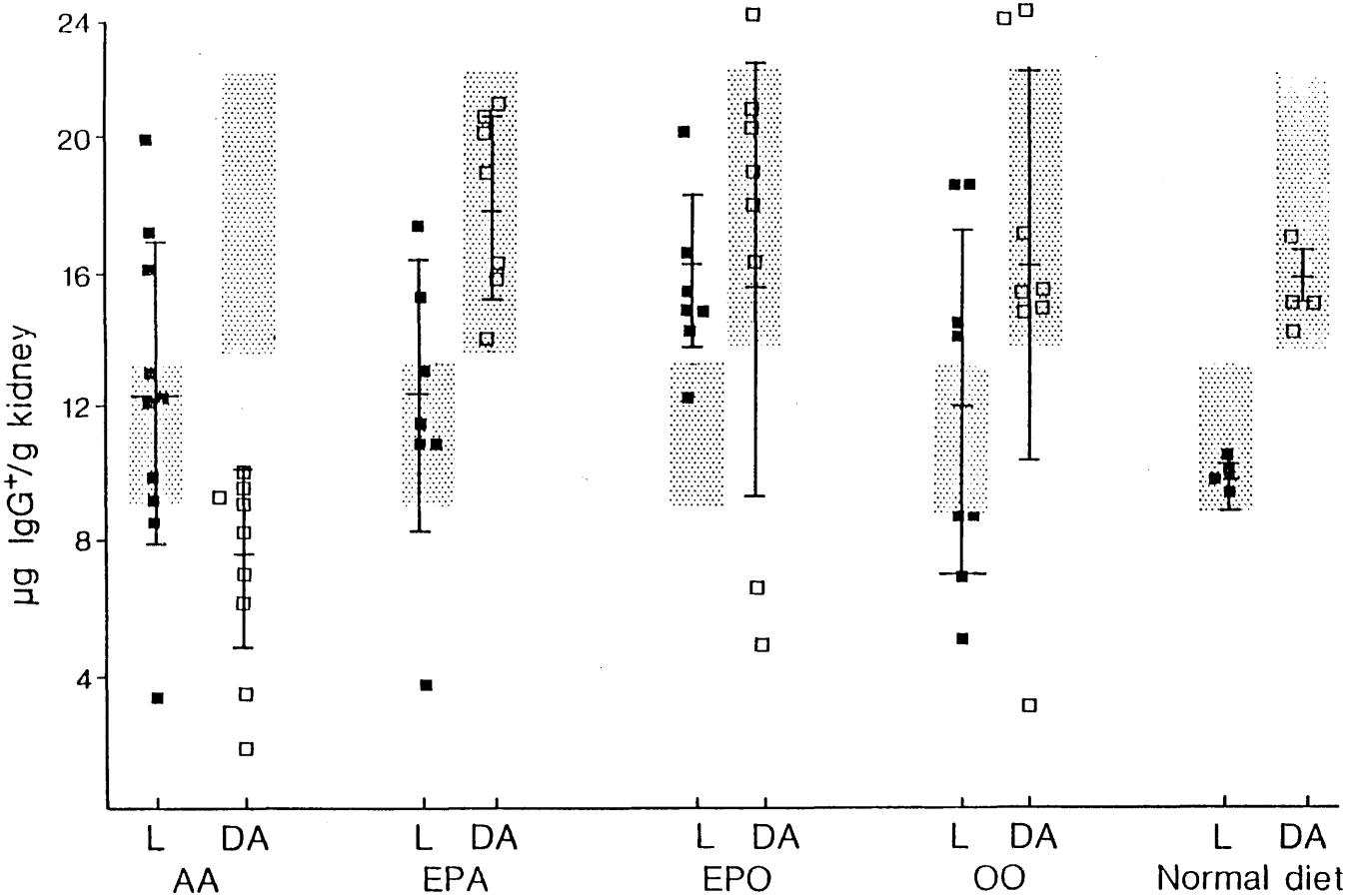


FIG 38 The renal uptake (μg/g of kidney tissue) of cationic IgG one hour after injection of 1mg cationic IgG is shown in rats fed fatty acid enriched diets for 6 weeks. Shaded areas represent the mean $\pm$ 2SD of rats on normal diets and is devised from the data given in Fig 13.

AA - Arachidonic acid
EPA - Eicosapentaenoic acid
EPO - Evening Primrose oil
OO - Olive oil

TABLE 14

GLOMERULAR LOCALISATION OF CATIONIC HUMAN IgG AND RAT IgG IN FATTY ACID DIET FED RATS

IF STUDIES

GROUP	L E W I S		D A	
	FITC ANTI-HUMAN IgG	FITC ANTI RAT IgG	FITC ANTI-HUMAN IgG	FITC ANTI RAT IgG
AA	+ cl ++ mes	+ cl + mes	++ cl +++ mes	+++ focal
EPA	+ mes + cl	++ mes	++ mes + cl	+++ mes
EPO	+ cl + mes	+ mes	++ cl + mes	++ mes
O.O	++ mes + cl	+ mes + cl	++ mes + cl	+ mes
NORMAL DIET	-ve	-ve	-ve	++ mes
CONTROL RATS	-ve	-ve	-ve	++ mes

cl = capillary loop

mes = mesangial

FIG 39a - GLOMERULAR IMMUNOFLUORESCENCE PATTERN AFTER STAINING WITH FLUORESCEINATED ANTIBODY TO HUMAN IgG (FITC anti human IgG) IN THE LEWIS STRAIN FED AN ENRICHED SAFFLOWER OIL DIET. (x350)

FIG 39b - GLOMERULAR IMMUNOFLUORESCENCE PATTERN AFTER STAINING WITH FITC ANTI HUMAN IgG IN THE DA STRAIN FED A SAFFLOWER OIL DIET. (x350)

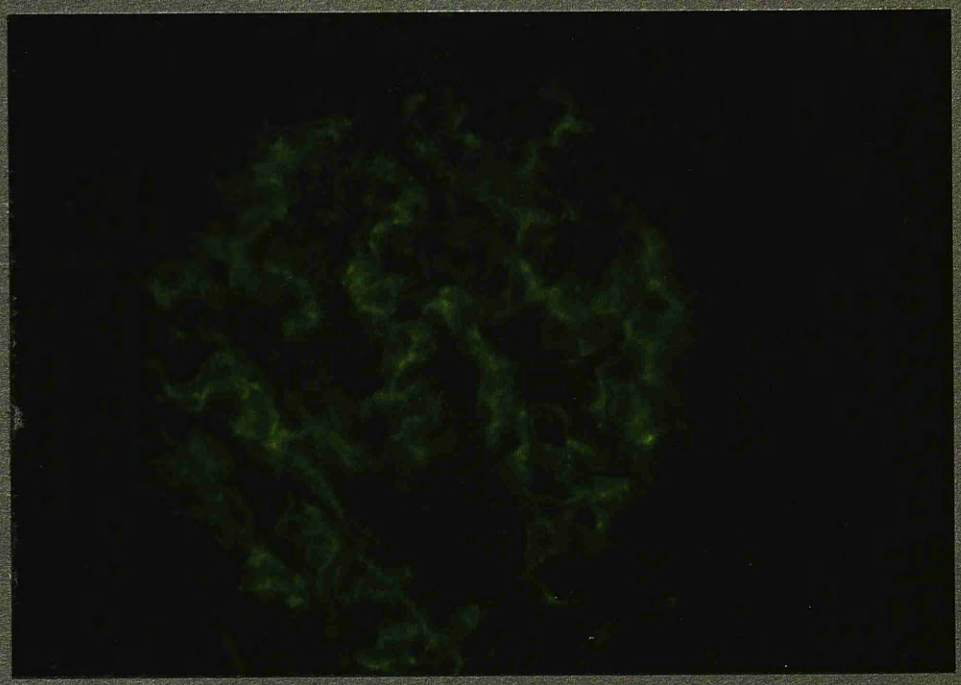


Fig39a

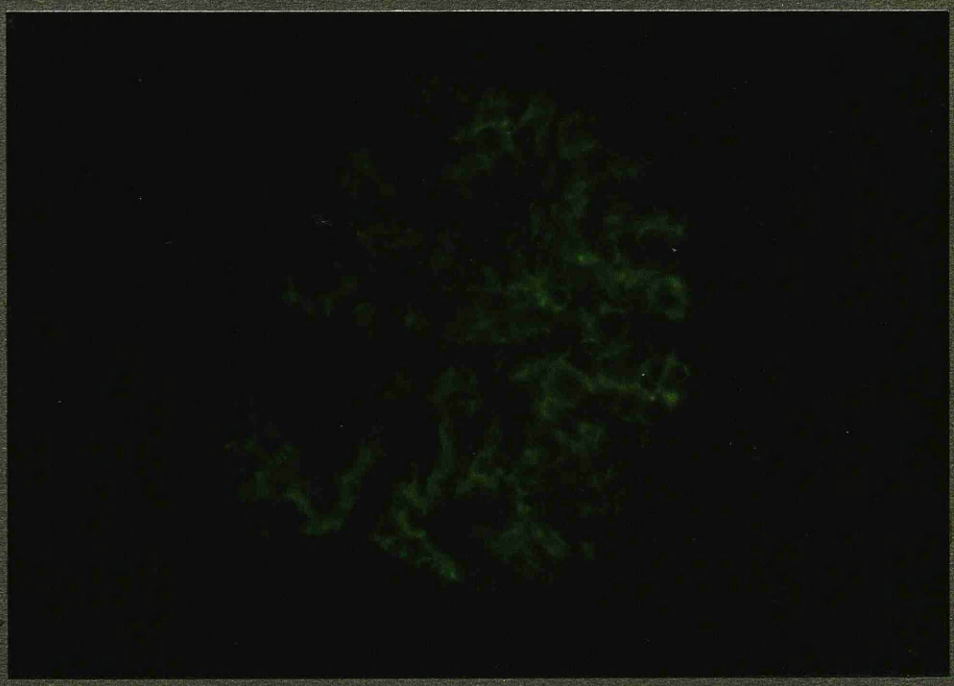


Fig39b

FIG 40a - GLOMERULAR IMMUNOFLUORESCENCE PATTERN AFTER STAINING WITH FITC ANTI HUMAN IgG IN THE LEWIS STRAIN FED AN ENRICHED EVENING PRIMROSE OIL DIET. (x350)

FIG 40b - GLOMERULAR IMMUNOFLUORESCENCE PATTERN AFTER STAINING WITH FITC ANTI HUMAN IgG IN THE DA STRAIN FED AN ENRICHED EVENING PRIMROSE OIL DIET. (x350)

Fig 40a

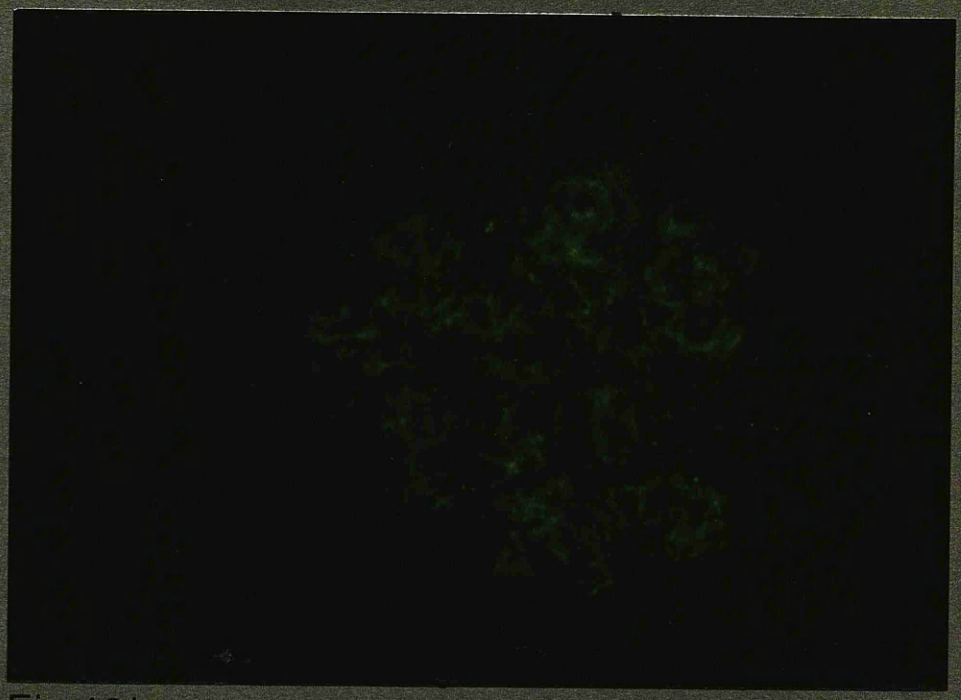


Fig 40b

FIG 41a – GLOMERULAR IMMUNOFLUORESCENCE PATTERN AFTER STAINING WITH FITC ANTI HUMAN IgG IN THE LEWIS STRAIN FED A NORMAL DIET. (x350)

