THE RENAL GLOMERULUS OF THE CAT: ITS NORMAL STRUCTURE AND THE DESCRIPTION OF A NOVEL GLOMERULAR LESION.

By

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Thesis submitted for the degree of Master of Science in the Faculty of Veterinary Medicine, University of Glasgow.

CONTENTS

DEDICATION

This thesis is dedicated

to the memory of my mother.

"Hon hoi theoi philousi

apothneskei neos"

Menander

ACKNOWLEDGEMENTS

I am indebted to my supervisor Professor N.G.Wright, for his guidance and assistance throughout the course of the work contained in this thesis and for his constructive criticism during the preparation of this manuscript.

My gratitude also goes to my colleagues in the Department of Veterinary Anatomy, both past and present, for their help, forbearance and co-operation during the course of this work.

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To Mr. Alan Bradley and the Pathology Animal House animal technicians thanks are also due for their exemplary care of the experimental animals.

I am also grateful to the University of Glasgow and to the Wellcome Trust for financial support during the period of this study.

Special thanks to L.B.R.

DECLARATION

I declare that all the work, techniques and photographs included in this study were carried out by myself. This is with the exception of Figures 1.7, 1.8, and 1.9 which were taken by Professor N.G.Wright in whose debt I am.

ERRATA

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152	18	- For convential read conventional
170	37	- For Goormatigh read Goormaghtigh

ABBREVIATIONS

T.E.M. : Transmission Electron Microscope / Microscopy.

S.E.M. : Scanning Electron Microscope / Microscopy.

P.B.S. : Phosphate Buffered Saline.

B.N.F. : Buffered Neutral Formalin.

G.N. : Glomerulonephritis.

G.B.M. : Glomerular Basement Membrane.

K HCl : Ketamine hydrochloride.

Na P : Sodium pentobarbitone.

EXPLANATORY TERMS

Focal : only a percentage of glomeruli affected.

Diffuse : all glomeruli equally affected.

SUMMARY

Familarity with the anatomy of the normal glomerulus would seem to be an essential pre-requisite to the understanding and the interpretation of the glomerular abnormalities as detected by light, electron microscopy and immunofluorescence both during and after pathological conditions.

As glomerulonephritis is now recognised as being an important feline nephropathy the need for such a base-line study of the normal feline glomerulus was highlighted.

In Chapter 1 of the present work, an attempt was made to elucidate the more important gaps in our understanding of the normal cat glomerulus. This was done with particular reference to a number of important parameters such as methods of fixation and embedding, section thickness and glomerular size variation within the kidney. In the course of this part of the work nine cats were found, at necropsy, to be suffering from a diffuse glomerular endothelial lytic lesion.

Chapter 2 provided a detailed study of the sequential autolytic changes taking place after death in the cat kidney. The time scale of this experiment ranging from five minutes to six days following the animals demise. Autolytic changes were seen to occur very rapidly after death, although even with TEM, there were significant areas of tissue where recognisable cytological integrity had been preserved as late as 60 hours after death.

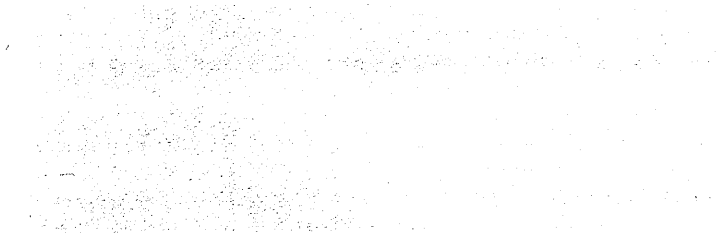
In Chapter 3 the neonatal kidney was examined at the time of birth and at three day intervals until 70 days. This study attempted, once again, to illuminate those areas which no previous study had examined in the cat. Thus it was found that the nephrogenic zone persisted as late as eight weeks after birth.

Chapter 4 dealt with those animals excluded from Chapter 1 due to their exhibiting a severe form of a glomerular endothelial lesion not previously described in the literature. This was a combined light, electron microscopy and immunofluorescence study. It revealed the complete destruction of the glomerular endothelium together with the partial destruction of the glomerular mesangium. This was accompanied by granular deposition of both IgG and C_3 in the glomerular capillaries.

In Chapters 5, 6 and 7 successive experimental attempts to elucidate the nature and occurrence of this novel lesion by examining both the physical parameters and the chemicals used in the primary study.

Overall, by histological, ultrastructural and immunofluorescence methods of examination, the lesion recreated in Chapters 6 and 7 was seen to be identical, if differing in severity, to that noted in spontaneously occurring cases in Chapter 4.

It was concluded that the anaesthetic agents employed in the present study, which are also used in general practice, were responsible for this novel lesion yet in an entirely random fashion. The consequences of this lesion occurring in the cat during routine and / or experimental procedures in which the structural detail of the glomerulus was under study were considered to merit further study.



INTRODUCTION

INTRODUCTION

Within the last two decades investigation of naturally-occurring renal disease in man and his domesticated animals has undergone major advances.

These have been brought about by the development of several investigative techniques such as renal biopsy and the increased use of the electron microscope. Furthermore, widespread application of immunofluorescence techniques has created an increased awareness and understanding of the pathogenesis of kidney disease through the realisation that immunologically-mediated injury to the kidney is an important pathogenic mechanism (Sakaguchi et.al., 1965; Germuth and Rodriguez, 1973).

In particular, these techniques have emphasised the importance of primary glomerulopathy, especially glomerulonephritis, in the overall spectrum of renal disease of man and animals (Fish et.al., 1971; Churg et.al., 1973).

Primary glomerulopathy is a renal disease -- though in reality a whole range of different conditions -- which initially acts upon the glomerulus but the morphological and functional abnormalities often progress to alterations in renal tubules, interstitium

and vasculature. Indeed, a major feature of the disease is leakage of protein into the urine and this is sometimes sufficient to cause severe hypoproteinaemia and oedema, characteristic of the nephrotic syndrome. In a proportion of cases where the severity of the disease is sufficiently great progressive renal failure may follow.

The apparent increase of spontaneous glomerulopathy in domestic animals, particularly in the dog and cat (see reviews by Osborne et.al., 1972; Osborne and Vernier, 1973; Osborne et.al., 1977; Slauson and Lewis, 1979), and the realisation that similar pathogenetic mechanisms to those in man are operating in these animals is shown by the substantial number of reports in the recent literature (Meadows, 1973; Lucke, 1978; Murray and Wright, 1974; Krakowka, 1979; Wright et.al., 1981 and Lucke, 1982).

However, despite these reports there has, with the exception of man and laboratory animals, only been a limited number of reports on the normal morphology of the renal glomerulus (Arakawa, 1970; Spinelli, 1976 and Osborne et.al., 1977).

Yet it would seem that such information must be of the utmost importance to the veterinary diagnostic pathologist who wishes to distinguish the abnormal from the normal.

In the case of the cat, which has been shown to be susceptible to a range of renal diseases similar to those of man, no up-to-date detailed information on the normal structure of the cat kidney is available (Zimmerman, 1935 (cited by Mueller, 1958)).

As membranous nephropathy, in particular, is emerging as an important disease of cats (Osborne and Vernier, 1973; Nash et.al., 1979; Wright et.al., 1981 and Lucke, 1982), a definitive study of the normal feline glomerulus would be of great value. Such a base-line study involving both light and electron microscopy would establish a representative morphological picture of the feline renal glomerulus to which further studies on spontaneous glomerular disease could be referred and compared.

The present study had the primary aim of establishing normal morphological parameters for the renal glomerulus of the cat from birth to adulthood. This involved the use of histological, transmission and scanning electron microscopical and immunofluorescence techniques.

A detailed account of the sequential autolytic changes which take place in the glomerulus was also carried out.

MATERIALS and METHODS

MATERIALS and METHODS

1. Source of Animals.

For the purpose of this study, 20 young adult cats, ranging in age from nine months to 18 months, were obtained from commercial sources specifically for the purpose of examining normal glomerular histological and ultrastructural parameters.

Prior to commencement of the study, haematological examination and urine biochemical analysis showed the animals to be healthy and free of subclinical or overt renal disease. Care was taken to ascertain whether or not the cats had evidence of any extra-renal disease.

2. Euthanasia.

The method of euthanasia employed was as follows; the animals were first sedated by an intramuscular injection of Ketamine hydrochloride ('Vetalar'; Parke, Davis & Co., Pontypool, Gwent) at a dose rate of 22 mg/kg body weight, into the quadriceps muscle mass. This preliminary injection of Ketamine was necessitated by the fractious nature of the animals used and for the saftey of the operator. After a period of some five to ten minutes when sufficient sedation was achieved, deep anaesthesia was induced by a slow intravenous injection, into the cephalic vein, of a 6% solution of sodium

pentobarbitone ('Sagatal'; May & Baker Ltd., Dagenham, England). Once the femoral pulse was only faintly discernible the axillary artery was severed and the animal exsanguinated.

3. Sampling of Tissues.

Following exsanguination, the abdomen was opened by a mid-line incision, the abdominal organs displaced to one side and both kidneys removed (see Fig. 1.1, Chapter 1). The kidneys were then halved longitudinally revealing cortex and medulla whereupon samples of kidney material were removed for histological and ultrastructural examination.

The interval between exsanguination and immersion in fixative never exceeded three minutes.

Transmission Electron Microscopy (TEM):

Samples from the renal cortex were diced into blocks no greater than 1mm. thick and immersed in Karnovsky's fixative (Karnovsky, 1965) (2% paraformaldehyde - 2.5% gluteraldehyde) at $4^{o}C$ for a minimum of one hour. Tissues were then washed in 0.1M cacodylate buffer before post-fixation in cacodylate-buffered 1% osmium tetroxide for a further

hour. Samples were then washed and subsequently dehydrated through a graded series of acetones (70%, 90%, 100%), cleared using propylene oxide and embedded in 'E mix' (EMScope, Ashford, Kent) which was polymerised overnight at 60°C.

lum. sections were prepared using an LKB pyramitome and stained with toluidine blue. Subsequently, after identification of glomeruli ultrathin sections were prepared with an LKB Mk.III ultramicrotome and collected on G300 copper grids (Polaron Equipment, Watford, England). These were stained using uranyl acetate and lead citrate (Millonig, 1961) and examined by means of a Hitachi HS 8 and latterly by a JEOL 100 CX II transmission electron microscope.

Scanning Electron Microscopy (SEM):

At the same time, thin slices of cortex, again no thicker than 2mm., were immersed in Karnovsky's fixative at 4°C for a minimum of 24 hours to be subsequently used for SEM studies. After fixation, specimens were trimmed to expose the surface to be examined, then washed in 0.1M cacodylate buffer and dehydrated through a series of acetones (70%, 90%, 100%) before being dried using a Polaron critical point drier. The tissues were then attached to aluminium stubs using conductive silver

paint, which was allowed to dry before the specimen was coated with a gold/palladium mixture using an EMScope sputter coater. These specimens were examined using a Philips SEM 501B scanning electron microscope.

Light Microscope studies :

Material for histological examination was immersed in 10% buffered neutral formalin for seven to ten days, then post-fixed in mercuric chloride-formal for an additional 24 hours. Tissue blocks, approximately 5mm. in thickness were then dehydrated through a series of phenol alcohols, cleared and finally embedded in paraffin wax. Sections were cut on a Leitz 1510 rotary microtome at 3um. and routinely stained with Mayer's haematoxylin and Putt's eosin. Other stains used as the occasion demanded included Masson's trichrome (collagen), Martius scalet blue (M.S.B.) (collagen and fibrin) and periodic acid-Schiff (basement membranes).

Histological slides were examined using a Leitz Laborlux 12 microscope and by means of a Wild Photoautomat MPS 45 unit and a Wild MPS 51S camera a photographic record of the histological findings was obtained.

Immunofluorescence :

Once tissue had been taken for ultrastructural and histological examination small blocks of renal cortex were removed and snap frozen in liquid nitrogen then stored at $-20^{o}C$ to be used later for immunofluorescence studies.

Frozen sections 4um. thick were cut on a Reichart-Jung series 2700 Frigocut freezing microtome (American Optical Co., London). The sections were allowed to come to room temperature then washed in isotonic phosphate buffered saline (pH 7.4) (PBS) before being fixed in acetone for five minutes. The sections were then stained with anti-cat immunoglobulin G (Evai Bios, Horsham, England) or anti-cat complement (C_3) (kindly supplied by Dr. Neil Gorman, University of Cambridge) congujated with fluorescein isothiocyanate (FITC). After further washing in PBS, they were examined by means of a Leitz Orthoplan fluorescence microscope equipped for incident light fluorescence.

4. Perfusion studies :

For comparison with tissues fixed by immersion, six kidneys were perfused with fixative. Here once the animal had been exsanguinated the abdominal cavity was opened to reveal the kidneys. The aorta was located and

followed to its junctions with the renal arteries. The aorta was then clamped, both proximally and distally to the renal arteries, and removed together with the renal arteries and kidneys.

Whilst one of the renal arteries was clamped-off the other was cannulated using a flexible nylon cannula (No. 12 gauge) through which physiological saline was injected, with the intention of clearing the kidney of residual blood, at a flow rate of approximately 2ml per minute. At the same flow rate, approximately 20ml of Karnovsky's fixative was then injected into the kidney. Any excess fixative escaped through the severed renal vein.

Material for ultrastructural studies was then removed from the perfused kidney and immersed in cold Karnovsky's fixative while material for histology was immersed in buffered neutral formalin. For direct comparison, samples were also taken from the un-perfused kidney in the manner previously described.

5. Preparation of Arterial Casts :

Corrosion casts of two kidneys were also prepared. This was achieved by flushing the renal circulation with PBS, as previously described, then injecting 15 ml of a mixture of Tensol A & B (I.C.I. Plastic Division, Welwyn

Garden City, England) in a ratio of 25 to 1 respectively via the cannulated artery.

When filling was complete the kidney was transferred to an incubator at 37°C for eight hours (or overnight), then immersed in a 30% solution of potassium hydroxide for three days. Once the digestion process was completed, the kidney was placed in a bath of running water in order to remove any crystallised potassium hydroxide. After drying at 37°C overnight small pieces of cortex were carefully removed for examination with the SEM as previously described.

CHAPTER 1

A HISTOLOGICAL AND ULTRASTRUCTURAL STUDY OF THE NORMAL CAT GLOMERULUS

INTRODUCTION

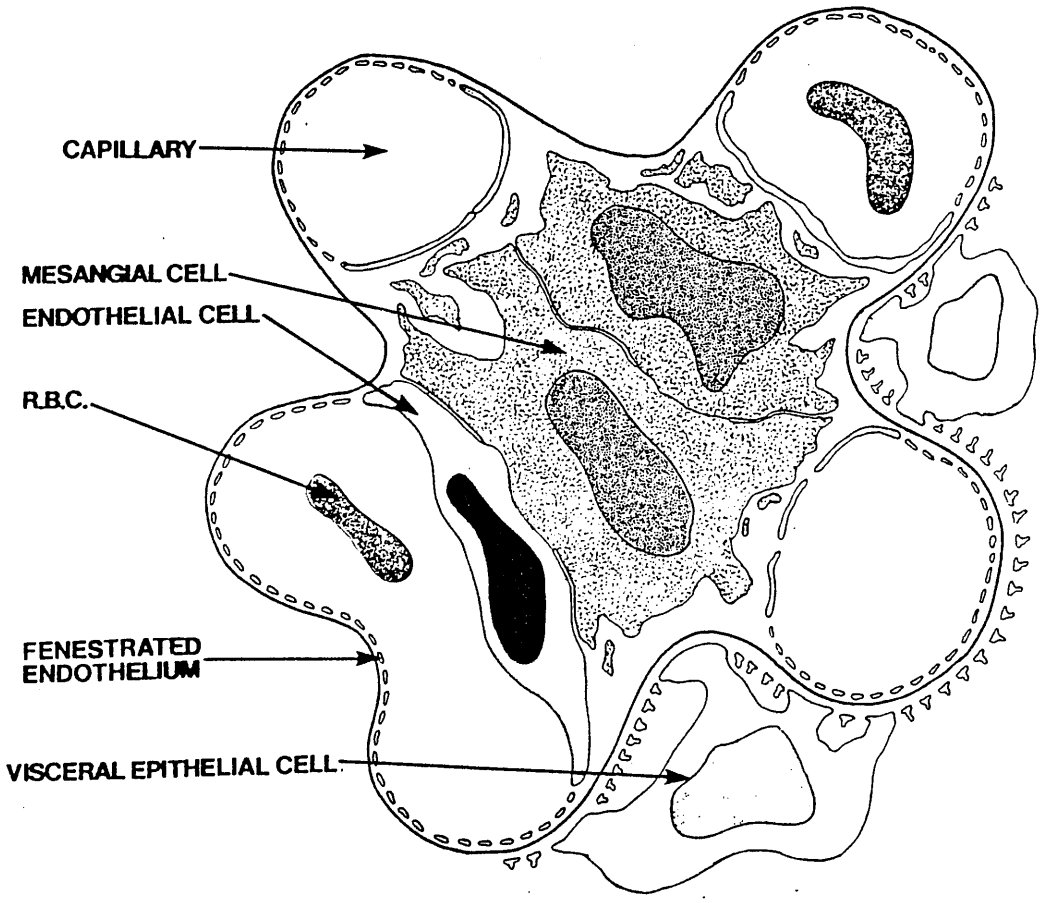
1. A brief review of the structure of the mammalian glomerulus:

The normal renal corpuscle as we know it today is a cluster of capillaries composed of three cell types, namely the capillary endothelial cell, the visceral epithelial cell, between which two there is a basement membrane, and a third cell type the mesangial cell which has associated with it a mesangial matrix.

Surrounding the glomerlus is a capsule, Bowman's capsule, lined with flat epithelial cells which are continuous with both the visceral epithelial cells and with the epithlial cells of the proximal convoluted tubule.

The basic structure of these components of the glomerulus can be seen in diagram 1.

Diagram 1.


CAPILLARY
MESANGIAL CELL
ENDOTHELIAL CELL
R.B.C.
FENESTRATED ENDOTHELIUM
VISCERAL EPITHELIAL CELL

To aid in the understanding of glomerular structure a brief historical background to the development of glomerular anatomy now follows:

The renal corpuscle was first discovered in 1669 by Marcello Malpighi (cited by Bowman, 1842) who by injecting the renal artery discovered a "curiously tufted spherical arrangement" of capillaries which were later to be named after him as the "Malpighian corpuscle". This arrangement of capillaries has subsequently become known as the renal corpuscle and contains the glomerulus. However, Malpighi considered the corpuscle to be a gland which gave rise to the uriniferous tube and from which urine was elaborated from the blood.

During the 19th. century several investigators, for example Ruysch and Albinus (cited by Bowman, 1842), extended this pioneering work by trying to show that the arteries supplying the Malpighian corpuscles were continuous with the uriniferous tubules. Conversely there were others, for example Schumlansky and Muller, (again cited by Bowman, 1842) who held the opposite opinion that the 'Malpighian bodies' and the 'uriniferous ducts' had no connection.

It was in this atmosphere of doubt and uncertainty that Bowman spent the years 1840 to 1842 inquiring into

the nature of the Malpighian corpuscle. He discovered, by means of the injection of precipitate-forming chemical solutions (namely potassium bichromate and lead acetate), that the afferent vessel entered the capillary tuft, where it split into a number of smaller vessels, only to rejoin to exit as the efferent vessel where it formed a capillary plexus surrounding the uriniferous tubules. It then re-united with other efferent vessels to leave the kidney as the renal vein (Bowman, 1842).

But perhaps Bowman's most notable discovery at this time was that the basement membrane of the uriniferous tube was expanded over the Malpighian tuft of capillaries to form its capsule. In the basment membrane of this capsule, which now bears his name, he could find no structure; however he considered that it was perforated by and not reflected over the afferent and efferent vessels of the Malpighian corpuscle which he considered were "bare" lying coiled freely within the space of Bowman's capsule (Bowman, 1842).

This concept that the vessels of the glomerular tuft lay "coiled freely" and were "bare" being a "simple, homogenous and transparent membrane" gained a number of followers. The most notable of whom was Henle (cited by McGregor, 1929) who with further examination stated that "the vessels in the glomerulus have the calibre of capillaries". Their framework being "a

structureless wall possessing elliptical nuclei". However, a great deal of investigation was carried out using silver stains and injections of argyrophilic materials in an attempt to describe these cells and their boundaries. Unfortunately these investigations proved unsuccessful although these boundaries could easily be demonstrated between the endothelial cells of the vas afferens and the vas efferens.

It was not until 1927, some 85 years later, that von Moellendorff (cited by McGregor, 1929) described the human glomerular endothelium as being a "sparsely nucleated layer having visible cytoplasm only where the nuclei lay".

In the 1940's and '50's the agreed view was that the endothelial cell was shaped like a tube of fairly short length. On cross section it appeared 'signet ring' shaped with a thin layer of cytoplasm extending around the capillary lumen from the mass of cytoplasm which contains the nucleus. This thin layer of cytoplasm lying adjacent to the basement consists of two plasma membranes of approximately 150A width separated by a 200 to 300A cytoplasmic space (Mueller, 1958).

This endothelial layer appeared to be discontinuous, as though perforated at regular intervals, to Hall and he named the structure the "lamina fenestrata" (Hall, 1954 a and b). He

subsequently renamed the structure the "lamina attenuata" due to doubts raised about his fixation techniques. However, Farquhar et.al. (1961) later confirmed the existence of these large pores or fenestrae and measured them at from 500 to 1000A in diameter. Although 60A thick diaphragms have been observed in the fenestrae of mouse glomerular endothelium (Rhodin, 1962) such diaphragms have not been observed in other species (Farquhar et.al., 1961; Latta, 1970).

The epithelial covering of this "simple, homogenous and transparent membrane" was first described by Gerlach in 1845 (cited by McGregor, 1929). He did this by examining glomeruli after having injected the kidney via the ureter. He further suggested that these cells were glandular in nature. Once again opinions differed as to the structure and function of these cells but in 1874 Heidenhain (cited by Clark, 1957), after examining several mammalian species, found that the epithelial cells formed a continuous layer over and between the capillary loops. This work was subsequently confirmed by several investigators until in 1886 Nussbaum (cited by Vimtrup, 1928) clearly distinguished between glomerular epithelium and the smaller endothelium of the frog glomerulus. He also recognised the continuity of the

glomerular and the parietal epithelium of the Bowman's capsule.

It was not until 1927 that von Moellendorff (cited by McGregor, 1929) described the epithelium, which he referred to as "deckzellen", as having long branching anastomosing processes which form a network over the capillary loops. As important to later studies he recognised the ability of these cells to transform into round process-less cells during pathological conditions (e.g. Seiler et.al., 1977).

These epithelial cells, or "deckzellen", have now been named "podocytes" and their processes as "foot processes". They are now seen to abut directly onto the glomerular basement membrane. Between each process there is a diaphragm of 200 to 300A termed the "slit membrane" (Yamada, 1955). The role of these slit membranes as a "primary filtration barrier" has been extensively reviewed by Farquhar (1975).

In recent years the introduction of scanning electron microscopy has done much to illuminate the topography of these interdigitating foot processes. The primary, secondary and tertiary arrangement of the processes has now been described in various mammalian species (Arakawa, 1970; Fujita et.al., 1970; Arakawa, 1971; Andrews, 1975 and Spinelli, 1976).

In the normal glomerulus there is, lying between the endothelium and the epithelium, a glomerular basement membrane (GBM) which was first described by Seng in 1871 (cited by McGregor, 1929), some thirty years after Bowman had rejected its existence. Seng also described the continuity of the GBM and the capsular basement membrane of Bowman's capsule.

Once again there were as many proponents of Seng's beliefs as there were opponents. For example Nussbaum (1886)(cited by Vimtrup, 1928) having searched unsuccessfully for a GBM concluded that the glomerular wall was comprised of only two layers namely the endothelial and epithelial cell layers.

However, Drasch (1877) and Ruhle (1897) (both cited by McGregor, 1929), both using digestion methods, were able to show the GBM, the capsular basement membrane and their junction. Subsequently with the advent of the latest staining techniques Regaud and Policard, (1903); Hueter, (1908); Gross, (1919) and Ohmori, (1921) (all cited by McGregor, 1929) all confirmed the presence of a GBM. Nevertheless controversy remained as to its existence. This controversy was not helped by the fact that whereas most basement membranes stain, like reticulin, with appropriate silver staining techniques the GBM was thought to be negative to silver impregnation with the normal glomerulus containing only

a small amount of argyrophilic fibres (Bensley and Bensley, 1930).

Subsequently it was discovered that certain aldehydes from carbohydrate containing material, in this case GBM, will selectively reduce an alkaline hexamine-silver salt mixture thus facilitating visualisation of the GBM (Gomori, 1946; Jones, 1957).

With the advent of the electron microscope the GBM was first seen as a homogenous layer without the same fibrillar nature as the capsular basement membrane (Mueller, 1958).

The description now accepted was first propounded by Rhodin (1955) when he described the GBM of the rat as having three separate layers namely a central dense layer, the lamina densa, of approximately 650A and two outer, less dense, layers the lamina rara externa and the lamina rara interna both of approximately 300A in size. The current status of the GBM has been reviewed by Crowell et. al. (1974) and Churg and Grishman (1975) and is largely unchanged from Rhodin's original observations.

The composition of the GBM has been extensively reviewed by Kefalides (1972). Basically, it has been shown to be a collagenous protein containing hetero-saccharide residues and disulphide linkages (Latta et.al., 1975; Latta and Johnston, 1976; Osborne

et.al., 1977). The peptide portion is formed, not surprisingly, by amino acids in a sequence similar to that of other organs (Albini et.al., 1979). By chemical comparison a striking similarity has been shown between the composition of the GBM in terms of carbohydrates and phospholipids in three species namely man, rat and the rabbit (Sachot et.al., 1975).

Recent histochemical studies of these peptides have shown them to contain large amounts of sialic and aspartic acid, both strongly polyanionic molecules (Venkatachalam and Rennke, 1978).

The significance of this charge structure has been shown experimentally whereby neutralisation of the anionic charge on the GBM, using polycations, has resulted in an increase in the urinary excretion of albumin and immunoglobulin G (Hunsicker et.al., 1982 and Vehaskari et.al., 1982) and has increased permeability to native substances, such as ferritin, which are anionic (Batsford et.al., 1980 and Barnes et.al., 1984).

It has been suggested that when the electrical charge on the GBM is neutralised anionic proteins, such as ferritin, albumin and insulin are adsorbed onto the GBM by hydrogen-ion bonding and thus clog the filter which reduces ultrafiltration (Kanwar and Rosenzweig, 1982). Whereas under normal conditions the sulphated polyanionic macromolecules prevent bonding and thus

maintain an efficient ultrafiltration (Gregor and Gregor, 1978). It has also been suggested that the disruption of this electrical charge structure on the GBM may contribute to the pathogenesis of glomerulonephritis (Border et.al., 1982). Indeed proteinuria has been recorded in rats as a result of electrostatic disruption of the GBM (Brenner et.al., 1978).

The glomerular mesangial cell and its associated intercellular matrix have been the subject of the greatest controversy since they were described in 1865 by Key (cited by McGregor, 1929). Key described "star-shaped" cells which he considered to be of a connective tissue type.

Briefly, the early workers in this field who saw these cells described them as being components of a connective tissue "stalk" which gave rise to the glomerular capillaries. It was not until 1933 when Zimmerman (cited by Mueller, 1958) propounded and explicitly described the "third cell" concept that the situation became clearer. By his use of specialised stains he was able to show that not all the cells within the confines of the GBM were endothelial in nature. However, this "third cell" type which he identified he described as being fibrocytic in nature despite the fact

that neither he nor any other anatomist found any evidence of collagen in the normal glomerulus. It was also he who first named these cells "mesangial cells" and this so-called connective tissue stalk the "mesangium".

More recently two different workers Goormaghtigh and Yamada described the mesangial cells as having similarities to smooth muscle cells but again they saw their function as controlling the support for the glomerular capillaries (Goormaghtigh, 1951 (cited by Jones, 1953) and Yamada, 1955).

Subsequent to these studies investigation of glomerular localisation and transport of macromolecular tracer substances have revealed that following their intravascular injection they were seen to be taken up by mesangial cell processes which can extend between endothelial cell cytoplasm and the GBM. The fate of these phagocytosed macromolecules is as yet uncertain but it has been suggested that there is a transport system within the mesangium which slowly moves macromolecules towards the base of the glomerulus where they are removed by the renal lymphatic system (Bernacerraf et.al., 1957; Ashworth and James, 1961; Farquhar and Palade, 1962; Latta and Maunsbach, 1962; Menafee et.al. ,1964; Crowell et.al., 1974; and Farquhar, 1975).

It has also been suggested that these macromolecules enter the mesangium through channels, containing mesangial matrix, between mesangial cells whereupon they are phagocytosed by individual mesangial cells.

This mesangial matrix comprises a band of amorphous, fibrillar, dense basement membrane-like material of, as yet, unknown composition and origin (Osborne et.al., 1977). Currently it has been proposed that it is formed, in part at least, following the turnover and renewal of the GBM (Walker, 1973) by the mesangial cells themselves (Romen et.al., 1976). However, immunologically speaking it possesses no cross-reactivity with GBM itself (Scheinmann et.al., 1974).

Recently, Burkholder (1982) has reviewed the numerous functions so far attributed to the mesangial cell. These functions include fibrocytic activity, smooth muscle contractility, phagocytosis, hormone reception, prostaglandin production, synthesis of mesangial matrix and proliferation in response to injury.

These mesangial cells are also continuous with the "Lacis" or pseudo-Meissnerian cells of the juxta-glomerular apparatus and may assist these cells in conjunction with the granular epitheliod cells in

controlling glomerular blood flow (Latta et.al., 1962 and Latta and Maunsbach, 1962).

Almost completely encircling these three cell types lies the epithelium of Bowman's capsule which is a thin flattened layer of cells intimately opposed to the capsular basement. This layer of cells, whose cell junctions are quite distinct (McGregor, 1929), is continuous with the proximal tubular epithelium and also with the visceral epithelium covering the capillaries.

Electron microscopical studies of these cells have revealed them as having few cytoplasmic organelles and apart from a few mitochondria scattered throughout the cytoplasm, they do not have the internal organisational complexity characteristic of the cells of the proximal tubule (Mueller et.al., 1955 and Yamada, 1955).

Despite their seemingly non-complex nature there is an accumulation of distinctively granular parietal cells encircling the entrance to the proximal tubule. These are known as the "peripolar cells" (Ryan et.al., 1979) and although their function is unclear they may exert a regulatory role on the glomerular filtrate.

2. The renal glomerulus of the cat :

During the past twenty years the cat has frequently been used as the experimental animal in functional studies of the kidney (Nissen and Galskov, 1972; Spielman and Osswald, 1977; Freidman and Roch-Ramel, 1977 and Jones et.al., 1979) yet little ultrastructural information is available in the literature. The majority of morphological studies of the renal glomerulus have been carried out in the rat, mouse and man (Arakawa, 1970; Spinelli, 1976; Osborne et. al., 1977).

Furthermore, the recently increasing numbers of reports of glomerulonephritis in the cat (Lucke and Hunt, 1965; Wright et.al., 1976; Nash et.al., 1979; Lucke et.al., 1980; Lucke, 1982) have highlighted the importance of an understanding of normal glomerular structure in this species not only at the light microscope level but also in view of the increasing use of transmission and scanning electron microscopy.

The histological examination of the cat kidney began more than 50 years ago with Zimmerman (1935) (cited by Mueller, 1958). However, since that initial study data from apparently normal feline kidneys that could be used for quantitative comparison has been meagre in the extreme. Indeed only two reports have

examined the normal glomerulus histologically and then only in relation to the autolytic changes occurring within the kidney (Mayer and Ottolenghi, 1947; Crowell and Leininger, 1976). Another deals with the preparation of the kidney for ultrastructural studies and again makes only scant reference to the glomerulus (Yun and Kenny, 1976). A third concentrates on the changes occurring during a wide range of pathological conditions. Even though this work uses a large number of healthy cats no attempt is made to describe or define the normal renal glomerulus (Lucke, 1968).

Other aspects of feline glomerular morphology such as overall number of glomeruli having been studied. The number of glomeruli in the cat has been calculated at 202,000 (Vimtrup, 1928), 184,000 and 214,500 (Kunkel, 1920) and 184,000 and 214,000 (Rytand, 1938).

In this chapter of the thesis the normal renal glomerulus of the adult cat will be examined by means of histological methods, transmission electron microscopy and scanning electron microscopy. The use of perfusion fixation techniques together with Tensol casting will also be described.

MATERIALS and METHODS.

1. Source of Animals.

Twenty adult cats were obtained from commercial sources to be used in this section of the study. Prior to their being used in the study detailed examinations, as previously described on page 6 were carried out.

Upon examination nine animals were found to have a hitherto unrecognised glomerular lesion. These animals were excluded from this part of the study and will be more closely examined in chapter 4.

2. Euthanasia.

The method of euthansia employed was as stated in the general Materials and Methods, pages 6-7.

3. Sampling of Tissues.

After euthanasia tissues were sampled as described on pages 7 - 10 of the general Materials and Methods section. All histological and ultrastructural techniques are also as previously stated.

A summary of the methods of investigation employed and the number of animals involved of those 11 remaining animals which were found to be clinically, macroscopically and histologically normal now follows :-

TABLE 1.1

Summary of Tissues Obtained.

Methods of Investigation		Number of Animals
Histology-Immersion		11
	Perfusion	4
T.E.M.-Immersion		11
	Perfusion	4
S.E.M.-Immersion		8
	Perfusion	4
Tensol Casting		2
Immunofluorescence		8

RESULTS

On examination of the kidneys of these 20 animals, it was found that nine had a glomerular lesion of a type not previously described in the literature. These animals were excluded from this part of the study and an in-depth examination of this condition will be reported later in this work (Chapter 4).

1. LIGHT MICROSCOPY

The aim of this section of the work was to ascertain the optimal thickness for paraffin-embedded kidney sections using both immersion and perfusion fixation techniques in order to study the normal morphology of the cat renal corpuscle.

a) Immersion fixation:

Six cats were utilised in this study. Figs. 1.2, 1.3 and 1.5 show glomeruli illustrating sections cut at 10, 6 and 2 um. respectively. It can be seen that in the 10um. section (Fig. 1.2) little glomerular detail could be determined due to the seemingly closed capillaries and a glomerular tuft which appeared hypercellular. This apparent hypercellularity comprised darkly stained nuclei whose cell type was difficult to identify. In the 6um. section (Fig. 1.3) the majority of the capillary loops appeared closed and again there was the impression

of hypercellularity which obstructed the overall microscopic view of glomerular morphology. In the 2um. section (Fig. 1.5), a level of thickness was reached where each and every cell type could be identified with certainty. The capillaries were patent and it was possible to examine the capillary loops directly to ascertain the detailed structural features.

b) Perfusion fixation:

With the present perfusion fixation method not all the glomeruli were evenly perfused, particularly those in the outer cortex. If one compares Figs. 1.3 and 1.4 it can be observed that Fig. 1.3 illustrates well perfused juxtamedullary glomeruli with patent capillaries the lumina of which are largely free of any debris. In addition, there is good differentiation of individual cell types. On the other hand Fig. 1.4 (also a perfusion-fixed specimen) shows an outer cortical glomerulus in which perfusion failed to produce adequate filling of the glomerular capillaries. It was also noticeable in this study that the deep juxtamedullary glomeruli were larger in size than those glomeruli of the mid and outer cortex.

Once again differences in section thickness affected the histological results of perfusion fixation. Any advantage accrued from perfusion fixation was lost by using sections of too great a thickness. This is

illustrated by Figs. 1.2 and 1.3, which show perfused juxtamedullary glomeruli sectioned at 10 and 6um. respectively with Fig. 1.5 showing similar glomeruli sectioned at 2um. The differences illustrated are identical to those observed using immersion fixation.

One area of note with perfusion fixation was that the urinary space was often enlarged compared to that for immersion fixation regardless of whether or not the glomerulus was from the outer or inner cortex.

c) Plastic lum. sections:

Fig. 1.6 shows a glomerulus which was perfusion fixed in Karnovsky's fixative prior to embedding.

On occasion a glomerulus was also able to be visualised accurately under immersion fixation conditions (see Fig. 4.3, Chapter 4). In both instances the capillaries were patent and detailed structural features could be examined within the capillary loops. However, the perfusion of kidney material used for lum. plastic sections and ultimately for TEM appeared to offer little advantage to the operator as the difference in the results obtained from these procedures for light microscopy is minimal, yet the additional technical problems are not inconsiderable.

2. TRANSMISSION ELECTRON MICROSCOPY (TEM)

Particular attention was paid to the major structural components of the feline renal corpuscle namely a) the visceral epithelium, b) the endothelium, c) the mesangium, d) the glomerular basement membrane (GBM) and e) the parietal epithelium of Bowman's capsule (see diagram; page 15).

a) Visceral epithelial cell:

From the main mass of the epithelial cell body, in which the nucleus and the majority of the cellular organelles were located, a number of primary cytoplasmic processes gave rise to numerous smaller secondary branches (Fig. 1.7). Tertiary, or 'foot', processes then arose from these branches and could be observed regularly arranged along the GBM. A single unit membrane, the 'slit membrane', was often observed running between adjacent foot processes (Fig. 1.10, also see Fig 2.12, chapter 2).

Organelles within the cytoplasm of this cell type showed little difference to that of other mammals. Golgi complexes were prominent being characteristically recognised as a stack of cylinders in longitudinal section or as a group of vesicles in transverse section. Both rough and smooth endoplasmic reticulum was present but only in small quantities. The former had a coating of ribosomes giving it its characteristic roughened

appearance. However, fragments of endoplasmic reticulum could also be found in any of the processes emanating from the main cell body. In addition, aggregates of ribosomes were commonly discovered throughout the cytoplasmic processes. Mitochondria were not conspicuous within either the cell body or its numerous processes.

b) Endothelial cell:

The endothelial cell lining the glomerular capillary appeared discontinuous as it encircled the capillary (Fig 1.8). This was due to the presence of fenestrae in the attenuated portion of the endothelial cytoplasm which extends away from the cell body which lay in the axial portion of the capillary loop. The plasma contained within the capillary loop appeared to have direct access to the lamina rara interna.

The cytoplasmic organelles of this cell appeared to be restricted, in the main, to the cytoplasmic mass containing the nucleus. However, small mitochondria could be found throughout the cell, even in the fenestrated portion, though in low numbers. Occasional membrane-bound 'dense bodies' could be found and many small pinocytotic vesicles could always be seen.

This attenuated portion of the cell appeared only to be approximately 400A thick with the fenestrae themselves measuring approximately 500 to 1000A in diameter.