FIG 41b – GLOMERULAR IMMUNOFLUORESCENCE PATTERN AFTER STAINING WITH FITC ANTI HUMAN IgG IN THE DA STRAIN FED A NORMAL DIET. (x350)

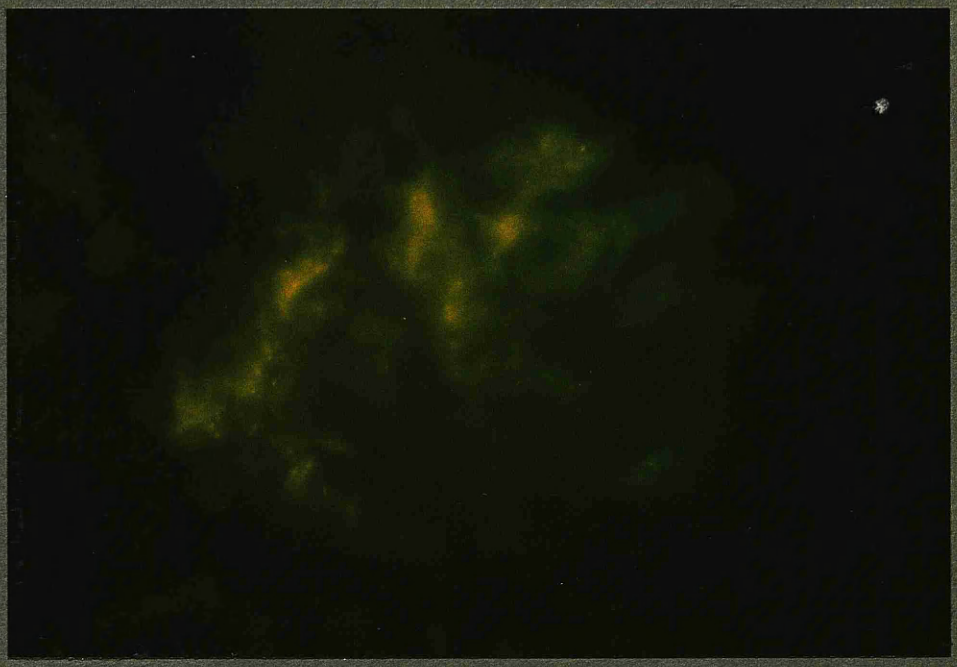


Fig41a

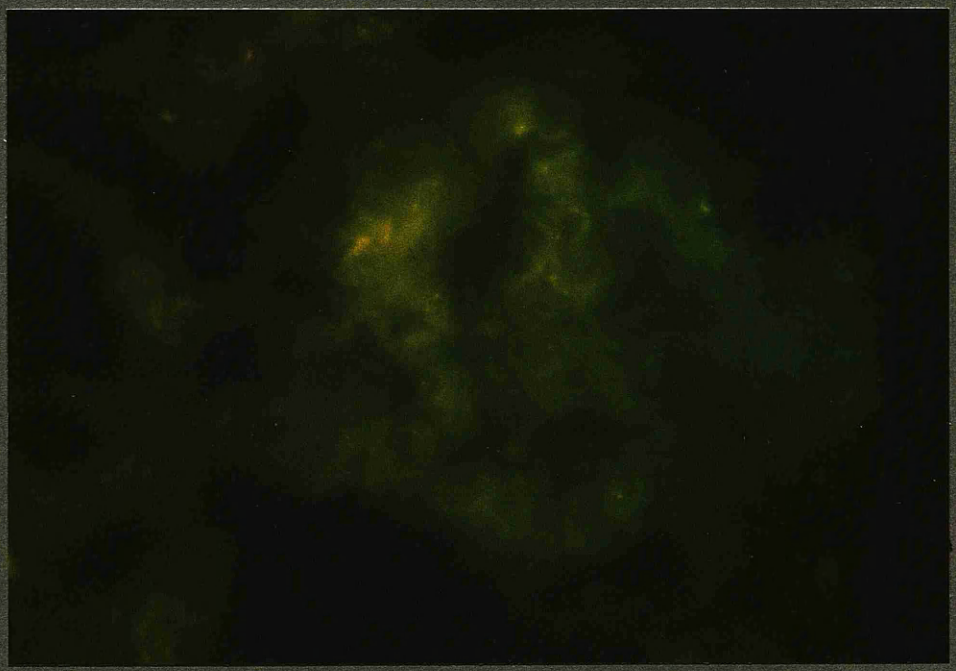


Fig 41b

4:2:3 Renal infusions of PGE_1 and PGI_2

Infusion of PGE_1 and PGI_2 by the protocol described previously did not change renal uptake of cationic IgG in Lewis rats but considerably decreased it in DA rats to approximately the same quantity as was found in Lewis rats (table 15). Blood pressure remained steady at 120 mmHg during both infusions in the 2 strains of rats.
The distribution of IgG^+ following PGE_1 or PGI_2 infusion is shown in Table 16. Glomerular immunofluorescence patterns of IgG^+ are shown in Figs 42 and 44, and Figs 43 and 45 following PGE_1 and PGI_2 infusions respectively.

RENAL UPTAKE OF CATIONIC IgG AFTER INFUSION OF PGE_1 or PGI_2

TABLE 15

INFUSATE	LEWIS	DA	LEWIS	DA
	MEAN $\mu g\ IgG^+ / g$ KIDNEY		RANGE	
GLYCINE BUFFER	13.1	20.0	8·51 - 13·67	19·5 - 23·3
PGI_2	11.1	13.2	7·80 - 12·96	10·4 - 14·8
PGE_1	12.07	14.6	10·81 - 13·70	12·88-15·80

Each value is the mean of 4 experiments

GLOMERULAR LOCALISATION OF HUMAN IgG AND RAT IgG AFTER PGE_1 OR PGI_2 INFUSION

I F STUDIES

TABLE 16

INFUSATE	LEWIS		DA	
	ANTI HUMAN IgG	ANTI RAT IgG	ANTI HUMAN IgG	ANTI RAT IgG
Buffer	weak mes	-ve	weak mes	+ mes
PGI_2	+ mes + cl	-ve	+ mes	+ mes
PGE_1	+ mes	-ve	+ mes	+ mes

mes = mesangial

FIG 42 - GLOMERULAR IMUNOFLUORESCENCE PATTERN OF HUMAN IgG IN THE LEWIS STRAIN AFTER INFUSION OF PGE_1. (x350)

FIG 43 - GLOMERULAR IMMUNOFLUORESCENCE PATTERN OF HUMAN IgG IN THE LEWIS STRAIN AFTER INFUSION OF PGI_2. (x350)

Fig 42

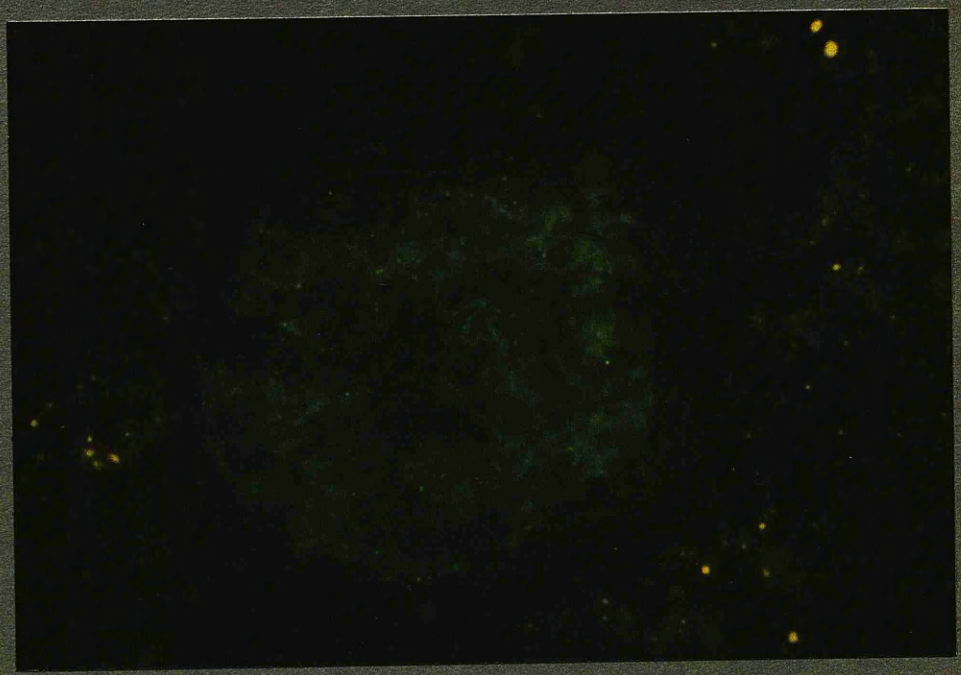


Fig 43

FIG 44 - GLOMERULAR IMMUNOFLUORESCENCE PATTERN OF HUMAN IgG IN THE DA STRAIN AFTER INFUSION OF PGE_1. (x350)

FIG 45 - GLOMERULAR IMMUNOFLUORESCENCE PATTERN OF HUMAN IgG IN THE DA STRAIN AFTER INFUSION OF PGI_2. (x350)

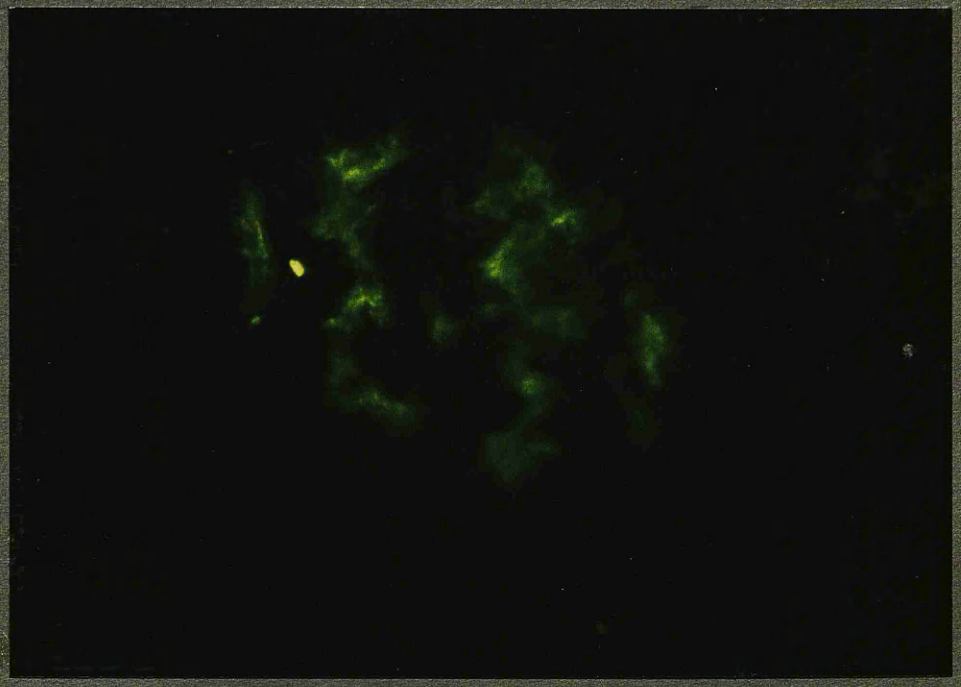


Fig 44

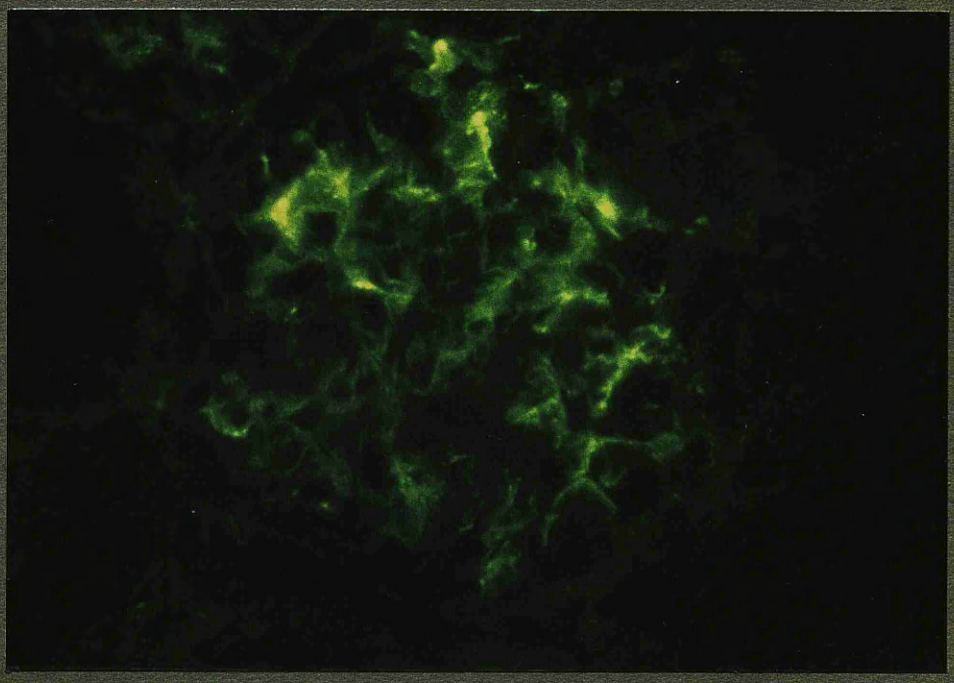


Fig 45

D I S C U S S I O N

CHAPTER 5

SECTION 5:1:1

Variations in glomerular properties

This study was designed to define genetically controlled differences in two glomerular properties which may influence the uptake of antigen and thereby affect an individual's susceptibility to some forms of glomerulonephritis.

Glomerulonephritis affects only a minority of people exposed to a particular cause such as endocarditis or penicillamine. The reasons why some individuals are susceptible are unknown. A strong inherited predisposition may be inferred by the close association with DR2 with Goodpasture's syndrome (152), DR 3 with membranous nephropathy in European patients (153) and DR4 with mesangial IgA disease in Japanese patients (154). Two strains of healthy male rats, Lewis and DA, were studied because the former is susceptible to Heymann nephritis and the latter is resistant, an observation which we have confirmed.

Cationic IgG and aggregated IgG were used as probe antigens because their deposition within the glomerulus has already been studied and is thought to depend on charge and mesangial uptake respectively (103,42). Cationic IgG was used to make a quantitative assessment of the fixed negative charge carried by glycoaminoglycans on the glomerular capillary wall. This charge is crucial in maintaining the impermeability of the glomerular basement membrane (GBM) to negatively charged molecules of the size of albumin (155) and to prevent clogging of the filtering surface by large negatively charged molecules (43). It is lost, or reduced, in most animal (156,157) and human nephropathies (158) associated with proteinuria and infusion of a polycation into the renal artery has been shown to cause proteinuria .

Therefore, inherited differences in the quantity of negative charges on the glomerular capillary wall may be an important determinant of the renal response to disease.

The renal uptake of cationic IgG by Lewis rats was significantly less than the renal uptake by DA rats at all times from 15 minutes to 24 hours (Fig 13). Native IgG (IgG^n) was used as a non-specific marker to eliminate differences in renal blood flow to or within the kidney between the strains so that the difference in uptake of IgG^+ and IgG^n is due to charge alone. Although the IgG^n uptake was also greater in DA rats, the difference (IgG^+ - IgG^n) confirmed that the specific uptake of IgG^+, due to its charge,was greater in DA rats. The specific uptake in Lewis rats was negligible as determined by this technique. However, this value may be falsely low as the clearance of IgG^+ due to charge on red cells and vascular endothelium was greater than that of IgG^n. The difference in renal uptake of IgG^n between strains, could be due to an increased uptake of IgG^n by the mesangium of DA rats. Previous studies have shown that cationic IgG deposits principally in the GBM (103). This was confirmed by immunofluorescent studies which showed a weaker GBM uptake in Lewis compared to DA rats. However, glomeruli were not separated to quantify precise differences.

Infusions of IgG^+ directly into the renal arteries of temporarily isolated left kidneys confirmed the difference between strains and showed that it was not due to a differential uptake of IgG^+ by other tissues. In fact, the specific uptake of IgG^+ by liver and spleen was also greater in DA rats, which implies that other cell surfaces of DA rats possess greater numbers of negatively charged glycoproteins. It is interesting that the difference was proportionally greater when the lowest dose of antigen (IgG^+) was used, whether given intravenously or into the renal artery.

Since antigen probably circulates in very low concentrations in clinical states, this observation may account for variation and susceptibility to some nephropathies. Another finding of clinical interest is that the charge on the GBM of male Lewis rats was less than that of female. The difference between sexes was not so marked for DA rats. Lewis rats are susceptible to Heymann nephritis of which membranous nephropathy is the equivalent in man which has a distinct male preponderance - as high as 5.3:1 in patients of Glasgow Royal Infirmary (154). However, it is not clear how, or if, a reduced charge predisposes to membranous nephropathy.

The second glomerular property examined in this study was the renal uptake of aggregated IgG. Previous studies have shown that a small proportion of intravenously administered aggregated IgG is deposited in the mesangium and this has been used as a test of mesangial uptake (42). Large molecules such as aggregated IgG enter the mesangium, through that part of the capillary where endothelial cells are in direct contact with mesangial cells or matrix because the GBM, which surrounds the rest of the capillary, is reflected onto adjacent capillaries (Fig 4). Studies using tracer molecules such as imposil have shown that mesangial traffic passes down the mesangial stalk to the hilum of the glomerulus. Thereafter, its route is unknown; it may enter the distal tubule, the distal arteriole, or more likely the renal lymphatic system. Alternatively, it may be degraded in the mesangium itself (160). One of the main functions of the mesangium is to remove debris from the capillary walls (43), as was shown by the removal of both IgG^{+} and aggregated IgG from the capillary loops into the mesangium in the immunofluorescent studies. It is interesting that IgG^{+} which is apparently bound to fixed negative

charges in the GBM is transferred to the mesangium at the same rate, as aggregated IgG (at least within the experimental limitations of this study). The reasons for this are not clear. The rate at which antigen is transferred from the capillary loop into the mesangium may be slowed by antibody binding to it while it is on the capillary wall (104). Therefore, slow transfer is more likely to lead to membranous deposits and quick transfer to mesangial deposits.

Whole kidney uptake of aggregated IgG was greater in DA than Lewis rats but the relative difference was not dose dependant (Figs 17 and 18). Immunofluorescent studies showed that aggregated IgG was on the GBM at 15 minutes and in the mesangium at 1 hour and was denser in the DA rat. It is probable that the increase in mesangial uptake of IgG^n seen in DA rats was also due to an increased mesangial uptake in that strain. However, it proved difficult to identify it by immunofluorescent studies presumably because it was not bound and was easily washed off. Traces of IgG^n were found in the mesangium when the slides were not washed before fixing.

Therefore, it has been shown that the glomeruli of healthy male Lewis and DA rats differ in that Lewis rats have a reduced functional glomerular capillary loop charge and a lesser mesangial uptake under basal conditions. However, the studies have not shown whether these properties are separate or interdependant. Nonetheless, these differences may be important factors in determining the susceptibility of the 2 strains of rat to various forms of glomerular injury.

CHAPTER 5

SECTION 5:1:2

Consequences of variations in glomerular properties in response to animal models of glomerular injury

The significance of the difference in glomerular properties between the two strains was examined by comparing their response to four models of glomerular injury. These were:

(a) Heymann nephropathy in order to confirm the reason for the original choice of the 2 strains.

(b) Chronic serum sickness induced by cationic HSA.

(c) Infusion of hexadimethrine (HDM) into the renal artery.

(d) Puromycin aminonucleoside nephropathy (PAN)

Heymann nephritis (Autologous immune complex nephritis)

The two strains were chosen because Lewis rats are susceptible to Heymann nephritis (HN) and DA rats are not. Other work has shown that strains of rats which are susceptible to HN share a particular MHC gene. It was shown that the Lewis rat carried the ($H - I^1$) histocompatibility haplotype and were highly susceptible, whereas DA rats which carried the ($H - I^a$) were resistant. Strains which shared the Lewis strain genetic background ($H - I^1$) were weakly susceptible to HN (161).

Heymann nephritis is the animal model of idiopathic membranous nephropathy and is characterised by subepithelial immune complexes made up of antigen, and autologous antibody to it (97,99). Characterisation of the antigen from brush border membranes of renal tubules showed that it consisted of a large glycoprotein that had a molecular weight of approximately 600,000 and was highly nephritogenic for the induction of HN (100).

It is thought that this antigen may be a constituent part of the epithelial cell membrane, however this remains controversial. Using the passive Heymann nephritis model (PHN), immune complexes were detected in the kidney after perfusion of antibody into the renal artery of an isolated kidney. As a single by-pass system was used to prevent the possibility of deposition of circulating immune complexes, it was hypothesised that the immune complexes were formed in situ with the antigen being an intrinsic part of the GBM (98). On the other hand it is possible that the antigen circulates in very low concentration and may be deposited in that site because of its physical properties. If the properties of the kidney are altered by infusion of puromycin aminonucleoside (PA) into one renal artery in such a way as to prevent systemic spillover, and the rat is then immunised with FX1A; then the complexes form in the mesangium, until the proteinuria induced by PAN disappears and thereafter the complexes are formed in the capillary loop. The unperfused kidney develops membranous complexes. Thus, this experimental data suggests that it is unlikely that the antigen is a constituent part of the epithelial cell membrane and altering the glomerular properties changes the site of antigen deposition (162).

The results shown in fig 27 are characteristic of active Heymann nephropathy. Proteinuria developed in Lewis rats 12 weeks after immunisation and when the animals were sacrificed at 30 weeks dense deposits, typical of immune complexes, were found in the epithelial side of the capillary loop. No proteinuria and no deposits were found in DA rats.

The difference in susceptibility of the two strains may occur for several reasons. The antigen may be absent in DA rats, or they may remain tolerant to it. It is also possible that the complexes do form but are removed because of the difference of glomerular properties.

This was not explored further, but instead other animal nephropathies were examined.

Chronic serum sickness

A model of chronic serum sickness described by Border et al (103) who demonstrated that repeated immunisation with cationic BSA caused a membranous nephropathy in rabbits, whereas native BSA led to the deposition or formation of mesangial immune complexes, was used. They had previously performed the same experiments in Sprague-Dawley rats, all of which developed a mesangial nephropathy whether the antigen was cationic or not (124). They hypothesised that the cationic charge of the antigen was important in its binding to the glomerular basement membrane. Oite reported that antibody fixing to antigen on the GBM delayed its transfer into the mesangium (104). Therefore, if Border's hypothesis that charge is an important factor in localising the antigen is correct, then the DA strain having the greater charge on the GBM should develop capillary loop deposits when challenged with a cationic antigen. But, if the rate of transfer from the capillary loop to the mesangium is more important and if this is a function of mesangial uptake, then the DA strain should develop mesangial lesions. In the event, Lewis rats with a lower charge and lesser mesangial uptake of cationic IgG developed membranous lesions and DA rats with a greater charge and greater mesangial uptake had mesangial lesions. It is of interest that similar mesangial deposits of IgG were found in all control DA rats. No deposits of HSA could be detected by immunofluorescence in either strain. This experiment suggests that the rate of transfer to the mesangium is important in determining the site at which complexes form.

Infusion of hexadimethrine

In order to test the significance of the reduction in negatively charged sites on the GBM of Lewis rats a polycation was infused intravenously. Hunsicker et al had shown the intravenous infusion of Hexadimethrine into rats caused fusion of epithelial foot processes and proteinuria and attributed this to masking of the glomerular polyanion (117).

Intravenous infusions of hexadimethrine caused proteinuria in Lewis but not DA rats confirming the significance of the the reduction in negative charge in the Lewis rat. Therefore, Lewis rats are more likely to develop proteinuria as a result of any mechanism which leads to the abolition or masking of charge.