Between the endothelial cell and the mesangium there was a cell membrane, though its presence was sometimes difficult to distinguish, however, this together with the differing appearance of their cytoplasmic matrices allowed these two cells to be easily distinguishable (Fig. 1.9).

c) Mesangial cell:

Between each capillary loop usually only one or two mesangial cells could be identified. These cells were bounded laterally by the GBM and at the axial portion of the capillary loops by the cell body of the endothelium. Frequently, mesangial cell cytoplasmic processes could be seen projecting either between the GBM and the endothelial cell, for a short distance, or simply projecting through the endothelial cell body into the capillary lumen (Fig. 1.9). These protrusions of the mesangial cells, the 'Intrakapillarhockerchen' (Zimmerman, 1933) (cited by Mueller, 1958) could also be seen as isolated cytoplasmic masses in the lumen of the capillaries (Also see Fig. 6.5, Chapter 6).

In the cytoplasm of these cells slender mitochondria; endoplasmic reticulum, mostly bounded by ribosomes, and occasional electron dense membrane-bound granules could be seen in varying quantities. In addition, numerous bundles of thin filaments were arranged along the axes of the cell processes. Towards

the periphery of the glomerular tuft, there appeared to be a diminution of mesangial cellular material though its structural pattern was the same as that at the base of the tuft.

The characteristic structure of the mesangial cell allowed them to be easily differentiated from both endothelial and epithelial cells. Moreover, any confusion with epithelial cells was rendered unlikely by virtue of the interposed GBM and the characteristic topography and processes of the epithelial cell.

An amorphous, relatively homogeneous material was observed to separate adjacent mesangial cells and, although channels have been found in this matrix in certain species (Latta et.al., 1960), no channels could be found in this series of cats.

d) Glomerular basement membrane:

The prominent GBM with its characteristic appearance of a thick electron dense central layer, the lamina densa, and two thinner electron lucent layers namely the lamina rara externa on which the epithelial foot processes were implanted and the lamina rara interna adjacent to the fenestrated endothelial cell, was easily identifiable (Fig. 1.10).

The basement membrane was shown to be continuous with the capsular basement membrane at their junction at the base of the tuft.

The thickness of the feline GBM was approximately 1000 - 1500 A.

e) Parietal epithelium:

The parietal epithelium was an extremely flattened layer of cytoplasm and had dense basilar clumps of parallel filaments resembling those in the proximal tubule with which it was continuous at the urinary pole.

However, examination of these cells showed them to have a few mitochondria scattered throughout the cytoplasm but no intricate organisation of internal structure such as characterises the cells of the proximal tubule. Similarly, no peripolar cells were found.

3. SCANNING ELECTRON MICROSCOPY

Observations were carried out on both juxtamedullary and outer cortical glomeruli with the scanning electron microscope, these areas being readily identifiable at low magnification prior to a high magnification study.

When the kidney was sectioned with a razor Bowman's capsules, renal corpuscles, uriniferous tubules and blood vessels were severed. Tissues prepared for SEM in this manner, therefore, afforded a good view of the topography of those areas. At low magnification, the cut surface revealed numerous renal corpuscles complete with

their glomeruli as well as empty capsules. For the most part, glomeruli were intact; however a few sectioned glomeruli could be found but were less obvious (Fig. 1.11).

a) Parietal epithelium:

The nucleated regions of the inner i.e. luminal parietal surface bulged into the cavity of Bowman's capsule. In certain cases the aperture of the proximal tubule could be observed.

A prominent space, the urinary or Bowman's space separated the parietal epithelium from the glomerulus (Fig. 1.11).

b) Visceral epithelium:

The visceral epithelium consisted of the podocytes with their interdigitating cell processes arising from large nucleated cell bodies and enveloping the capillary loops (Fig. 1.12).

The arm-like processes (primary processes) arising from any given cell body usually gave off secondary processes which in turn terminated in tertiary processes. The relative lengths of these processes together with the angles at which they arose varied considerably. Additionally, the diameters of these processes varied and often a process appeared to flatten before giving rise to other processes.

Occasionally, the processes arising from one of

these arms fused with another process from the same cell; however none could be found fusing with processes from any other cell (Figs. 1.12 and 1.13).

The tertiary (or foot) processes which emerged from all the podocyte processes and occasionally from the cell body itself were as polymorphic as the major processes. They varied considerably in their lengths and diameters and usually exhibited tapered or irregular bulbous ends. When traced to their cell of origin it was found that the foot processes from one cell always interdigitated with foot processes from another cell (Fig. 1.13).

Slender microvilli on both the visceral epithelial cell body and its processes together with shorter projections from these areas could also be seen (Fig. 1.12).

c) Endothelium:

Severed glomeruli allowed visualisation of the lumina of glomerular capillaries and their relationship to the GBM and to the visceral epithelium.

At high magnification, the fenestrated nature of the attenuated portion of the endothelial cell could be discerned. These pores ranged in size from 500 to 1000A and were usually separated by a space of approximately 800 A (Fig. 1.14).

On the luminal capillary surface there were many

long, branching thickenings on the endothelial cell which appeared to form a support network for the thin fenestrated endothelium. The surfaces of this network are relatively sparsely covered with microprojections found on the free surfaces of the visceral epithelium (Fig. 1.14).

4. ARTERIAL CASTS

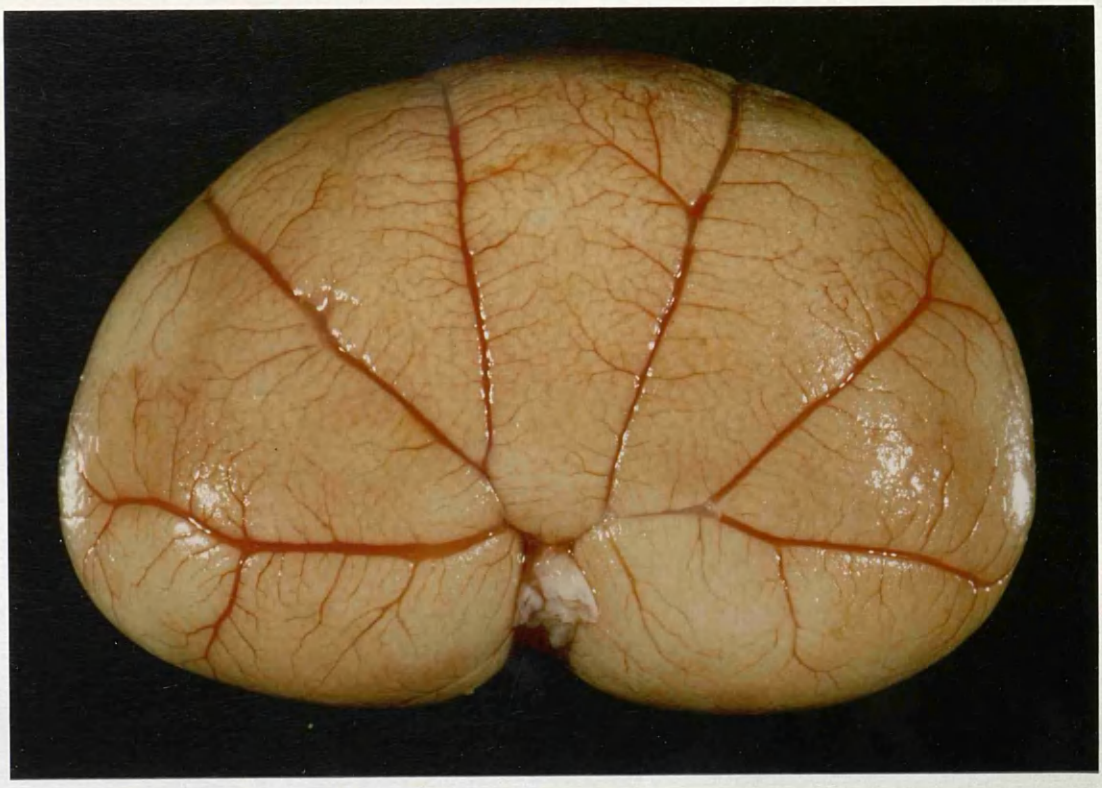
Casts of renal glomeruli, and their afferent and efferent arterioles, successfully demonstrated the general morphology of the renal glomerulus. The afferent arteriole was seen to arise from the interlobular artery and then divide into three or four capillary branches as it entered the glomerular tuft. Extensive branching of these primary capillaries was observed with numerous anastomoses occurring between the capillaries and their branches.

The branches rejoined to form the smaller efferent vessel which emerged from the vascular pole of the capillary tuft only to divide into a network of peritubular capillaries.

No vascular shunts leading directly between the afferent and efferent vessels could be demonstrated using this method of investigation (Figs. 1.15 and 1.16).

Figure 1.1
Cat Kidney
Fresh Specimen
(x 4)

Figure 1.2
Normal Cat Glomerulus
10 um. thick
Inner Cortex
Note the apparent hypercellularity
H & E (x 300)



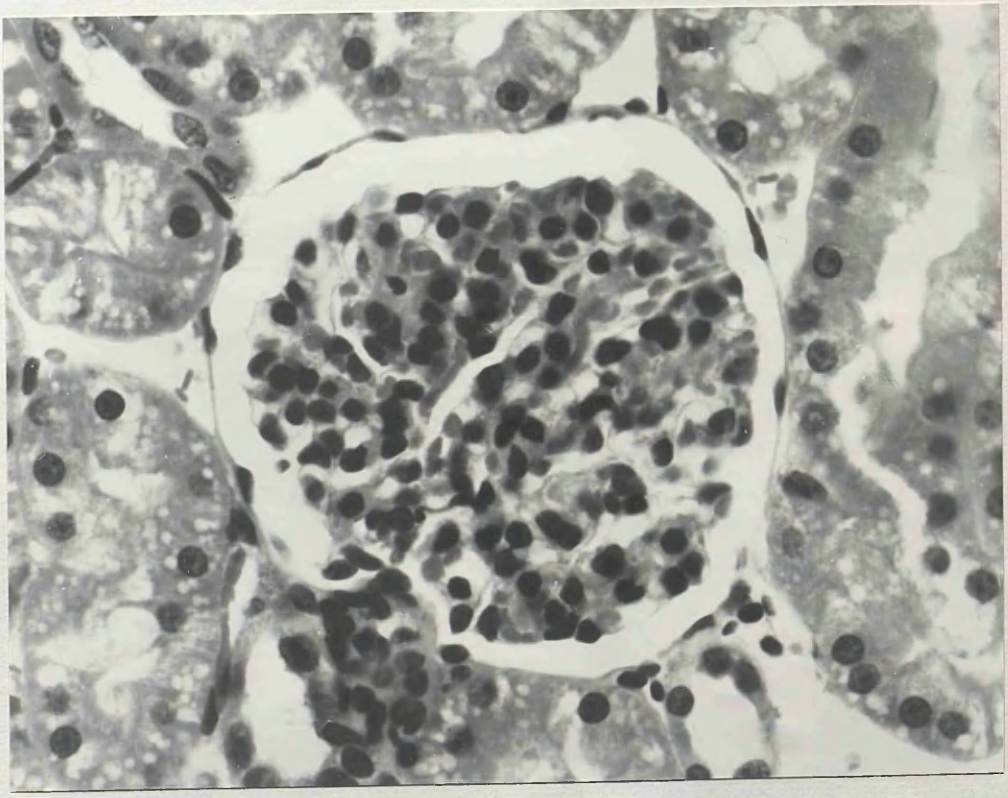
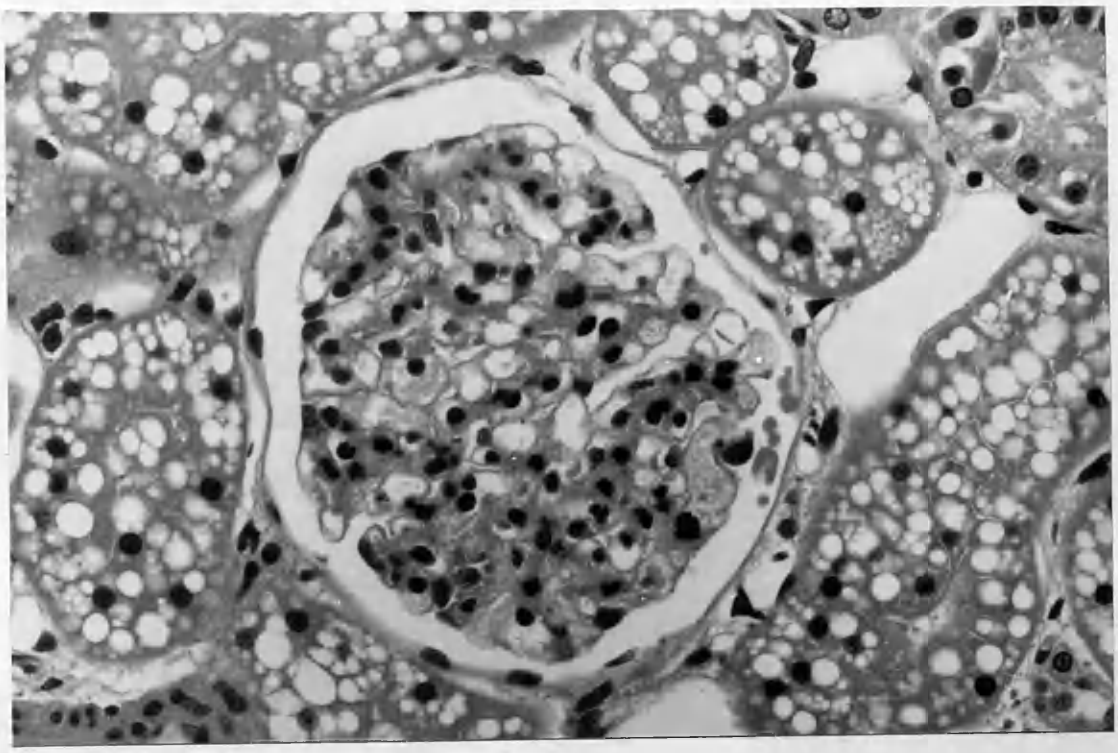


Figure 1.3
Normal Cat Glomerulus
6 um. thick
Inner Cortex
Note the increased visualisation over
Fig. 1.2 due to the decrease in thickness
H & E (x 300)

Figure 1.4
Normal Cat Glomerulus
6 um. thick
Outer Cortex
Note the smaller dimension as
compared to Fig. 1.3
H & E (x 300)



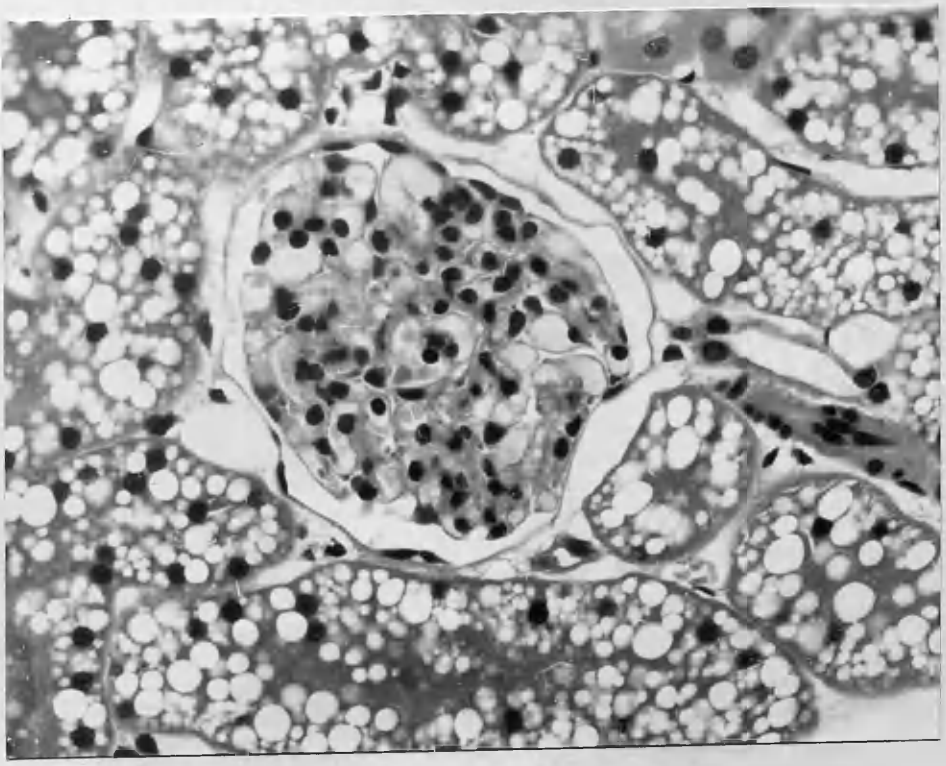


Figure 1.7
Visceral Epithelial Cell (v)
Capillary (c)
Podocytic Processes (arrows)
T.E.M. (x 5000)

Figure 1.8
Endothelial Cells (e)
Fenestrated Endothelium (arrows)
Red Blood Corpuscle (rbc)
Capillary (c)
T.E.M. (x 5000)


c
v

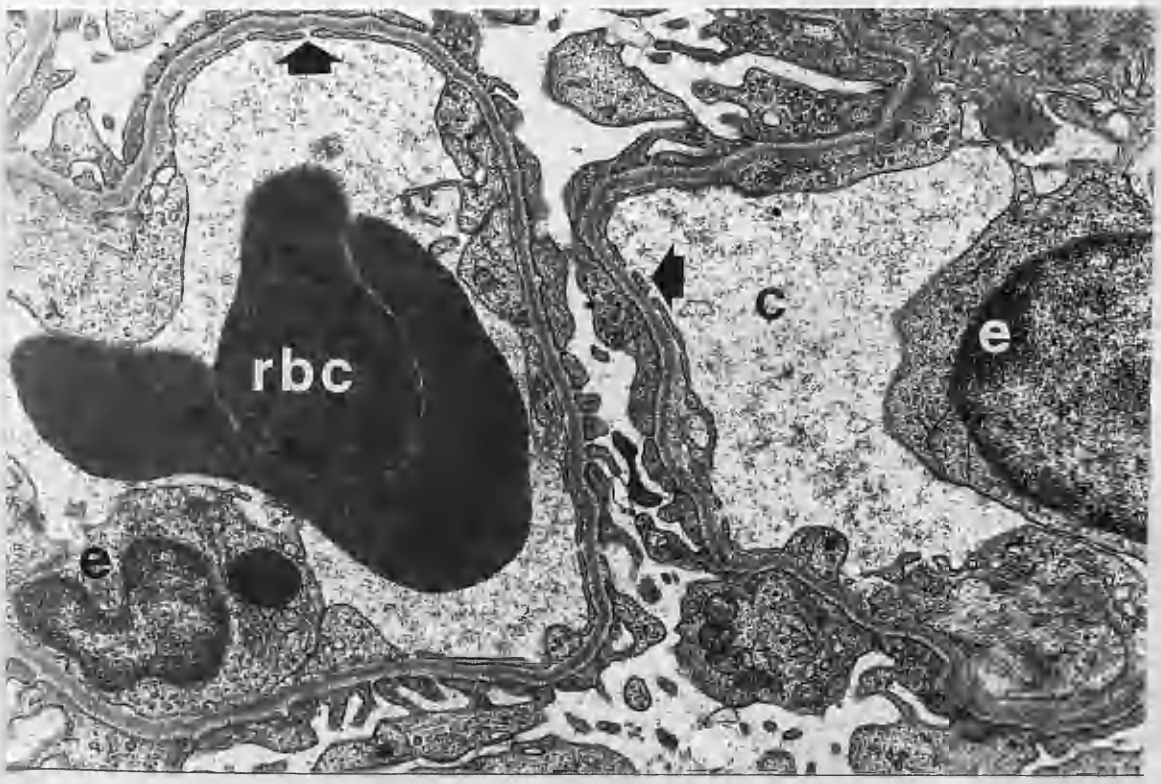
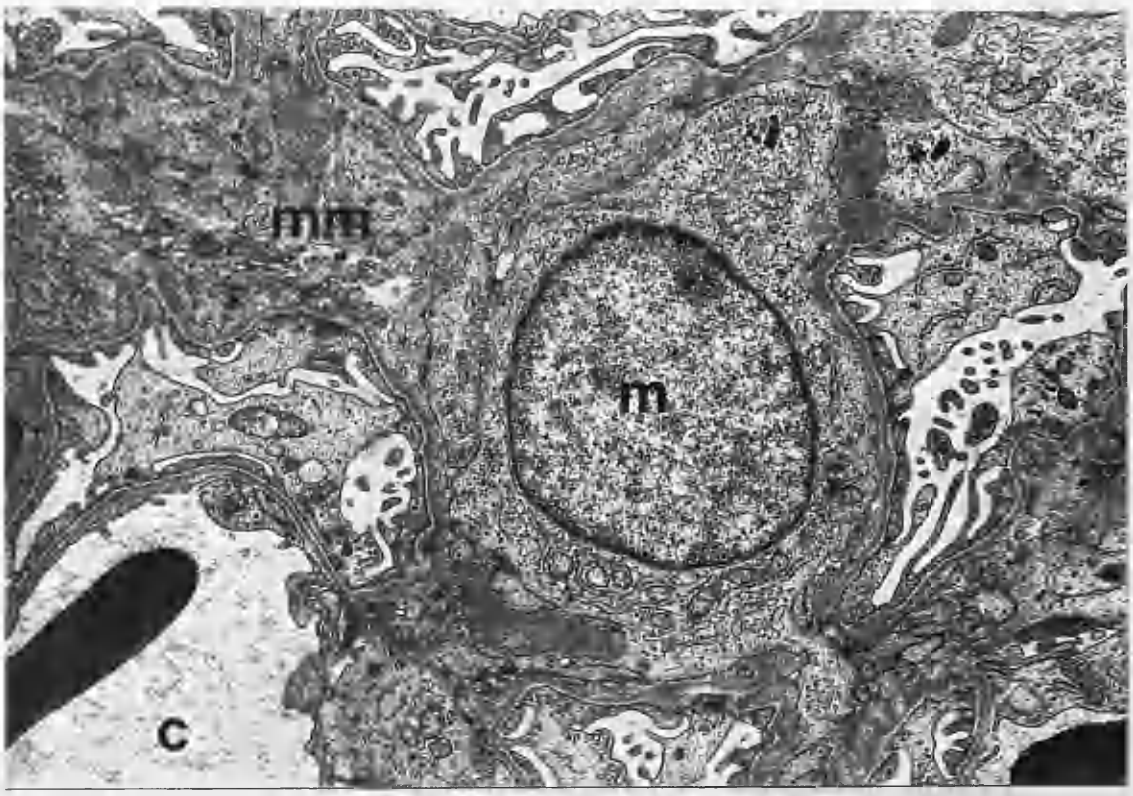

rbc
c
e
e

Figure 1.9
Mesangial Cell (m)
Mesangial Matrix (mm)
Capillary (c)
T.E.M. (x 5000)

Figure 1.10
Glomerular Basement Membrane (gbm)
Endothelial Fenestrations (arrow 1)
Epithelial Slit Membrane (arrow 2)
T.E.M. (x 20000)


mm
m
c

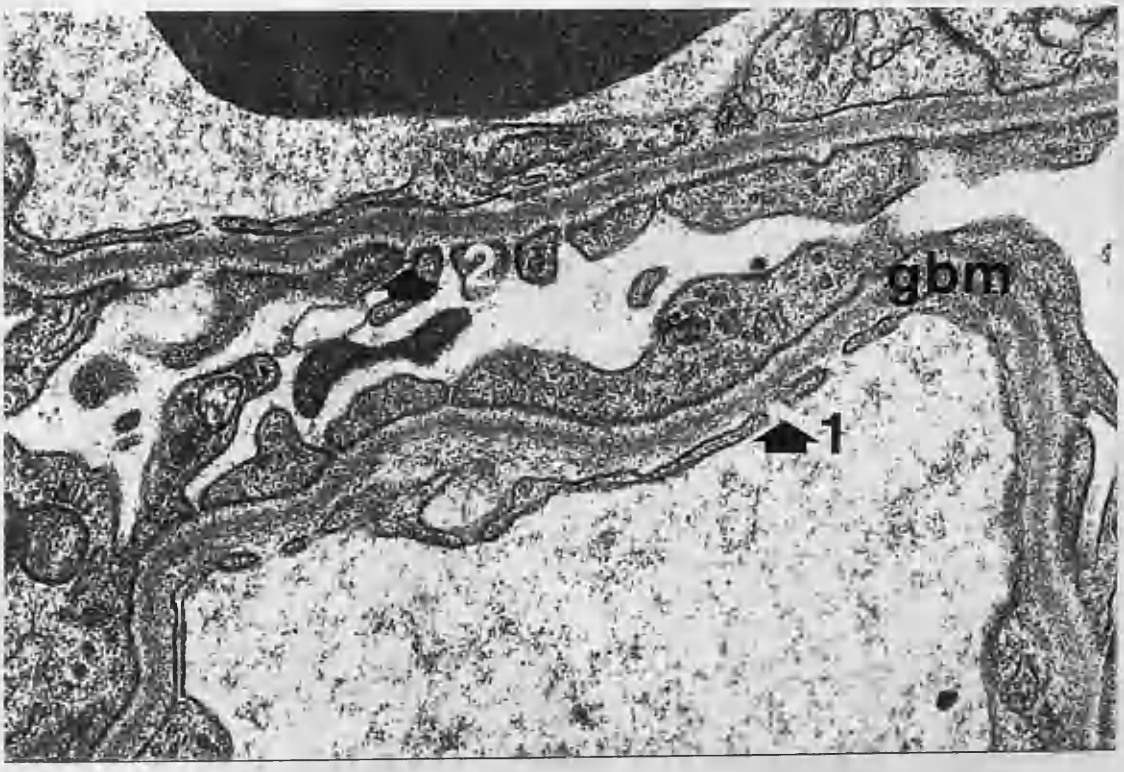

2
gbm
1

Figure 1.11
Normal Cat Glomerulus
Bowman's space (asterisk)
Sectioned capillaries (arrows)
S.E.M. (x 640)

Figure 1.12
Visceral Epithelial investment
of glomerular capillaries
Cell body (asterisk)
S.E.M. (x 2500)

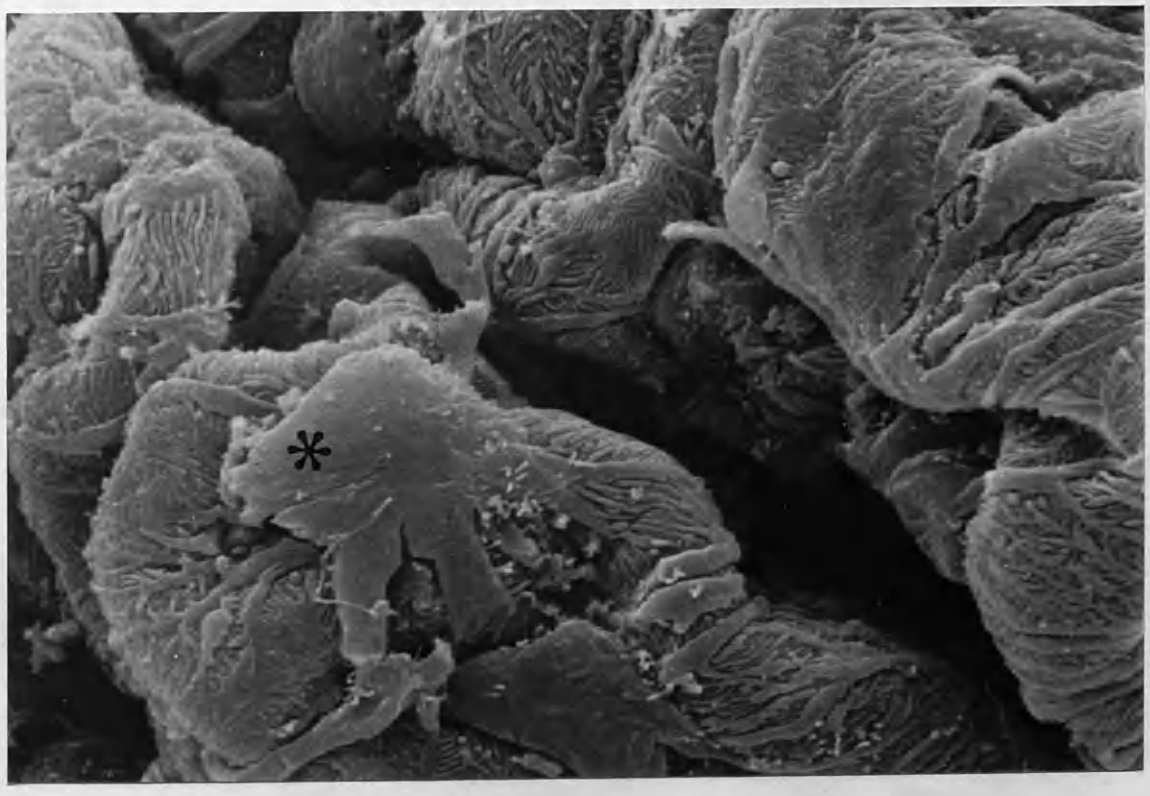
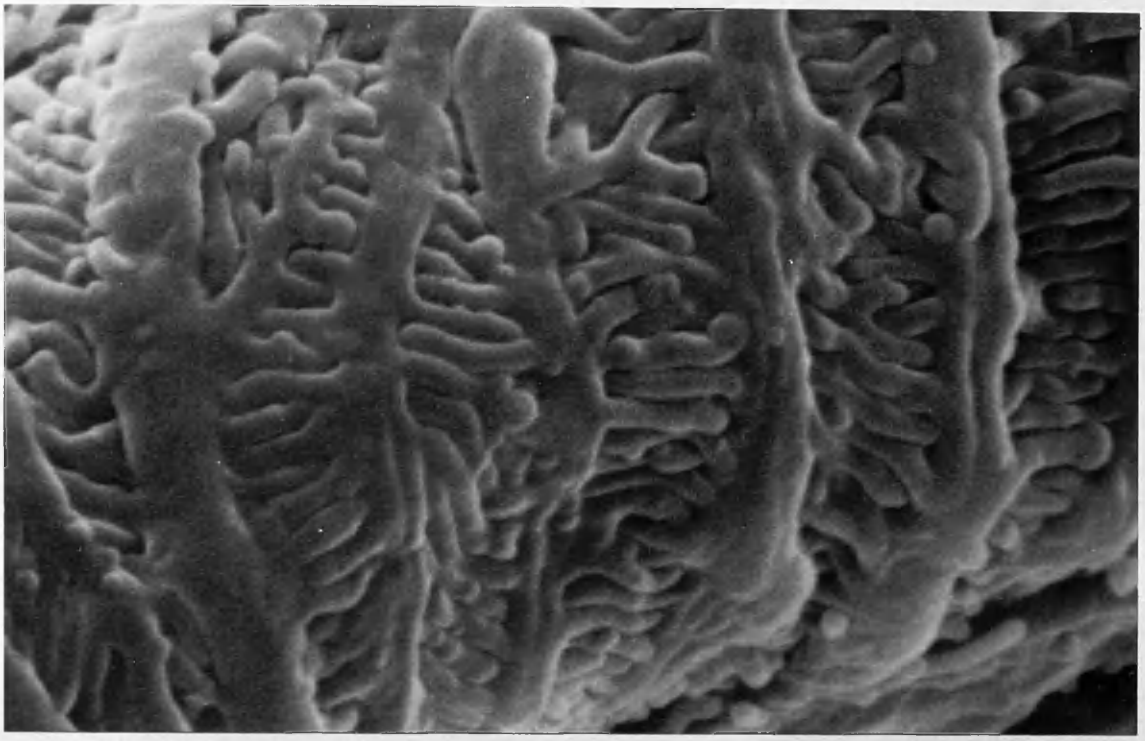


Figure 1.13
Visceral Epithelial Cell
Primary, Secondary and
Tertiary Podocytic Processes
S.E.M. (x 20,000)

Figure 1.14
Endothelial Cell
Fenestrated Endothelium
Note supporting ridges
(asterisk)
S.E.M. (x 40,000)



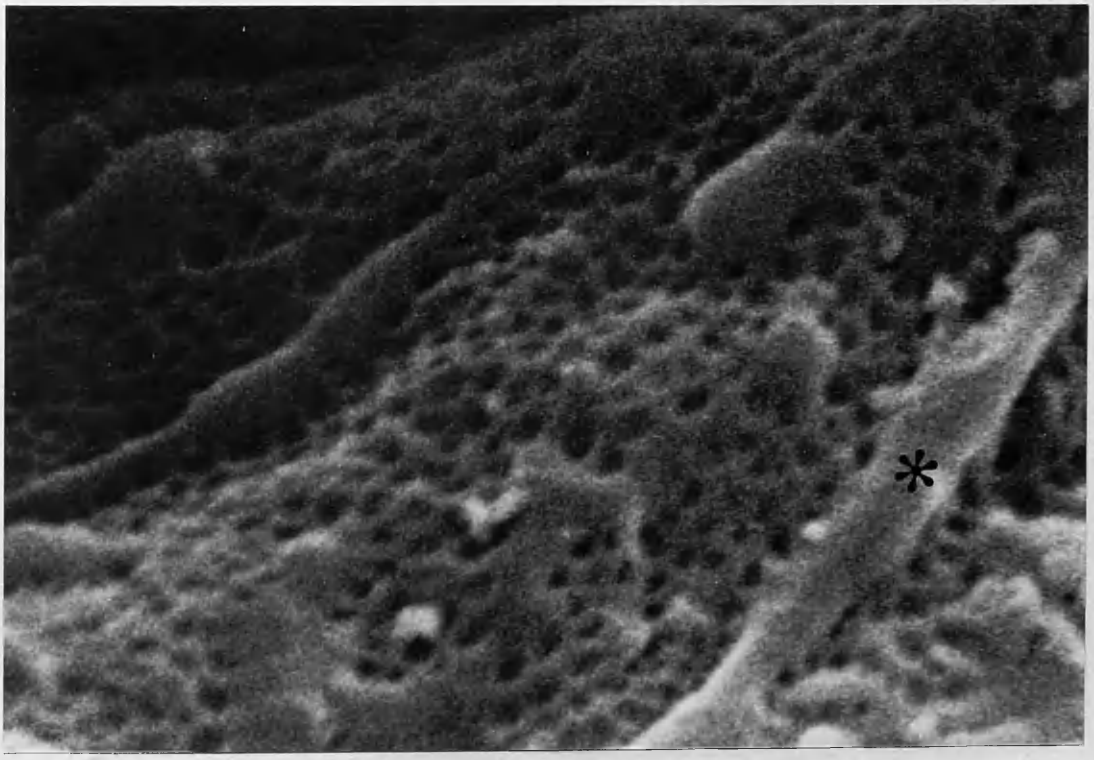
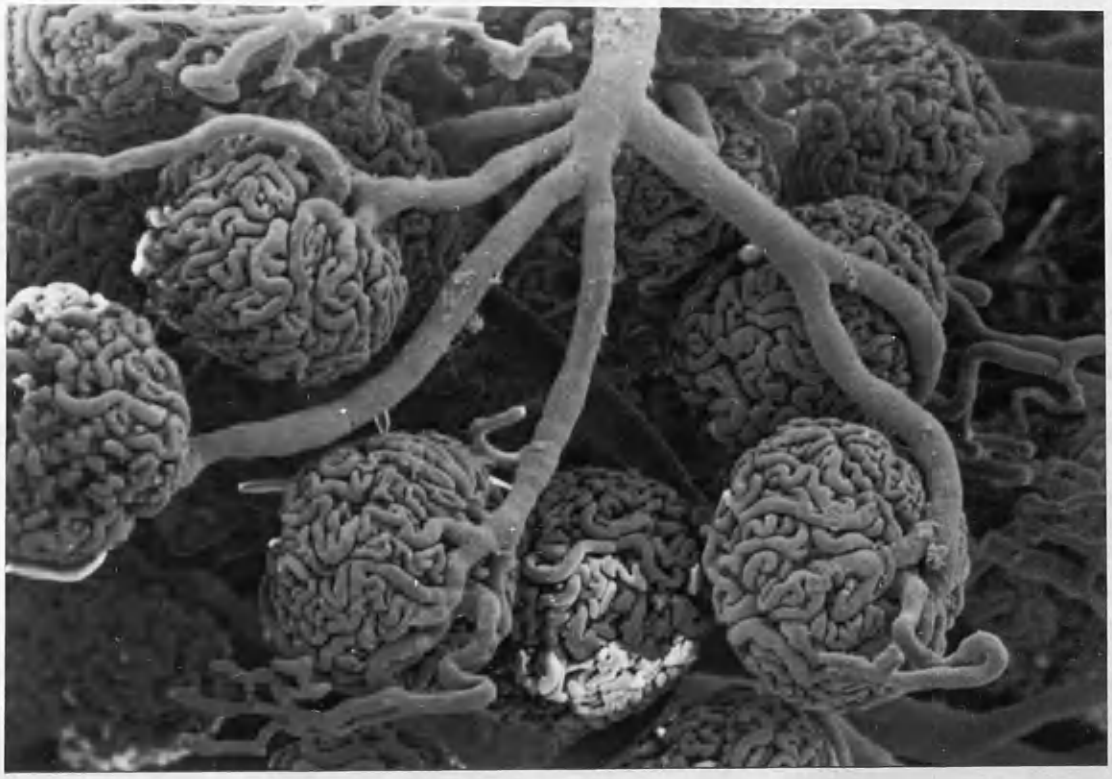
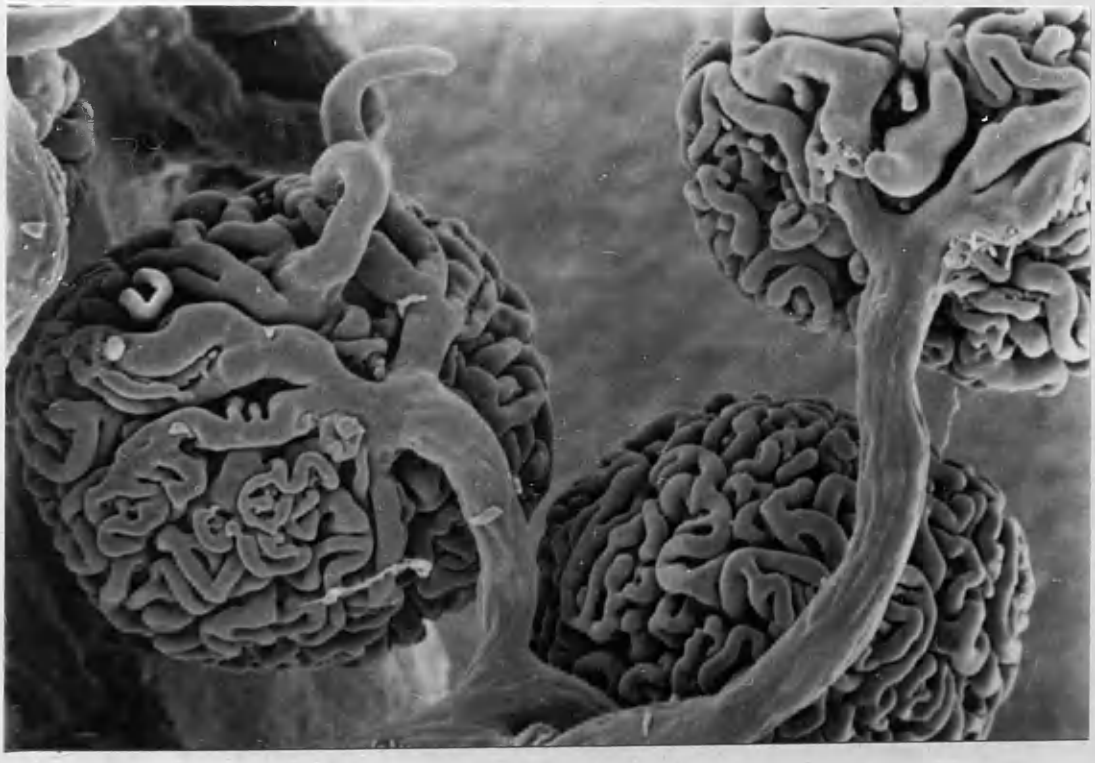


Figure 1.15
Tensol cast: Interlobular artery supplying a group of glomeruli
S.E.M. (x 160)

Figure 1.16
Tensol cast: afferent arterioles can be seen branching into a number of lobular vessels
S.E.M. (x 320)





DISCUSSION

The two methods of fixation described, both of which were successful in preserving renal structures, were immersion and perfusion fixation. The comparison made in the present study showed that, although both methods resulted in fixation which was more than adequate for both light and transmission microscopy, perfusion fixation appeared to be superior to immersion fixation fixation in respect of the patency of glomerular capillaries which was achieved. This patency facilitated differentiation of glomerular cells using light microscopy.

However, perfusion fixation did have its disadvantages namely i) the capillary lumina and urinary space were sometimes artificially widened; ii) small numbers of glomeruli in the outer cortex appeared not to be perfused at all. Thus the technical difficulties presented by perfusion fixation could in some cases outweigh the advantages gained.

However, despite these difficulties perfusion fixation remains the optimal method of choice particularly for TEM and SEM studies.

In the present investigation, the role of section thickness in the study of the renal glomerulus with the light microscope was highlighted. In the thickest of the

sections examined, i.e. 10um., glomeruli appeared to be hypercellular and even when the kidney was fixed by perfusion, glomerular detail was obscured by the sheer mass of tissue. In 6um. sections, the ease of cellular differentiation was better; however, overall, the advantages of good fixation were lost due to the thickness of the section. It is interesting to note that in most of the previous histological studies on the renal glomerulus, sections of this thickness were routinely used (e.g. McManus, 1948 and Bulger et.al., 1979).

For light microscopy, using paraffin-embedded material the 2um. section appeared to be optimal as it allowed good visualisation of glomerular capillaries and cellular differentiation was made relatively easy.

The use of 1um. plastic-embedded sections, whilst offering the thinnest section available for light microscopy, had distinct disadvantages. For example the fact that only a small area of kidney cortex could be examined at any one time with only a relatively small number of glomeruli present increased the technical difficulties.

The ultrastructural findings presented in this chapter were the first detailed examination of the normal feline renal glomerulus. As such, they have

provided a basis for future studies of the cat glomerulus with a view to evaluating the morphological changes which accompany various kidney disorders.

Previous ultrastructural studies of the glomerulus have been carried out in the dog (Mueller et.al., 1955; Movat and Steiner, 1961; Crowell et.al., 1974; Bulger et.al., 1979), rat (Pease and Baker, 1950; Griffith et.al., 1967; Bulger et.al., 1974), mouse (Yamada, 1955) and man (Pak Poy, 1958; Bulger et.al., 1967 and Jorgensen and Bentzon, 1968).

This study has confirmed that the cat kidney lies within the general pattern of mammalian kidney ultrastructural morphology and has emphasised the similarity of structure to that of mammals in general.

Likewise, the present study also provided the first detailed SEM views of the normal cat glomerulus and as such provided a basis for evaluating morphological changes which will be described later in this work.

Whilst the majority of SEM studies, to date, have concentrated on the rat there have been sufficient examinations of other mammalian kidney material for a clear picture of the SEM morphology of the mammalian kidney in general to have been formed. One area of controversy still remaining concerns the arrangement of the visceral epithelium and its processes. Thus Buss and

and Kronert (1969) described the interdigitating foot processes as arising from either different podocytes or from the same parent cell. However, in 1970 Fujita et.al., like Buss and Kronert using the rat, indicated that neighbouring foot processes arose invariably from different podocytes.

The present work is in agreement with the findings of Fujita et.al..

The presence of slender microvilli and smaller projections on both the podocyte cell body and its processes were also noted. This is in agreement with the works of both Buss and Kronert (1969) and Fujita et.al.(1970) who described these features in the rat and rabbit.

Other features such as cytoplasmic bridges, which have been given a role in the anchorage of the foot processes, have been described as running between adjacent interdigitating foot processes in the rat by Buss and Kronert (1969) and Andrews and Porter (1974). However Fujita et.al.(1970) and Arakawa (1970) denied their existence. The present work was in agreement with the former workers.

Andrews and Porter (1974) were the first authors to suggest that although when viewed by the SEM adjacent foot processes seemed to be irregularly spaced the TEM revealed that the portion of any single foot process in

contact with the basal lamina varied to such an extent that adjacent foot processes were actually separated by a remarkably uniform gap. However, the present work failed to concur with this observation.

Despite the large number of light microscopical and ultrastructural reports on the mammalian kidney, there have been relatively few reports of cilia in the glomeruli of higher vertebrates. The most notable of these was undertaken by Latta et.al. (1961) who reported finding single cilia on the parietal epithelial cells of Bowman's capsule in the rat. This work has, more recently, been confirmed for the parietal epithelium of the rat (Tyson and Bulger, 1972) and for man (Tisher et.al., 1966).

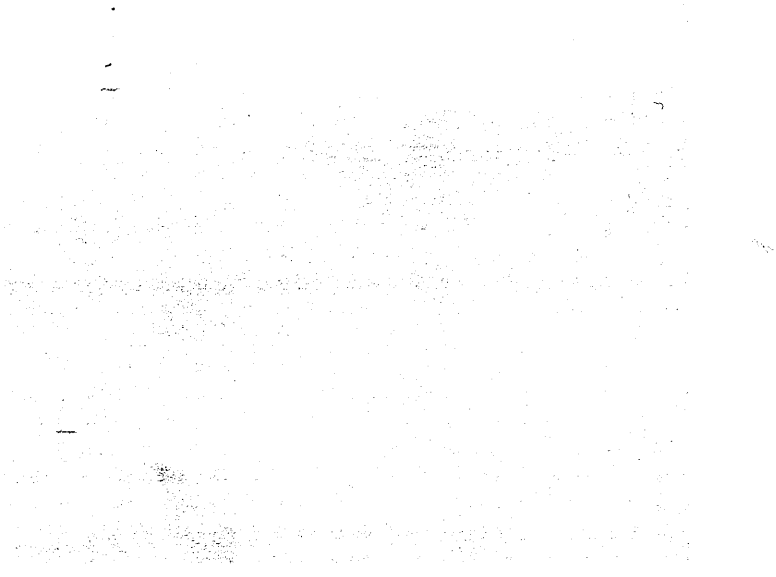
The present work found no cilia emanating from the parietal epithelium, thus agreeing, at least in part, with the studies of Latta et.al. (1961).

The SEM observation of corrosion casts of blood vessels enabled a detailed morphological study of the microcirculation patterns of the feline glomerulus. However, the reliability of vascular casting using high viscosity resins has been questioned by some workers (Ljungqvist, 1963). The main reason for doubt lies in the possibility of incomplete filling and consequent misinterpretation of the vascular features perfused with

resin.

However, the present investigation found no justification for these doubts as glomerular capillaries appeared to be evenly filled. Leakage of cast resin from ruptured capillaries did not occur and, as has been described, even the most delicate of anastomosing interlobular channels were easily examined.

In the present study no interarteriolar shunts were found in the cat glomerulus thus concurring with the observations of Spinelli et.al. (1972) and Mohammed (1985) who also failed to detect direct afferent / efferent arteriolar anastomoses in normal dogs.



CHAPTER 2

A HISTOLOGICAL AND ULTRASTRUCTURAL STUDY OF THE NEONATAL CAT GLOMERULUS

INTRODUCTION

General morphological events in the development of the mammalian kidney are well established (see reviews by Patten (1953), Arey (1954) and Potter (1965)) and require only brief recapitulation here:

Three successive "kidneys" are formed in mammalian embryos namely the pronephros, mesonephros and metanephros. To a certain extent these distinct structures overlap not only chronologically but also morphologically; that is the metanephros which forms the definitive kidney in all amniotes forms before the mesonephros disappears and similarly the mesonephros begins to form before the pronephros regresses completely.

Moreover, each of these sequential systems pre-empts certain components of its precursor so that the final urinogenital system is a montage derived from antecedent pairs of organs. From the beginning of human nephrogenesis at seven to eight weeks of embryonic life until it ceases the developmental pattern of individual glomeruli follows similar lines viz:

In the earliest stages of development individual ampullae surrounded by fairly well-delineated masses of nephrogenic cells are present immediately beneath the renal capsule. These ampullae go on to produce the

collecting tubule system whilst the nephrogenic caps condense into oval masses in which a central cavity appears. This structure is known as the renal vesicle which undergoes elongation and differentiation to produce an entire nephron.

It does this by firstly developing an elongated lumen and then by differential growth a pair of invaginations are found in the wall of the cell mass. As these invaginations enlarge the characteristic 'S-shaped' structure is formed within which precursor cells of the future renal corpuscle cell types can clearly be distinguished. Primitive capillaries then invade this structure with capillary growth proceeding at a rapid rate and with lobulation of the capillaries within the primitive glomerulus being particularly well marked even at an early stage. The base of the glomerulus now begins to constrict so that only the afferent and efferent vessels may pass through the constriction, thus forming the classical glomerular shape. At the end of this process the glomerular capillaries are covered by an epithelial investment continuous with that comprising Bowman's capsule which is, in turn, continuous with that of the collecting tubule (Osathanondh and Potter, 1963a and b).

The capillaries in the functional glomerulus are formed by the proliferation and repeated splitting of

the original invading capillary (Osathanondh, unpublished information, as cited by Potter, 1965). The complicated vascular pattern of the mature glomerulus is produced as a result of several generations of such splittings. This pattern is entirely variable but it generally consists of two main branches with two or three later generations of branches arising from each major branch (Vimtrup, 1928) each of which may communicate with one another at any level of the glomerulus (Potter, 1965).

These glomeruli formed during the first twenty to twenty-second weeks of foetal life constitute those that will lie in the juxtamedullary region of the cortex in the mature foetus. Further development of the glomeruli in the cortex will take place radially with growth proceeding periferally.

In the rat where growth of the kidney has been investigated more extensively than in any other species it has been shown that only approximately one third of the numbers of glomeruli that comprise the adult kidney are present at birth (Kittelson, 1917 and Arataki, 1926) with glomeruli continuing to be formed for 100 days after birth. Though the majority are formed within the first three to four weeks of life.

In this animal the oldest and largest glomeruli are in the cortico-medullary region of the kidney with the

youngest situated in the outer cortex immediately beneath the capsule and once again growth continues peripherally as in the general mammalian description.

Apart from the development of glomeruli there are gradual and progressive changes within the renal glomerulus throughout its life. For example, in the human infant the glomerular basement membrane measures only 1000 A (Vernier, 1961). It gradually thickens until at approximately three years of age it measures 2850 $\pm$ 550 A (Vernier et.al., 1958). After this time there is very little change in thickness with adult figures quoted at 2700 A (Farquhar, 1959) to 3500 A (Bergstrand and Bucht, 1958).

In the glomerulus of the rat the aging process is reflected in the relative thicknesses of the lamina densa and the laminae rarae in that the lamina densa is seen to become thicker at the expense of the laminae rarae with aging (Ashworth et.al., 1960).

Whilst it was beyond the scope of this thesis to examine the development taking place in the embryonic kidney the aim of this section of the present work was to elucidate the development occurring within the neonatal animal.

To this end, neonatal kidneys were examined by means of light and electron microscopy to ascertain how closely the feline glomerular development compared with development in the general mammalian glomerulus.

The present study also served to provide the first detailed scanning electron microscopical views of the neonatal feline glomerulus.

MATERIALS and METHODS

1. Source of Animals.

Six adult cats which were found to be pregnant during their physical examination were used in this experiment to provide suitable numbers of kittens. These animals were either unwanted strays or cats which owners wished euthanasia for personal reasons. A summary of the number of litters and the numbers of kittens per litter is given in Table 2.1. In Table 2.2 a summary of the intervals at which litter-mates were sacrificed after birth is shown.

2. Euthanasia.

The method of euthanasia was as follows: the kittens were injected intra-peritoneally with a computed dose of a 20% solution of sodium pentobarbitone ('Euthatal'; May & Baker, Dagenham, England) at the rate of 1ml/kg. Once the femoral pulse was only faintly discernible, the axillary artery was severed and the animal exsanguinated.

3. Sampling of Tissues.

Following exsanguination, the abdomen was opened by a mid-line incision, the abdominal organs displaced and both kidneys removed. The kidneys were then hemisected

longitudinally revealing the cortex and medulla whereupon samples were removed for histological and ultrastructural examination.

Once again the interval between exsanguination and immersion in fixative never exceeded three minutes.

Due to the small size of the kidneys and their renal arteries no perfusion fixation was attempted.

The methods of histological and ultrastructural examination were all as previously described in the section on general Materials and Methods.

TABLE 2.1

Summary of Kittens per Litter

Cat No.	No. of Litters	No. per Litter
1	1	7
2	1	5
3	1	6
4	1	7
5	1	4
6	1	6

TABLE 2.2

Summary of Sampling Times.

Cat No.	Litter	Time(days)	Cat No.	Litter	Time(days)
2.1	1	0	2.19	2	36
2.2	3	1	2.20	3	39
2.3	2	2	2.21	2	42
2.4	1	3	2.22	4	45
2.5	3	4	2.23	5	48
2.6	4	5	2.24	4	51
2.7	1	6	2.25	6	54
2.8	4	7	2.26	5	57
2.9	4	8	2.27	2	60
2.10	1	9	2.28	6	63
2.11	1	12	2.29	4	66
2.12	1	15	2.30	3	69
2.13	6	18	2.31	5	70
2.14	1	21	2.32	5	3 Mths.
2.15	3	24	2.33	6	4
2.16	2	27	2.34	6	5
2.17	4	30	2.35	6	6
2.18	3	33			

RESULTS

Whilst maturation of the kidney proceeds at differing rates within differing individuals the following account details the general developments occurring during the period of the investigation.

1. Histological findings:

At birth and up to three days of life the nephrogenic zone could easily be observed as being a wide band extending as far as the juxta-medullary region (Fig. 2.1). There were a small number of primitive glomeruli in this area which were characterised by a prominent cuboidal epithelium (Fig. 2.4). In these glomeruli, only a portion of the capillaries present were fully patent. However, in this deep cortical region a number of well developed apparently fully functional glomeruli were also present. The latter contained patent capillaries which were observed to contain erythrocytes (Fig. 2.5).

In the nephrogenic zone extending to the outer cortex, below the capsule, numerous stages of developing glomeruli could be seen. These included numerous metanephric caps, vesicles and 'S-shaped' structures (Figs. 2.2 and 2.3).

From three days onwards the nephrogenic zone

gradually reduced radially from the deep cortex outwards. The nephrogenic zone remained prominent even at 21 days; however the numbers of functional glomeruli only slowly increased so that by day 18 almost 25% of the cortex had functioning glomeruli. These glomeruli, though, were still considered not quite mature, as could be determined by their basophilic cuboidal visceral epithelium, their hypercellular appearance and the presence of only a small number of patent capillaries (Fig. 2.4).

By 24 days, the nephrogenic zone had narrowed to be confined within the outer cortical zone. The mid and outer cortical regions still contained developing hypercellular glomeruli with few patent capillaries. However, many more glomeruli with patent capillaries could now be seen in the deeper cortical regions although a proportion remained hypercellular in appearance. At this stage, mature functioning glomeruli now comprised approximately 50% of the total number of glomeruli.

By 55 days, the nephrogenic zone had disappeared with the percentage of functioning glomeruli having increased to more than 80% of the total glomerular numbers. However, many less well developed glomeruli were still to be found scattered throughout the mid- and outer-cortex (Fig. 2.6).

By 65 days, mature well developed glomeruli predominated, however, even at this stage of development 5 - 10% of the glomeruli present still showed cuboidal visceral epithelium and had few patent capillaries.

At three months of age, development of the kidney apparently was still not complete with a small proportion of still apparently functionally immature glomeruli persisting in the outer cortex.

After the age of four months these apparently functionally immature glomeruli were not in evidence in any of the animals examined.

2. T.E.M. findings:

The earliest distinguishable renal corpuscle comprised of a mass of loosely arranged undifferentiated cells. Between these cells lay a small amount of amorphous material, the primitive GBM (Fig. 2.7). This can be seen in cross-section in Fig. 2.8. At this stage the primitive GBM has condensed into a more recognisable form and the previously undifferentiated cells are beginning to differentiate (Fig. 2.8).

As development progressed, a cleft formed between the developing glomerular tuft and a single layer of cells which were later to form the parietal epithelium (Fig. 2.9). For a period of time these cells assumed a cuboidal appearance which had no distinctive feature;

however, later they became stretched and tenuous in order to cover the entire parietal aspect of the developing corpuscle (Fig. 2.10).

The developing glomerular tuft also quickly differentiated in appearance. Its cells were characterised by an increased density of nuclear and cytoplasmic proteins, abundant poorly defined mitochondria and occasional Golgi bodies.

The visceral epithelial processes quickly initiate the process of applying themselves along the developing capillary network (Fig. 2.11). The development of the GBM also took place at a similar rate whereby it now occupied a position between the developing endothelial network and the developing visceral epithelium (Fig. 2.12; also see Fig. 2.18)

3. S.E.M. Findings:

Using the SEM the early stages of glomerular formation can be observed ranging from vesicles, where the invasion by a single capillary had only just begun (Fig. 2.13) through the classical S-shaped structure (Fig. 2.14) to the more complex structures where the S had doubled back on itself several times (Fig. 2.15).

The least derived glomeruli, which were derived from the S-shaped structures observed by light microscopy, appeared as morular structures contained

within a Bowman's capsule. The prominence of the cuboidal visceral epithelium in this structure, previously seen by both light and transmission electron microscopy, was well illustrated by use of the SEM (Fig. 2.16).

In the earliest stages of development individual capillaries were not discernible; however, they became more noticeable as development of the visceral epithelial podocytes progressed. Fig. 2.17 shows this transition of the cuboidal epithelium into the form previously described in Chapter 1. At high power the flattened sheet of the visceral epithelium could be seen to form the primary, secondary and tertiary processes so characteristic of the mature form of this cell type (Fig. 2.18).

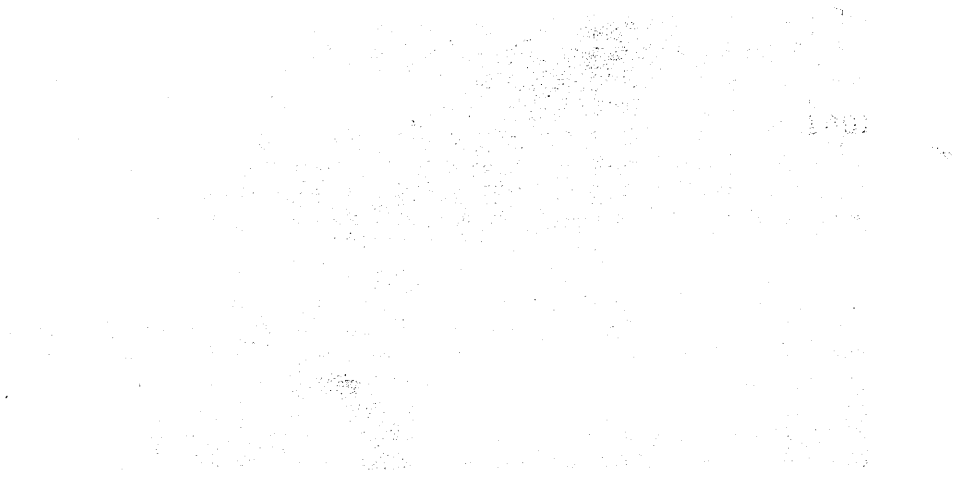


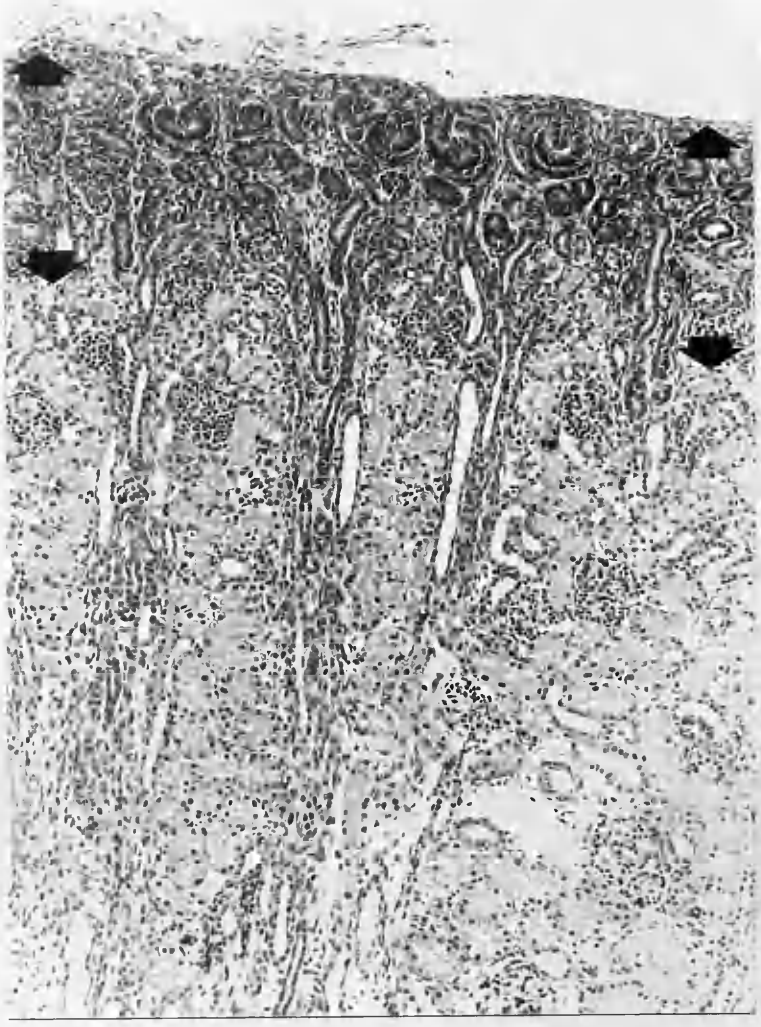
TABLE 2.3

Summary of Results.

Time	Nephrogenic Zone	Glomeruli
0-12 Days	Extends as far as Cortico-medullary junction.	5%
13-24	Slowly Reducing Radially	25%
25 Days-2 Mths.	Peripheral yet still prominent	50%
		80%
2-4	Disappeared	90-95%
4-6	"	100%

Figure 2.1
Nephrogenic Zone
Neonate
Arrows indicate extent
of Nephrogenic Zone
H & E (x 60)

Figure 2.2
Early S-Shaped primitive
nephron with ascending
collecting tubule (arrow)
H & E (x 300)



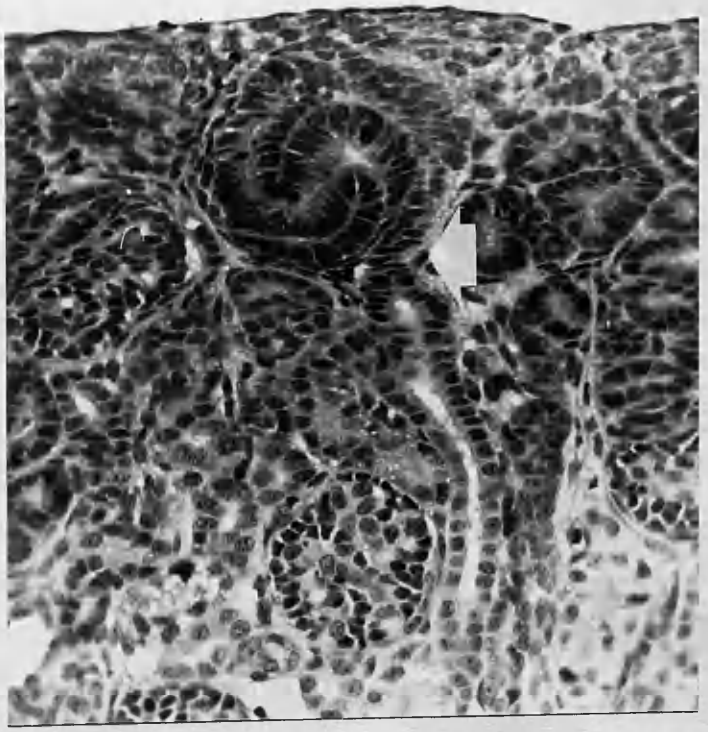
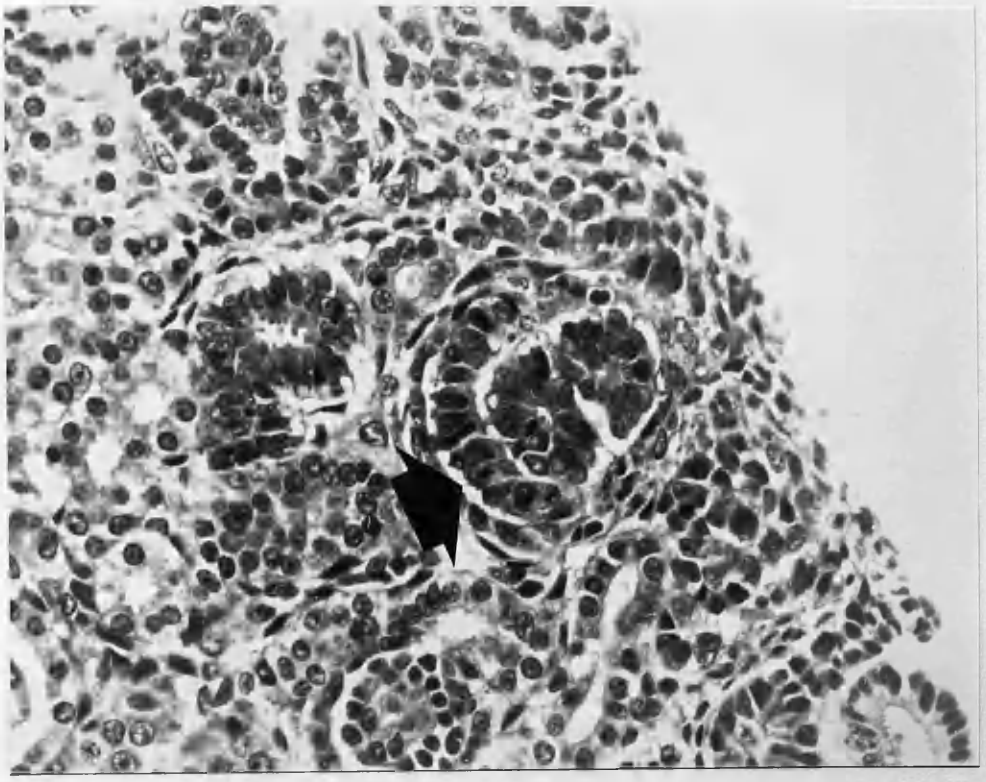


Figure 2.3
Primitive glomerulus
Bowman's space appearing
Cuboidal epithelium (arrow)
H & E (x 300)

Figure 2.4
Transitional glomerulus
Cuboidal epithelium giving
way to visceral epithelium
Note juxtaposition of developing
tubule with the ascending
collecting tubule (arrow)
H & E (x 300)



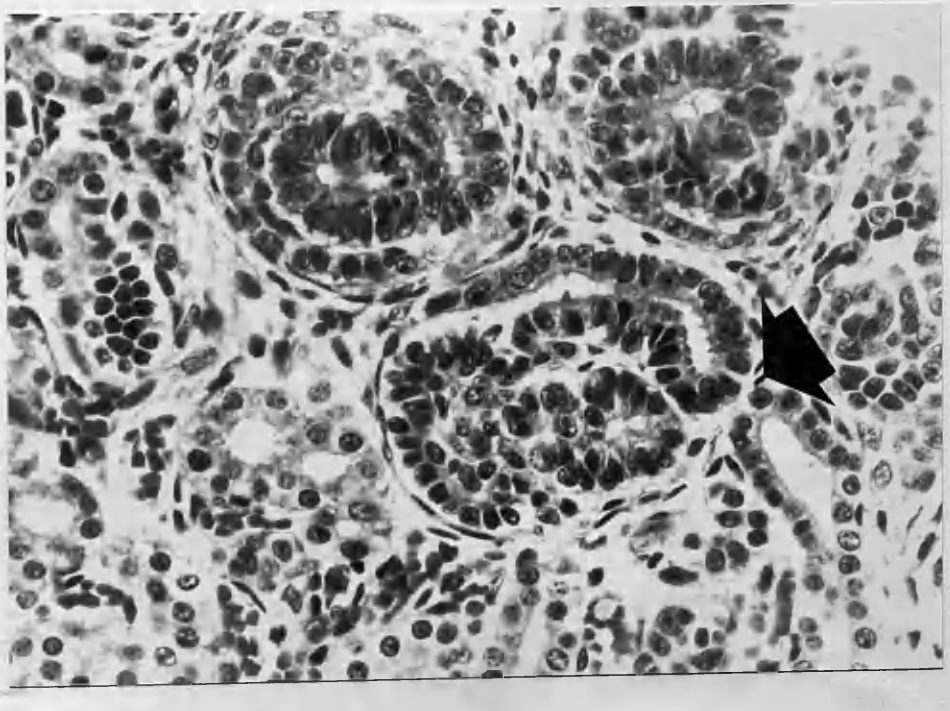
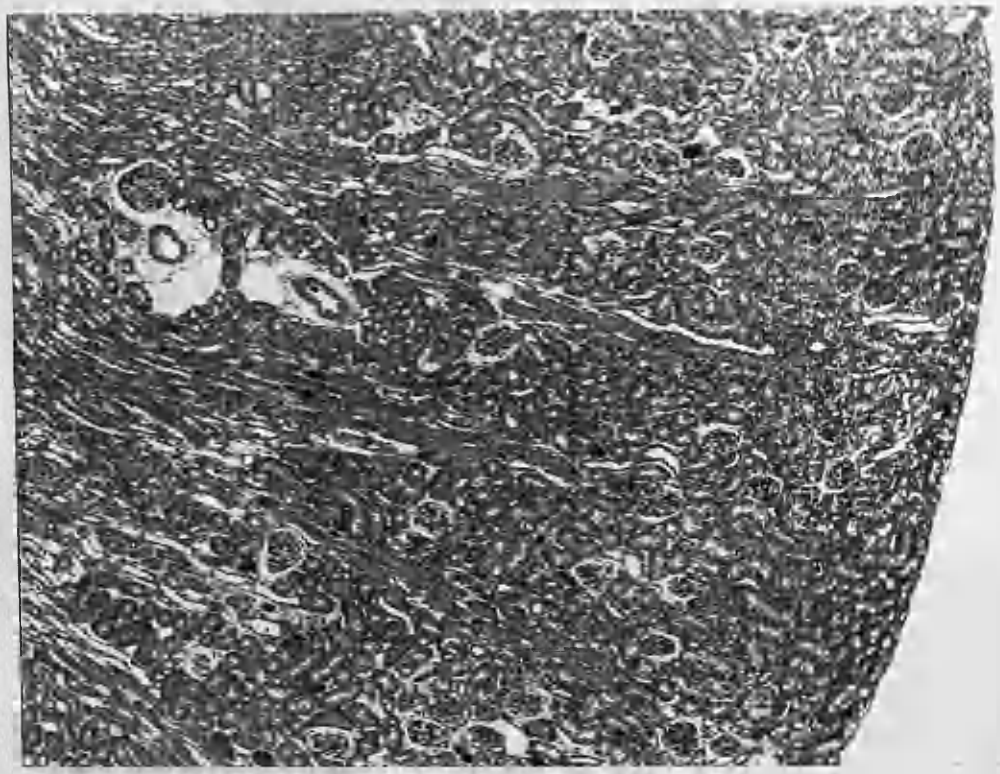


Figure 2.5
Functional glomerulus
Deep cortical
Note patent capillaries
H & E (x 300)

Figure 2.6
Normal cat kidney
Day 60
Note absence of
nephrogenic zone
H & E (x 60)



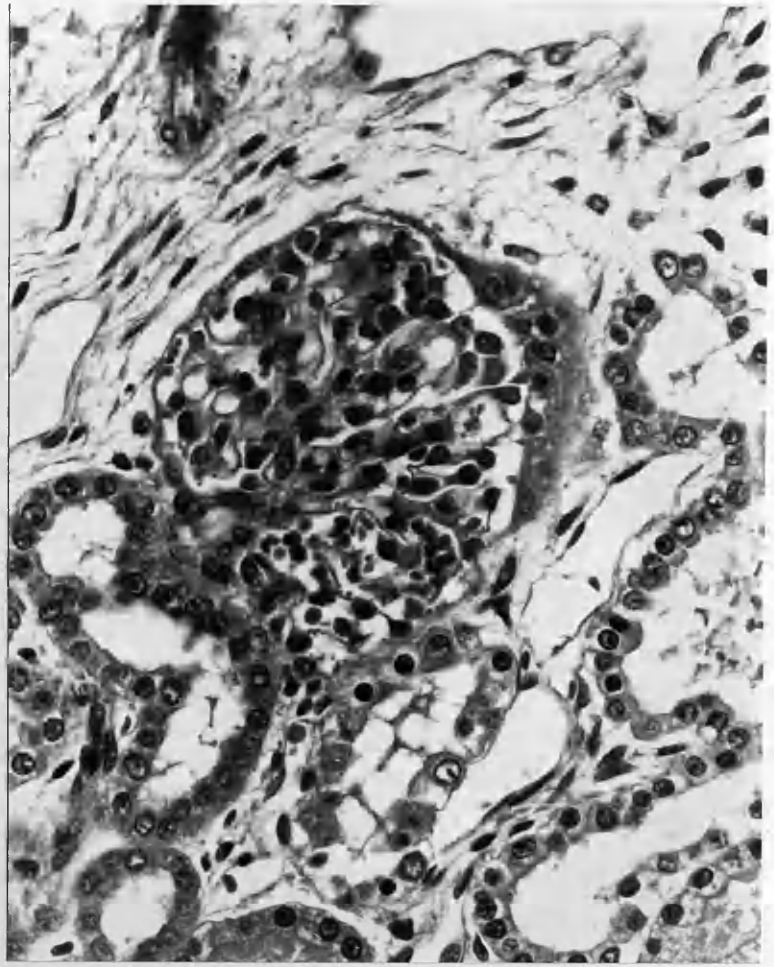
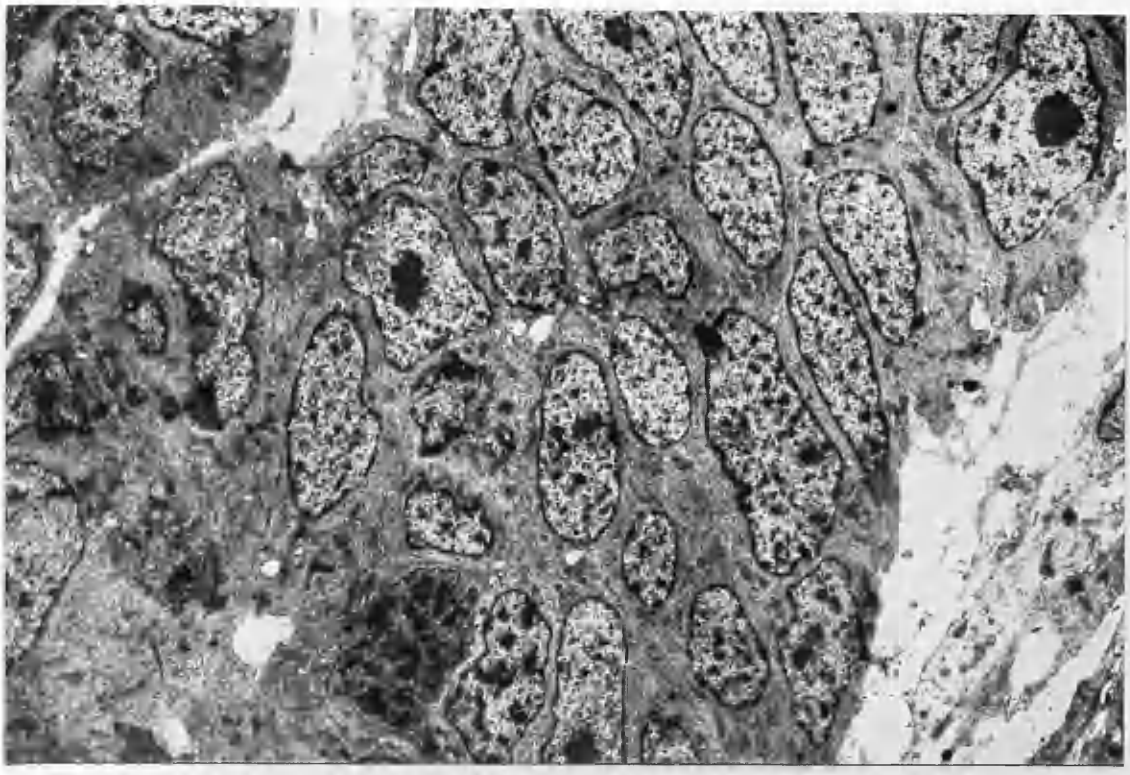


Figure 2.7
Tangential section through
S-Shaped vesicle
TEM (x 3000)

Figure 2.8
Developing glomerulus
Pre-capillary invasion
Note peripherally developing
blood vessels (arrows)
and primitive GBM (gbm)
TEM (x 3000)