This hypothesis was supported by the finding that intravenous injection of cationic IgG caused a greater loss of radiolabelled HSA into the urine in Lewis compared to DA rats (table 10). In order to assess how quickly this change in glomerular permeability occurred, HSA^n was given 15 minutes before sacrifice and the renal uptake of HSA (HSA^n) measured. The increase in uptake of HSA^n following IgG^+ given at various times before sacrifice (HSA_c - HSA_s) is an index of glomerular capillary permeability. The results (table 12) showed that an increase in uptake of HSA^n did not occur at 15 minutes, but had occurred by 1 hour in Lewis rats and persisted for 24 hours. No increase in uptake of HSA occurred in DA rats until three hours and even then it was minimal. It is interesting to note the time similarity between this experiment and the HDM infusion in which proteinuria started 40 minutes after the start of the infusion. However, in the former case it was not proven that increase in HSA uptake by the kidney was due to HSA crossing of the GBM, but no other explanation seems probable.

These three experimental models which either caused proteinuria or increased uptake of HSA after infusion of a cation, again suggests that a decreased charge on the GBM of Lewis rats is of biological importance in disease.

Infusion of puromycin aminonucleoside (PA)

The final animal model studied was that of puromycin aminonucleoside nephrosis of which the pathogenesis is not understood. Experimental evidence has shown that PAN causes proteinuria in rats by a direct effect on the kidney and takes five days to do so; therefore, suggesting that it is not a direct effect on the charge, but more likely a change in the metabolism of the glomerulus, perhaps by causing a decreased production in glycoaminoglycans or production of a local proteinuric factor. Proteinuria was transferred by transplantation of the kidney from rats with established proteinuria, to normal rats which had not received puromycin aminonucleoside thus confirming that this substance has a direct effect on the kidneys and does not involve systemic factors (163).

DA rats developed twice as much proteinuria compared to Lewis rats (Fig 35). Previous experiments have shown that DA rats have an increased mesangial uptake of circulating proteins and other molecules compared with Lewis rats and this may be the reason for the increased proteinuria in DA rats. An increased uptake of PA by mesangial cells would lead to a greater dose of PA per glomerulus and greater proteinuria. Since the actual mechanism of PAN is not known the significance of these experiments is difficult to interpret. However, it is interesting that there is a variation of such magnitude between the two strains.

SECTION 5:1:3

CLINICAL APPLICATION OF ANIMAL STUDIES

Variation in charge on red cells in patients subject to various human glomerulopathies

In this study, quantitative analysis examination of the negative charge on the surface of red cells was undertaken in three groups of patients, while their nephropathy was in complete or partial remission Previous studies had shown a correlation between the red cell charge and the charge of the glomerular capillary wall (GCW) in the two strains of rat. The glomerular polyanion maintains the impermeability of the GCW to negatively charged molecules the size of albumin and it is reduced in almost all animal and human nephropathies associated with proteinuria. The red cell charge may be reduced by the same processes which lead to loss of glomerular polyanion, as was shown by Levin, who demonstrated a reduction in children during the nephrotic phase of minimal change nephropathy. To determine the charge unaffected by the disease process, patients were studied when in complete or partial remission as defined by a 24 hour urine protein excretion of less than 2.2g. The results showed that there was a significant difference in the surface charge of red cells of patients with IgAN compared with that of red cells of patients with MCN or IMN. The values for the three patient groups all fell within the normal control range. These results provide indirect evidence for a similarity between the kidneys of patients with IMN and Lewis rats. Both have a lower red cell charge than patients with IgAN or DA rats respectively; both are liable to form capillary loop immune complexes and to develop heavy proteinuria. Therefore, it is probable that patients with IMN and MCN have a lesser charge on their glomerular capillary wall than patients with IgAN and this is the probable cause

of their susceptibility to proteinuria. The pathogenesis of proteinuria in MCN is thought to result from an electrochemical change of the glomerular cell wall perhaps caused by release of a factor which increases vascular permeability (164). Lymphocytes of patients with IgAN, release as much vascular permeability factor as do lymphocytes of patients with MCN, but proteinuria may be less because of denser polyanion on their glomerular cell wall. Another consequence of the difference in charge on cell and membrane surfaces may be at the site at which antigen deposits. The site at which antigen is fixed by antibody in the kidney has been shown to depend on the physical properties of the antigen. Equally important and not previously considered are the properties of the glomeruli themselves. As previously discussed one antigen has been shown to cause membranous deposits in one kidney and mesangial deposits in the other if the properties of the latter have been changed by infusion of puromycin aminonucleoside (162).

The reason why Lewis rats are susceptible to membranous nephropathy remains unclear. Lewis rats differ from DA rats not only in the charge of their red blood cells and glomerular capillary wall but also in their mesangial uptake, which is the mechanism which maintains the glomerular barrier free of filtration residues. A reduced mesangial uptake, may delay the removal of antigen and thus lead to the formation of capillary loop immune complexes, whereas a greater mesangial uptake would lead to mesangial complex formation. This would imply a link between reduced charge and reduced mesangial uptake. If charge indirectly controls mesangial uptake, then modulations of mesangial uptake may offer a new approach to the therapy of these nephropathies.

CHAPTER 5

SECTION 5:1:4

Modulation of glomerular properties by prostaglandins

Inherited glomerular properties have been shown to influence glomerular response to different injuries. Lewis rats, with a lower capillary wall charge and lower mesangial uptake, are prone to the formation of membranous immune complexes and heavy proteinuria. On the other hand DA rats are prone to mesangial complexes and resistant to the development of proteinuria. Differences in charge may not offer a therapeutic opportunity although mesangial uptake has been shown to vary. It is possible that a lower charge, if present on platelets and vascular endothelium, may predispose to more collisions between the two and thus cause release of more prostacyclin (PGI_2) to prevent platelet aggregation and endothelial damage. This possibility was supported by the increased rate of release of PGI_2 by aortic rings of normal Lewis rats compared with DA rats. It was proposed that increased release of PGI_2 would lead to reduced mesangial uptake. This hypothesis would associate low charge with reduced mesangial uptake. Mesangial uptake is increased in puromycin aminonucleoside nephrosis (PAN), nephrotoxic serum nephritis both of which are associated with loss of glomerular cell wall charge and by angiotensin II (147,165,174). Angiotensin II has been shown to increase mesangial uptake of an intravenously administered molecule, keyhole-limpet haemocyanin (78). All these factors also lead to mesangial cell contraction, which may be the mechanism by which mesangial cell uptake is increased. The measurements of mesangial uptake made in the study however, do not discriminate between rate of removal from the capillary loop into the mesangium and the rate of removal from the mesangium.

Human glomeruli synthetize mainly $PGI_2$and also some TxB_2, PGE_1 and

$PGF_{2\alpha}$ (166,167). On the other hand, the principal prostaglandin produced by the glomeruli of the Sprague Dawley rat is PGE_1 (168). Human mesangial cells produce predominantly PGI_2 in culture (169) similar to the isolated human glomerulus. The physiological significance of prostaglandins produced by the glomerulus is uncertain. One hypothesis is that they have an important role in autoregulation of renal blood flow and in compensatory mechanisms following renal damage. The effect on mesangial uptake is unknown but PGI_2, PGE_1 cause mesangial cell contraction through increased synthesis of angiotensin II (AII)(170). Beierwaltes et al exposed cultures of isolated glomeruli to arachidonic acid enriched media and noted not only a marked increase in prostaglandin synthesis, but also a significant release of renin demonstrating a direct local prostaglandin-renin interaction within the glomerulus (171). Therefore, an increase in glomerular prostaglandin production leads to an increase in angiotensin II synthesis, which in turn increases mesangial uptake perhaps by mesangial contraction. Infused 3H AII localises primarily in the mesangial region of rat glomeruli, and specific receptors for AII on mesangial cells have been identified (172). AII causes contraction of cultured glomerular mesangial cells in vitro (173) and AII infusion in vivo results in a lowering of the glomerular capillary ultrafilatration coefficient (174).

Prostaglandins have also been used in the treatment of some forms of glomerulonephritis. Administration of PGE_1 has been shown to ameliorate the evolution of the nephropathies in NZB/W and MRL/1 mice. The mechanism by which this is achieved is unknown, but has been ascribed to the effect of PGE_1 on the immune response (135). A thromboxane synthetase inhibitor was shown to greatly reduce the proteinuria caused by adriamycin (175). Recent reports have demonstrated beneficial effects of dietary polyunsaturated fatty acids

on immune complex forms of glomerulonephritis. A menhaden oil diet, rich in eicosapentaenoic acid, protected female NZB/WF_1 mice from autoimmune disease. Injections of pharmacological doses of prostaglandins have been shown to have beneficial effects in various models of renal disease. Intravenous infusion of PGE_1 improved renal function in patients with chronic renal failure by increasing creatinine clearance by 29%. The benefit seem to have been sustained for 1.5-7 months after the course of the PGE_1 infusions. There was no significant change in 24 hour urinary protein excretion. It was hypothesised that the improvement in renal function with PGE_1 may be related to its vasodilatory action (176).

Studies were performed to examine the effect on renal uptake of cationic IgG either by direct infusion of PGI_2 or PGE_1 or by an enriched fatty acid diet. The rationale for using cationic IgG was that it had already been shown that it bound to the GBM at 15 minutes and by 1 hour had begun to move into the mesangium and its site of deposition could be assessed by immunofluorescence. Therefore, the renal uptake at 1 hour is a measure of mesangial uptake. For these reasons the rats were sacrificed at 1 hour after intravenous injection of cationic IgG to measure variation of mesangial uptake. The polyunsaturated fatty acids (PUFA) derivatives used in the study are precursors of oleic, linoleic and eicosapentaenoic acids, which in turn are precursors of the mono (177) di (178) and trienoic (179) prostaglandins respectively (Fig 46). Fig 38 shows the renal uptake of cationic IgG in rats fed on one of four fatty acids enriched diets. Arachidonic acid caused a marked decrease in renal uptake in DA rats which remained unchanged in Lewis rats. This may be the result of the higher PGI_2 production in normal Lewis rats. No consistent change of renal uptake occurred in either strain fed on the enriched EPA