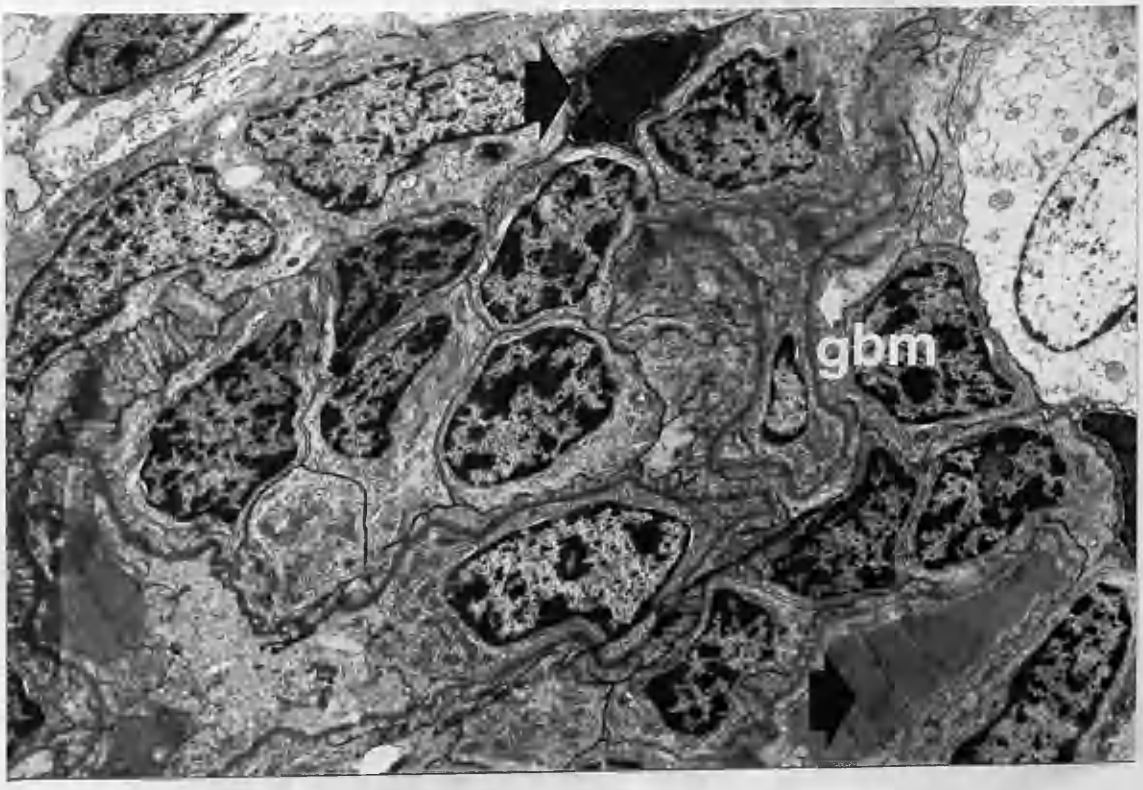
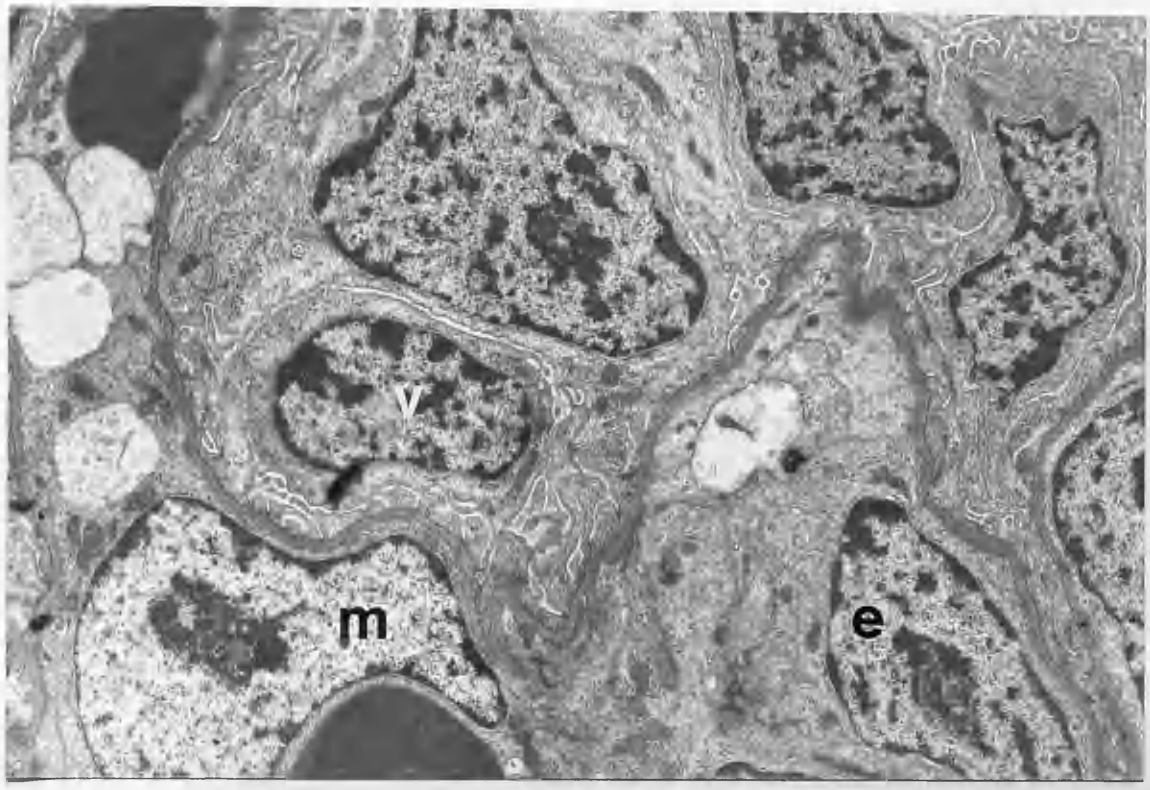

gbm

Figure 2.9
Developing glomerulus
Cell types differentiating
Note visceral epithelium (v)
endothelial cell (e) and Mesangial cell (m)
TEM (x 3000)

Figure 2.10
Developing glomerulus
Further differentiation of cell types
Note the cuboidal visceral
epithelium with foot processes (v)
Parietal epithelium flattening (p)
Capillary with red blood cell (c)
Endothelial cell (e) and Mesangial cell (m)
TEM (x 3000)


v
m
e

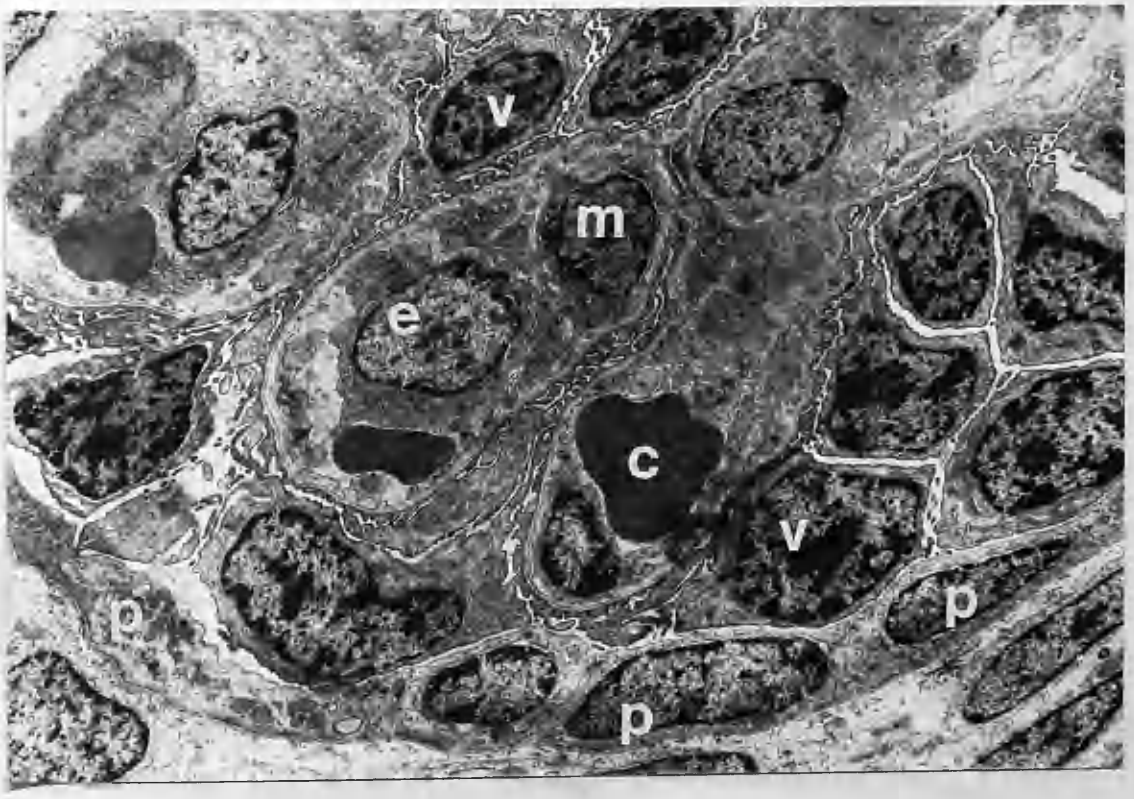

v
m
e
c
v
p
p
p

Figure 2.11
Developing endothelium (e) and
visceral epithelium (v)
Note split GBM (arrow)
Capillary (c) and urinary space (u)
TEM (x 5000)

Figure 2.12
Split GBM (asterisk)
Tertiary podocytic processes (p)
Note slit membranes (arrow 1)
endothelium with fenestrae (arrow 2)
Capillary (c) and urinary space (u)
TEM (x 20,000)


u
v
v
v
c
e

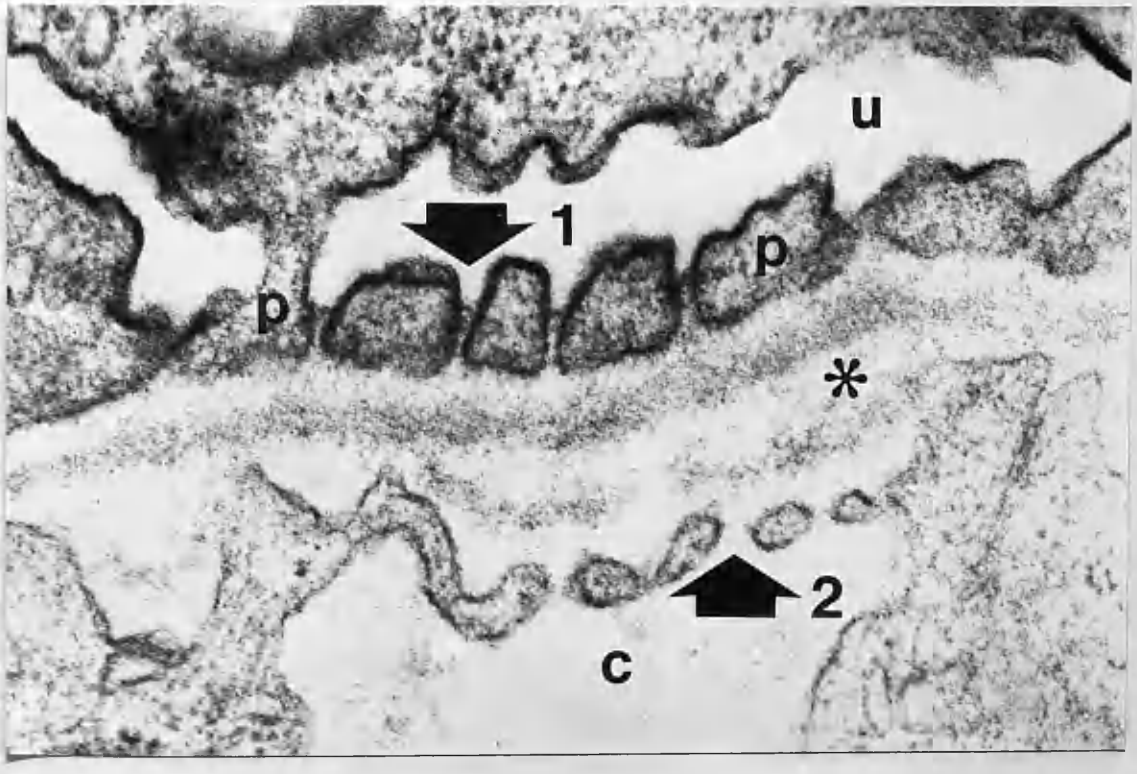

u
1
p
p
*
2
c

Figure 2.13
Renal vesicle
Note invagination formed by
the developing capillary (asterisk)
SEM (x 320)

Figure 2.14
S-Shaped structure
As the capillary develops torsional
forces transform vesicle into S-shape
SEM (x 320)

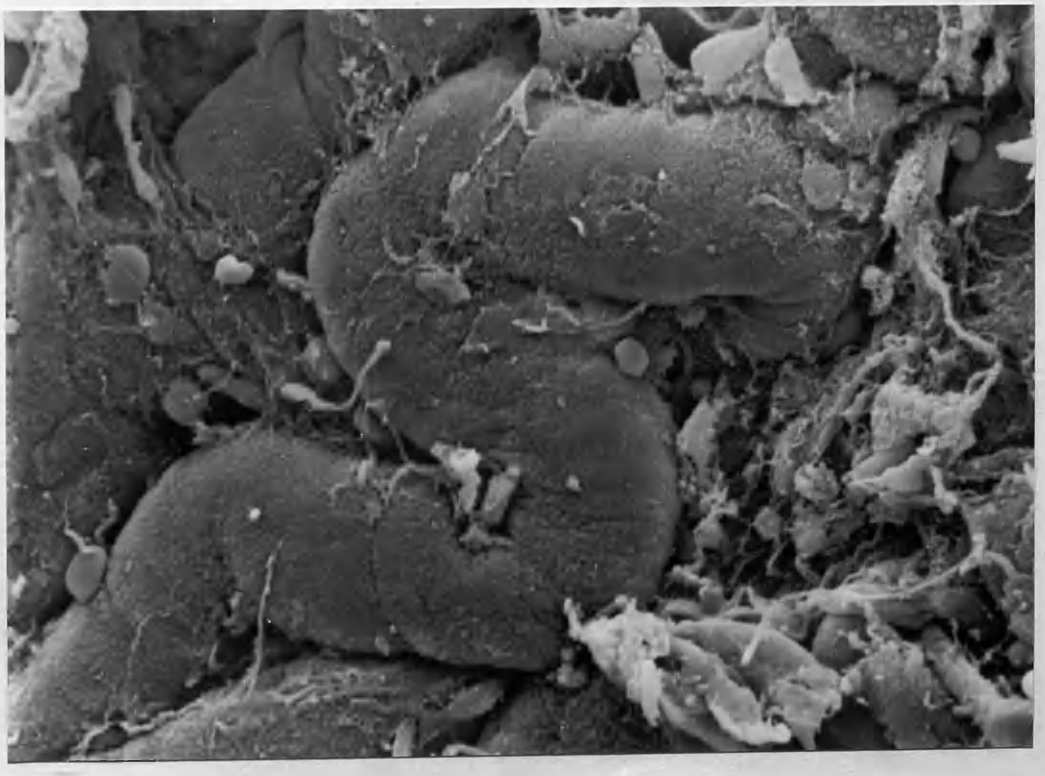


Figure 2.15
Developing S-shaped structure
Note increase in complexity
SEM (x 320)

Figure 2.16
Cuboidal epithelium (arrow)
This surrounds the primitive glomerulus
SEM (x 640)

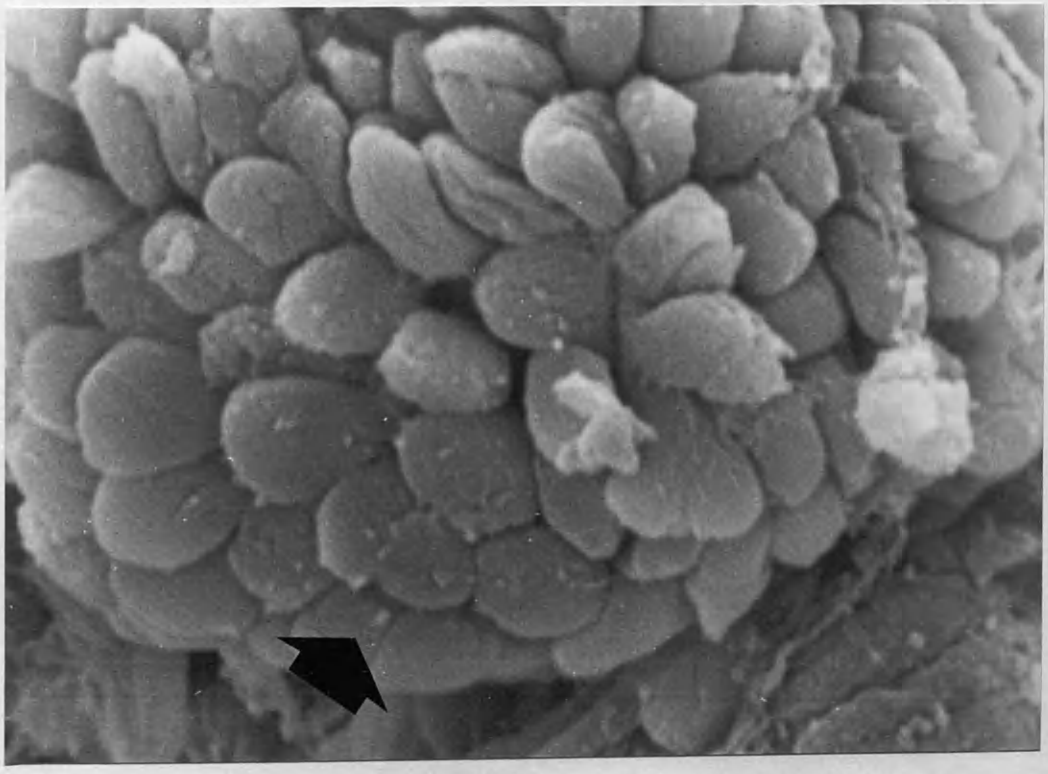
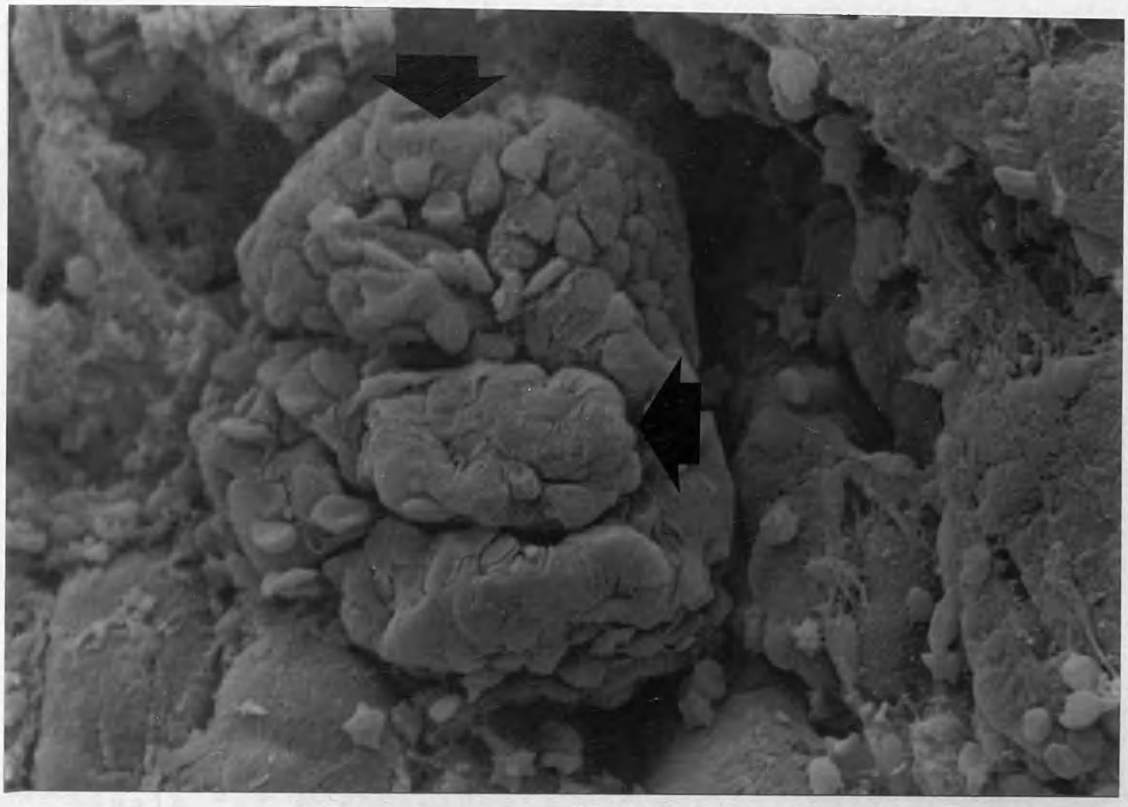
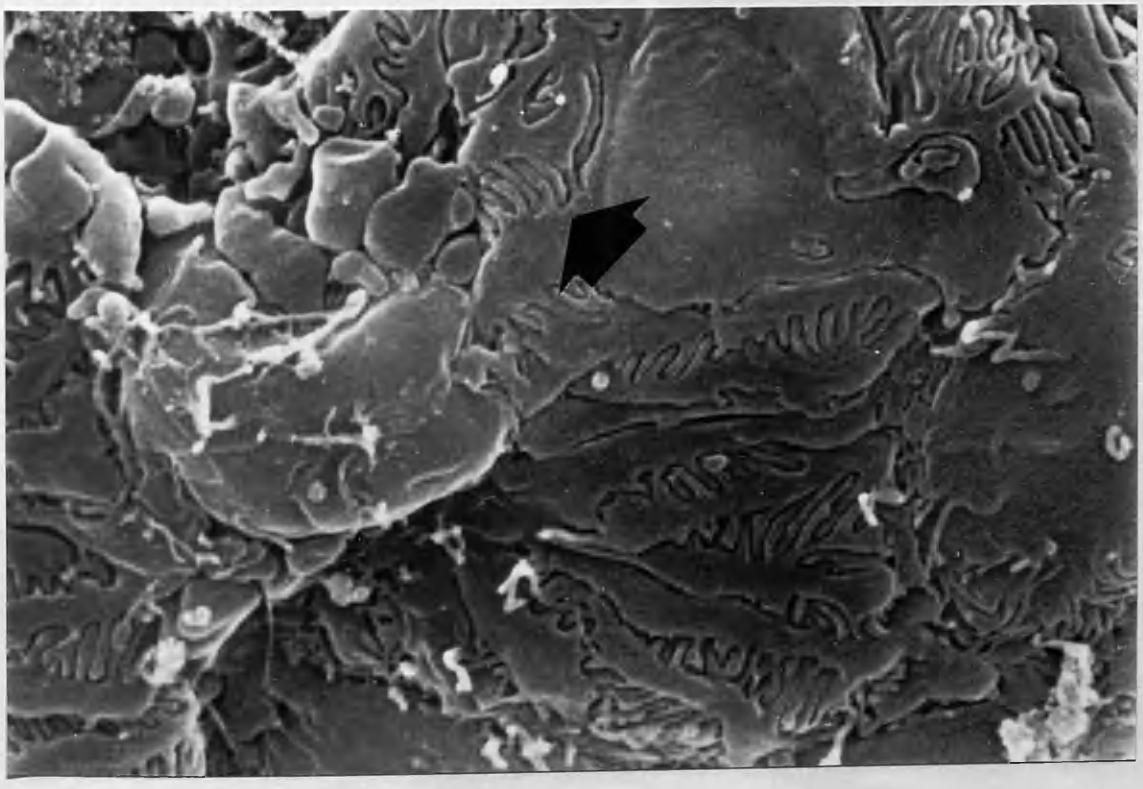


Figure 2.17
Transitional glomerulus
Cuboidal epithelium maturing into
formal visceral epithelium (arrows)
SEM (x 160)

Figure 2.18
Visceral epithelium
Developing podocytic processes (arrow)
SEM (x 2500)





DISCUSSION

In the cat, as in many other species having a short gestation period, the kidneys are still in a state of development at the time of birth. In the peripheral cortex the nephrogenic zone was seen to persist with aggregates of mesenchymal cells, vesicles and 'S-shaped' bodies in close apposition to the distal ends of the collecting ducts. It is these collecting duct endings which are thought to be the transmitters of inductive signals for nephrogenesis (Gruenwald, 1952; Grobstein, 1957). Deeper in the cortex the glomeruli were in a more advanced state of differentiation but only those in the most juxta-medullary areas did the glomeruli appear well developed. This situation lasted until approximately the eighth week of life during which time the formation and development of new glomeruli was taking place in the outer cortex.

This situation was similar to the reports of the development of glomeruli in dog and the rat. In the former the total number of functional glomeruli did not increase after 23 days of life (Horster et.al., 1971). These workers stated categorically that nephrogenesis did not take place in the Beagle after three weeks of age. This conclusion was based on three propositions: i) that glomerular numbers fell within the range of values

for mature dogs, ii) that the constancy of these findings was maintained during the subsequent post-natal period, and iii) there was an absence of any visible nephrogenic tissue.

In the rat, although several widely quoted early works have suggested that the formation of new nephrons continues for several days or even weeks after birth (Kittleson, 1917; Arataki, 1926) in actual fact recent reports using more up to date methods have concluded that nephrogenesis terminates within a few days of life (Larsson, 1975; Kazimierczak, 1978; Kazimierczak, 1980), and that the adult number of nephrons is already established at that time.

MacDonald and Emery (1959) have postulated kidney maturation, in man, as occurring in three phases namely i) a nephrogenic phase which may continue until 44 weeks after conception but is usually over by the 36th. week of gestation, ii) a second phase where the full complement of glomeruli are present; however almost all are immature (this lasts until the individual reaches three to five years of age) and iii) a third phase which generally lasts from the third to the twelfth year of life and in this period there is a steady increase in the proportion of fully mature glomeruli.

The development of the capillaries within the differentiating glomerulus lasts for a considerable

period after nephrogenesis finishes. In different species this period changes; In the ten day old rat the best differentiated glomeruli contain relatively few capillary loops within the lobules of the adult glomerulus (Kazimierczak, 1980). On the other hand, in the newborn human it has been reported that the vascular pattern of the developing glomerulus was almost complete by the time of basal constriction of the renal corpuscle (Potter, 1965).

In the present work a middle ground position of these two extremes has been adopted. This was seen where the best differentiated glomeruli in the juxta-medullary region contained a high proportion of patent capillaries; yet those developing nephrons in the nephrogenic zone were at a 'pre-capillary invasion' stage.

The constriction of the base of the developing glomeruli brings the afferent and efferent vessels into apposition to form the vascular pole. It is at this site that Ljungvist (1964, 1975) described, in the juxta-medullary glomeruli of man, rabbit and the rat, direct shunts between these vessels, thus forming a by-pass to the glomerulus.

However, the presence of these shunts could not be confirmed by either SEM studies of glomerular casts in the rat (Murakami, 1972) or in the adult dog (Spinelli

et.al., 1972; Mohammed, 1985) or in the adult cat as presently described or by a combined TEM/SEM study in the rat (Kazimierczak, 1980).

The structural development of the glomerulus as described by Trabucco and Marquez (1952) i.e. that of a single vessel bent in an acute angle and then simply evaginating into a multiple blind ending saccular tubular arrangement also failed to be confirmed in the present study.

This leaves an apparent contradiction in the cat in that the glomeruli appear to be fully functional at approximately the eighth week of life, and, as the nephrogenic zone was no longer present at this time, nephrogenesis had apparently finished. However the size and weight of the kidneys at this stage of the cats life is only a fraction of their eventual size and weight in the fully mature cat. This problem would seem to be answered by Horster et.al. (1971) who described a similar situation in the dog where, from the end of nephrogenesis until the termination of their experimentation (i.e. at 74 days), the tubular volume increased by 235% whereas glomerular volume increased by a mere 33%. This is also in agreement with the situation found in man by Fetterman et.al. (1965). However, this change in the relative proportions of tubular and glomerular volumes has not been seen to cause any

functional imbalance as determined by micropuncture analysis (Horster and Valtin, 1971). Thus it would seem reasonable to assume that the size difference between the immature cat, where nephrogenesis has ended, and the mature cat is due only to the tubular development and the disproportionate increase in tubular as compared with glomerular volumes.

It has been stated, by Wilmer (1941) and Ljungquist (1963), that except for the juxta-medullary glomeruli no other glomeruli have functioning efferent vessels until birth or at least until late foetal life. However, for this opinion to be correct there would have to be two separate stages in glomerular development i.e. pre-formation of the juxta-medullary glomeruli and a period after their development. It would also imply stasis of the blood within the capillaries of this second group of glomeruli. The present study concurs with that of Potter (1965) in the rejection of this theory. The grounds for this rejection being that no evidence of this phenomenon has been produced other than by Wilmer and Ljungquist to lend support to their argument that renal development differs so dramatically at any one particular stage of the developmental process and secondly that blood may enter the glomeruli yet fails to find any exit for its return to the circulation.

CHAPTER 3

A HISTOLOGICAL AND ULTRASTRUCTURAL STUDY OF THE AUTOLYTIC CAT GLOMERULUS

INTRODUCTION

Renal tissue cannot always be obtained immediately after an animal dies or is destroyed, even under optimal conditions.

The morphological changes which occur due to autolysis must therefore be known in order to differentiate these from any changes occurring during pathological processes.

These autolytic alterations have been studied by several authors in a number of mammalian species. For example Osvaldo *et.al.* (1965), using the rat kidney as their model, sampled kidney tissue samples at intervals after death for histological examination. A further study dealt with the ultrastructural changes taking place (Cook *et.al.*, 1965). Likewise, in the dog Crowell *et.al.* (1974) performed a combined histological and ultrastructural study in biopsy, perfused and *in situ* autolysed kidneys. Langlinais (1981) also studied the autolytic changes occurring in the dog by using the SEM. This latter study was carried out as part of a larger study on several other species of laboratory animals.

Indeed, as would be expected, the majority of the autolytic studies have concentrated on laboratory animals e.g. Hanssen (1960a and b) and Trump *et.al.* (1962) using the laboratory mouse; Cook *et.al.* (1965),

Latta et.al. (1965) and Osvaldo et.al. (1965) employing the rat; Mullink and Feron (1967) and Crowell et.al. (1974) who concentrated on the dog.

However, only two reports of autolytic changes occurring in feline glomeruli can be found in the literature, namely the contributions of Mayer and Ottolenghi (1947) and Crowell and Leininger (1976). The work of Mayer and Ottolenghi was concerned only with the "protrusion", or reflux, of proximal tubular epithelium back into Bowman's space at the time of, or shortly after, death. This study, however, was only part of a much larger study of the same phenomenon in the dog kidney. The Crowell and Leininger study using both conventional histological methods and transmission electron microscopy compared the kidneys of normal cats with those of several abnormal cats, suffering from membranous glomerulonephritis, as well as describing the autolytic changes occurring in normal kidneys.

Information collected from this part of the study was aimed at expanding upon these initial findings by the use of more precise investigative techniques. In particular, the present study sought to establish more thoroughly the glomerular autolytic changes in the feline kidney as detected by light microscopy and transmission electron microscopy as well as, for the first time, scanning electron microscopy.

MATERIALS and METHODS

1. Source of Animals.

Four young adult domestic, short-hair cats were obtained from commercial sources specifically for the purpose of examining the histological and ultrastructural changes taking place in the autolytic kidney.

Physical and biochemical examinations carried out prior to the commencement of the experiment showed these cats to be normal and disease free a fact which was subsequently confirmed by histological examination of necropsy specimens.

2. Euthanasia.

The method of euthanasia was as previously stated on pages 6-7 of the general Materials and Methods.

3. Sampling of Tissues.

Following euthanasia, tissues were sampled in the manner as previously described (pages 7-10).

From each of the four cats both kidneys were removed immediately after euthanasia. As before, the kidneys were incised longitudinally to reveal both the cortical and medullary areas and samples were taken immediately to act as '0 hour' control specimens. The

remainder of the kidneys were then stored at 4°C during the post mortem interval. Samples were then taken at five minute intervals for the first hour then at 30 minute intervals for the following six hours. Subsequent to that time further samples were taken every six hours for six days (i.e. up to 144 hours).

These samples provided material for histology, transmission and scanning electron microscopy studies with all the histological and ultrastructural techniques employed being as previously described.

A summary of the tissue samples taken and the method of investigation employed on these four animals now follows:

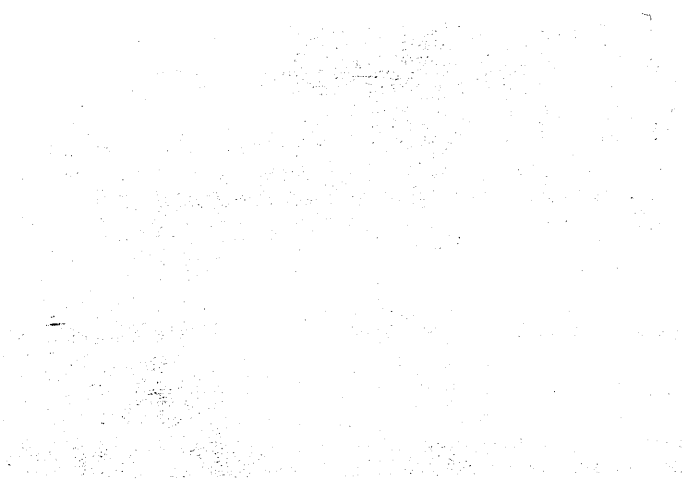


TABLE 3.1

Summary of Sampling Times.

Cat No.	Sample Times after Death (mins.)
3.1	5, 10, 15, 20, 25, 30.
3.2	35, 40, 45, 50, 55, 60.
	Sample Times after Death (hrs.)
3.3	0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6.
3.4	12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, 78, 84, 90, 96, 102, 108, 114, 120, 126, 132, 138, 144.

RESULTS

Although the nature and severity of autolytic changes often varied in different glomeruli within the same tissue section, nevertheless the pattern of morphological alterations was similar at each time interval in all four cats.

1. Light Microscopy

No histological changes were detected until 20 minutes after excision. At this time the glomeruli appeared normal apart from a small number which showed tubular epithelial reflux into the urinary space (Fig. 3.1).

This tubular epithelial reflux extended, with increasing time after death, to the remainder of the glomeruli so that by 24 hours all glomeruli showed tubular epithelial debris to a greater or lesser extent in the urinary space. Fig. 3.2 illustrates the extent to which the reflux had reached by 12 hours after excision of the kidneys with Fig. 3.3 showing the amount of tubular epithelial reflux, together with the consequent splitting of the glomerulus, at approximately 36 hours.

In a similar fashion margination of nuclear chromatin progressed, at different rates within different glomeruli, and together with some nuclear

shrinkage, resulted in the appearance of pyknotic nuclei by 12 hours. This time scale applied to visceral epithelial cells. Mesangial and endothelial cells, whose nuclei normally appear smaller and darker before autolysis commences, showed nuclear pyknosis more rapidly in the shorter time of six hours.

After 24 hours a proportion of nuclei began to show karyorrhexis, where the nuclear material fragments, and this condition had extended to virtually all nuclei by 48 hours.

The capillary lumina also appeared to be partially occluded during this time. Beginning at 30 minutes, glomerular capillaries were noticed to contain swollen endothelial and mesangial cell cytoplasm. Indeed, a small number were completely closed by one hour; however, for the most part they remained open until approximately 24 hours when all appeared completely occluded.

The swollen processes of the mesangial cells appeared to protrude through the endothelial cells thus contributing to the cytoplasmic profiles filling the capillary lumina.

By 54 - 60 hours the normal histological configuration of the glomerulus was completely lost and the urinary space almost completely filled with tubular epithelial debris (Fig. 3.4).

The red blood cells present in the vessels began, by about two hours to alter their normal configuration to show discoid forms with cup-shaped forms appearing at eight hours in the larger vessels. In smaller vessels which contain swollen mesangial and endothelial cytoplasm the red blood cells are packed tightly into solid masses and by 24 hours only swollen haemolysed forms may be identified.

2. Transmission electron microscopy.

As with convential light microscopy, reflux of tubular epithelium into the urinary space began to be noticeable as early as ten minutes after death (Fig. 3.5). Reflux was present in the great majority of glomeruli by 24 hours and continued thereafter eventually affecting all the glomeruli to some extent.

In the visceral epithelial cells distinct changes began at approximately ten - 20 minutes at which time slight swelling of some of the epithelial foot processes could be seen (Figs. 3.5 and 3.6). Nevertheless some foot processes remained normal until approximately 60 hours when general epithelial cytoplasmic disintegration had developed (Fig. 3.8).

The cytoplasmic organelles within the epithelium also underwent change. The mitochondria began to swell and lose their electron density within the first hour,

although a small number could be observed to have changed little even after 24 hours. In the endoplasmic reticulum vesicles had formed by four hours post- mortem and the endoplasmic reticulum as a whole had decreased in numbers and size by eight hours. By one hour the Golgi bodies were prominent and had distended cisternae. While at four hours myelin figures could be found frequently associated with the degenerating Golgi bodies. Nuclei were observed to develop pyknosis by four to eight hours with margination of chromatin a prominent feature. On the surface of these cells numerous microvillous processes were present at eight hours at which time there were also increased numbers of irregular cellular processes.

The basement membrane appeared normal until approximately 24 hours when the lamina densa began to thicken. The total thickness of the basement membrane was measured as increasing from approximately 1400 A to approximately 1600 A (Compare Fig. 3.5 with Figs. 3.9 and 3.10).

Within four hours the endothelial cytoplasm had begun to enlarge and occupy the capillary lumina thus compressing any erythrocytes contained within them (Fig. 3.6). However, the fenestrated portion of the cytoplasm persists even when the lumen of the capillary was filled with these cytoplasmic profiles. At this time many of

the nuclei had become pyknotic. Pyknosis being complete in an endothelial nucleus by eight hours. By 24 hours erythrocytes and pyknotic nuclei became difficult to distinguish. The mesangial cells underwent similar changes in a similar time scale as those that had taken place in the endothelial cells. However, the mesangial processes which could be seen protruding through the endothelium into the capillary lumina, the so-called 'Intrakapillarhocherchen', were seen to undergo a marked swelling which became noticeable at ten minutes and was quite prominent at 60 minutes. These swollen processes contributed to the occlusion of the capillary lumina with cytoplasmic profiles yet remained distinct from endothelial profiles (Fig. 3.7).

Complete disintegration of glomerular structure was observed any time after 24 hours post mortem as illustrated by Figs. 3.9 and 3.10 taken at 48 and 96 hours after excision respectively.

3. Scanning electron microscopy.

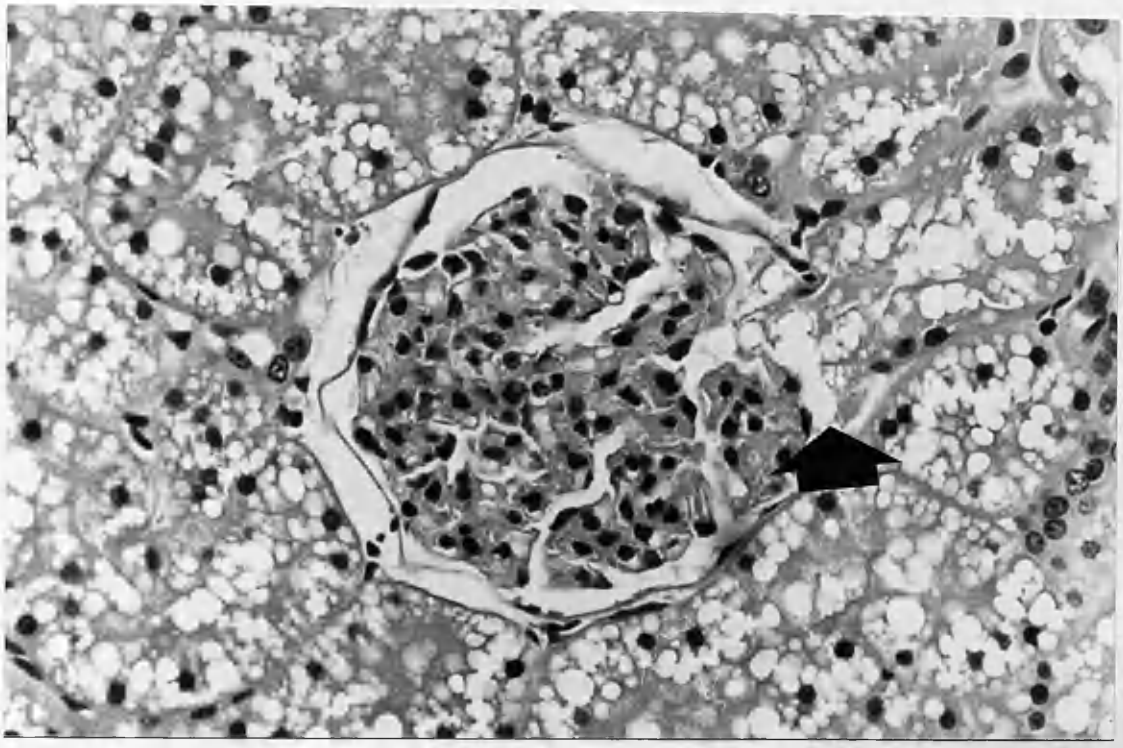
Using the SEM several distinct changes in glomerular morphology were observed. The more immediate changes included i) the constriction of capillary loops, ii) the appearance of blunt or rounded microvilli on the surfaces of the cell bodies of the visceral podocytes and iii) the formation of 'blebs' of various shapes and

sizes on the surface of the podocyte and its processes. These changes began to appear at 20 minutes and remained constant in severity and distribution until 24 hours (and afterwards). Subsequent changes in the visceral epithelium included fusion of adjacent podocytes as well as adjacent podocyte processes. This erosion of podocyte integrity exposed intracellular contents and also exposed the GBM in patches (Fig. 3.11).

At a later stage i.e. after 24 hours the epithelial surface of most capillaries was transformed into a roughened sheet-like structure without any discernible features. Nevertheless occasional patches of glomerular capillaries still showed normal visceral epithelial features (Fig. 3.12).

Figure 3.1
Tubular epithelial reflux
(arrow) (30 mins PM)
H & E (x 300)

Figure 3.2
Tubular epithelial reflux
(arrow) (12 hrs PM)
H & E (x 300)



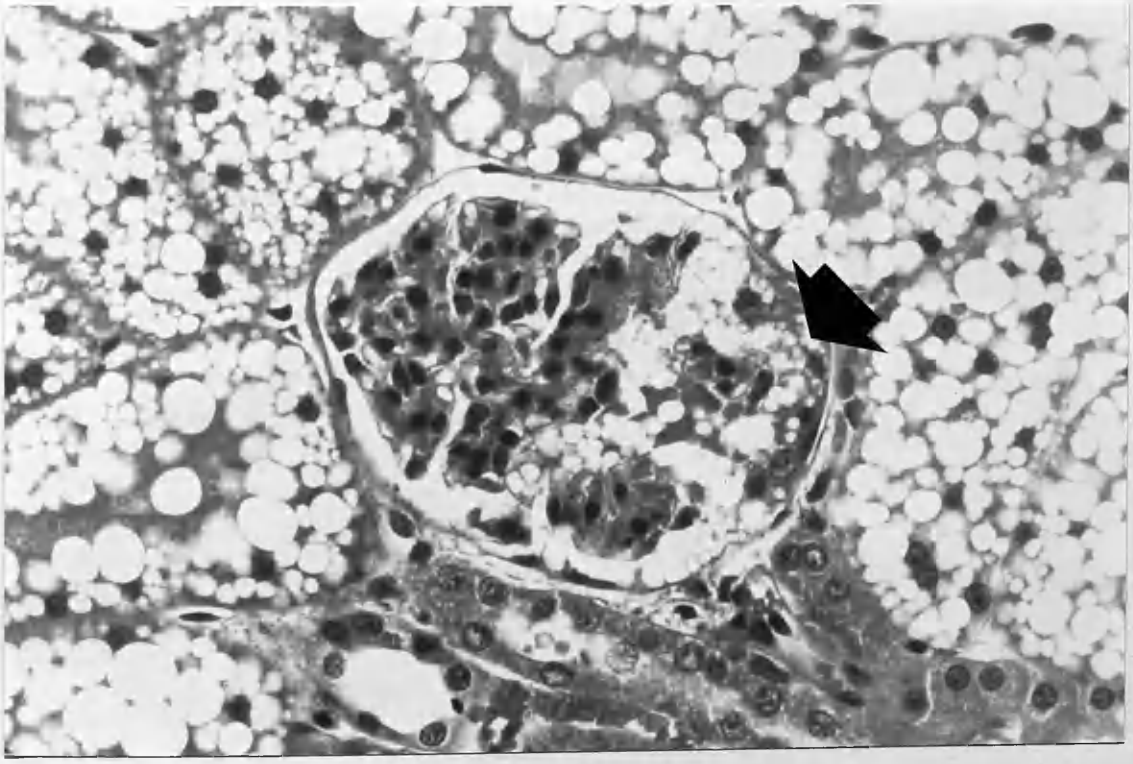
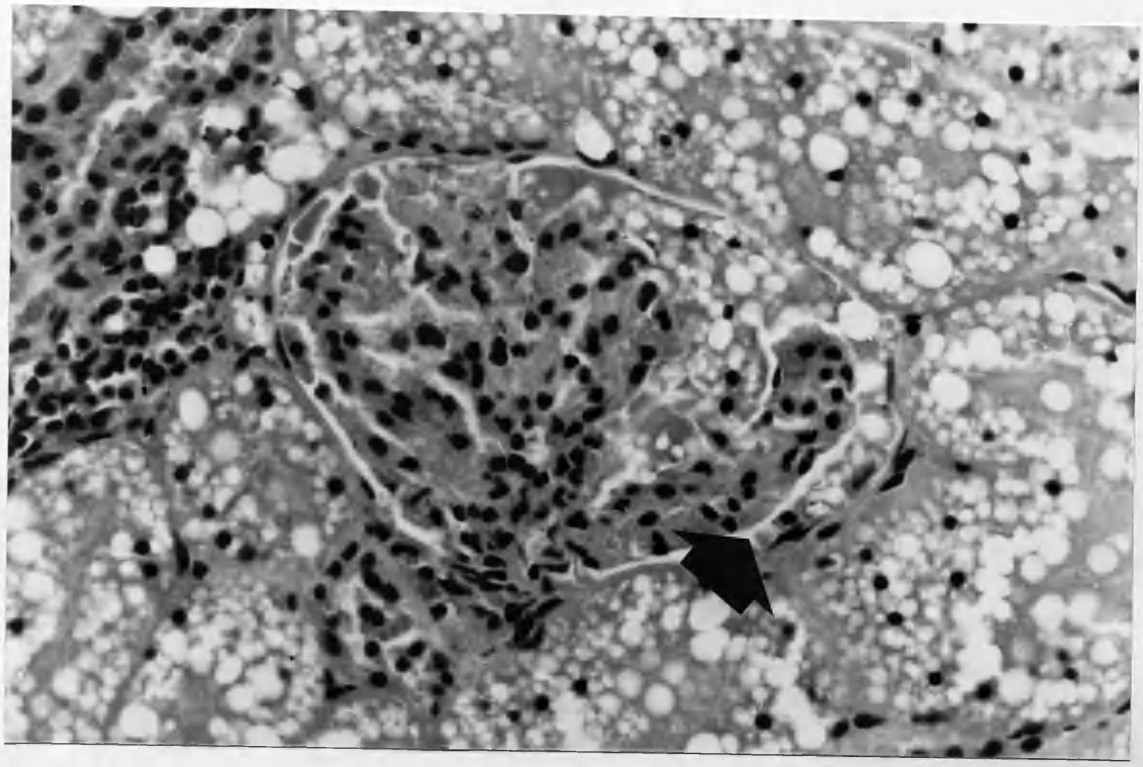


Figure 3.3
Tubular epithelial reflux
Note glomerular splitting and
capsular disruption (arrow)
(36 hrs PM)
H & E (x 300)

Figure 3.4
Glomerular disintegration
Note nuclear pyknosis
(60 hrs PM)
H & E (x 300)



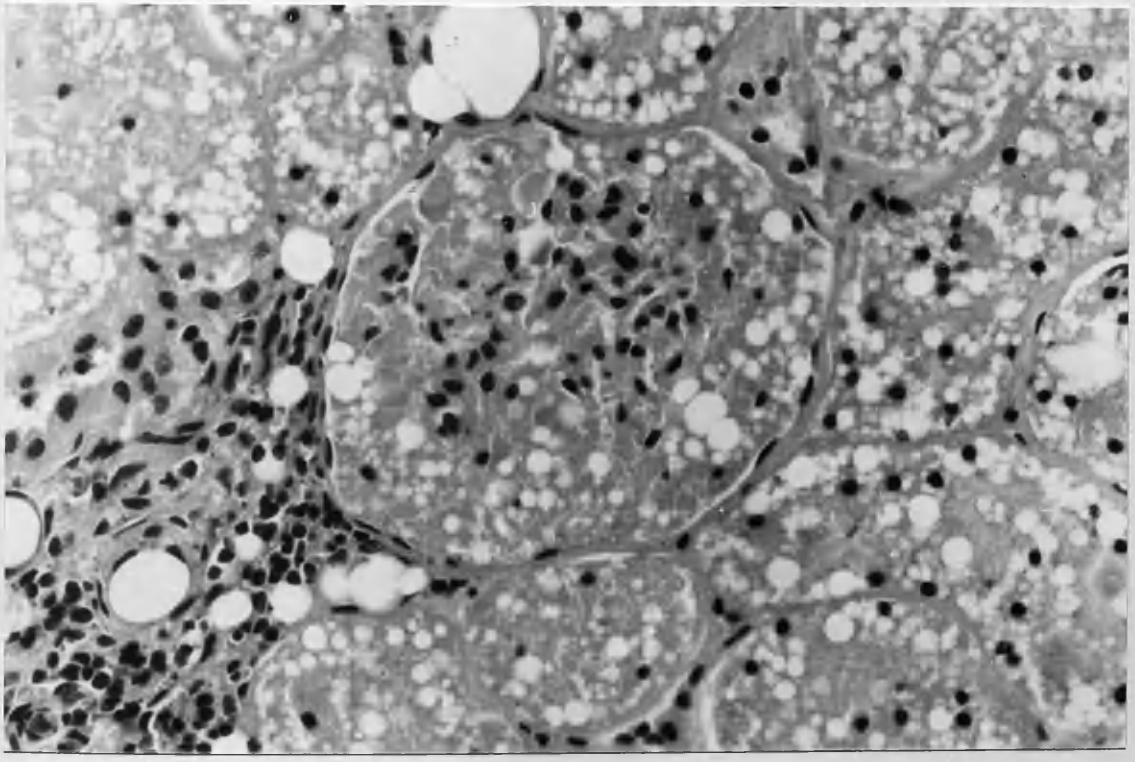


Figure 3.5
Tubular epithelial reflux
into urinary space (u) (arrows)
visceral epithelium (v), capillaries (c)
(10 mins PM)
TEM (x 5000)

Figure 3.6
Tubular epithelial reflux
into urinary space (u) +
filling of capillaries (c) with debris
(arrows)
(30 mins PM)
TEM (x 2000)


c
c
u
y
c
c

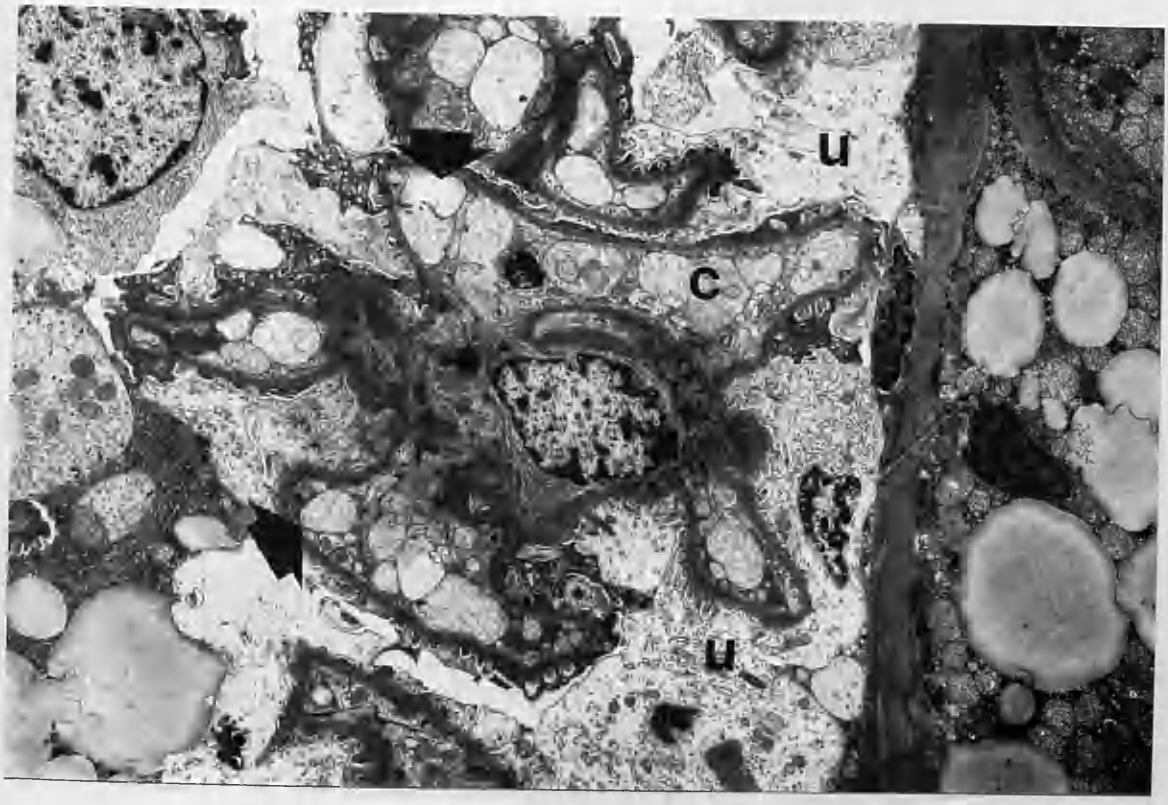
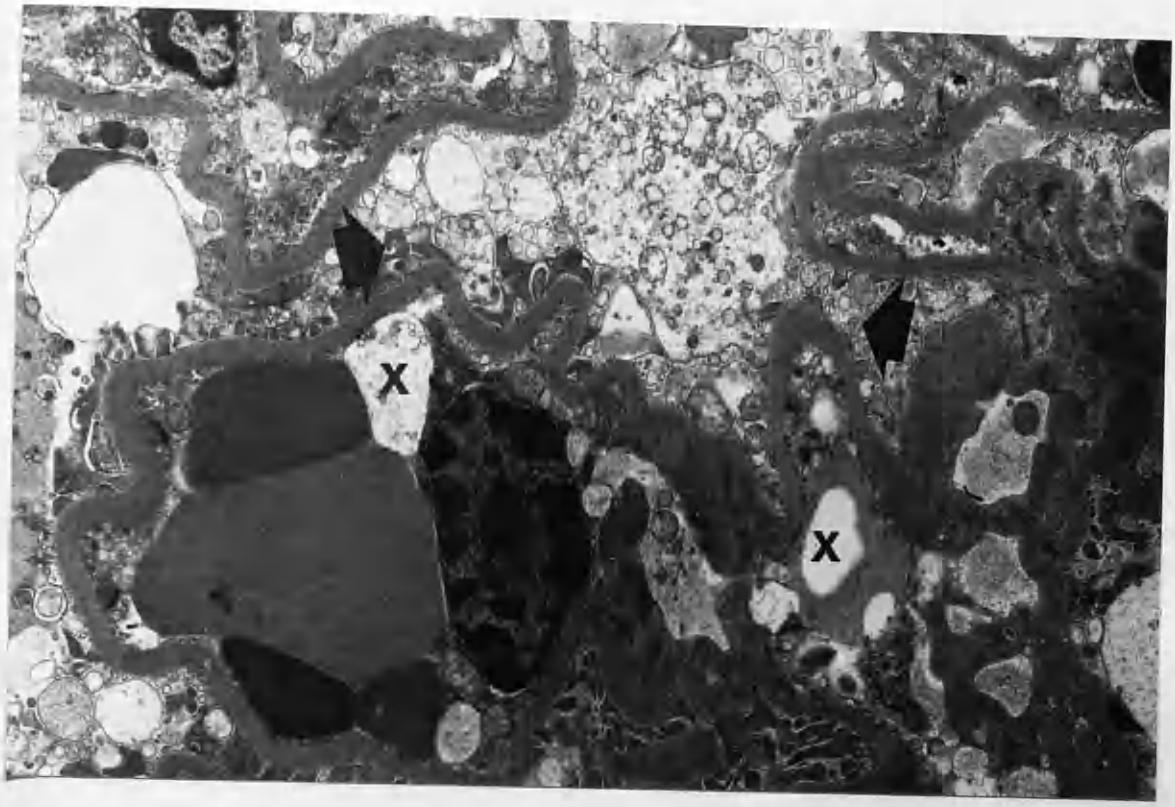

u
c
u

Figure 3.7
Capillary occlusion (x)
Visceral epithelial disintegration (arrows)
(60 mins PM)
TEM (x 4000)

Figure 3.8
Patchy preservation of
foot processes (arrow)
(60 hrs PM)
TEM (x 3000)


X
X

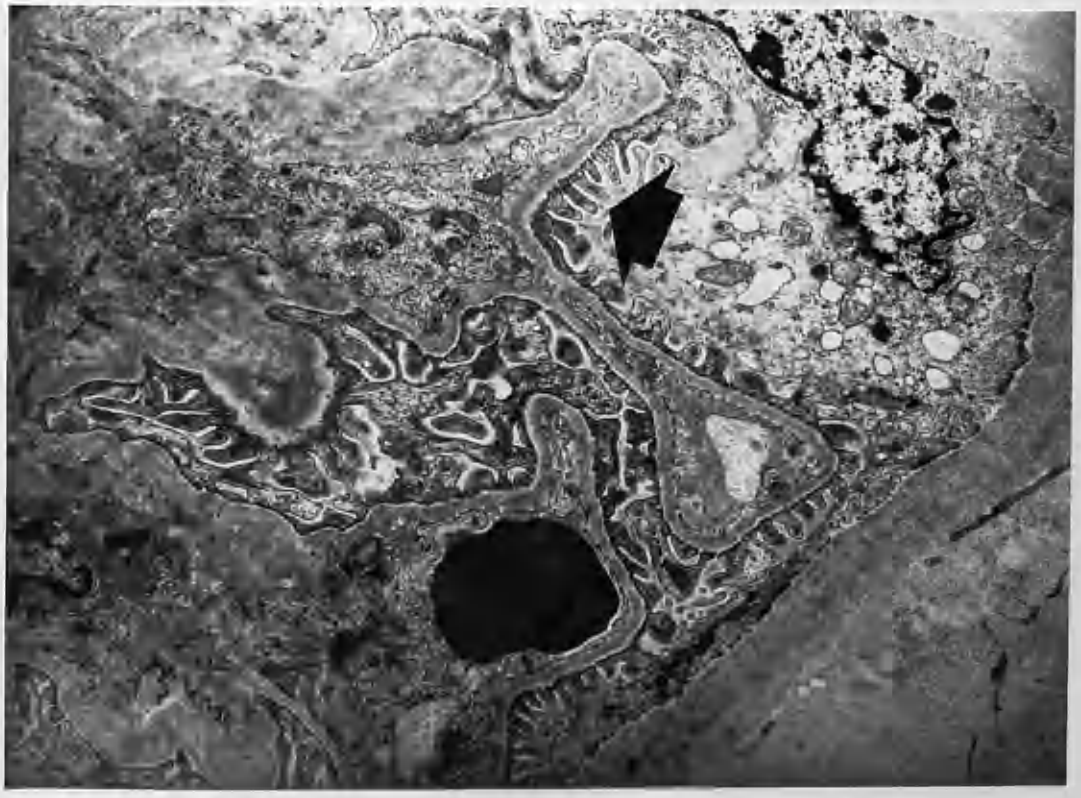
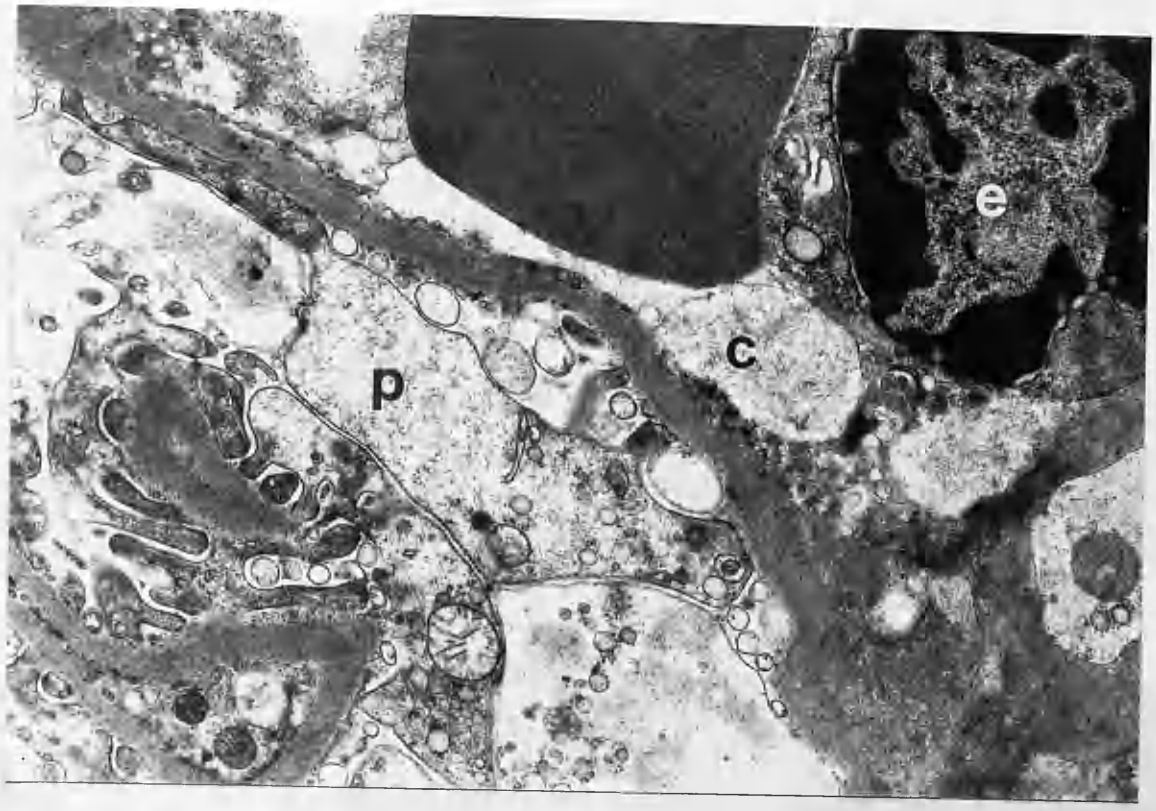


Figure 3.9
Glomerular disintegration
Endothelial cell (e), capillary (c)
Remnants of epithelial podocyte (p)
(48 hrs PM)
TEM (x 6000)

Figure 3.10
Glomerular disintegration
Note pyknotic nuclei (n)
and occluded capillaries (c)
(96 hrs PM)
TEM (x 3000)


e
c
p

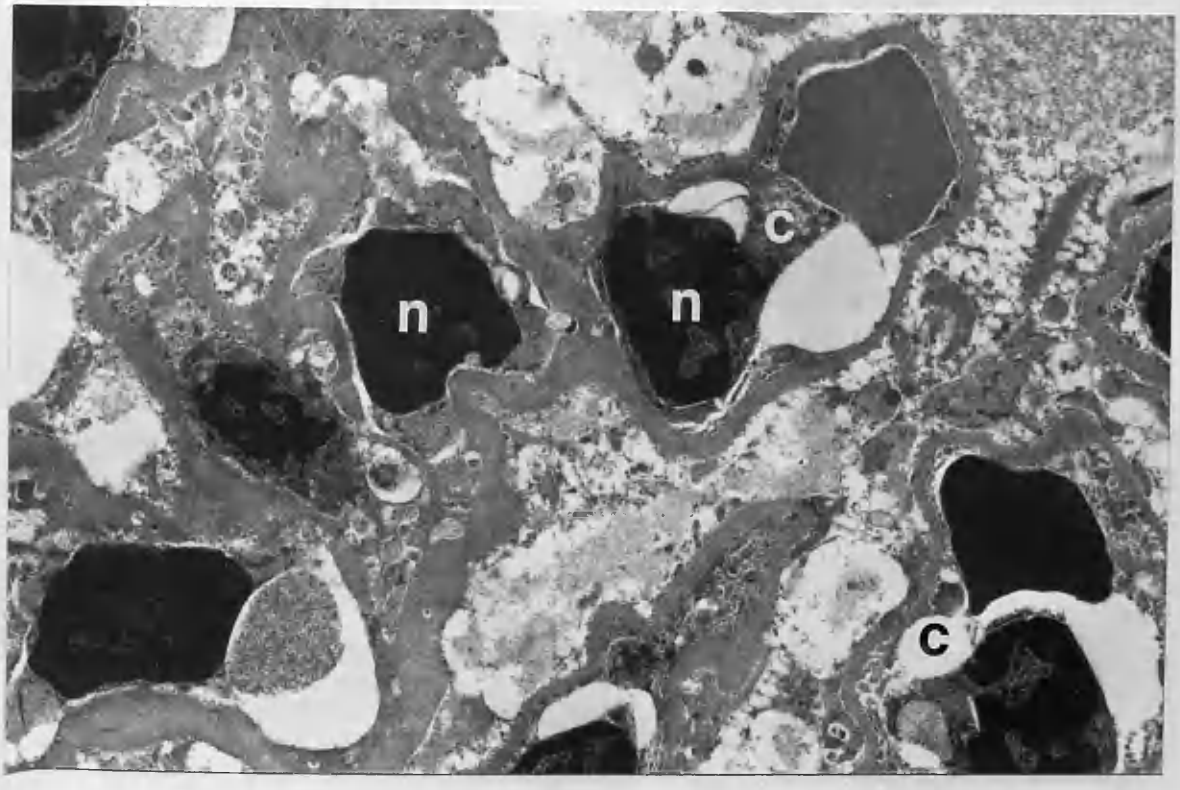
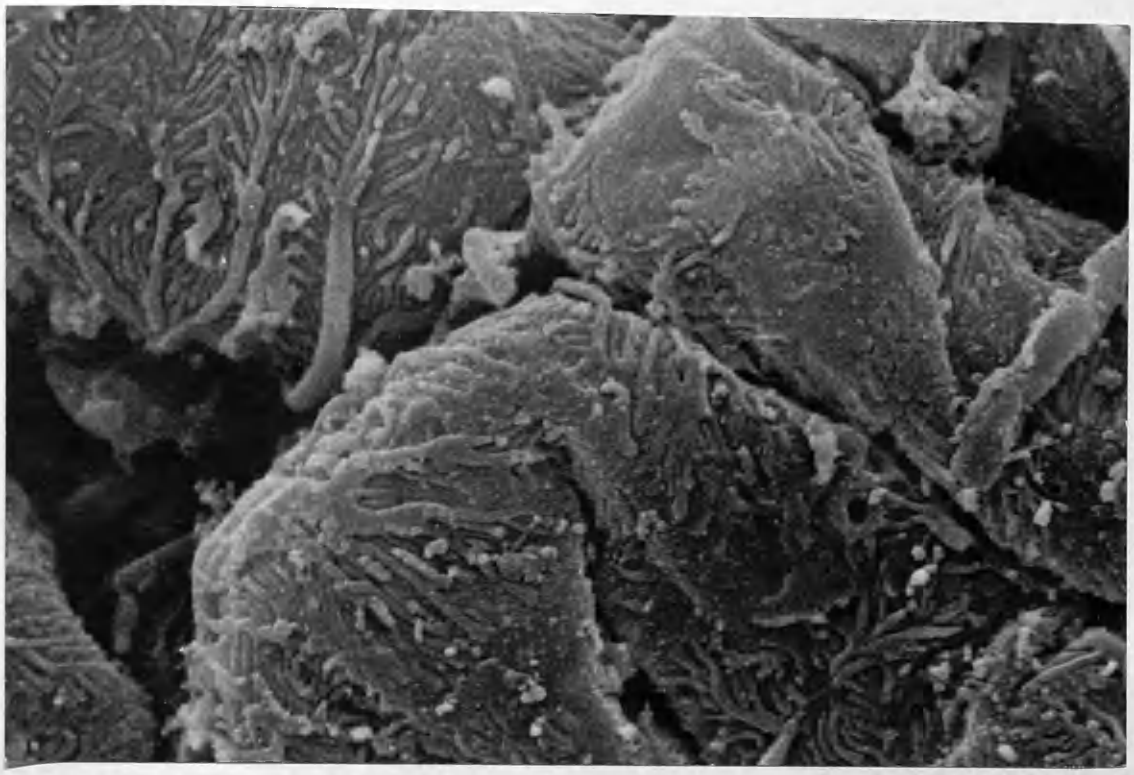
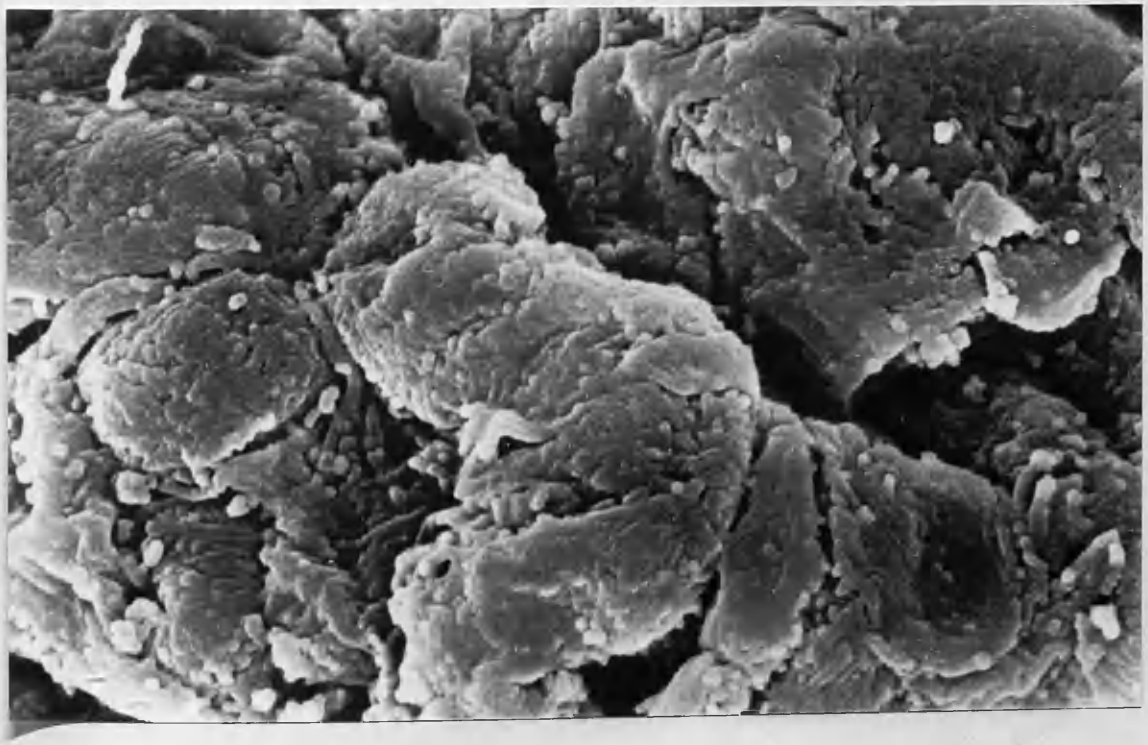

c
n
n
c

Figure 3.11
Podocytic erosion
Note loss of process integrity
(12 hrs PM)
SEM (x 160)

Figure 3.12
Podocyte disintegration
(> 24 hrs PM)
SEM (x 320)





DISCUSSION

Of fundamental importance for the understanding of pathologic processes is the relationship of autolytic vis-a-vis disease changes operating in the tissues at the same time. It is clearly essential to establish the sequential autolytic changes in normal tissue in order to evaluate with accuracy pathological change in similar tissues fixed and processed by the same methods. The major stimulus for the initiation of autolytic changes is the removal from the circulation of the kidneys and the subsequent anoxia. Moreover trauma as a result of over-handling the tissue has been shown previously to be a major contributary factor to the development of the autolytic changes (Cook et.al., 1965).

The histological findings in the present study began with the observation of tubular epithelial reflux into Bowman's space at approximately 20 minutes whereas the same condition in the sections prepared for electron microscopy appeared as early as ten minutes which would tend to confirm the similar findings of Cook et.al. (1965) and their suggestion that the additional manipulation involved in the preparation of tissues for electron microscopy resulted in both earlier and increased reflux.

This tubular epithelial reflux is, as the name

suggests, due to the detachment of the uppermost part of the proximal tubular epithelial cells and has also been observed to develop in the dog and rat under normal post-mortem conditions (Mullink and Feron, 1967). However, it has also been seen to occur due to intravital ischaemia in dogs and rats (Mullink and Feron, 1967) and has been found in acute and sub-acute nephritis in humans (Dixon et.al., 1971).

The swelling of the visceral epithelial podocytes seen in this study was accompanied by the swelling of its cytoplasmic processes; however, the foot processes resisted the autolytic changes much longer. This is in agreement with Cook et.al. (1965) who worked on the rat as well as Crowell et.al. (1974) and Mohammed (1985) both using the dog who noted a "remarkable preservation" of the foot processes and the GBM despite the early disintegration of the endothelial cells and the cell bodies of the visceral epithelial cells.

In these earlier studies as well as in the present study morphologically recognisable foot processes were distinct as late as 60 hours post-mortem despite the fact that the cell bodies had long disappeared.

Other features observed during autolysis in the present study have also been described in other previous studies: i) the myelin figures observed within the visceral epithelium after several hours of autolysis

have also been observed during uranium poisoning in the rat (Benscome et.al., 1959). ii) development of microvilli and/or irregular surface 'blebs' during autolysis have also been described in human glomerular disease by Latta (1960) (cited by Cook et.al., 1965) and Jackson et.al. (1962). It has been suggested by these authors that this development is at least part of a degenerative phenomenon. iii) margination of chromatin was observed by both light and electron microscopy in all types of glomerular cell and appeared to be a forerunner of pyknosis; indeed, even when the nucleus was seen to shrink the granules of marginated chromatin usually remain peripherally clumped. This phenomenon has been observed in other animals used in similar autolytic studies such as the rat (Osvaldo et.al., 1965 and Cook et.al., 1965) and the dog (Crowell et.al., 1974). iv) the swelling of the mesangial cells is thought to be the main explanation why the capillary lumina are filled by cytoplasmic debris. This swelling has been postulated as explaining the 'Intrakapillarhocherchen' first described by Zimmerman (1933) (cited by Mueller, 1958) and several subsequent authors. For example, they have been termed 'colliculi' (Yamada, 1955), 'balloon swelling' (Bergstrand, 1957) and 'diverticulums' (Michelson, 1962). Moreover, similar structures have been described in temporary renal ischaemia (Thoenes, 1962) (cited by

Cook et.al., 1965) as well as in pathological conditions in man and rats (Miller and Bohle, 1957 and Latta, 1960 (cited by Cook et.al., 1965)). The latter include acute glomerulonephritis (Vernier et.al., 1958; Movat et.al., 1962 and Strunk et.al., 1964), diabetes (Farquhar et.al. 1959 and Suzuki et.al., 1963) and toxaemia during pregnancy (Pollack and Nettles, 1960; Mautner et.al., 1962 and Wakamori et.al., 1962).

The morphology of autolytic change appears a little less dramatic when viewed with the SEM as compared with the ultrastructural changes seen when using the TEM. Indeed, a small proportion of glomeruli appeared normal even as late as 48 hours.

In visceral epithelial cells undergoing autolysis the development of surface irregularities together with the enlargement of podocytic processes began at 20 minutes. Although primary and secondary processes were seen to fuse at this time and lose any semblance of orderly interdigitation some foot processes remained morphologically recognisable and attached to the GBM as late as 60 hours.

Direct comparison of the present study with the more recent of the only two other reports dealing with autolytic changes in the cat (Crowell and Leininger, 1976) is not possible due to the differences in the parameters examined, for example the time scale of their

examination is shorter and their method of storage of the kidney is different from the present study. However, these workers did describe herniation of the tubular epithelium into Bowman's space yet did not give a time scale to its appearance or a scale of severity to the phenomenon. This herniation had been previously described by Mayer and Ottolenghi (1947) for the cat as part of a larger study in the dog; however, again the amount of reflux was not correlated with time after death.

CHAPTER 4

A HISTOLOGICAL AND ULTRASTRUCTURAL INVESTIGATION INTO A NOVEL GLOMERULAR LESION

INTRODUCTION

During the investigation into the normal feline glomerular morphology as previously reported in Chapter 1, a hitherto unrecognised glomerular lesion was detected.