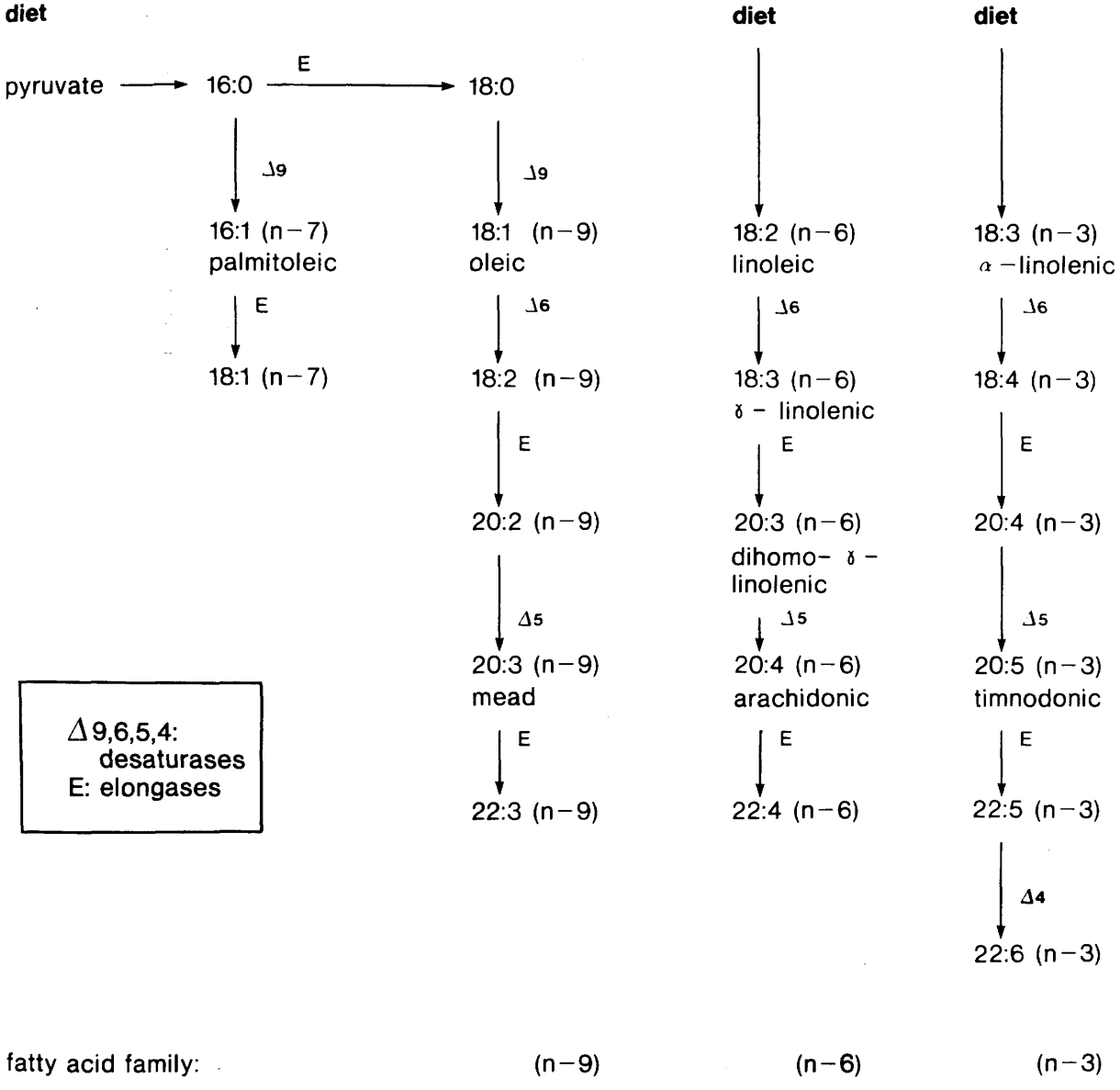


FIG 46 - MAJOR BIOSYNTHETIC PATHWAYS OF FATTY ACIDS

diet. Diets enriched with EPO caused an increased renal uptake in Lewis rats but no change in DA rats. Direct infusion of PGI_2 or PGE_1 caused a decreased renal uptake in DA rats but no change in Lewis rats (ie) comparable to results with AA enriched diet (table 15) Interestingly, immunofluorescent studies showed that glomerular uptake, particularly mesangial uptake, increased with all diets and also PGE_1 and PGI_2 infusions. This discrepancy between total kidney uptake and increased glomerular uptake of cationic IgG is not understood. Infusion of PGE_1 caused a marked increase in mesangial deposition of antigen, but following PGI_2 infusion, IgG^+ was deposited both in the mesangium and around the capillary loops. This difference occurrred in both Lewis and DA rats. The role of the higher production of PGI_2 by the vascular endothlelium of Lewis rats in their susceptibility to capillary loop immune complex formation has not yet been investigated further.

Prostaglandins including PGI_2 and PGE_1, have been shown to enhance glomerular monocyclic nucleotide production (170) which in turn is capable of stimulating renin release and angiotensin II production which is known to increase mesangial uptake. Therefore, it seems likely that the increase in mesangial uptake is due to the ability of prostaglandins to stimulate local intrarenal angiotensin II production via the generation of cAMP and thus cause mesangial contraction. How and if mesangial contraction leads to increased mesangial uptake remains contoversial. Ward et al suggested that increased mesangial uptake may be due to an increased percolation of plasma through mesangial intercellular channels. Infusions of high doses of angiotensin II have been shown to enlarge the intercellular channels in the mesangium (180). This may be a direct effect of mesangial cell contraction. Whatever the mechanism, the results in this study show

that infusion of prostaglandins clearly enhances mesangial localisation of antigen. It remains to be determined whether this result can be extrapolated to infer that kidneys subjected to the intraglomerular effects, that is increased mesangial uptake, might be prone to develop cumulative mesangial localisation of high molecular weight materials naturally present in the circulation as was seen in normal healthy DA rats. If so, the rate of progression of glomerular lesions in any of the human or animal diseases characterised by prominent mesangial accumulation of abnormal materials such as diabetic nephropathy, may be susceptible to modulation by angiotensin II, prostaglandins and their precursors or antagonists. Some support for this comes from the study by Hamazaki (181). Twenty patients with IgA nephropathy were divided into two groups, one group treated with fish oil (1.6g EPA) for one year and the other was not. After one year, renal function had not changed significantly in the EPA group, although, the controls had significantly deteriorated. It was hypothesised that EPA might be a safe and useful agent to stop the progression of IgA nephropathy. Prostanoids may, therefore, justify further investigation to evaluate their role in modulating mesangial flow.

CONCLUSION

The results of the experiments described in this thesis would suggest a new hypothesis in the pathogenesis of immune complex glomerulonephritis. Earlier hypotheses had postulated that the kidney was an innocent victim due to the high hydrostatic pressure of its' capillary bed. Failure to eliminate antigen was an indication of immune deficiency which was thought to be a precondition for the persistence of circulating immune complexes. It was later shown that many forms of animal glomerulonephritis could be produced by in situ immune complex formation and that the site of deposition of the antigen depended on its' properties.

In this thesis it has been shown that glomeruli possess properties which vary between two strains of rat. These properties may account for differences in the expression of various forms of experimental glomerular injury. Low charge on the glomerular capillary wall was associated with low mesangial uptake and high charge associated with high mesangial uptake. Experiments have yet to be performed to see if there is a causal link between the two properties.

Mesangial uptake can be modulated by a variety of stimuli including the prostanoids, as was demonstrated in this thesis. Mesangial uptake is a result of movement of molecules into the mesangium from the capillary loop and degradation or removal from the mesangium. If the movement of complexes into the mesangium could be enhanced this might be of benefit to patients with capillary loop deposits. Similarly, if the removal of complexes from the mesangium could be accelerated, this may be useful in the treatment of patients with mesangial immune complex diseases. Indirect evidence that the differences found

between Lewis and DA rats, may resemble those between patients with Idiopathic membranous nephropathy and IgA nephropathy (provided by the studies of the red blood cell membrane charges) suggests that such investigation would be relevant in man.

APPENDIX

Buffers

Standard dialysis buffer (working buffer)

17.55g Sodium chloride (NaCl)

0.40g Ethylene diaminetetra-acetic acid (EDTA)

Made up to 2 litres with distilled water - pH 8.6.

Buffer A - (protein modification buffer)

3.28g. di sodium hydrogen phosphate (Na_2HPO_4)

8.77g Sodium chloride (NaCl)

Made up to 1 litre with distilled water - pH 5.5.

Isoelectric focusing reagents

Fixing solution

5% Sulphosalicylic acid

10% Trichloroacetic acid

in distilled water.

Destaining solution

35% Ethanol

10% Acetic acid

in distilled water.

Staining solution

0.2% coomassie blue

disolved in destaining solution.

PROTEIN ASSAYS

Urine total protein using the Rice method

Principle Protein is precipitated from urine with phosphotungstic acid and assayed using the Biuret reagent.

Sensitivity The limit of detection is 3mg/100ml and the standard curve is linear to 200mg/100ml.

REAGENTS

1	Tsuchiya's reagent	To 365ml absolute alcohol in a 500ml beaker, add 10ml deionised water and 25ml conc hydrochloric acid. Dissolve 7.5g phosphotungstic acid in this solution.
2	Biuret reagent	1.5g $CuSO_4$ $5H_2O$ (AR) are dissolved in approximately 500ml deionised water followed by 6.0g Sodium Potassium Tartrate (AR). Add with stirring 30g NaOH (AR). Dilute to 1 litre.
3	Blank reagent	As above omitting 1.5g $CuSO_4$ $5H_2O$.

STANDARDS

Prepare standards 10-200mg/100ml using Human albumin fraction V (Miles laboratories) suitably diluted with 0.9% NaCL.

METHOD

Test the urine with Labstix and dilute as necessary with saline. Label pairs of conical centrifuge tubes for Test (T) and Blank (B). Pipette 2ml of urine samples and standards into appropriate pairs of

tubes. Add 2ml Tsuchiya's reagent to all tubes with shaking, mix and incubate at 56 ^{o}C in water bath for 15 minutes. Centrifuge at 2500rpm for 10 minutes and aspirate off supernatant. Add 1ml absolute ethanol to all tubes and mix on a whirlimixer. Centrifuge for 5 minutes and remove supernatant. Wash again with 1ml ethanol and remove supernatant. To all tubes labelled 'T' add 4ml Biuret reagent and 4ml of blank reagent to all tubes labelled 'B! Ensure all precipitate is dissolved by mixing on a whirlimixer. Leave for 30 minutes for full colour development. Read absorbance at 540nm. Blanks are read against Blank reagent and tests against Biuret reagent. Prepare a standard curve of corrected absorbance against concentration. Report results to 2 decimal places, ie g/vol.

LOWRY PROTEIN ASSAY

Principle

This method of protein assay combines the Biuret reaction and the Folin ciocalteu reaction. In the Biuret reaction, a solution of protein is treated with Cu^{++} ions in a moderately alkaline medium and a blue coloured chelate complex is formed between the Cu^{++} ion and the carbonyl and imine groups of the peptid bonds. Folin ciocalteu reagent is used to measure phenolic compounds and to assay Tyrosine, which has a phenol side chain. This reagent oxidises the phenolic compounds, itself being reduced from yellow to blue.

The Lowry reaction is about 100 times as sensitive as the Biuret method, but a number of buffers containing amino groups, or substituted amino groups (eg TRIS, glycine amide), interfere by producing a colour.

REAGENTS

1 Stock reagents

i) Solution A 2% Na_2CO_3 in 0.1N NaOH

ii) Solution B 2% Na K Tartrate in deionized water

iii) Solution C 1% Cu SO_4 in deionized water

iv) Folin ciocalteu reagent.

2 Working reagents

i) Biuret reagent:- To 100mls solution A, add 1ml solution B and 1ml solution C.

ii) Working Folin ciocalteu reagent:- dilute the stock reagent 1:1 with deionized water.

STANDARDS

i) Stock standard - Human serum albumin - 1mg/ml.
Store at -15 °C.

ii) Working standards

A working standard curve in the range 0-100ug is prepared by taking 0, 50, 75 and 100µl of stock standard and adjusting the final volume to 400µl with deionized water as shown in Table 1.

TABLE 1

Volume of stock standard (µl)	Volume of deionized H_2O (µl)	Final protein concentration (µg)
-	400	-
25	375	25
50	350	50
75	325	75
100	300	100

Sample preparation

Any sample requiring dilution should be diluted using deionized water and the final volume adjusted to 400ul.

Quality control

Bovine serum albumin, 0.6mg/ml. 100ul of the control is taken and the volume adjusted to 400µl with deionized water.

Method

i) To 400µl of standard, control and sample, add 2mls Biuret reagent.

ii) Vortex and stand for 10 minutes.

iii) Add 200µl of working folin ciocalteu reagent.

iv) Vortex and stand for 30 minutes.

v) Read OD at 750nm.

vi) Plot OD_{750} vs concentration of standards. Read unknowns from the standard curve.

REFERENCES

1 Gabriel, R (1981).
Renal Anatomy and Physiology.
In: Renal Medicine.
Bailliere Tindal, Ch2, 3-23

2 Braus, H (1924).
In: Anatomie des Menschen.
Springer-Verlag Berlin.

3 Rouillier, C (1969).
General anatomy and histology of the kidney.
In: The Kidney, Morphology, Biochemistry, Physiology.
ed. Rouillier, C and Muller, A F, Vol 1.

4 De Wardener, H E (1985).
Structure of the kidney.
In: The Kidney.
Churchill Livingston Ch1, 1-12

5 Farquhar, M G (1981).
The glomerular basement membrane.
In: Cell Biology of Extracellular Matrix.
ed. Hay, E, Ch11, 335-378.

6 Latta, H, Maunsbach, A B and Madden S C (1960).
The centrolobular region of the renal glomerulus studied by electron microscopy.
Journal of Ultrastructure Research, 57, 65-67.

7 Vernier, R L, Resnick, J S and Mauer, S M (1975).
Recurrent hematuria and focal glomerulonephritis.
Kidney International, 7, 224-231.

8 Bohrer M P, Baylis, C, Humes, H D, Glassock, R J, Robertson, C R and Brenner, B M (1978).
Permselectivity of the glomerular capillary wall, facilitated filtration of circulating polycations.
Journal of Clinical Investigation, 61, 72-78.