The purpose of this section of the work was to conduct a detailed histological, immunofluorescence and ultrastructural study of this novel form of glomerulopathy.

MATERIALS and METHODS

1. Source of Animals.

Nine adult cats were obtained from commercial sources as part of a larger group of 20 animals which were the subject of a study concerning the normal feline glomerular morphological parameters as described in Chapter 1.

Prior to their use detailed examinations, as previously described in the section on general Materials and Methods were carried out. The outcome of these examinations showed there to be nothing to distinguish these nine animals from the remainder of the initial group of 20 animals.

2. Euthanasia.

The method of euthanasia employed for these nine animals was again no different from that used on the initial group of animals and is described in full in the general Materials and Methods (pages 6-7).

As before, the animals were exsanguinated by severing the axillary artery as soon as the femoral pulse was only faintly discernible. This was done to ensure adequate exsanguination and the consequent enhancement of histological preparations.

3. Sampling of Tissues.

Following exsanguination, the abdomen was opened by a mid-line incision and the abdominal organs displaced to allow easy removal of both kidneys. Tissue samples were then taken, as previously described, for both histological and ultrastructural studies. In addition small blocks of fresh renal cortex were removed and snap-frozen by immersion in liquid nitrogen (LN_2). They were then stored at $-20^{o}C$ to be subsequently used in immunofluorescence studies as previously described (pages 7-10).

All the tissues used were prepared for examination by the methods previously stated and these remain unchanged.

RESULTS

1. Histological findings:

All nine cats showed a marked diffuse glomerulopathy characterised by necrosis of the glomerular endothelial and mesangial cells together with the appearance of granular necrotic debris within the lumina of the glomerular capillaries. It was noticeable that all the glomeruli were involved to an equal extent (Figs. 4.1 and 4.2).

The nuclei of the endothelial cells together with a proportion of mesangial cells were quite definitely pyknotic whereas those cells comprising the visceral epithelium appeared largely unchanged. The destructive effect suffered by the glomerular endothelium was subsequently confirmed by use of 1 um. plastic sections (Fig. 4.3).

In marked contrast, the histological appearance of the tubules remained unchanged with the tubular epithelial cells remaining well preserved likewise, the tubular capillaries were unaltered.

A search for any occlusive vascular lesion of the renal microcirculation proved fruitless and apart from the glomerular lesions the kidneys were in all other respects normal.

2. TEM findings:

The most striking feature observed in these nine cases was lysis of the glomerular endothelial cells with detachment of whole segments of necrotic endothelial cytoplasm from the GBM (Fig.4.4). In many capillary loops, the GBM was completely denuded of any endothelial covering with the lumina of capillaries often completely filled with cytoplasmic debris. Pyknotic nuclei from these disrupted cells could also be found floating free in the capillary lumina (Fig. 4.5).

In addition, swollen processes of mesangial cells were seen to be extruding through the axial region of the capillary loops into the lumina where they intermingled with the detached profiles of the endothelial cytoplasm. The mesangial cells from which these processes arose showed varying degrees of necrosis with many of them having pyknotic nuclei (Fig. 4.6).

In marked contrast, the visceral epithelial podocytes, although somewhat swollen, showed relatively little change with the normal orderly arrangement of the podocytic processes remaining undisturbed except for an occasional patchy area of swelling or fusion (Fig. 4.7).

The GBM, too, was for the most part quite normal though there were occasional areas of thickening.

3. SEM findings:

When viewed by the SEM the glomeruli appeared, to all intents and purposes, normal. There was little outward indication that these glomeruli were suffering from a severe endothelial lesion apart from an occasional area of epithelial podocyte fusion. Surrounding tubules in the remainder of the kidney were also normal in appearance (Figs. 4.8 and 4.9).

Probably due to the preparative techniques no luminal deposits of debris were found in sectioned glomeruli; likewise due to the lesion itself there was a complete loss of the fenestrated epithelium.

4. Immunofluorescence findings:

These are summarised in Table 4.1.

Eight out of the nine cases were found to have a marked granular deposition of complement (C_3). The distribution of this C_3 was such that it appeared to lie within the capillary loops rather than along the GBM or in the mesangium (Figs. 4.10).

Similar deposits of immunoglobulin G (IgG) were also found in these cases and, here again, a similar granular deposition pattern was observed yet at a diminished level of intensity (Fig 4.11).

Also noted in several of these animals (five out of nine) was a patchy distribution of C_3 staining a small

number of tubules of the affected kidneys in a linear fashion. The significance of this finding is, as yet, unclear as no tubular lesion could be found in similar areas within histological prepartions.

A retrospective examination, by immunofluorescence, of the other eleven animals in the original group of 20 (Chapter 1) using both anti-cat C_3 and IgG showed no deposition of either of these two reagents either within the glomeruli.

TABLE 4.1

Summary of Immunofluorescence results.

Cat No.	Immunofluorescence *	
	C_3	IgG
1.2	3+	2+
1.3	2+	1+
1.4	3+	2+
1.6	2+	1+
1.8	1+	1+
1.9	-	1+
1.13	3+	$\pm$
1.14	2+	2+
1.17	3+	1+

* The deposits were graded $\pm$ to 4+ according to their severity.

Note: $\pm$ represents the minimum change observed using immunofluorescence.

Figure 4.1
The glomerular lytic lesion.
Note the granular debris in
capillary lumina (arrows).
H & E (x 300)

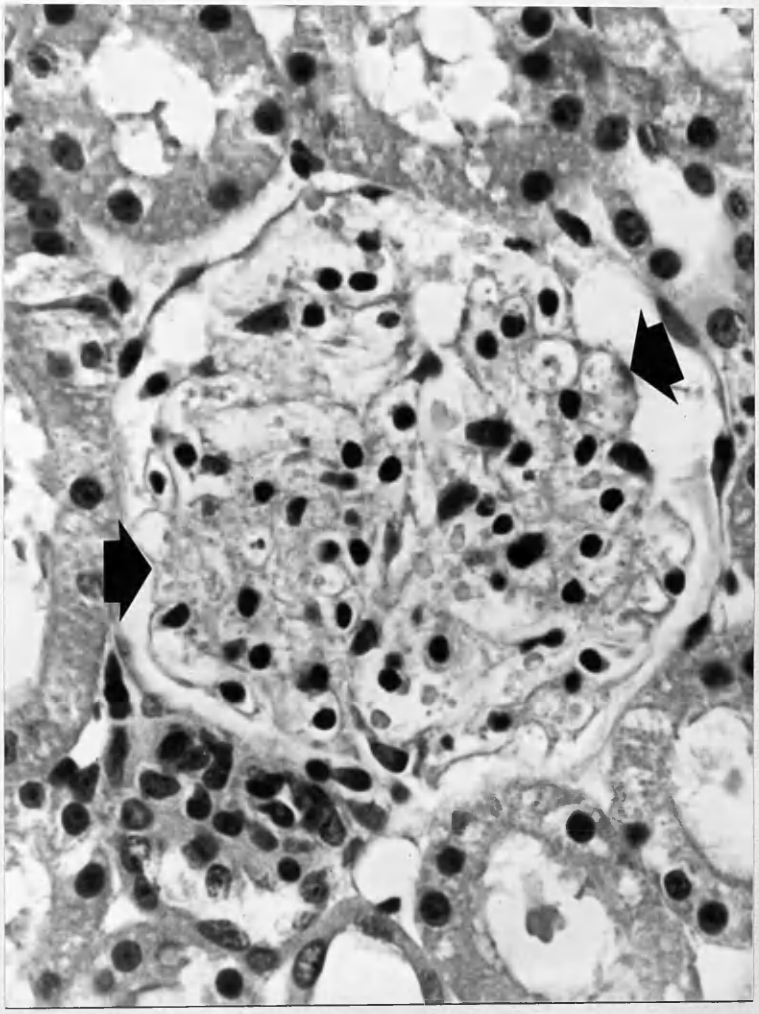
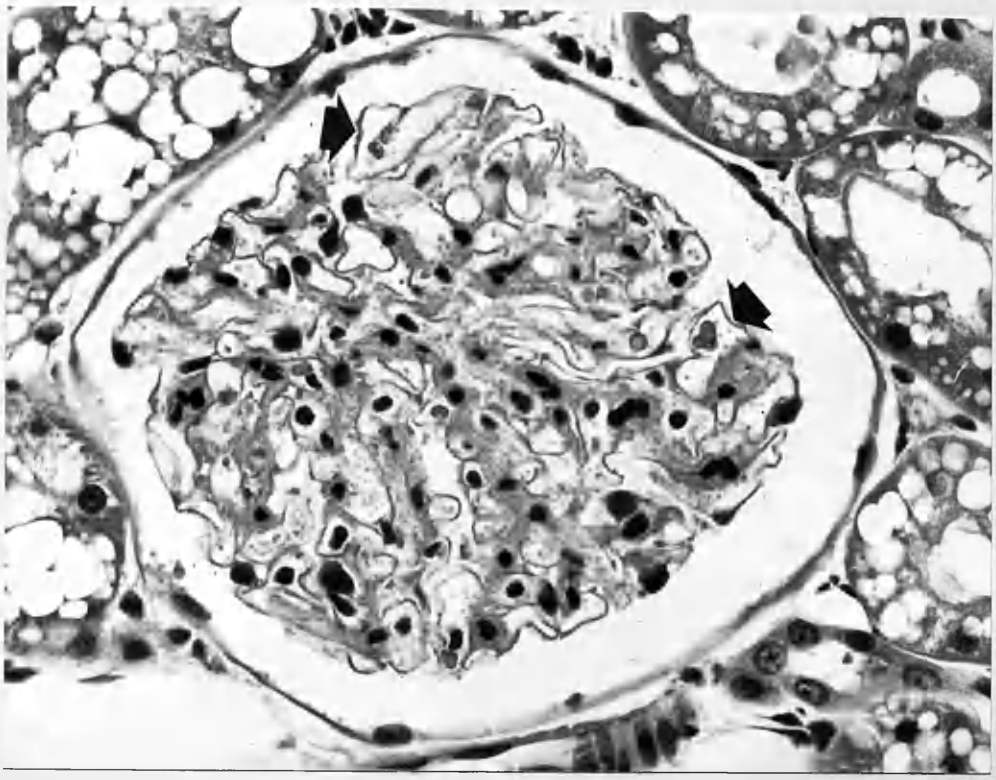


Figure 4.2
Lytic lesion.
Note the intact GBM and
the cellular disruption (arrows).
H & E (x 300)

Figure 4.3
Lytic lesion.
Note the complete disruption
of many mesangial areas (arrows).
Toluidine blue lum.plastic (x 300)



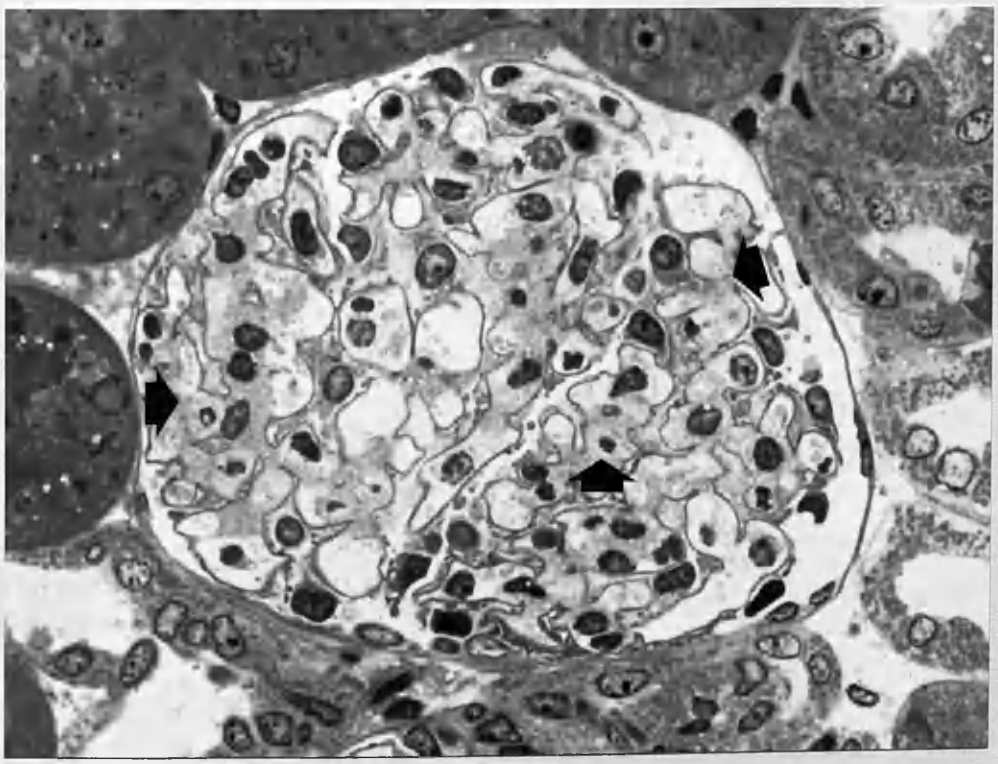
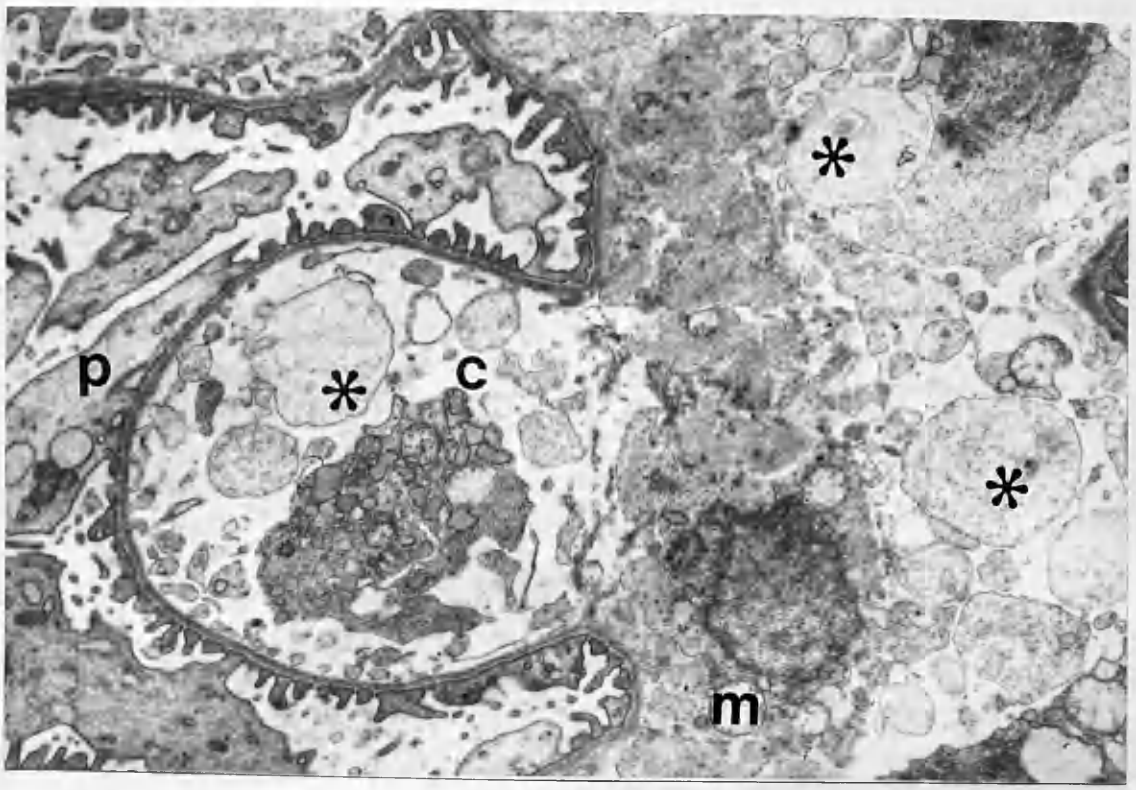


Figure 4.4
Lytic lesion.
Note the absence of endothelium;
capillary (c), debris (asterisks)
and remains of mesangial cell (m).
Note intact podocytic processes (p).
TEM (x 4000)

Figure 4.5
Lytic lesion.
Note occlusion of capillary (c)
with capillary debris (asterisks).
Note also the unaltered
podocytic processes (arrow).
TEM (x 4000)


p
c
m

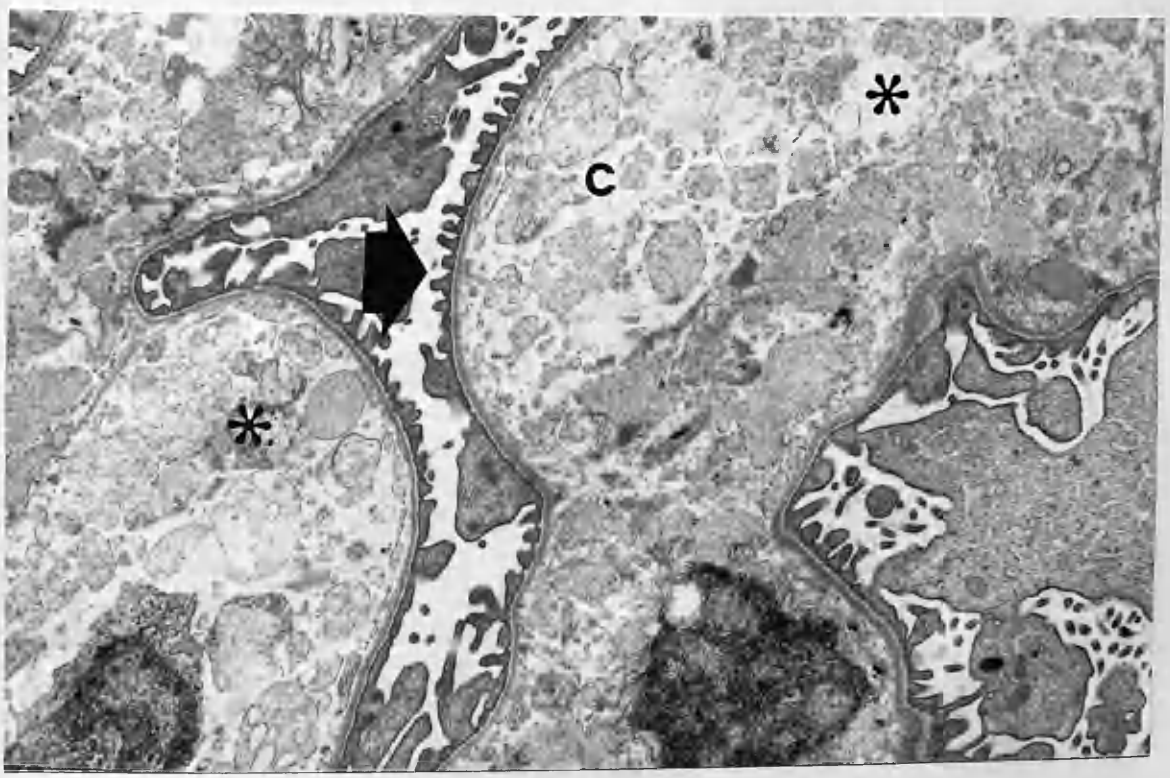
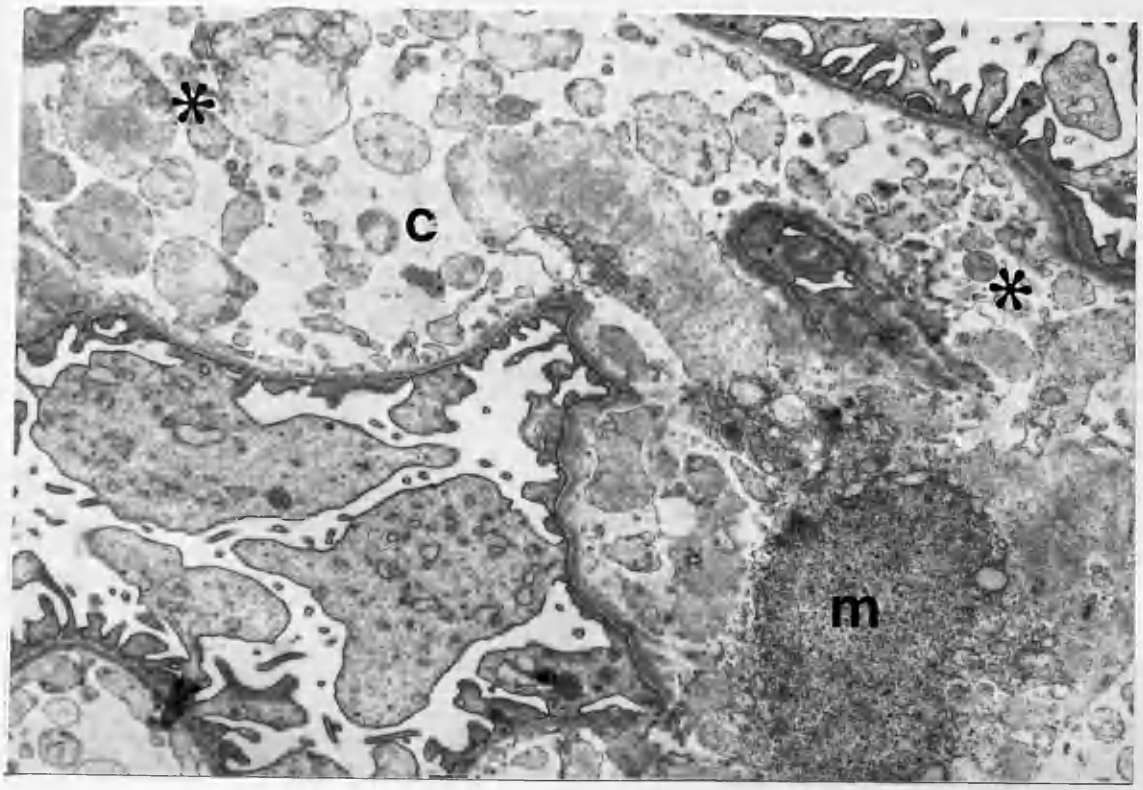

c

Figure 4.6
Lytic lesion.
Note the mesangial destruction (m) and
the absence of fenestrated endothelium.
Debris fills the capillary (asterisks).
TEM (x 4000)

Figure 4.7
Lytic lesion
Note the visceral cell (v) and
its unaltered processes.
Capillaries contain cellular debris (c).
TEM (x 4000)


*
c
*
m

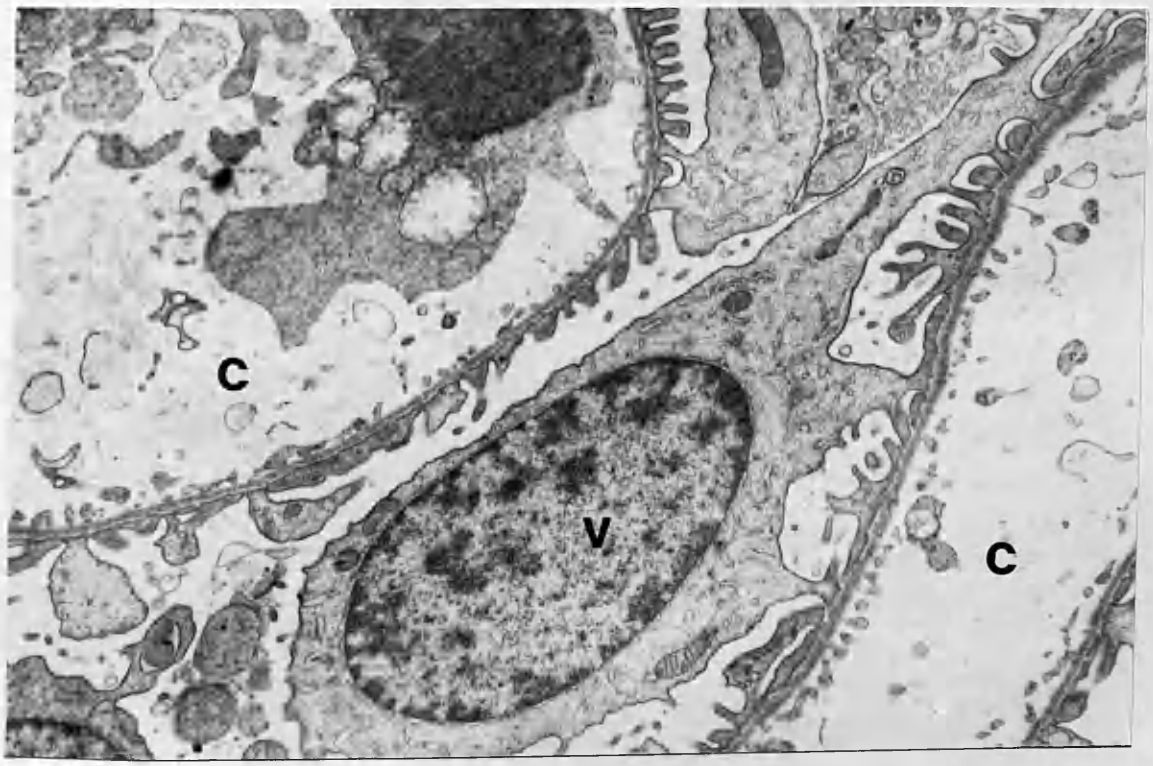
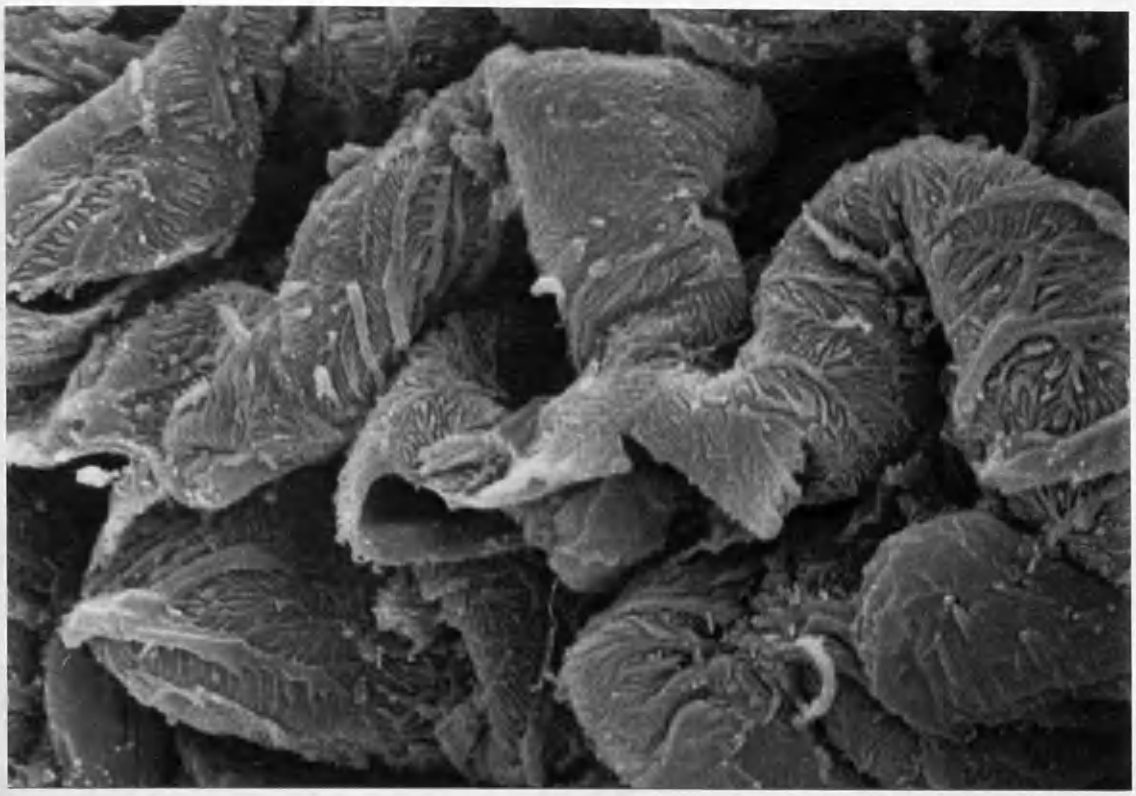

c
v
c

Figure 4.8
Lytic lesion.
Visceral epithelium-
unaltered from normal.
SEM (x 2500)

Figure 4.9
Lytic lesion.
Visceral epithelium-
Cell body (c) and processes (p)
unaltered from normal.
SEM (x 5000)



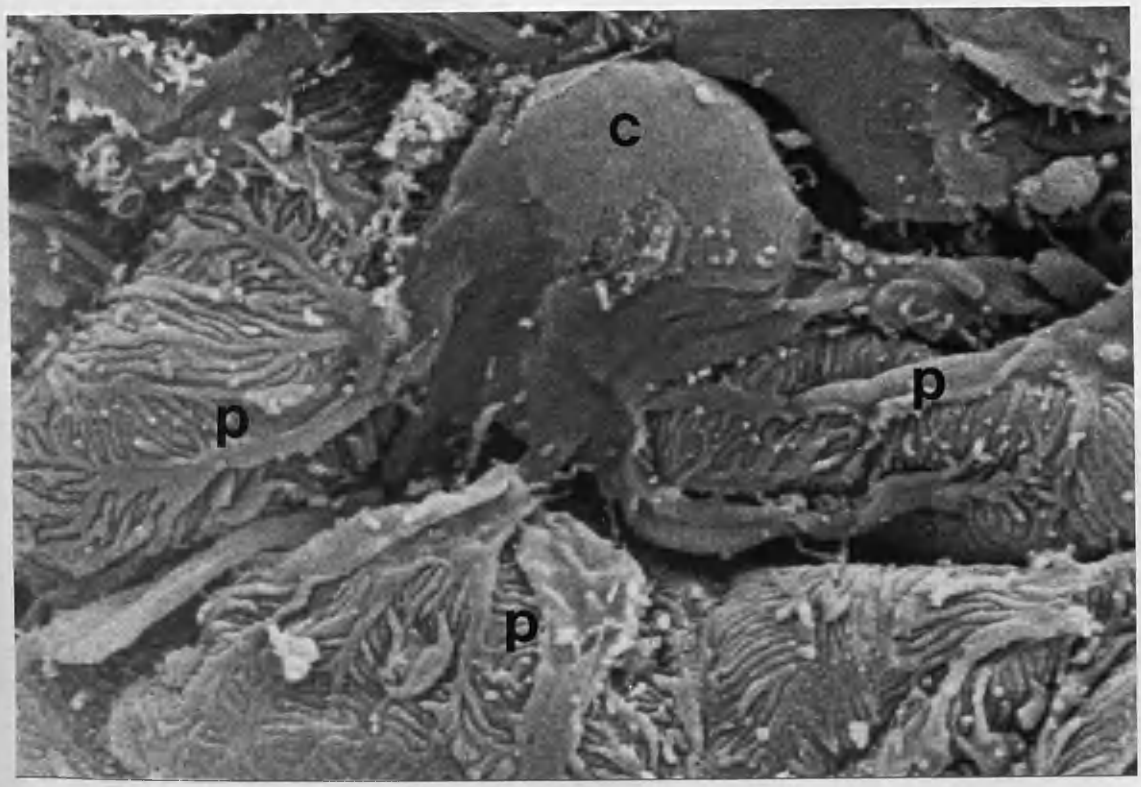
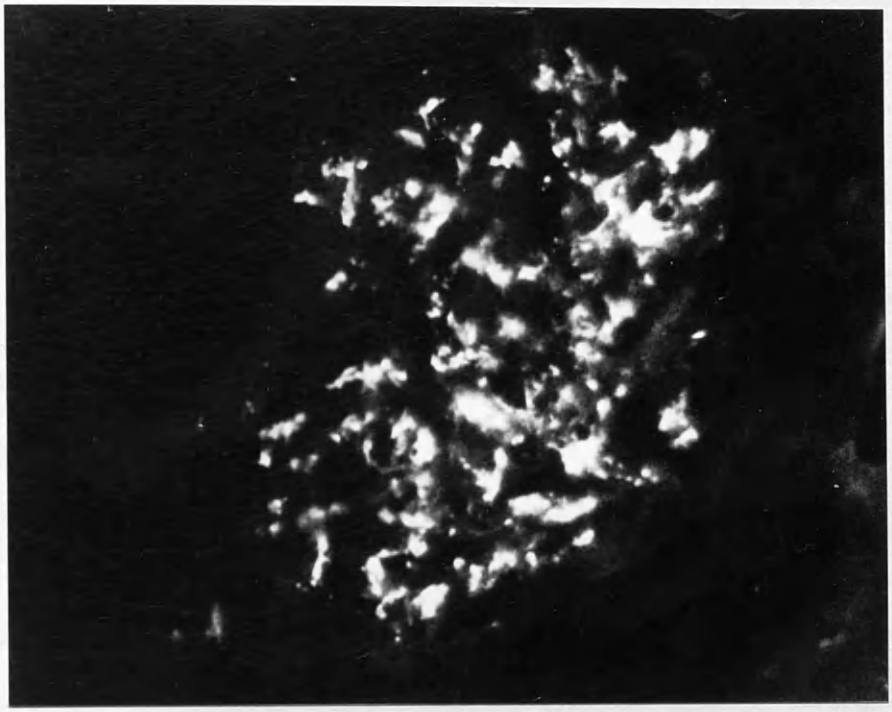
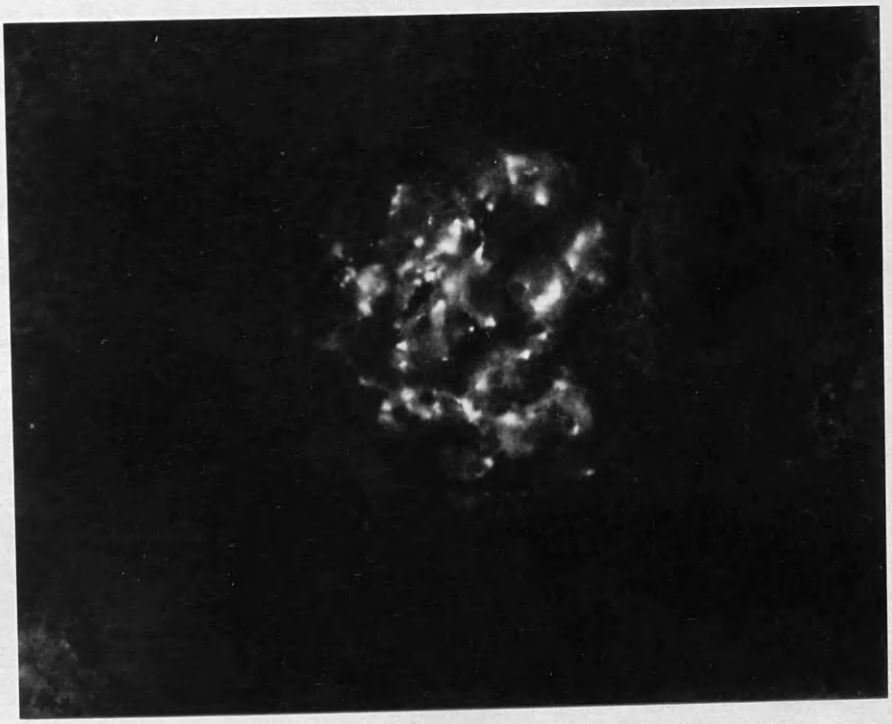

c
p
p
p

Figure 4.10
Glomerular complement deposition.
Note the granular deposits of C_3
Immunofluorescence (x 250)

Figure 4.11
Glomerular IgG deposition.
Note the granular deposits of IgG.
Immunofluorescence (x 250)





DISCUSSION

In these nine animals, a severe necrotising lesion of the glomerular endothelium and to a lesser extent of the glomerular mesangium was identified.

The ultrastructural features of this lesion left no doubt as to the recent and severe nature of the endothelial damage. Indeed, if the lesion had occurred at some time prior to euthanasia subsequent tubular epithelial deterioration must certainly of have been expected, given the severity of the glomerular pathology.

Furthermore, the speed at which the kidneys were fixed for both conventional light microscopy and electron microscopy makes it unlikely that the lesion was a simple autolytic change. This was especially true as the cortical tubules, which are much more sensitive to anoxia than the glomeruli (Latta et.al., 1965) were themselves well preserved.

The only feature common to all nine affected cats, and, incidentally to the other 11 of the initial study group, was the fact that they had received both K HCl and Na P , the latter within three minutes prior to the removal and fixation of the kidneys.

For the purposes of general anaesthesia in the cat the manufacturers of K HCl ('Vetalar'; Parke, Davis) recommend that the anaesthetic should not be given in the same syringe as barbiturate due to the formation of insoluble precipitates within the syringe (Veterinary Data Book, 1984). This was tested by the author in vitro and found to be the case (see Chapter 7, Materials and Methods, page 132).

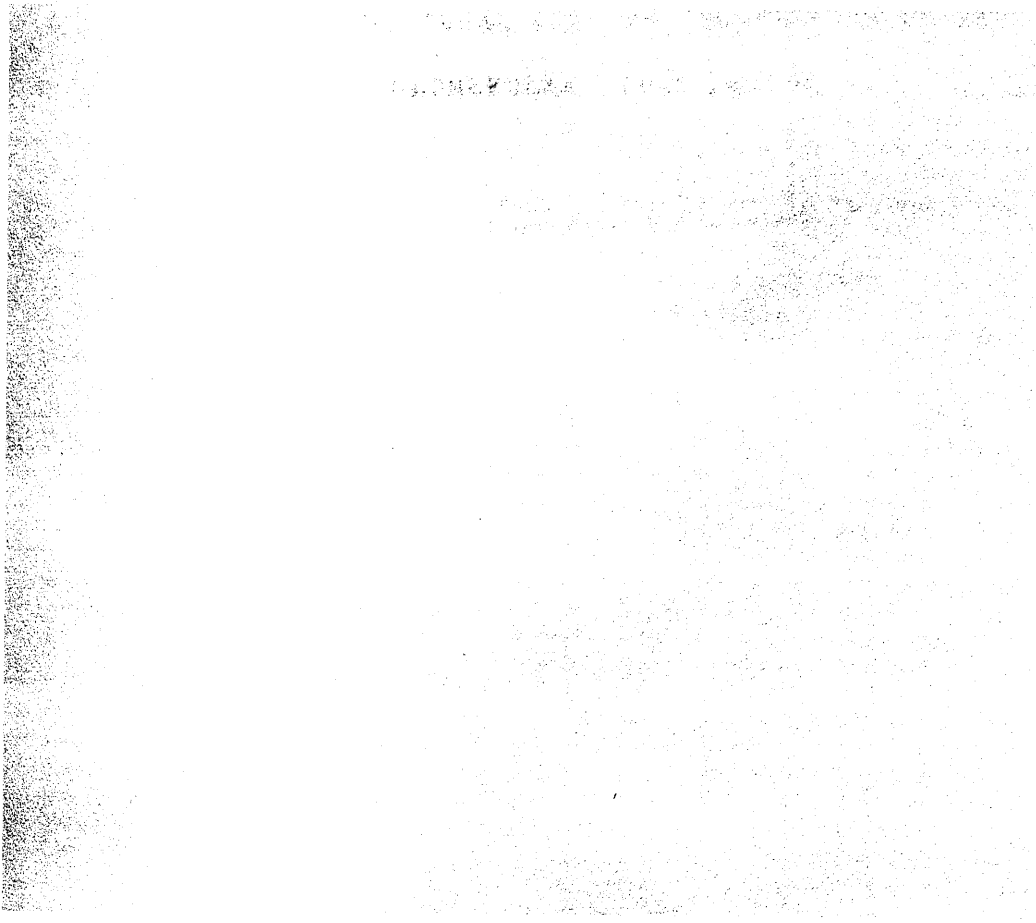
However the use of the two drugs in combination in the cat (or indeed in primates) is not discouraged. Indeed there is the statement that in a proportion of cases it is necessary to supplement K HCl with barbiturates following the primary administration of K HCl although suitable adjustments to the dose level of barbiturate are recommended in accordance with the general principles of veterinary anaesthesia.