9 Rennke, H G and Venkatachalam, M A (1977).
Glomerular permeability: In vitro tracer studies with polyanionic and polycationic ferritins.
Kidney International, 11, 44-53.

10 Kelley, V E and Cavallo, T (1977).
Glomerular permeability, ultrastructural studies in New Zealand black/white mice using polyanionic ferritin as a molecular probe.
Laboratory Investigation, 37, 265-275.

11 Kuhn, K, Ryan G B, Hein, S J, Galaske, R G . and Karnovsky M J (1977).
An ultrastructural study of the mechanisms of proteinuria in rat nephrotoxic nephritis.
Laboratory Investigation, 36, 375-387.

12 Farquhar, M G, Wissig, S L and Palade, G E (1961).
Glomerular permeability, I. Ferritin transfer across the normal glomerular capillary wall.
Journal of Experimental Medicine, 113, 47-66.

13 Graham, R C and Karnovsky, M J (1966).
Glomerular permeability. Ultrastructural cytochemical studies using peroxidases as protein tracers.
Journal of Experimental Medicine, 124, 1123-1134.

14 Karnovsky, M J and Ainsworth, S K (1972).
The structural basis of glomerular filtration.
In: Advances in Nephrology, Vol 2.
ed. Hamburger, J, Crosnier, J and Maxwell, M H, 35-60.
Chicago, Yearbook Medical Publishers.

15 Karnovsky, M J, Venkatachalam, M A, Graham, R C Jr, and Cotran, R S.
Ultrastructural basis of glomerular permeability to macromolecules.
In: Proceedings of the IV International Congress on Nephrology, Stockholm, Vol 1.
ed. Alwall, N, Berglund F and Josephson.

16 Venkatachalam, M A, Karnovsky, M J, Fahimi, H D and Cotran, R S (1970).
An ultrastructural study of glomerular permeability using catalase and peroxidase as tracer proteins.
Journal of Experimental Medicine, 132, 1153-1167.

17 Caulfield, J P and Farquhar, M G (1974).
The permeability of glomerular capillaries to graded dextrans. Identification of the basement membrane as the primary filtration barrier.
Journal of Cell Biology, 63, 883-903.

18 Chang, R L S, Deen, W M, Robertson, C R and Brenner, B M (1975).
Permselectivity of the glomerular capillary wall: III. Restricted transport of polyanions.
Kidney International, 8, 212-218.

19 Rennke, H G, Cotran, R S and Venkatachalam, M A (1975.)
Role of molecular charge in glomerular permeability. Tracer studies with cationized ferritins.
Journal of Cell Biology, 67, 638-646.

20 Caulfield, J P and Farquhar, M G (1978).
Loss of anionic sites from the glomerular basement membrane in aminonucleoside nephrosis.
Laboratory Investigation, 39, 505-512.

21 Michael, A F, Blau, E and Vernier, R L (1970).
Glomerular polyanion. Alteration in aminonucleoside nephrosis.
Laboratory Investigation, 23, 649-657.

22 Latta, H, Johnston, W H and Stanley, T M (1975)
Sialoglycoproteins and filtration barriers in the glomerular capillary wall.
Journal of Ultrastructure Research, 51, 354-376.

23 Seiler, M W, Rennke, H G, Venkatachalam, M A and Cotran R S (1977).
Pathogenesis of polycation-induced alterations ('fusion') of glomerular epithelium.
Laboratory Investigation, 36, 48-61.

24 Caulfield, J P and Farquhar, M G (1976).
Distribution of anionic sites in glomerular basement membranes: Their possible role in filtration and attachment.
Proceedings of the National Academy of Sciences of the United States of America, 73, 1646-1650.

25 Kanwar, Y S and Farquhar, M G (1979a).
Anionic sites in the glomerular basement membrane. In vivo and in vitro localization to the laminae rarae by cationic probes.
Journal of Cell Biology, 81, 137-153.

26 Lindahl, U and Hook, M (1978).
Glycosaminoglycans and their binding to biological macromolecules.
Annual Review of Biochemistry, 47, 385-417.

27 Rosenzweig, L J, Kanwar, Y S and Farquhar, M G (1982)
Removal of sulfated (heparan sulfate) or nonsulfated (hyaluronic acid) glycosaminoglycans results in increased permeability of the glomerular basement membrane to ^{125}I-BSA .
Laboratory Investigation, 47, 177-184.

28 Kanwar, Y S, Linker, A and Farquhar, M G (1980).
Increased permeability of the glomerular basement membrane to ferritin after removal of glycosaminoglycans (heparan sulfate) by enzyme digestion.
Journal of Cell Biology, 86, 688-693.

29 Farquhar, M G and Kanwar, Y S (1982).
Functional organization of the glomerulus,
In: Immune Mechanisms in Renal Disease.
eds. Cummings, N B, Michael, A F and Wilson, C B.
Plenum Press, New York.

30 Hernandez, A M and Amenta, P S (1983).
The basement membrane in pathology.
Laboratory Investigation, 48, 656-677.

31 Kazimierczak, J (1971).
Development of the renal corpuscle and the juxtaglomerular apparatus.
Acta Pathology & Microbiology, Scandinavia, Supplement 218, Section A.

32 Zimmerman, K W (1933).
Uber den bau des glomerulus der saugerniere.
Weitare Mitteilunger, Z, Mikr-anat Forsch, 32, 176.

33 Latta, H and Maunsbach, A B (1962).
Relations of the centrolobular region of the glomerulus of the juxtaglomerular apparatus.
Journal of Ultrastructure Research, 6, 562-578

34 Latta, H, Maunsbach, A B and Madden, S C (1960).
The centrolobular region of the renal glomerulus studied by electron microscopy.
Journal of Ultrastructure Research, 4, 455-472.

35 Farquhar, M G and Palade, G E (1962).
Functional evidence for the existence of a third cell type in the renal glomerulus. Phagocytosis of filtration residues by a distinctive 'third' cell.
Journal of Cell Biology, 13, 55-87.

36 Michael, A F, Nevins T E, Raij, L, Keane, W F and Scheinman, J I (1979).
Macromolecular transport in the glomerulus
In: Contemporary issues in nephrology, Vol 3, Ch 7, 167-213.
Ed. Curtis B Wilson, Churchhill Livingston.

37 Schreiner, G F, Kiely, J M and Cotran, R S (1981).
Characterization of resident glomerular cells in the rat expressing Ia determinants and manifesting genetically restricted interactions with lymphocytes.
Journal of Clinical Investigation, 68, 920-931.

38 Schreiner, G F, and Cotran, R S (1982).
Localization of an la-bearing glomerular cell in the mesangium.
Journal of Cell biology, 94, 483-488.

39 Ausiello, D A, Kreisberg, J I, Roy, C and Karnovsky, M J (1980).
Contraction of cultured rat glomerular cells of apparent mesangial origin after stimulation with angiotensin II and arginine vasopressin.
Journal of Clinical Investigation, 65, 754-760.

40 Harper, P A, Robinson, J M, Hoover, R L, Wright, T C and Karnovsky, M J (1984).
Improved methods for culturing rat glomerular cells.
Kidney International, 26, 875-880.

41 Leiper, J M, Thomson, D, MacDonald, M K (1977).
Uptake and transport of imposil by the glomerular mesangium in the mouse.
Laboratory Investigation, 37, 562-533.

42 Michael, A F, Keane, W F, Raij, L, Vernier, R L, Mauer, S M (1980).
The glomerular mesangium.
Kidney International 17, 141-154.

43 Kanwar, Y S, Rosenzweig, L J (1982).
Clogging of the glomerular basement membrane.
Journal of Cell Biology, 93, 489-494.

44 Vernier, R L and Birch-Andersen, A (1963).
Studies of the human fetal kidney. II. Permeability characteristics of the developing glomerulus.
Journal of Ultrastructure Research 8, 66-88.

45 Elema, J D, Hoyer, J R and Vernier, R (1976).
The glomerular mesangium: Uptake and transport of intravenously injected colloidal carbon in rats.
Kidney International 9, 395-406

46 Mauer, S M, Fish, A J, Blau, E B and Michael, A F (1972).
The glomerular mesangium. I. Kinetic studies of macromolecular uptake in normal and nephrotic rats.
Journal Clinical Investigation, 51, 1092-1101.

47 Michael, A F, Fish, A J and Good, R A (1967)
Glomerular localization and transport of aggregated proteins in mice.
Laboratory Investigation, 17, 14-29.

48 Herbet, L A, Allhuser, L, Koethe, S (1978).
Some hemodynamic determinants of immune complex trapping by the kidney.
Kidney International 14, 452-465

49 Michael, A F, Fish, A J, Good, R A (1967).
Glomerular localization and transport of aggregated proteins in mice.
Laboratory Investigation, 17, 14-29.

50 Takamiya, H, Batsford, S, Kluthe, R, Vogt, A (1979).
Comparison of the handling of ferritin and ferritin-protein conjugates by the glomerular mesangium
Laboratory Investigation, 40, 18-24.

51 Germuth, F G, Rodriguez, E (1973).
Immunopathology of the renal glomerulus.
(Little Brown, Boston).

52 Cohen, S, Vernier, R L, Michael, A F (1983)
The effect of charge on the renal distribution of ferritin.
American Journal of Pathology, 110, 170-181.

53 Batsford, S R, Weghaupt, R, Takamiya, H, Vogt, A (1985).
Studies on the mesangial handling of protein antigens.
Nephron, 41, 146-151.

54 Glasser, R J, Velosa, J A, Michael, A F (1977).
Experimental model of focal sclerosis: 1. Relationship to protein excretion in aminonucleoside nephrosis.
Laboratory Investigation, 36, 519-526.

55 Velosa, J, Glasser, R J, Nevins, T E, Michael, A F (1977).
Experimental model of focal sclerosis: II. Correlation with immunopathologic changes, macromolecular kinetics and polyanion loss.
Laboratory Investigation, 36, 527-534.

56 Berger J (1969).
IgA glomerular deposits in renal disease.
Transplantation proceedings, 1, 939-944.

57 Perico, N, Schieppati, A, Mecca, G, Rossi, E C and Remuzzi, G (1982).
Prostacyclin and renal disease.
Clinical Nephrology, 18, 111-119.

58 Sim, F F, Taylor, B M, McGuire, J C, Wong, P Y (1981).
Metabolism of prostaglandins in the kidney.
Kidney International, 19, 760-770.

59 Schlondorff, D, Ardaillou, R (1986).
Prostaglandins and other arachidonic acid metabolites in the kidney.
Kidney International, 29, 108-119.

60 Lee, J B, Crowshaw, K, Takman, B H, Attrep, K A, Gougoutas, J Z (1967).
Identification of prostaglandins E_2, F_2 and A_2 from rabbit kidney medulla.
Biochemistry Journal, 105, 1251-1260.

61 Lote, C J and Haylor, J (1986).
Renal prostaglandins.
In: Advances in Renal Physiology.
Ed. C J Lote, Croom Helm, Ch5, 138-179.

62 Dunn, M J, Staley, R S and Harrison, M (1976).
Characterization of prostaglandin production in tissue culture of rat renal medullary cells.
Prostaglandins, 12, 37-49.

63 Grenier, F C and Smith W L (1978).
Formation of 6-Keto PGF_1 by collecting tubule cells isolated from rabbit renal papillae.
Prostaglandins, 16, 759-772.

64 Schlondorff, D, Zanger, R, Satriano, A, Folkert, V W and Eveloff, J (1982).
Prostaglandin synthesis by isolated cells from the outer medulla and from the thick ascending loop of Henle rabbit kidney.
Journal of Pharmacology and Experimental Therapeutics, 233, 120-124.

65 Kreisberg, J I, Karnovsky, M J and Levine, L (1982).
Prostaglandin production by homogeneous cultures of rat glomerular epithelial and mesangial cells.
Kidney International, 22, 355-9.

66 Sraer, J, Seiss, W, Dray, F and Ardaillou, R (1983).
Regional differences in in vitro prostaglandin synthesis by the rat kidney.
In: Prostaglandins and the kidney.
eds. Dunn, M J, Patrono, C and Cinotti, G A.
Plenum, New York, pp 41-52.

67 Terragno, N A, Terragno, D A, Early, J A, Roberts, M A and McGiff, J C (1978).
Endogenous prostaglandin synthesis inhibitor in the renal cortex. Effects on production of prostacyclin by renal blood vessels.
Clinical Science, 55, 1995-2025.

68 Folkert, V W and Schlondorff, D (1977).
Prostaglandin synthesis in isolated glomeruli.
Prostaglandins, 13, 873-892.

69 Bright, R (1827).
Reports of medical cases, selected with a view of illustrating the symptoms of cures of diseases by a reference to morbid anatomy.
Longman, Orme, Brown and Green, London.

70 Wilks, S (1853).
Cases with Bright's disease with remarks.
Guy's Hospital Reports, 2nd series, 8, 232.

71 Volhard, I and Fahr, T (1914).
Die Brightsche Nierenkrankheit, Berlin: Springer

72 Ellis, A (1942).
Natural history of Bright's disease: clinical histological and experimental observations.
Lancet, 1:1, 34-36

73 Iverson, P and Brun, C (1951).
Aspiration biopsy of the kidney.
American Journal of Medicine II: 324-330

74 Wilson, C B and Dixon, F J (1976).
The renal response to immunological injury.
In the Kidney, ed, Brenner, B M and Rector F C Jr
p838. Philadelphia: W B Saunders.

75 Dixon, F J (1962).
The role of antigen-antibody complexes in disease.
Harvey Lectures, 58, 21-31.

76 Germuth, F G (1953).
Comparative histologic and immunologic study in rabbits of induced hypersensitivity of serum sickness type.
Journal of Experimental Medicine, 97, 257-282.

77 Barcelli, U, Rabbmacher, R, Ool, YM, Ool, BS (1981).
Modification of glomerular immune complex deposition in mice by activation of the reticuloendothelial system.
Journal of Clinical Investigation, 67, 20-27.

78 Ward, D M and Blantz R C (1981).
Uptake of macromolecules by the glomerular mesangium is increased by angiotensin II (Abstract).
Clinical Research, 29, 479.

79 Barnes, J L, and Venkatachalam, M A.
Glomerular interactions of exogenous and endogenous polycations.
In: The Pathogenicity of Cationic Proteins.
ed. Lambert, PP and Bergmann, P.

80 PetersD K, and Lachmann, P J (1974).
Immunity deficiency in pathogenesis of glomerulonephritis.
Lancet, 1, 58-60.

81 Coleman, T H, Forristal, J, Kosaka, T and West, C D (1983).
Inherited complement component deficiences in membrano-proliferative glomerulonephritis.
Kidney International, 24, 681-690.

82 Alpers, J H, Steward, M W and Soothill, J F (1972).
Differences in immune elimination in inbred mice.
Clinical and Experimental Immunology, 12, 121-132.

83 McGinley, E, Martin, W, Henderson, N
and Boulton Jones, J M (1986).
Defective splenic reticuloendothelial function in idiopathic membranous nephropathy.
Clinical and Experimental Immunology, 56, 295-301.

84 Batsford, S, Oite, T, Takamiya, H and Vogt, A (1980).
Anionic binding sites in the glomerular basement membrane.
Possible role in the pathogenesis of immune complex glomerulonephritis.
Renal Physiology, 3, 336-340.

85 Vogt, A (1984).
New aspects of the pathogenesis of immune complex glomerulonephritis: formation of subepithelial deposits.
Clinical Nephrology, 21, 15-20.

86 Pussel, B A, Lockwood, C M, Scott, D M, Pinching, A J
and Peter, D K (1978).
Value of immune complex assays in diagnosis and management.
Lancet, I, 359-363.

87 Cochrane, C G (1963).
Studies on the localization of circulating antigen-antibody complexes and other macromolecules in vessels.
Journal of Experimental Medicine, 118, 489-502.

88 Arthus, M (1903).
Injection repetees de serum de chevel chez le lapin.
Compte Rendus Des Seances de la Societe
de Biologie, 55, 817-820.

89 Schick, B (1907).
Die nachkrankheiten des scharlachs.
Jahrbf Kinderheit, 65, 132-173

90 Couser, W G, Stilmant, M M, Darby, C (1976).
Autologous immune complex nephropathy: 1 Sequential study of immune complex deposition, ultrastructural changes, proteinuria and alterations in glomerular sialoprotein.
Laboratory Investigation, 34, 23-30.

91 Heymann, W, Hackel, D B, Harwood, J, Wilson, S G F and Hunter, J L P (1959).
Production of nephrotic syndrome in rats by Freunds adjuvants and rat kidney suspension.
Proceedings of the Society for Experimental Biology and Medicine, 100, 660-666.

92 Edgington, T S, Glassock, R J, Dixon, F J (1967).
Autologous immune complex pathogenesis of experimental allergic glomerulonephritis.
Science, 155, 1432-1434.

93 Naruse, T, Fukasawa, T, Hirokawa N, Oike, S, Miyakawa, Y (1976).
The pathogenesis of experimental membranous glomerulonephritis induced with homologous nephritogenic tubular antigen.
Journal of Experimental Medicine, 114, 1347-1362.

94 Abrass, C K, Border, W A, Glassock, R J (1977)
Demonstration of circulating immune complexes (CIC) in autologous immune complex nephritis (AICN) in rats (abst).
Kidney International, 12, 508.

95 Naruse, T, Fukasawa, T, Umegae, S, Oike, S, Miyakawa, Y (1978).
Experimental membranous glomerulonephritis in rats. Correlation of ultrastructural changes with the serum level of autologous antibody against tubular antigen.
Laboratory Investigation, 39, 120-127.

96 Barabas, A Z, Lannigan, R (1974).
Induction of an autologous immune complex glomerulonephritis in the rat by intravenous injection of heterologous anti-rat kidney tubular antibody.
1. Production of chronic progressive immune complex glomerulonephritis.
British Journal of Experimental Pathology, 55, 47-55.

97 Feenstra, K, Lee, R V D, Greben, H A, Arends, A and Hoedemaeker, P J (1975).
Experimental glomerulonephritis in the rat induced by antibodies directed against tubular antigens:
1. The natural history: A histologic and immunohistologic study at the light microscopic and ultrastructural level.
Laboratory Investigation, 32, 235-242.

98 Couser, W G, Steinmuller, D R, Stilmant, M M, Salant, D J and Lowenstein, L M (1978)
Experimental glomerulonephritis in the isolated perfused rat kidney.
Journal of Clinical Investigation,62,1275-1287.

99 Van Damme, B J C, Fleuren, G J, Bakker, W W, Vernier, R L and Hoedemaeker, P J (1978).
Experimental glomerulonephritis in the rat induced by antibodies directed against tubular antigens:
IV. Fixed glomerular antigens in the pathogenesis of heterologous immune complex glomerulonephritis.
Laboratory Investigation, 38, 502-510.

100 Makker, S P and Singh, A K (1984).
Characterization of the antigen (GP600) of Heymann nephritis.
Laboratory Investigation, 50, 287-293.

101 Kerjaschki, D and Farquhar, M G ((1983).
Immunocytochemical localization of the Heymann nephritis antigen (GP300) in glomerular epithelial cells of normal Lewis rats.
Journal of Experimental Medicine, 157, 667-685.

102 Neale, T J, Woodroffe, A J and Wilson, C B (1984).
Spontaneous glomerulonephritis in rabbits. Role of a glomerular capillary antigen.
Kidney International, 26, 701-711.

103 Border, W A, Ward, H J, Kamil, E S and Cohen, A H (1982).
Induction of membranous nephropathy in rabbits by administration of an exogenous cationic antigen:
Demonstration of a pathogenic role for electrical charge.
Journal of Clinical Investigation, 69, 451-461.

104 Oite, T, Batsford, S R, Mihatsch, M J, Takamiya, H and Vogt, A (1982).
Quantitative studies of in situ immune complex glomerulonephritis in the rat induced by planted cationized antigen.
Journal of Experimental Medicine, 155, 460-474.

105 Ward, H J, Cohen, A H and Border, W A (1984).
In situ formation of subepithelial immune complexes in rabbit glomerulus: Requirement of a cationic antigen.
Nephron, 36, 257-264.

106 Vogt, A, Batsford, S, Rodriguez-Iturbe, B and Garcia, R (1983).
Cationic antigens in poststreptococcal glomerulonephritis.
Clinical Nephrology, 20, 271-279.

107 Lange, K, Seligson, G and Cronin, W (1983).
Evidence for the in situ origin of poststreptococcal glomerulonephritis: Glomerular localization of endostreptosin and the clinical significance of the subsequent antibody response.
Clinical Nephrology, 19, 3-10.

108 Vogt, A, Rohrbach, R, Shimizu, F, Takamiya, H and Batsford, S (1982).
Interaction of cationized antigen with rat glomerular basement membrane: In situ immune complex formation.
Kidney International 22, 27-35.

109 Gallo, G R, Caulin-Glaser, T and Lamm, M E (1981).
Charge of circulating immune complexes as a factor in glomerular basement membrane localization in mice.
Journal of Clinical Investigation, 67, 1305-1313.

110 Agodoa, L Y C, Gauthier, V J and Mannik, M (1985).
Antibody localization in the glomerular basement membrane may precede in situ immune deposit formation in rat glomeruli.
Journal of Immunology, 134, 880-884.

111 Fleuren, G, Grond, J and Hoedemaeker, P J (1980).
In situ formation of subepithelial glomerular immune complexes in passive serum sickness.
Kidney International, 17, 631-637.

112 Koffler, D, Schur, P H and Kunkel, H G (1967).
Immunologic studies concerning the nephritis of systemic lupus erythematosus.
Journal of Experimental Medicine, 126, 607-623.

113 Izui, S, Lambert, P H, and Miescher, P A (1976).
In vitro demonstration of a particular affinity of glomerular basement membrane and collagen for DNA: A possible basis for local formation of DNA - anti-DNA complexes in systemic lupus erythematosus.
Journal of Experimental Medicine 144, 428-443.

114 Chan, E K L, Boyd, N D and Fritzler, M (1983).
In vivo anionic immune complex deposition in the murine glomerulus (Abs).
Clinical Research, 31, 448.

115 Cavallo, T, Goldman, M, Graves, K and Lambert, P H (1983).
Altered glomerular permeability in the early phase of immune complex nephritis.
Kidney International, 24, 632-637.

116 Camussi, G, Tetta, C Segoloni, G, Koda, R and Vercellone, A (1982).
Localization of neutrophil cationic proteins and loss of anionic charges in glomeruli of patients with systemic lupus erythematosus.
Clinical Immunology and Immunopathology, 24, 299-314.

117 Hunsicker, L G, Shearer, T P and Shaffer, S J (1981).
Acute reversible proteinuia induced by infusion of the polycation hexadimethrine.
Kidney International, 20, 7-17.

118 Michael, A F, Keane, W F, Raij, L, Vernier, R L and Mauer, S M (1980).
The glomerular mesangium (editorial review).
Kidney International, 17, 141-154.

119 Batsford, S R, Rohrbach, R, Takamiya, H Kluthe, R and Vogt, A (1979).
Autoantibodies specific for the glomerular mesangium and Bowman's capsule in man.
Clinical Nephrology, 12, 163-167.

120 Seelig, H P, Seelig, R and Roth, E (1975).
Antibodies reacting with the glomerular mesangium. Isolation and immunopathology.
Virchows Archives Annals of Pathology and Analytical Histology 366, 313-330.

121 Mauer, S M, Sutherland, D E R, Howard, R J, Fish, A J, Najarian, J S and Michael, A F (1973).
The glomerular mesangium: III. Acute immune mesangial injury: A new model of glomerulonephritis.
Journal of Experimental Medicine, 137, 553-570.

122 Couser, W G and Salant, D J (1980).
In situ immune complex formation and glomerular injury. (editorial review).
Kidney International, 17, 1-13.

123 Cohen, S, Vernier, R L and Michael, A F (1983).
The effect of charge on the renal distribution of ferritin.
American Journal of Pathology, 110, 170-181.

124 Border, W A, Kamil, E S, Ward, H J and Cohen A H (1981).
Antigenic charge as a determinant of immune complex localization in the rat glomerulus.
Laboratory Investigation, 40, 442-449.

125 Golbus, S M and Wilson, C B (1979).
Experimental glomerulonephritis induced by in situ formation of immune complexes in the glomerular capillary wall.
Kidney International, 16, 148-157.

126 Adler, S, Salant, D J, Dittmer, J E, Rennke, H G, Madio, M P and Couser, W G (1983).
Mediation of proteinuria in membranous nephropathy due to a planted glomerular antigen.
Kidney International, 25, 807-815.