There is no doubt that, in the present study, deeper anaesthesia than normal was employed in order to ensure satisfactory exsanguination prior to death. Thus the dose of barbiturate would have been beyond the usual employed for general anaesthetic purposes.

Nevertheless, it seems highly likely that the glomerulopathy described above in euthanased cats was associated, in some way, with an intravascular interaction between K HCl , or an associated compound resulting from its detoxification in the liver, and Na P

with a resultant cytotoxicity for glomerular endothelial and mesangial cells.

The deposition of complement, in the detectable form of C_3, via an 'alternative pathway' i.e. without the presence of a 'triggering antibody' or an antibody/antigen complex within affected glomeruli would tend to support this view.



CHAPTER 5

A HISTOLOGICAL AND ULTRASTRUCTURAL INVESTIGATION OF THE INDIVIDUAL CHEMICAL COMPONENTS OF THE GLOMERULAR LYTIC LESION

INTRODUCTION

In an attempt to examine the effects of the constituent drugs involved in the lesion described in Chapter 4, a method was required to obtain kidney material from the adult cat using each drug independently of the other. However, both drugs present problems with respect to their use in the cat;

a) Ketamine hydrochloride (K HCl) is a rapid- onset anaesthetic which can be administered via an intramuscular route making it ideal for use in the cat, especially if the animal is at all fractious. However the anaesthetic state produced does not fit into the conventional classification of the stages of anaesthesia but instead produces a state of unconsciousness which has been termed 'dissociative' anaesthesia. This, in effect, acts as a block to sensory input into the brain although protective reflexes such as coughing or swallowing are maintained.

It is, however, relatively short acting with the recipient becoming ataxic at five - ten minutes with anaesthesia lasting not longer than 30 - 40 minutes depending on the particular individual's general condition and age.

b) Sodium pentobarbitone (Na P) this drug is currently available in two differing preparations such

as 'Euthatal' (May and Baker, Dagenham, England), a 20% solution of sodium pentobarbitone, whose use is primarily for rapid euthanasia and as such is not suitable for the controlled anaesthesia leading to exsanguination as used in this study. Another preparation in common use is 'Sagatal' (May and Baker, Dagenham, England), a 6% solution, which can be used either as a sedative or a general anaesthetic depending on the dose administered.

Unfortunately the problem with the use of this drug is in its administration. The manufacturer's recommendation for administration of the drug is by intravenous injection; indeed they suggest that though the computed dose should be used the drug should only be administered to effect, administering the dose slowly and thus giving time for assessment of the degree of narcosis or anaesthesia to be judged until the required depth is attained.

However, the cat is not the most helpful of patients to administer such a drug to, hence the widespread use of K HCl as a simple, yet effective, means of rapid-onset anaesthesia.

For the purpose of this section of the work, two experiments were carried out. The aim of these was to test the separate effect(s) of K HCl and Na P in two groups of cats.

MATERIALS and METHODS

Prior to the commencement of these two experiments each cat was examined, weighed and samples of blood via jugular puncture, and urine via manual expression of the bladder, were taken. Full haematological and biochemical examinations were performed to assess normal functional parameters.

EXPERIMENT 1: The effect of K HCl alone.

The 11 cats were primarily sedated by an intramuscular injection of K HCl, into the quadriceps muscle mass, using the manufacturer's recommended dose (22 mg/kg.). After five - ten minutes a second similar dose of K HCl was given. Subsequent to these two injections, further amounts of K HCl were administered intravenously in order to achieve profound anaesthesia of sufficient depth for exsanguination to be carried out. However, all attempts to sedate animals to a level of anaesthesia sufficient to carry out painless euthanasia, using only K HCl, was unsuccessful.

In order to overcome the insufficient anaesthesia/ analgesia obtained by K HCl a revised experimental method using percutaneous renal biopsy was employed.

This was done after a period of seven to ten days to allow the cats an adequate recovery period. This method had the advantage of being a relatively straightforward and safe procedure in the cat which allows assessment of the effects of K HCl on the kidney at normal levels of anaesthesia.

These 11 animals were given a single injection (22mg/kg.), again into the quadriceps muscle mass, of K HCl and then the left flank was prepared for biopsy by either shaving or plucking a portion of the animals fur adjacent to where the kidney would be positioned during biopsy. The animal's flank was then cleaned by washing with antiseptic.

Once sufficiently anaesthetised an incision was made in the animals flank within the shaved area and a single blind percutaneous biopsy of the left kidney performed. This was carried out using a 4.5 inch 'Tru-Cut' disposable biopsy needle. (Travenol Laboratories Inc., Deerfield, Illinois, U.S.A.) The biopsy specimen was then divided into three portions with one each being fixed, by immersion, for histology and transmission electron microscopy by the methods previously detailed in the general Materials and Methods. The remaining portion was then snap frozen in liquid nitrogen and stored at $-20^{o}C$ for subsequent investigation using immunofluorescence.

A description of this biopsy method has been reported elsewhere (Nash et.al., 1983).

EXPERIMENT 2: The effect of Na P alone.

For the purpose of anaesthesia six adult cats were used; each cat was manually restrained by at least two animal technicians prior to intravenous injection, via the cephalic vein, of a 6% solution of Na P at the dose rate of 1 ml/ 2kg; by this method the animal was sufficiently deeply anaesthetised for exsanguination to be painlessly accomplished. Exsanguination was carried out by severing the axillary artery.

As before, the abdomen was opened by a mid-line incision, the abdominal organs displaced and the kidneys removed. Once again, tissues were fixed by immersion for morphological and ultrastructural studies by identical methods to those previously described. Similarly small blocks of renal cortex were snap-frozen in liquid nitrogen before being stored at $-20^{o}C$ for subsequent immunofluorescence studies.

All tissues were prepared for examination by the methods previously stated in the general Materials and Methods (pages 7-10).

RESULTS

EXPERIMENT 1: The effect of K HCl alone.

1. Clinical findings:

The anaesthetic was well tolerated by all the cats used in this chapter. In each case sufficient relaxation was induced to allow easy location, positioning and biopsy of the kidney. During recovery no complications were encountered and though the animals were ataxic during the first two hours post biopsy they had, in the majority of cases, regained the power of their limbs by four hours post biopsy. Certainly, by twenty four hours post biopsy, full recovery to normal movement and appetite was observed. No evidence of any pain whether localised renal or generalised abdominal was observed post biopsy. The rectal temperatures also remained normal over the length of the experiment.

The majority of the animals (nine out of 11) showed evidence of haematuria after the biopsy procedure, though this had been absent prior to the biopsy; however, in only three cases did this haematuria persist after 48 hours post-biopsy.

2. Histological, TEM and immunofluorescence findings:

In nine of the 11 animals used in this experiment biopsy material obtained contained sufficient glomeruli for assessment. The average number of glomeruli per sample being 13.8 for the 9 animals.

No evidence of any renal lesion was observed in the biopsy material taken for histology. Indeed, the glomeruli present in the sections appeared entirely normal with no indication of any endothelial lesion or mesangial involvement. This was subsequently confirmed by use of the TEM where again no evidence of any endothelial or mesangial damage could be observed.

With immunofluorescence all the biopsy specimens proved to be non-reactive against anti-cat immunoglobulin G (IgG) and complement (C_3).

EXPERIMENT 2: The effect of Na P alone.

Histological, TEM, SEM and immunofluorescence findings:

As for the group of cats where K HCl alone was used there was no evidence of any glomerular lesion in the histological specimens obtained from necropsy material.

Similarly no abnormalities in structure were observed when the kidney samples were examined by electron microscopy. There was no evidence of any endothelial or mesangial damage of the type described in Chapter 4. Likewise, in all respects the samples appeared normal when viewed by the SEM.

The results of the immunofluorescence testing of these frozen kidney samples were again as would be expected from normal kidneys i.e. having no deposition of C_3 or IgG within the glomeruli.

DISCUSSION

The initial finding of this section was that the manufacturers claim that "K HCl, at the recommended dose of 22 mg/kg. body weight intramuscularly will give satisfactory restraint with profound analgesia" was found to be only partially correct in that though sedation was sufficient to effect a renal biopsy satisfactory anaesthesia, even at large doses, was not profound enough for euthanasia.

Secondly, the findings that each anaesthetic agent acting individually in the cat caused no glomerular lesions lends further support to the hypothesis that the two chemicals are, in some manner, interacting when introduced into the cat.

There are only a small number of possible alternatives to explain the course of events observed in the affected cats. Firstly, that the interaction occurred intravascularly with the resultant combination of the two drugs being deposited in the kidney. Alternatively as K HCl requires a certain period of time (say five - ten minutes) to achieve anaesthesia, it is possible that some breakdown product was produced which reacted with the barbiturate before, again intravascularly, being deposited in the kidney.

A third possibility is that the K HCl is, in some way, 'fixed' in situ within the glomeruli where it then reacted with the barbiturate.

The aim of the following section of this work was aimed at ascertaining which, if any, of these explanations is the most likely.

CHAPTER 6

AN INVESTIGATION INTO THE CHANGING PHYSICAL PARAMETERS INVOLVED IN THE INDUCTION OF THE GLOMERULAR LYTIC LESION.

INTRODUCTION

With the finding of the necrotising glomerular lesion described in Chapter 4 and its subsequent absence when the two individual chemical components of the system were tested, a further series of trials were thought necessary. This was done in order to examine whether or not the lesion was dose dependent or whether or not the interval between administration of the initial drug ketamine hydrochloride (K HCl) and the second drug sodium pentobarbitone (Na P) contributed to the development of the lesion.

For this reason, several differing regimes of drug dosage and time interval between the administration of the two drugs were employed in an attempt to artificially induce the lesion.

MATERIALS and METHODS

1. Source of Animals.

As previously described, all the animals used in this chapter, which were unwanted strays destined for humane destruction, were thoroughly examined both clinically and biochemically prior to the commencement of the study. A summary of the numbers of animals used in each group is given in Table 6.1.

2. Methods of Drug Administration:

Group 1: The influence of the time interval between the administration of K HCl and Na P.

In this group, a series of 17 cats were sedated by an intramuscular injection of K HCl at the recommended dose rate of 22 mg/ kg. A time interval ranging from five to 50 minutes was then allowed to elapse prior to the intravenous administration of a 6% solution of Na P, again at the recommended dose of 1 ml/ 2 kg. body weight. The duration of this time interval is detailed for each animal in Table 6.2. Subsequent to the administration of Na P the animals were exsanguinated when the femoral pulse was only faintly discernible, this taking no more than five minutes in the majority of cases.

Group 2: The effect of an increased dosage of K HCl.

In this group, a series of six cats received the appropriate dose of K HCl in order to induce initial sedation. This was followed by a second similar dose, in this case given intravenously; this second dose was not sufficient to induce profound anaesthesia necessary for exsanguination. A period of 15 minutes was then allowed before intravenous administration of a 6% solution of Na P. As previously stated, the animals were exsanguinated when the femoral pulse was only faintly discernible.

Group 3: The effect of an increased dose of Na P.

In this group six cats were employed. Once the K HCl had been administered to the recipient, a 'sub-lethal' dose of Na P was given intravenously after a time interval of 15 minutes had elapsed. A second similar dose of Na P was then given very slowly by the same route. Although the cats were now in deep anaesthesia, a ten minute time interval was allowed prior to exsanguination.

Group 4: The effect of multiple doses of K HCl.

In this group only three animals were used. The animals were again sedated by an injection of K HCl at 22 mg/kg. followed, at intervals of 45 minutes, by

repeated intramuscular doses. This was carried on for six hours at which time a 6% solution of Na P was administered to achieve anaesthesia of the required depth for exsanguination to be commenced.

Group 5: The effect of multiple doses of Na P.

A series of six cats were given the recommended dose of K HCl (22 mg/kg.), again intramuscularly. The animals were then kept under deep anaesthesia using multiple doses of a 6% solution of Na P. This procedure was carried on at regular intervals over a period of six hours before a lethal dose of Na P was administered.

Group 6: The effect of an increased concentration of Na P.

In this group the K HCl (22mg/kg.) was given intramuscularly to the 12 animals used. This was followed by an intravenous injection of 'Euthatal' (May and Baker, Dagenham, England), a 20% solution of Na P. In this group cardiac arrest occurred in less than ten seconds. A summary of the time intervals between administration of K HCl and Na P is given in Table 6.3.

3. Sampling of Tissues.

Immediately each animal had been exsanguinated, the abdomen was opened by a mid-line incision, the abdominal organs displaced and the kidneys removed.

Tissue samples were then taken for histology, TEM, SEM and immunofluorescence by the methods previously detailed.

All the samples obtained were prepared for examination by the methods previously described in the general Materials and Methods section.

TABLE 6.1.

Summary of Cat numbers in each Group.

Group	No. of Animals
1	17
2	6
3	6
4	3
5	6
6	12

TABLE 6.2.

Summary of time intervals (in mins.)(Group 1)

Cat No.	Time	Cat No.	Time
6.1	5	6.10	25
6.2	5	6.11	25
6.3	5	6.12	30
6.4	10	6.13	30
6.5	10	6.14	35
6.6	15	6.15	40
6.7	15	6.16	45
6.8	20	6.17	50
6.9	20		

TABLE 6.3

Summary of time intervals (in mins.)(Group 6)

6.39	5	6.45	20
6.40	5	6.46	20
6.41	10	6.47	25
6.42	10	6.48	25
6.43	15	6.49	30
6.44	15	6.50	30

RESULTS

The results given below are summarised in Tables 6.4 to 6.9.

Group 1: The influence of the time interval between the administration of K HCl and Na P.

Two of the 17 animals used in this group developed a severe necrotising glomerulopathy as described in Chapter 4, yet at widely differing time intervals (namely ten and 30 minutes respectively) between administration of the two drugs. As can be seen from Figs. 6.3 and 6.7 the glomerular lesion in these two animals was diffuse involving all glomeruli and was identical to that previously graded as 'severe'.

In a further four animals a less severe lesion was found which, again, involved all glomeruli (Figs. 6.1, 6.2, 6.4, 6.5 and 6.6). This less severe or " mild" lesion, which was not described previously in Chapter 4, was characterised on histological examination by the presence of large amounts of cytoplasmic debris in the capillary lumina together with alterations of the glomerular endothelium itself. The latter alteration comprised of swelling of the endothelial cytoplasm and consequent occlusion of the glomerular capillary. Large scale detachment of endothelial cytoplasm from the GBM

was, however, not observed. In a few instances, glomerular mesangial cells were also to be seen to be swollen and this contributed to the occlusion of the glomerular capillary lumen.

These observations were subsequently confirmed by use of the T.E.M. (Figs. 6.4, 6.5 and 6.6).

With the S.E.M., views of glomerular capillaries showed some swelling of the visceral epithelium, however, this was extremely limited in its distribution (Figs. 6.8 and 6.9).

A summary of the immunofluorescence results is given in Table 6.4. The tabulated findings show that the two cats which exhibited the "severe" form of the glomerulopathy had large amounts of granular deposition of IgG and C_3 in all glomeruli (See Figs. 4.10 and 4.11, Chapter 4). The animals with the "mild" form of the lesion also showed a positive, albeit diminished, response to staining with anti-cat IgG and C_3 (Figs. 6.10 and 6.11). The remainder of the animals in this group were immunofluorescence negative.

Group 2: The effect of an increased dosage of K HCl.

Of the six animals used in this group, five showed no evidence of any glomerular lesion. The remaining animal exhibited the "mild" form of the lesion as described in group 1.

No animal in this group showed any deposition of either IgG or C_3 within the glomeruli (Table 6.5).

Group 3: The effect of an increased dose of Na P.

Only one of the six animals used in this group exhibited the "severe" form of the lesion and, again, this had a diffuse distribution (Figs. 6.3 and 6.7). A further animal showed the "mild" lesion again involving all glomeruli with swollen endothelial profiles occluding the capillary lumina (Fig. 6.5).

Once again only the "severe" case showed granular deposition of IgG and C_3 with the "mild" case and the non-affected cases eliciting no response (Table 6.6, also see Figs. 4.10 and 4.11, Chapter 4).

Group 4: The effect of multiple doses of K HCl.

Only one of the three cats used in this group developed the "severe" necrotising glomerulopathy with an associated granular deposition of IgG and C_3 (See Figs. 4.10 and 4.11, Chapter 4).

The two remaining cats had histologically normal glomeruli and immunofluorescence failed to show deposition of either IgG or C_3 (Table 6.7).

Group 5: The effect of multiple doses of Na P.

Only one of the six cats exhibited the "severe" necrotising glomerular lesion and this was focal in distribution. In affected glomeruli, heavy deposits of luminal debris and complete necrosis of the endothelial lining was noted (Fig. 6.3). One further animal showed a diffuse but "mild" form of the lesion where the capillary lumina contained numerous cytoplasmic profiles, yet the endothelium remained attached to the GBM (Fig. 6.6).

The two affected animals both exhibited a reaction to testing with anti-cat IgG and C_3, nevertheless despite the difference in intensity of the lesion in both animals the reaction was diffuse and of comparable intensity (Table 6.8).

Group 6: The effect of an increased concentration of Na P.

Of the 12 cats comprising this group, nine had varying degrees of capillary luminal debris. Two of these animals were found to have the "severe" necrotising form of the lesion and exhibited diffuse

glomerular lesions characterised by swelling, detachment and necrosis of the glomerular endothelium.

All seven remaining affected animals were defined as showing a milder lesion with diffuse capillary endothelial, and to a lesser extent, mesangial swelling without, however, any evidence of capillary endothelial necrosis.

Despite the frequency of occurrence of capillary endothelial swelling or necrosis there was no correlation between the severity of the lesions and the time interval between the administration of K HCl and Na P.

Immunofluorescence tests showed that five of the nine affected animals, including the two animals which had "severe" necrotising glomerulopathy, had diffuse granular deposits of IgG and C_3 in their glomeruli, whilst the remaining four affected animals were immunofluorescence negative (Table 6.9).

TABLE 6.4

Summary of Results (Group 1- The influence of the time interval between the administration of K HCl and Na P)

Cat No.	Debris	Immunofluorescence* IgG	C_3
6.1	None	1+	±
6.2	Mild	1+	±
6.3	None	-	-
6.4	Mild	2+	1+
6.5	Severe	3+	2+
6.6	None	-	-
6.7	None	-	-
6.8	Mild	±	±
6.9	None	-	-
6.10	None	-	-
6.11	None	-	-
6.12	Severe	4+	3+
6.13	None	-	-
6.14	None	-	-
6.15	None	-	-
6.16	None	-	-
6.17	Mild	±	±

TABLE 6.5

Summary of Results (Group 2- The effect of an increased dosage of K HCl)

Cat No.	Debris	Immunofluorescence* IgG	C_3
6.18	None	-	-
6.19	None	-	-
6.20	Mild	-	-
6.21	None	-	-
6.22	None	-	-
6.23	None	-	-

TABLE 6.6

Summary of Results (Group 3- The effect of an increased dosage of Na P)

6.24	None	-	-
6.25	None	-	-
6.26	None	-	-
6.27	Mild	-	-
6.28	Severe	3+	1+
6.29	None	-	-

TABLE 6.7

Summary of Results (Group 4- The effect of multiple doses of K HCl)

Cat No.	Debris	Immunofluorescence* IgG	C_3
6.30	Severe	4+	2+
6.31	None	-	-
6.32	None	-	-

TABLE 6.8

Summary of Results (Group 5- The effect of multiple doses of Na P)

6.33	None	-	-
6.34	Severe	3+	2+
6.35	None	-	-
6.36	None	-	-
6.37	Mild	3+	2+
6.38	None	-	-

TABLE 6.9

Summary of Results (Group 6- The effect of of an increased concentration of Na P)

Cat No.	Debris	Immunofluorescence* IgG	C_3
6.39	Mild	1+	1+
6.40	Mild	1+	1+
6.41	Severe	3+	2+
6.42	None	-	-
6.43	Mild	1+	±
6.44	Mild	2+	±
6.45	None	-	-
6.46	Mild	-	-
6.47	None	-	-
6.48	Severe	3+	1+
6.49	Mild	-	-
6.50	Mild	-	-

* These immunofluorescence results were graded ± to 4+ according to their severity.

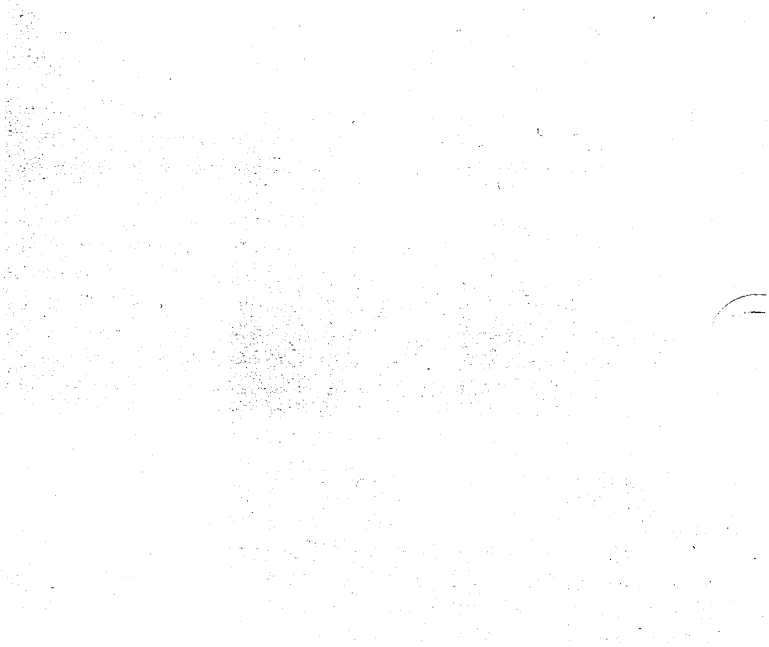


Figure 6.1
'Mild' non-lytic lesion
Note the capillary debris (arrow)
although a proportion of capilaries
appear normal
H & E (x 300)

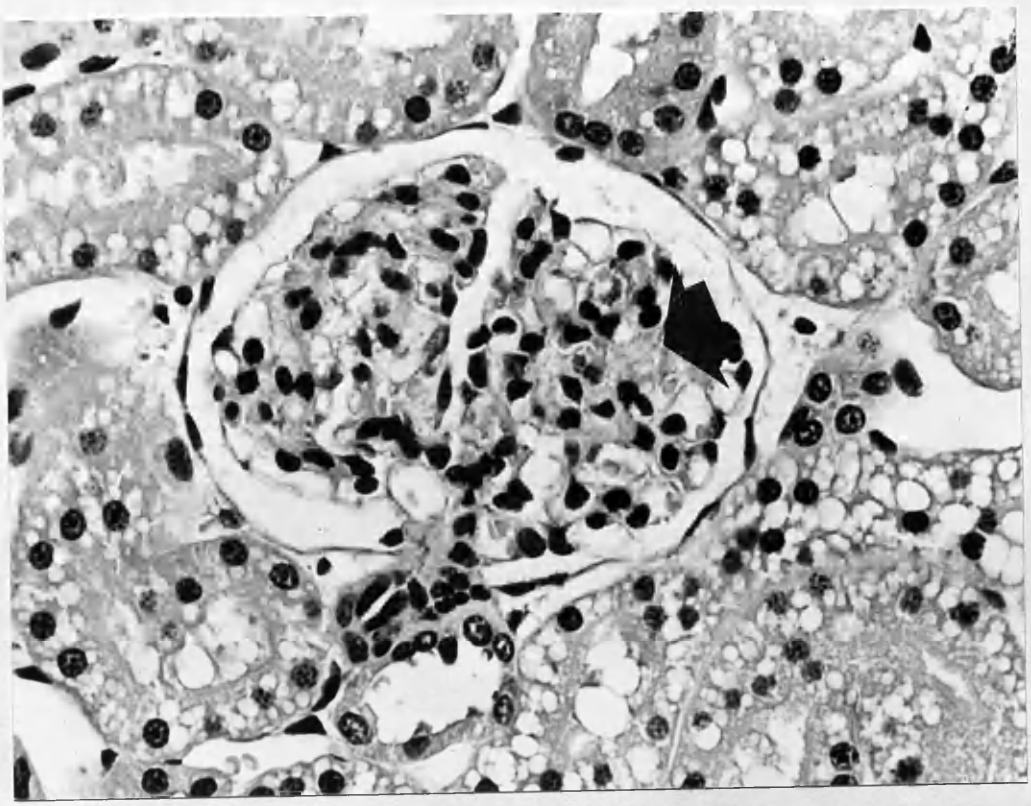
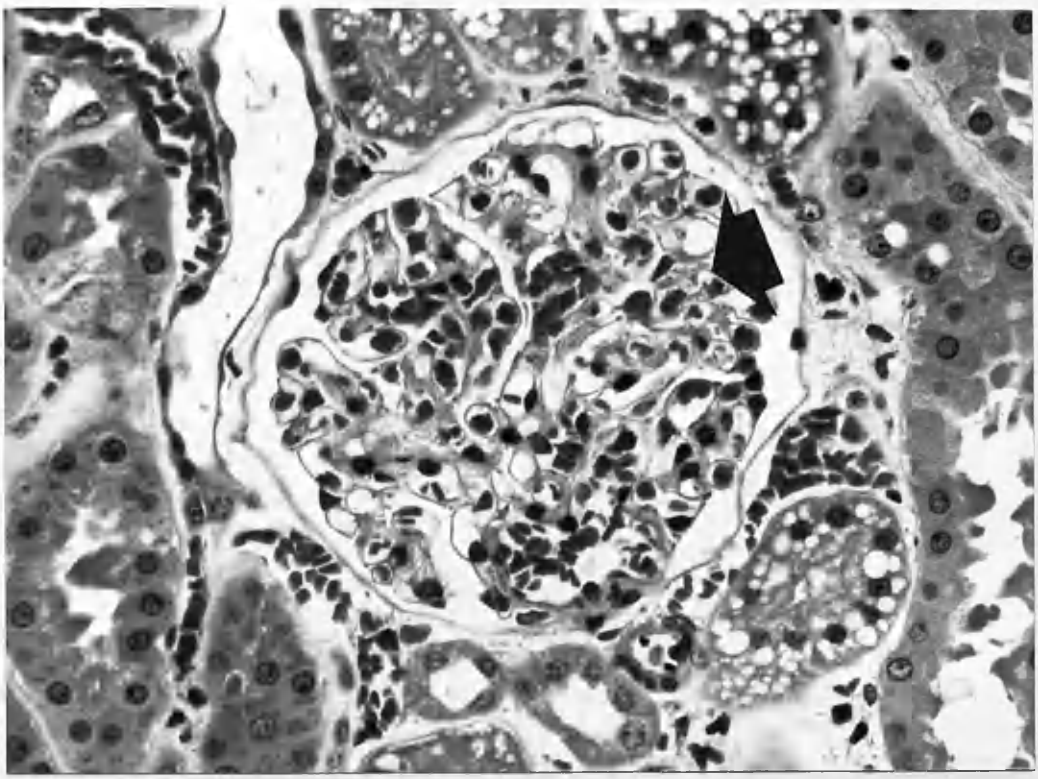


Figure 6.2
'Mild' non-lytic lesion
Note the significant amounts of
capillary debris (arrow)
H & E (x 300)

Figure 6.3
Lytic lesion
Note the severe disruption
of the glomerulus
H & E (x 300)



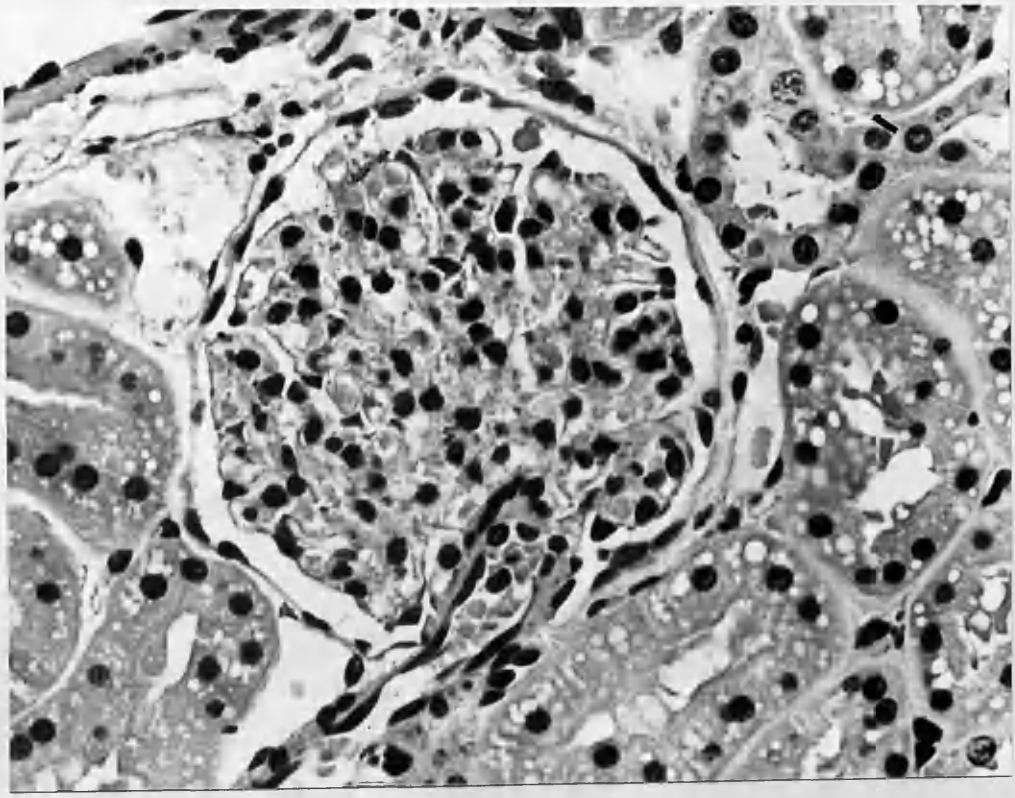
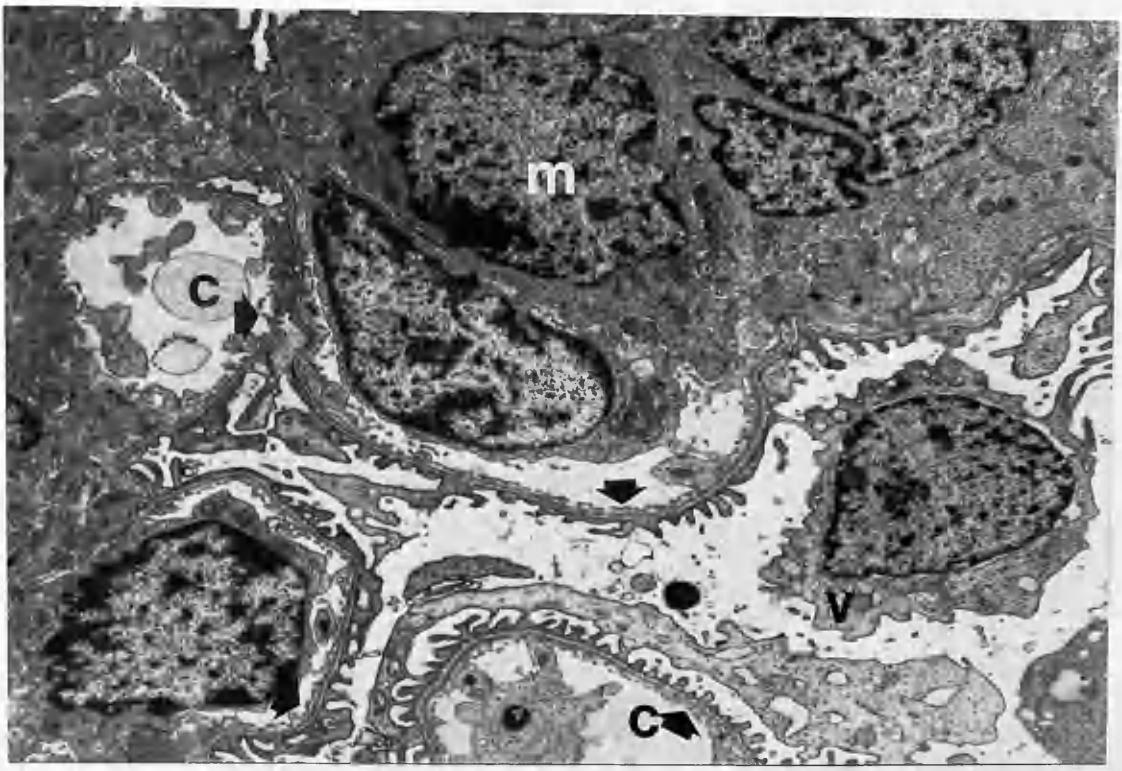


Figure 6.4
'Mild' non-lytic lesion
Note the amounts of capillary debris (c)
and the intact endothelium (arrows).
visceral epithelium (v) mesangial cell (m)
TEM (x 2000)

Figure 6.5
'Mild' non-lytic lesion
Note- mesangial processes (arrow 1)
and the unaltered foot processes (arrow 2)
TEM (x 3000)


m
c
v
c

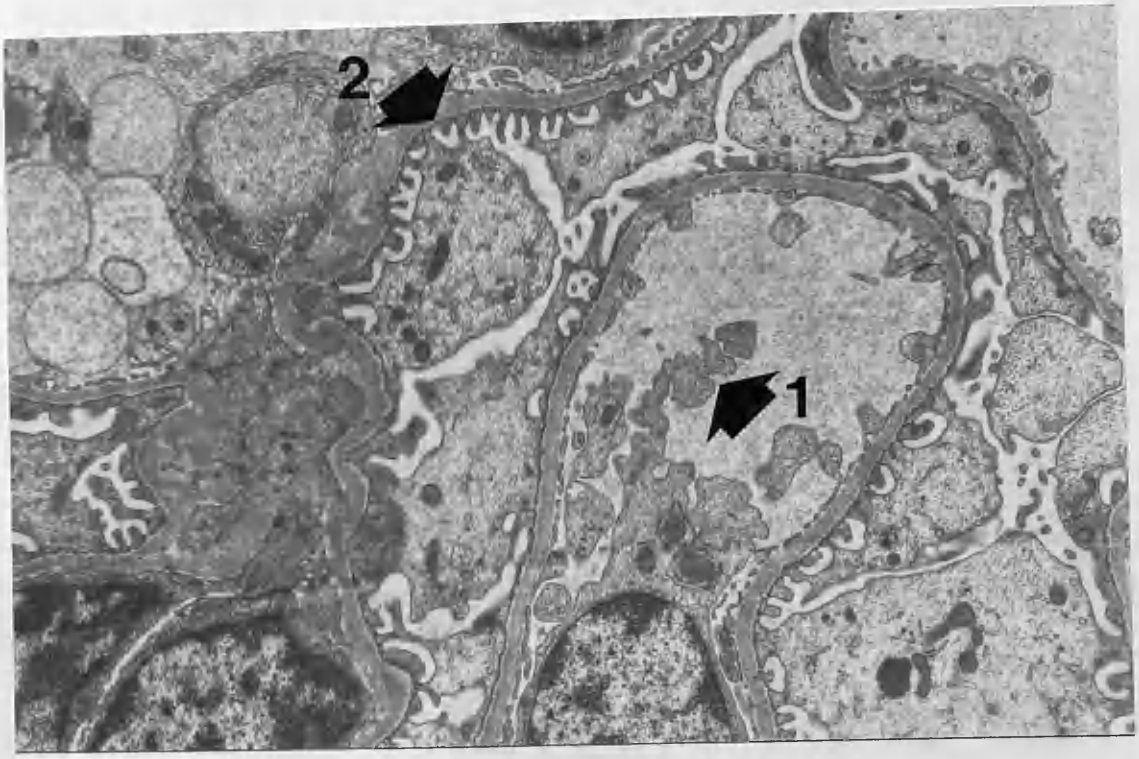
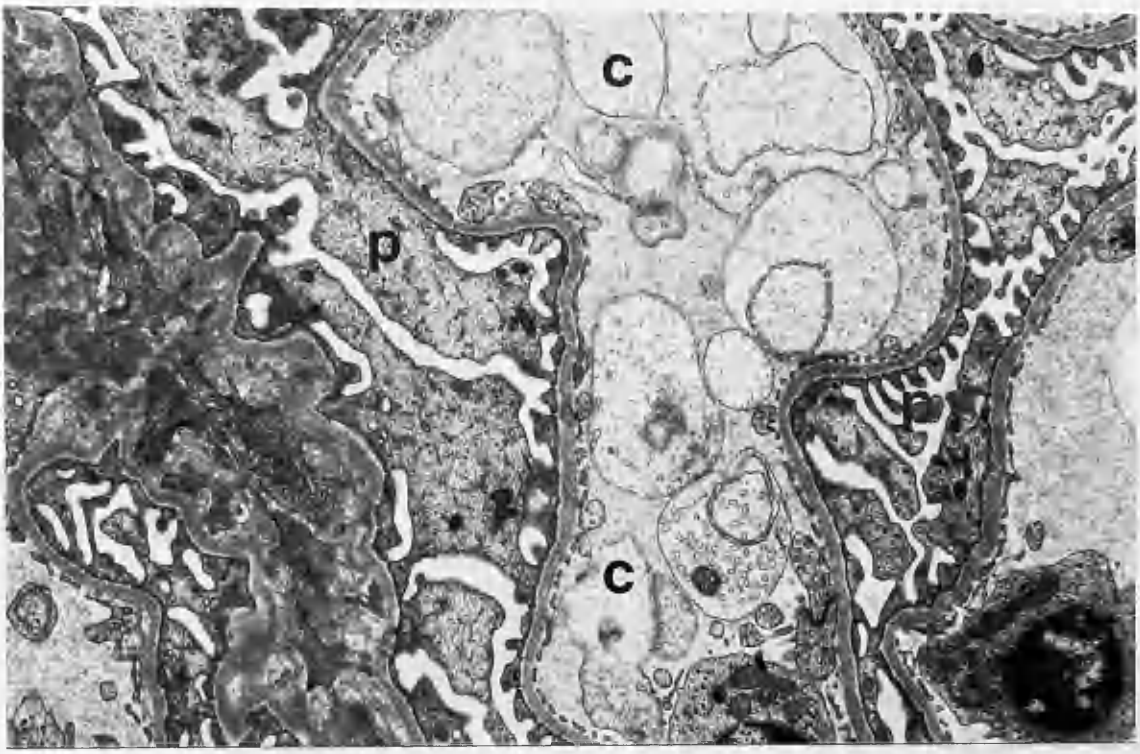