127 Matsuo, S, Caldwell, P, Brentjens, J and Andres G (1985).
Nephrotoxic serum glomerulonephritis induced in the rabbit by anti-endothelial antibodies (abs).
Kidney International, 27, 217.

128 Cines, D B, Lyss, A P, Reeber, M B and Dehoratius, R J (1984)
Presence of complement-fixing anti-endothelial antibodies in systemic lupus erythematosus.
Journal of Clinical Investigation, 73, 611-625.

129 Okudaira, K, Searles, R P, Ceuppens, J L Goodwin, J S and Williams, R C Jr (1982).
Anti-la reactivity in sera from patients with systemic lupus erythematosus.
Journal of Clinical Investigation, 69, 17-24.

130 Arisz, L, Donker, A J, Brentjens, J R and Van der Hem, G K (1976).
The effect of indomethacin on proteinuria and kidney function in the nephrotic syndrome.
Acta Medica, Scandinavia, 199, 121-125.

131 Donker A J, Brentjens, J R, Van der Hem, G K and Arisz, L (1978).
Treatment of the nephrotic syndrome with indomethacin.
Nephron, 22, 374-381.

132 Lianos, E, Alavi, N, Venuto, R, Brentjens, J and Brentzel, C (1981).
Effects of prostaglandin inhibition on protein excretion and renal hemodynamics in patients with proteinuria.
Kidney International, 19, 131.

133 Tiggler, R G W L, Hulme, B, and Wijdeveld, P G A B (1979).
Effect of indomethacin on glomerular permeability in the nephrotic syndrome.
Kidney International, 16, 312-321.

134 Zurier, R B, Damjanov, I, Sayadoff, D M and Rothfield, N F (1977).
Prostaglandin E_1 treatment of NZB/NZW F_1 hybrid mice. II. Prevention of glomerulonephritis.
Arthritis and Rheumatism, 20, 1449-1456.

135 Winkelstein, A, and Kelley, V E (1981).
Effects of PGE_1 in murine models of SLE: changes in circulating immune complexes.
Clinical Immunobiology and Immunopathology, 20, 188-192.

136 Prickett, J D, Robinson, D R and Steinberg, A D (1983).
Effects of dietary enrichment with eicosapentaenoic acid upon autoimmune nephritis in female NZB x NZW/F_1 mice.
Arthritis and Rheumatism, 26, 133-139.

137 Kelley, V E, Izui, S and Strom, T B (1984).
Fish oil diet reduces cyclooxygenase metabolites and suppresses lupus nephritis in MRL-lpr mice.
IXth International Congress of Nephrology, Los Angeles,274A

138 Kher, V, Barcelli, U, Weiss, M and Pollak, V E (1985).
Effects of dietary linoleic acid enrichment on induction of immune complex nephritis in mice.
Nephron, 39, 261-266.

139 Kimberly, R P, and Plotz P H (1977)
Aspirin-induced depression of renal function.
New England Journal of Medicine, 296, 418-424.

140 Ciabattoni, G, Cinotti, G A, Pierucci, Simonetti, B M, Manzi, M, Pugliese, E, Barsotti, P, Pecci, G, Taggi, F and Patrono, C (1984).
Effect of sulindac and ibuprofen in patients with chronic glomerular disease. Evidence for the dependence of renal function on prostacyclin.
New England Journal of Medicine, 310, 279-283.

141 Blum, M, Bauminger, S, Algutt, A, Kisch, E, Ayalon, D and Aviram, A (1982).
Urinary prostaglandin E_2 in chronic renal disease.
Clinical Nephrology, 15, 87-89.

142 Niwa, T, Maeda, K, Naotsuka, Y, Asada, H, Kobayashi, S, Yokoyama, M, Kawaguchi, S and Shibata, M (1982).
Improvement of renal function with prostaglandin E_1 infusion in patients with chronic renal disease.
Lancet II, 687.

143 Danon, D, Goldstein, Y, Marikovsky, Y and Skutelsky, E (1972)
Use of cationized ferritin as a label of negative charges on cell surfaces.
Journal of Ultrastructure Research, 38, 500-510.

144 Whaley, K, Lappin, D, McPhaden, A R, Riches, W H and Sandilands, E P (1983).
A comparison of the effects of heat aggregated and chemical cross-linked IgG on monocyte C2 production.
Immunology, 49, 457-461.

145 McFarlane, A S (1958).
Efficient trace labelling of proteins with iodine.
Nature (London), 53.

146 Lowry, O H, Rosebrough, N F, Farr, A L and Randall, R J (1951).
Protein measurement with the Folin phenol reagent.
Journal of Biological Chemistry, 193, 265-275.

147 Hoyer, J R, Mauer, S M and Michael, A F (1975).
Unilateral renal disease in the rat
I. Clinical, morphologic and glomerular mesangial functional features of the experimental model produced by renal perfusion with aminonucleoside.
Journal of Laboratory and Clinical Medicine, 85, 756-768.

148 Levin, M, Smith, C Walkers, M D S, Gascoine, P and Barra, H M (1985).
Steroid-responsive nephrotic syndrome: a generalised disorder of membrane negative charge.
Lancet, 2, 239-242.

149 Edgington, T S, Glassock, R J and Dixon, F J (1968).
Autologous immune complex nephritis induced with renal tubular antigen. I. Identification and isolation of the pathogenetic antigen.
Journal of Experimental Medicine, 127, 555-572

150 Rice, E W (1975).
Improved biuret procedure for routine determination of urinary total proteins in clinical proteinuria.
Clinical Chemistry, 21, 398-401.

151 McLaren, M, Belch, J J F, Forbes, C D and Prentice, C R M (1985).
Development of a radioimmunoassay for the measurement of prostacyclin metabolites in unextracted plasma.
Thrombosis Research, 37, 177-183.

152 Rees, A J, Peters, D K, Amos, N, Welsh, K I and Batchelor, J R (1984).
The influence of HLA linked genes on the severity of acute GBM antibody mediated nephritis.
Kidney International, 26, 444-450.

153 Klouda, P T, Acheson, E J, Goldby, R S, Lawler, W, Manos, J, Dyer, P A, Harris, R, Mallick, N P and Williams, G (1979).
Strong association between idiopathic membranous nephropathy and HLA-DRW3.
Lancet 2, 770-771.

154 Kashiwabara, H, Shishido, H, Tomura, S, Tuchida, H and Miyajima, T (1982).
Strong association between idiopathic membranous nephropathy and HLA-DR4 antigen.
Kidney International, 22, 377-382.

155 Brenner, B M, Hostetter, T H and Humes, H D (1978).
Molecular basis of proteinuria of glomerular origin.
New England Journal of Medicine, 298, 826-833.

156 Michael, A F, Blau, E and Vernier, R L (1970).
Glomerular polyanion: alteration in aminonucleoside nephrosis.
Laboratory Investigation, 23, 649-657.

157 Kreisberg, J I, Wayne, D B and Karnovsky, M J (1979).
Rapid and focal loss of negative charge associated with mononuclear cell infiltration early in nephrotoxic serum nephritis.
Kidney International, 16, 290-300.

158 Blau, E B and Haas, J E (1973).
Glomerular sialic acid and proteinuria in human renal disease.
Laboratory Investigation, 28, 477-481.

159 Mactier, R, Boulton Jones, J M, Payton, C D and McLay, A.
Membranous nephropathy in the West of Scotland.
Quarterly Journal of Medicine (In Press).

160 Sterzel, R B, Perfelto, M, Biemesderfer, D and Kashgarian, M (1983).
Disposal of ferritin in glomerular mesangium of rats.
Kidney International, 23, 480-488.

161 Stenglein, B Thoenes, G H, and Gunther, E (1978).
Genetic control of susceptibility to autologous immune complex glomerulonephritis in inbred rat strains.
Clinical Experimental Immunology, 38, 88-94.

162 Couser, W G, Hoyer, J R, Stilmant, M M, Jermanovich, N B and Belok, S (1978).
The effect of aminonucleoside nephrosis on immune complex localization in autologous immune complex nephroapthy in rats.
Journal of Clinical Investigation, 61, 561-572.

163 Hoyer, J R, Ratte, J, Potter, A H and Michael A F (1972).
Transfer of aminonucleoside nephrosis by renal transplantation.
Journal of Clinical Investigation, 51, 2777-2780.

164 Bakker, W W, Beukhof, J R, van Luijk, W H J and Van der Hem, G K.
Vascular permeability factor in IgA nephropathy.
Clinical Nephrology, 18, 165-167.

165 Mauer, S M, Fish A J, Day, N and Michael A F (1974).
The glomerular mesangium II studies of macromolecular uptake in nephrotoxic nephritis in rats.
Journal of Clinical Investigation, 53, 431-439.

166 Stahl, R A, Paravicini, M and Schollmeyer, P.
Angiotensin II stimulation of PGE_2 and 6 keto PGF_1 formation by isolated human glomeruli.
Kidney International, 26, 30-34.

167 Sraer, J, Foidart, J, Chansel, D, Mahieu, P, Kouznetzova, B and Ardaillou, R (1979).
Prostaglandin synthesis by mesangial and epithelial glomerular cultured cells.
FEBS Letters, 15, 420-424.

168 Scharschmidt, L A and Dunn, M J (1983).
Prostaglandin synthesis by rat glomerular mesangial cells in culture. Effects of angiotensin II and arginine vasopressin.
Journal of Clinical Investigation, 71, 1756-1764.

169 Ardaillou, N, Hagege, Y, Nivez, M P, Ardaillou, R and Schlondorff, D (1985).
Vasoconstrictor-evoked prostaglandin synthesis in cultured human mesangial cells.
American Journal of Physiology, 248, 240-246.

170 Dousa, T P, Shah, S V and Abboud, H E (1980).
Potential role of cyclic nucleotides in glomerular pathophysiology.
In: Advances in Cyclic Nucleotide Research, edited by P Hamet and H Sands. New York: Raven, 12, 285-299.

171 Beierwaltes, W S, Schryver, S, Olson, P and Romero, J (1980).
Interaction of the prostaglandin and renin-angiotensin systems in isolated rat glomeruli.
American Journal of Physiology, 239 (Renal Fluid Electrolyte Physiology,8), 602-608.

172 Skorecki, K L, Ballermann, B J, Rennke, G H and Brenner, B M (1983).
Angiotensin II receptor regulation in isolated renal glomeruli.
Federation Proceedings, 42, 3064-3074.

173 Ausiello, D A, Kreisberg, J I, Roy, C and Karnovsky, M J (1980).
Contraction of cultured rat glomerular cells of apparent mesangial origin after stimulation with angiotensin II and arginine vasopressin.
Journal of Clinical Investigation, 65, 754-760.

174 Peach, M J (1981).
Molecular actions of angiotensin.
Biochemical Pharmacology, 30, 2745-2751

175 Remuzzi, G, Imberti, L, Rossini, M, Morelli, C, Carminati, C, Cattaneo, G M and Bertani, T (1985).
Increased glomerular thromboxane synthesis as a possible cause of proteinuria in experimental nephrosis.
Journal of Clinical Investigation, 75, 94-101.

176 Barcelli, U and Pollak, V E (1985).
Is there a role for polyunsaturated fatty acids in the prevention of renal disease and renal failure?
Nephron, 41, 209-212.

177 Kernoff, P B A, Willis, A L, Stone, K J, Davies, J A and McNicol, G P (1977).
Antithrombotic potential of dihomo-gamma-linolenic acid in man.
British Medical Journal ii, 1441.

178 Dupont, J, Mathias, M M and Connally, P T (1980).
Effects of dietary essential fatty acid concentration upon prostanoid synthesis in rats.
Journal of Nutrition, 110, 1695-1702.

179 Ferretti, A, Schoene, N W and Flanagan, V P (1981).
Identification and quantification of prostaglandin E_3 in renal medullary tissue of three strains of rats fed fish oil.
Lipids, 16, 800-804.

180 Olivetti, G, Kithier, K, Giacomelli, F and Wiener, J (1981).
Glomerular permeability to endogenous proteins in the rat: Effects of acute hypertension.
Laboratory Investigation, 44, 127-137.

181 Hamazaki, T, Tatenos and Shishido, H (1984).
Eicosapentaenoic acid and IgA nephropathy.
Lancet, I, 1017-1018.