2
1

Figure 6.6
'Mild' non-lytic lesion
Characteristic circular profiles contained within the capillaries (c) Note the largely unaltered podocytic processes (p)
TEM (x 3000)

Figure 6.7
Lytic Lesion
Endothelial destruction within capillaries (c) although parietal (p) and epithelial cells (e) remain unaltered
TEM (x 3000)


c
p
p
c

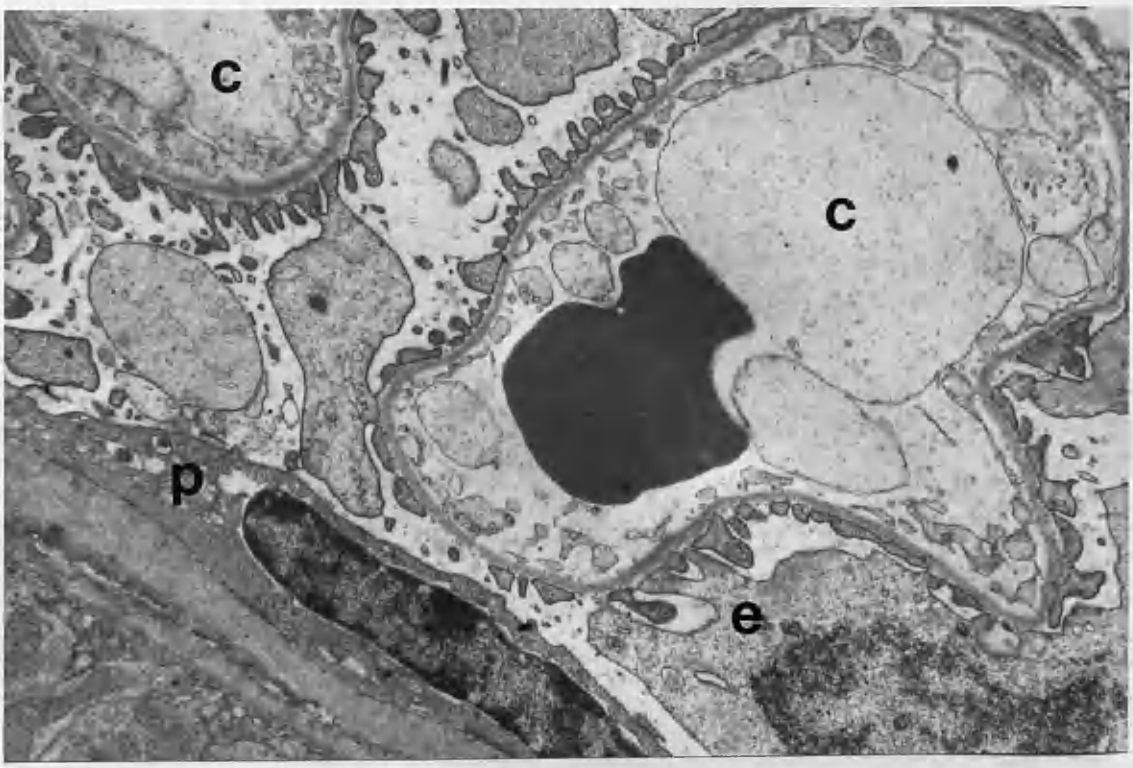
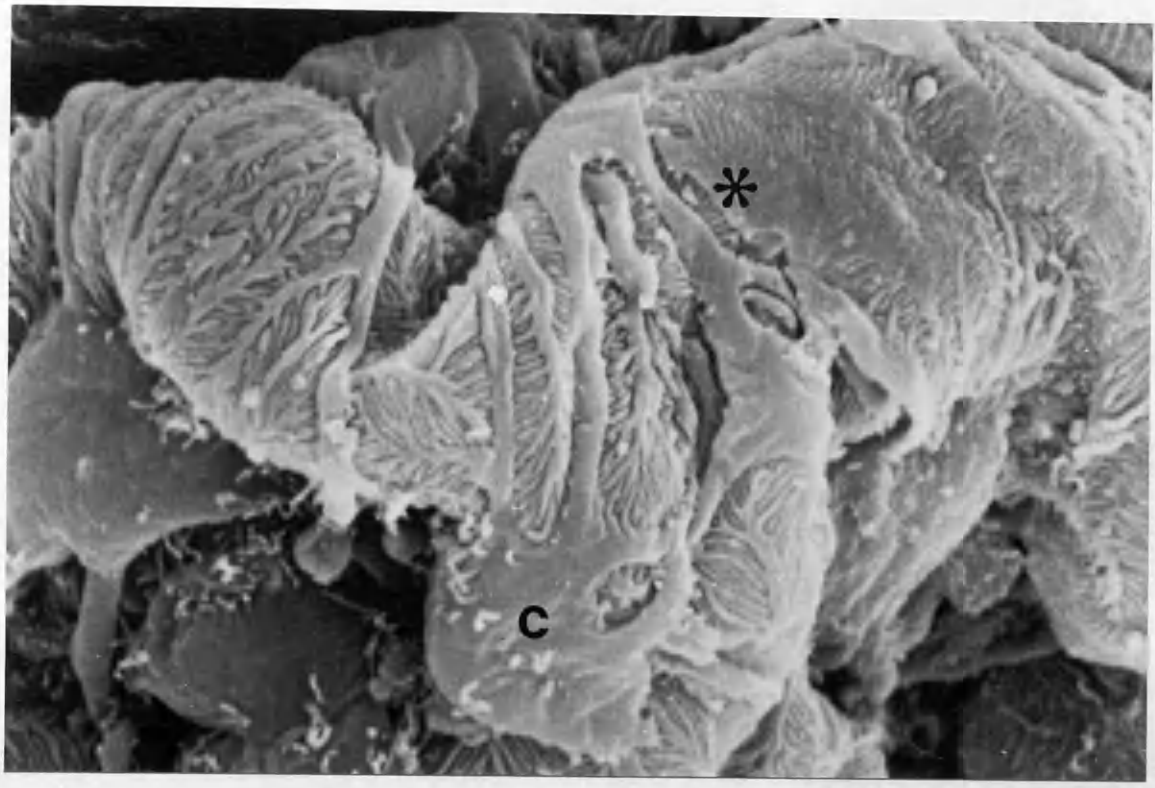

c
c
p
e

Figure 6.8
Unaltered visceral epithelium with processes radiating away to encircle the capillary (cell body (c))
Note the small area of podocytic fusion (asterisk)
SEM (x 2500)

Figure 6.9
Visceral epithelium cell body (c)
Note normality of processes (p) and the small area of podocyte fusion (asterisk)
SEM (x 10,000)


*
c

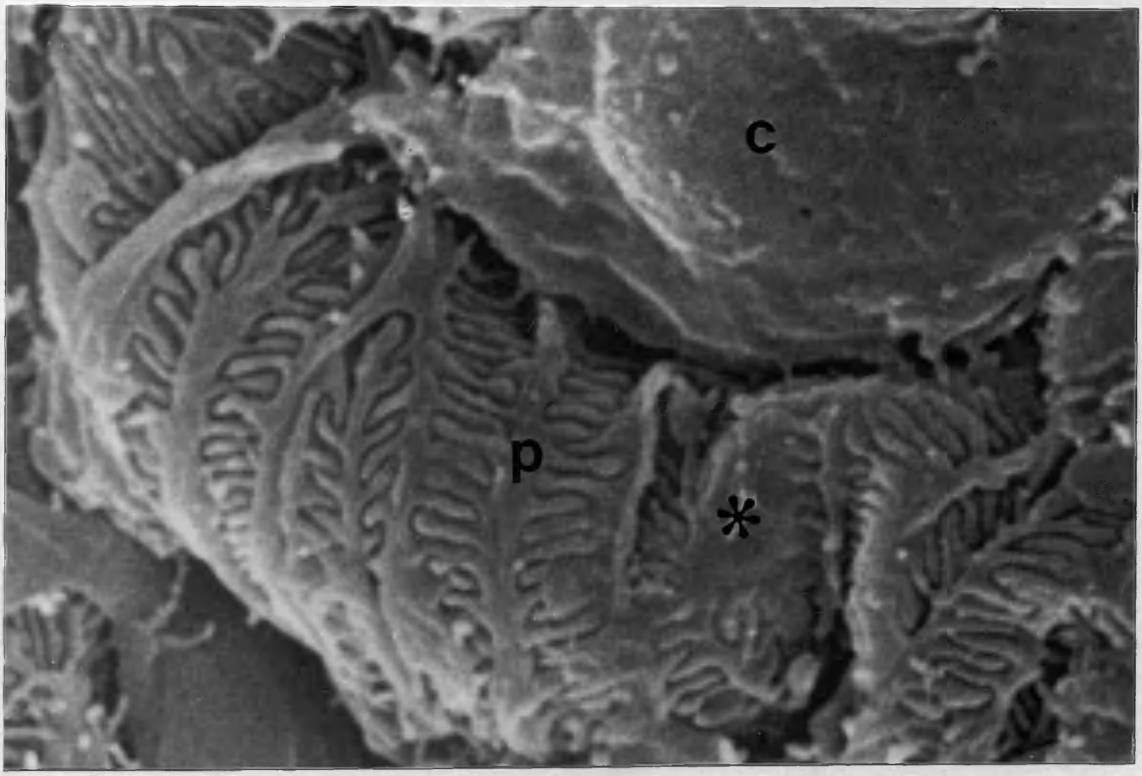
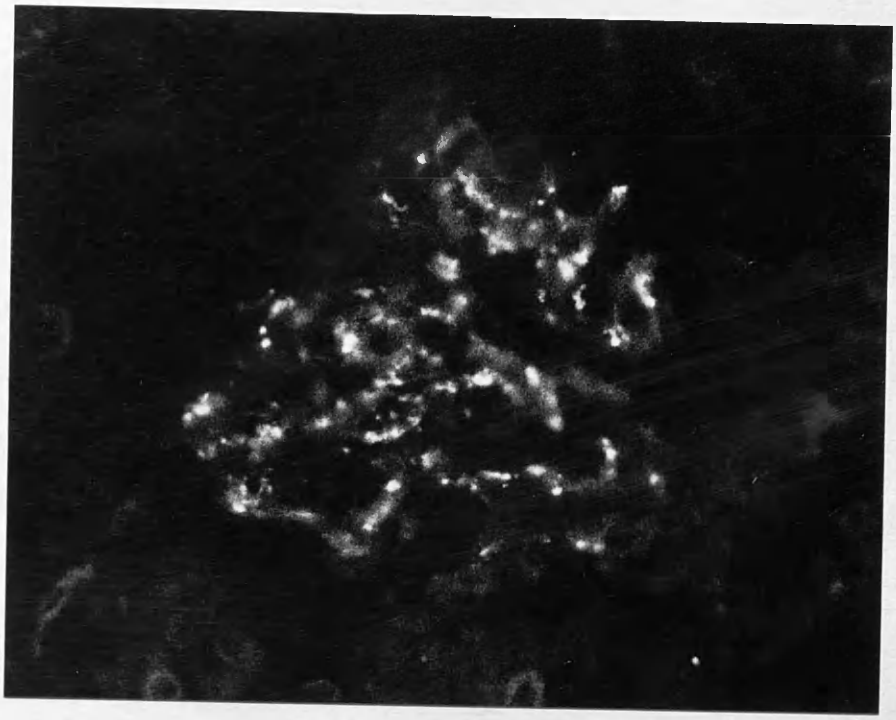
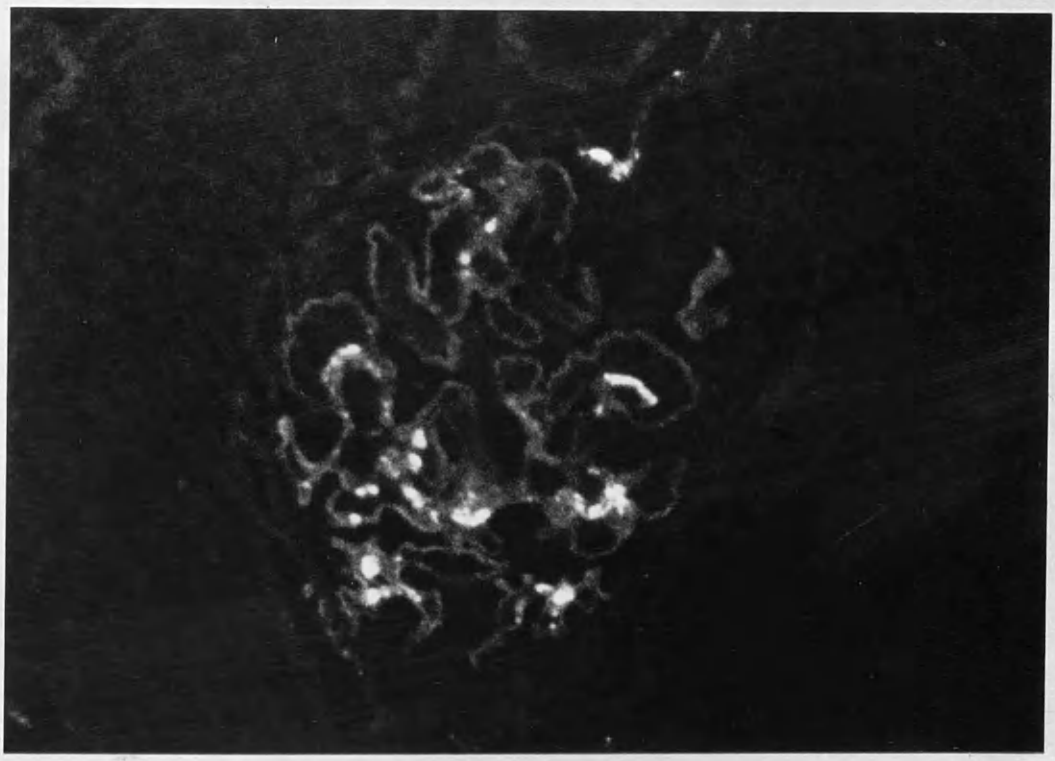

c
p
*

Figure 6.10
'Mild' non-lytic lesion
C_3 deposition
Note granularity of deposits
Immunofluorescence (x 250)

Figure 6.11
'Mild' non-lytic lesion
IgG deposition
Note granularity of deposits
Immunofluorescence (x 250)





DISCUSSION

In Group 1 where an increased time interval was allowed between the primary sedation with K HCl and deep anaesthesia using Na P, two of the 17 cats exhibited the "severe" necrotising lesion as first described in Chapter 4.

A further four animals suffered from a milder form of this lesion where although the endothelium remained intact, nevertheless cytoplasmic debris, sometimes in considerable amounts, was found in capillary lumina.

Two main findings emerge from the results of this group: a) the majority of the 17 animals in this group showed no signs of endothelial damage and b) there did not seem to be any correlation between the times of administration of the two drugs and the development of glomerular endothelial damage, as in all six affected animals the interval between administration of the two drugs differed.

This would suggest that there is no particular time after the administration of K HCl at which subsequent administration of Na P can be considered safe i.e. with the production of deep anaesthesia without the possibility of producing the glomerular endothelial lesion.

In the second group where a higher dose of K HCl

than normal was employed, again no detectable pattern for the occurrence of the lesion was observed. An important consideration of the experimental protocol of this group was that a particularly fractious animal might well receive a larger than usual dose of K HCl in order to initiate more rapid sedation.

Of the six cats used in this group only one showed any evidence of glomerular damage and that was considered only to be mild. This affected animal was treated no differently from the remainder in the group and there was no evidence to support the view that the occurrence of the lesion was anything other than a random event.

In the third group, a "double-dose" of barbiturate was employed in an attempt to simulate the situation whereby an animal was insufficiently anaesthetised for exsanguination to be commenced. Again no pattern to the initiation of the lesion was shown with only two of the six cats in the group showing any signs of glomerular damage. One of these exhibited a mild form of the lesion with considerable amounts of debris to be found within the capillary loops, whereas the other affected animal showed the severe diffuse necrotising lesion.

In the fourth group of three cats given multiple doses of K HCl, one animal examined showed the severe diffuse lesion and had heavy deposits of debris

contained within the glomerular capillaries. However, the other two animals examined showed none of these changes and appeared completely normal. Thus a high concentration of K HCl over a protracted time scale did not appear to predispose to the appearance of the severe necrotising lesion.

In Group 5 which involved repeated doses of Na P after one initial dose of K HCl, only one animal exhibited the severe necrotising lesion with its accompanying heavy deposits of debris in the capillary lumina. One other animal showed a much less severe degree of capillary luminal debris deposition with the remainder of the animals within the group proving to be completely normal.

In the final group of 12 cats where a higher concentration of Na P was employed (a 20% solution compared with the 6% solution previously used) nine animals used showed either the mild or severe forms of the glomerular lesion with corresponding amounts of glomerular capillary debris.

A 20% solution of Na P is primarily intended for euthanasia rather than anaesthesia and, as such, animals being sacrificed subsequent to experimentation might well receive this strength of Na P as a quick and efficient means of euthanasia. The disadvantages of the increased likelihood of the lesion itself appearing or

at least the presence of not inconsiderable amounts of glomerular debris should be apparent to any research carried out in the kidney using this drug regime.

Thus, it can be concluded that although the two drugs in combination do cause a cytotoxic glomerular lesion the timing of the administration of one drug with respect to the other is not critical to the initiation of the lesion. From the final group of animals it would also appear that the relative concentrations of the two drugs are also important.

However, despite attempts to elucidate the mechanism of the lesion by varying the concentrations of the two drugs and the time interval between administration the lesion would appear to occur in an entirely random fashion and be entirely variable in its effect in any particular animal.

CHAPTER 7

A HISTOLOGICAL AND ULTRASTRUCTURAL STUDY INTO THE USE OF PRE-FORMED PRECIPITATES OF K HCL AND Na P IN THE INDUCTION OF THE GLOMERULAR LYTIC LESION

INTRODUCTION

In Chapter 6 it was concluded that the glomerulopathy was induced by some form of combination of the drugs K HCl and Na P intravascularly and that this had led to a cytotoxic effect on the endothelium of the glomerular capillaries.

Although the manufacturers of K HCl have stated that, due to the formation of insoluble precipitates, the two drugs should not be administered either from or in the same syringe, they actually recommend the combined use of the two drugs in the same individual animal. In Chapters 4 and 6 attempts to induce the lesion by varying the dosages of the two drugs and by varying the time intervals between administration of the two drugs and exsanguination led to a variable response. It was considered worthwhile, therefore, to investigate the outcome of introducing pre-formed insoluble K HCl and Na P precipitates directly into the circulation.

To eliminate any possible 'sensitising' or 'targetting' effect the initial dose of K HCl might have had on the glomeruli, the use of Halothane (May and Baker, Dagenham, England) (a commonly used gaseous anaesthetic agent) was also tested.

MATERIALS and METHODS

1. Source of Animals.

The cats used in this chapter of the work were again stray unwanted animals due for humane destruction. As in previously described experiments, these animals were thoroughly examined prior to the commencement of this study.

2. Pre-formed precipitates of K HCl and Na P.

These were prepared prior to injection by mixing K HCl at the rate of 22 mg/kg. body weight with 6% Na P, at 1 ml / 2 kg. body weight in a test-tube. This mixture immediately became cloudy due to the formation of insoluble precipitates. This meant that a 2kg. animal would receive 1.5 ml of 'precipitate'.

This precipitate was even more evident when the 20% preparation of Na P (Euthatal) was employed as compared to the 6% preparation of Na P (Sagatal) (Fig. 7.1).

3. Experimental groups .

Group 1: Intravenous administration of precipitates.

The six cats in this group were first sedated by a single intramuscular injection of K HCl (at the dose rate of 22mg/kg.) into the quadriceps muscle mass. After

a period of five - ten minutes when sufficient sedation was achieved, each animal was weighed and the specific quantity of the pre-formed precipitates calculated for each individual animal was injected into the circulation via the cephalic vein. After a short period of time (usually five - ten minutes) when the femoral pulse was only faintly discernible the axillary artery was severed and the animal exsanguinated.

Group 2: Intracardiac administration of precipitates.

As in Group 1, the animals were first sedated by an intramuscular injection of K HCl. Again after five - ten minutes when sedation was satisfactory an individually measured quantity of the pre-formed precipitates was injected directly into the left ventricle of the heart. Once deep anaesthesia had been achieved and the femoral pulse only faintly discernible, the axillary artery was severed and the animal exsanguinated.

Group 3: Administration of precipitates into the renal artery.

As in Groups 1 and 2 the six cats that constituted this group were first sedated by an intramuscular injection of K HCl. This was followed, when the animal became more manageable by an injection of Na P to

produce a deeper anaesthesia.

Using surgical procedures the abdomen of each animal was opened by a mid-line incision. The abdominal organs were displaced and the aorta and renal arteries located. One of the renal arteries, usually the one leading to the right kidney, was then cannulated using a 12 gauge nylon intravenous cannula and this was tied in place by cotton thread. At the same time the renal vein was clamped using artery forceps. Again, an individually computed dose of pre-formed precipitates was slowly introduced directly into the renal artery.

After a short time interval of two - three minutes the animal was exsanguinated by severing the axillary artery.

Group 4: Halothane alone.

Six cats were euthanised using Halothane (May and Baker, Dagenham, England). Each was placed in an enclosed wooden box, fitted with an observation window. The container was then filled with Halothane gas by passing a stream of air (at atmospheric pressure) through a glass flask containing liquid Halothane. The stream of air saturated with gaseous Halothane was then piped from the flask into the observation box through a one-way valve. The animal within the box could be observed until deep anaesthesia had been attained. At

that time (usually ten - 15 minutes) the animal was removed from the box and exsanguinated by severing the axillary artery.

Group 5: Halothane plus Na P.

With the second group of six cats the same basic procedure was followed, only the depth of anaesthesia was somewhat lighter than that required for the cats in Group 1. When a satisfactory degree of anaesthesia was achieved, the animal was removed from its container and injected intravenously with a 6% solution of Na P calculated to produce deep anaesthesia. Five - ten minutes after administration of the Na P when the femoral pulse was only barely discernible the animal was exsanguinated by severing the axillary artery.

Group 6: Halothane plus intracardiac precipitates.

A further six cats were deeply anaesthetised with Halothane. The animals were then weighed and the amount of K HCl and Na P precipitate calculated by the same method as previously described. They were then inoculated intracardially with this measured quantity of pre-formed precipitates. These precipitates were then given slowly into the left ventricle. In five - ten minutes when the femoral pulse was only barely discernible exsanguination was again carried out by

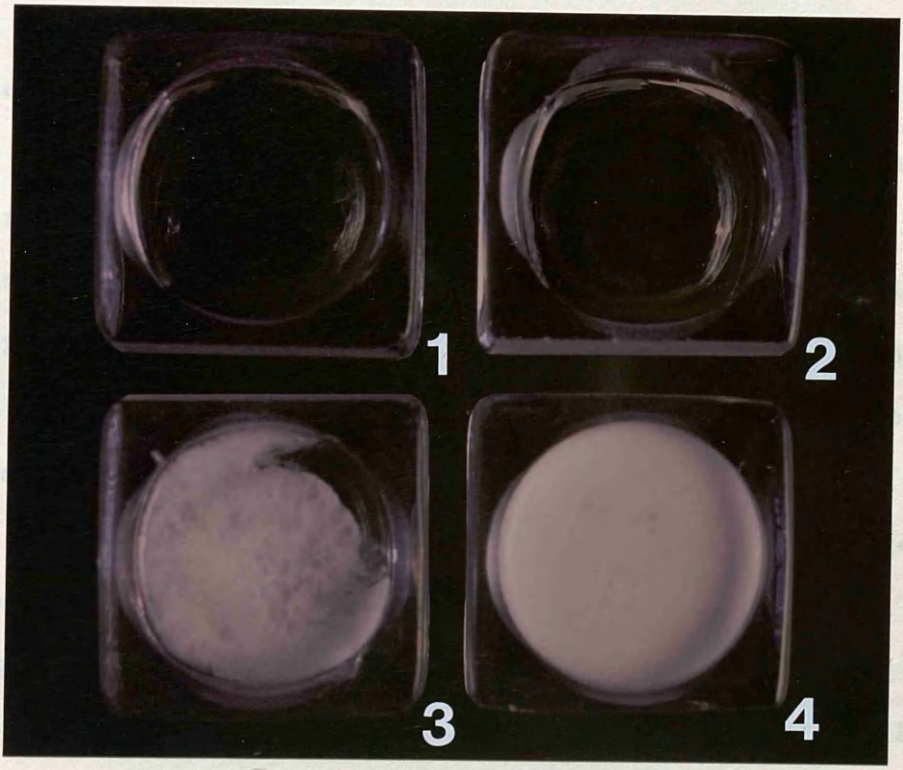
severing the axillary artery.

4. Sampling of Tissues.

Following exsanguination, the abdomen was opened (if it had not been so already during experimentation, as in Group 3) both kidneys were removed and samples were taken for histological, TEM, SEM and immunofluorescence studies in the manner as previously described in the general section on Materials and Methods.

In the third group samples for further examination were taken from each kidney and processed separately. Thus the kidney not having recieved the pre-formed precipitates acted as a "normal" control for the kidney which had recieved the precipitates.

Figure 7.1
1. Ketamine hydrochloride
2. Sodium pentobarbitone
3. K HCl + 6% Na P
4. K HCl + 20% Na P
Note the increased flocculency of 4. over 3.


1
2
3
4

RESULTS

A summary of these results is given in Tables 7.1 to 7.6.

Group 1: Intravenous administration of precipitates.

In this group, four of the six cats showed varying degrees of glomerulopathy. Two of these four animals exhibited only mild amounts of cytoplasmic debris in the capillary lumina. This "mild" form of glomerulopathy was identical to that first described in Chapter 6 (compare Figs. 7.2 and 7.3 with Figs. 6.1 and 6.2).

This was subsequently confirmed by use of the TEM (compare Figs. 7.6 and 7.7 with Figs. 6.4 and 6.5, Chapter 6).

The other two affected cats, however, had large amounts of debris within their capillary loops and this comprised both of the remains of endothelial and mesangial cell types together with profiles of the pre-formed precipitates (Fig. 7.4). This "severe" form of glomerulopathy was the same as that previously described in Chapters 4 and 6. In one of these cats the lesion was focal being restricted to only a proportion of the glomeruli (approximately 60%) with the remaining 40% of glomeruli being unaffected, whereas in the other

"severe" case, a diffuse form of the 'lytic lesion' was evident. All four cats showed granular deposits of IgG and C_3 in all glomeruli although the intensity of fluorescence was less in the two "mild" cases.

The kidneys of the remaining two animals proved to be entirely free from capillary debris and immunofluorescence examination failed to detect any deposition of IgG or C_3 (Table 7.1).

Group 2: Intracardiac administration of precipitates.

In this group of six cats only one animal did not show any evidence of glomerulopathy.

Of the remaining five cats, two developed a "severe" diffuse lytic lesion similar to that first described in Chapter 4. The other three cats showed the "mild" form of the glomerulopathy with luminal cytoplasmic debris but intact endothelium. All of the glomeruli were affected more or less equally.

The two animals exhibiting the diffuse lytic lesion both showed a strongly positive reaction to immunofluorescence testing for IgG and C_3. However, only two of the remaining three affected animals showed a positive reaction (Table 7.2).

Group 3: Administration of precipitates into the renal artery.

In this group of six cats the cannulated kidney which received the pre-formed precipitates was seen to contain very heavy amounts of debris deposited within its glomerular capillary loops together with a diffuse necrotising lesion even more severe than that first described in Chapter 4 (Fig. 7.5).

The histological findings of severe lytic damage to the endothelial cells and to the majority of the mesangial cells was confirmed by TEM (Figs. 7.8 and 7.9). This damage had led to the destruction of these two glomerular cell types and the consequent occlusion of the glomerular capillaries with their necrotic cellular contents. However, once again, the visceral epithelium appeared largely unscathed and only patchy areas of effacement of the foot processes were seen. Fig. 7.9 shows the severity of the lesion in this group whereby the visceral epithelium has been damaged. There was also some swelling of these processes to be observed with the SEM but, again, this was patchy in distribution (See Figs. 6.8 and 6.9, Chapter 6).

In only one of the non-cannulated kidneys (which received no pre-formed precipitates yet did receive both K HCl and Na P independently) was there evidence of a severe diffuse lytic glomerulopathy with moderately

large amounts of cytoplasmic debris in the capillary lumina. The remaining non-cannulated kidneys all appeared normal both by histological and ultrastructural examination.

When tested by immunofluorescence all of the cannulated kidneys proved strongly positive for IgG and C_3 as did the contralateral non-cannulated kidney from cat 7.16 which also showed a diffuse necrotising lesion (Figs. 7.10 and 7.11). However, of the remaining non-cannulated kidneys only one was positive, although fluorescence was less intensive than in the animals with the lytic lesion (Tables 7.3A and 7.3B).

Group 4: Halothane alone.

The kidneys of all six cats, which were anaesthetised using Halothane alone, were histologically normal and this finding was subsequently confirmed by ultrastructural examination. The only feature which distinguished these animals from those recorded in Chapter 1 was the presence of increased numbers of erythrocytes to be found within the capillary loops.

Immunofluorescent staining for IgG and C_3 proved negative in all cases (Table 7.4).

Group 5: Halothane plus Na P.

As in the previous group, no evidence of the necrotising glomerulopathy or of any deposition of cytoplasmic debris within the capillary loops was found on histological and ultrastructural examination. Likewise, immunofluorescence tests failed to detect IgG or C_3 in any animal (Table 7.5).

Group 6: Halothane plus intracardiac administration of precipitates.

In each of the six cats in this group small amounts of cytoplasmic debris were to be found in the glomerular capillary loops, sufficient for the glomerulopathy to be classified as "mild". However, there was no evidence of severe damage to the endothelial and mesangial cell types.

Immunofluorescence tests showed granular deposits of IgG and C_3 in all six animals (Table 7.6, also see Figs. 6.10 and 6.11, Chapter 6).

TABLE 7.1

Summary of Results (Group 1- Intravenous administration of precipitates)

Cat No.	Debris	Immunofluorescence* IgG	C_3
7.1	Severe	2+	1+
7.2	Severe	2+	1+
7.3	Mild	1+	1+
7.4	Mild	1+	1+
7.5	None	-	-
7.6	None	-	-

TABLE 7.2

Summary of Results (Group 2- Intracardiac administration of precipitates)

7.7	Severe	3+	1+
7.8	Severe	3+	1+
7.9	Mild	2+	1+
7.10	Mild	1+	$\pm$
7.11	Mild	-	-
7.12	None	-	-

TABLE 7.3A

Summary of Results (Group 3- Administration of precipitates into the renal artery (cannulated))

Cat No.	Debris	Immunofluorescence* IgG	C_3
7.13	Severe	3+	1+
7.14	Severe	3+	1+
7.15	Severe	2+	2+
7.16	Severe	1+	2+
7.17	Severe	3+	1+
7.18	Severe	2+	1+

TABLE 7.3B

Summary of Results (Group 3- Administration of precipitates into the renal artery (non-cannulated))

7.13	None	-	-
7.14	None	-	-
7.15	None	1+	$\pm$
7.16	Severe	2+	1+
7.17	None	-	-
7.18	None	-	-

TABLE 7.4

Summary of Results (Group 4- Halothane alone)

Cat No.	Debris	Immunofluorescence* IgG	C_3
7.19	None	-	-
7.20	None	-	-
7.21	None	-	-
7.22	None	-	-
7.23	None	-	-
7.24	None	-	-

TABLE 7.5

Summary of Results (Group 5- Halothane plus Na P)

7.25	None	-	-
7.26	None	-	-
7.27	None	-	-
7.28	None	-	-
7.29	None	-	-
7.30	None	-	-

TABLE 7.6

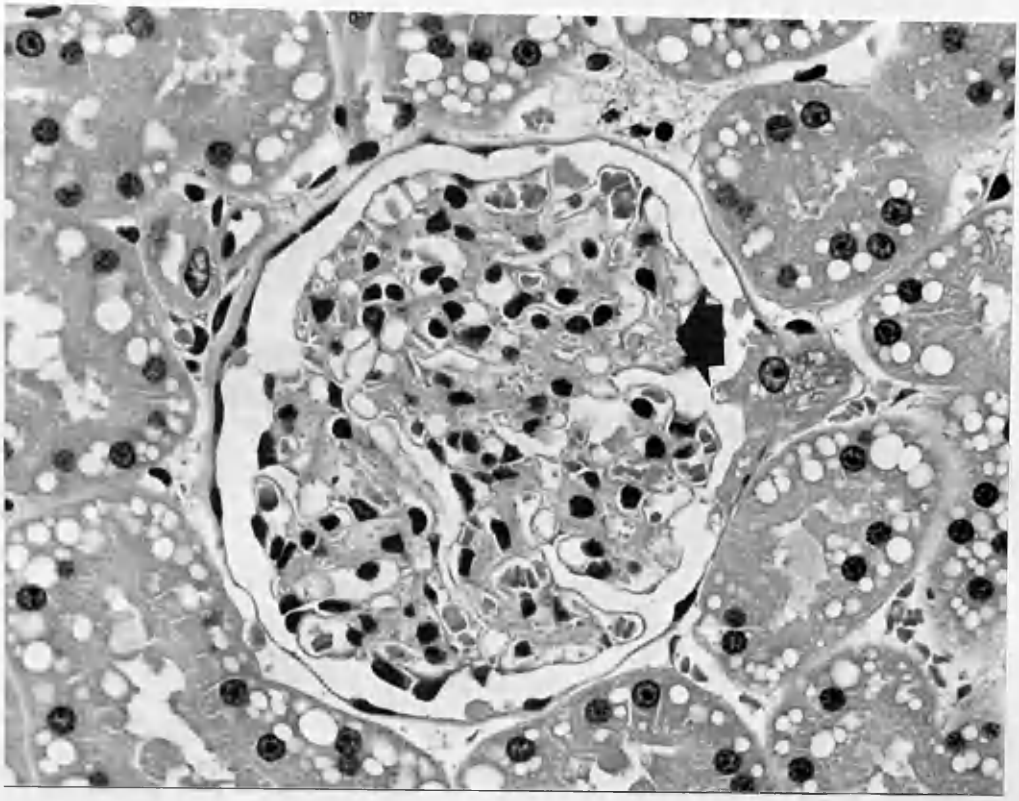
Summary of Results (Group 6- Halothane plus intracardiac administration of precipitates)

Cat No.	Debris	Immunofluorescence* IgG	C_3
7.31	Mild	2+	1+
7.32	Mild	2+	1+
7.33	Mild	1+	1+
7.34	Mild	2+	2+
7.35	Mild	3+	1+
7.36	Mild	1+	1+

* These immunofluorescence results were graded $\pm$ to 4+ according to their severity.

Figure 7.2
'Mild' non-lytic lesion
Note the capillary debris (arrow)
although a proportion of capillaries
appear normal
H & E (x 300)

Figure 7.3
'Mild' non-lytic lesion
Note the increased amounts of debris
as compared to Fig. 7.2 (arrows)
H & E (x 300)



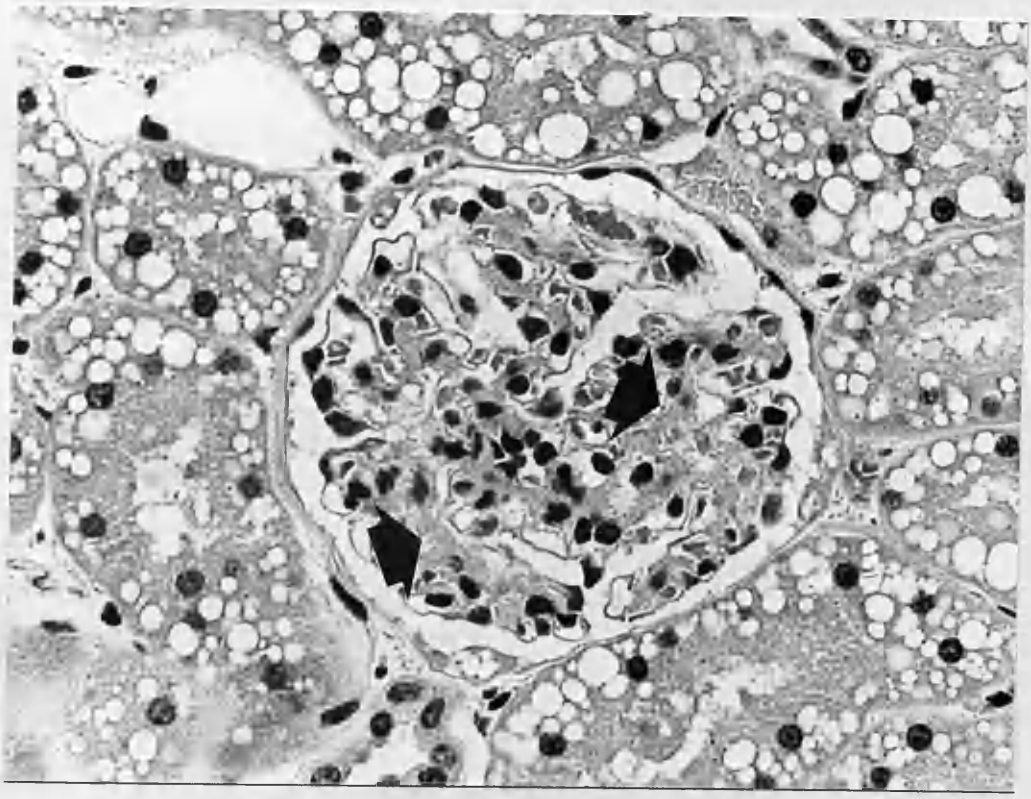
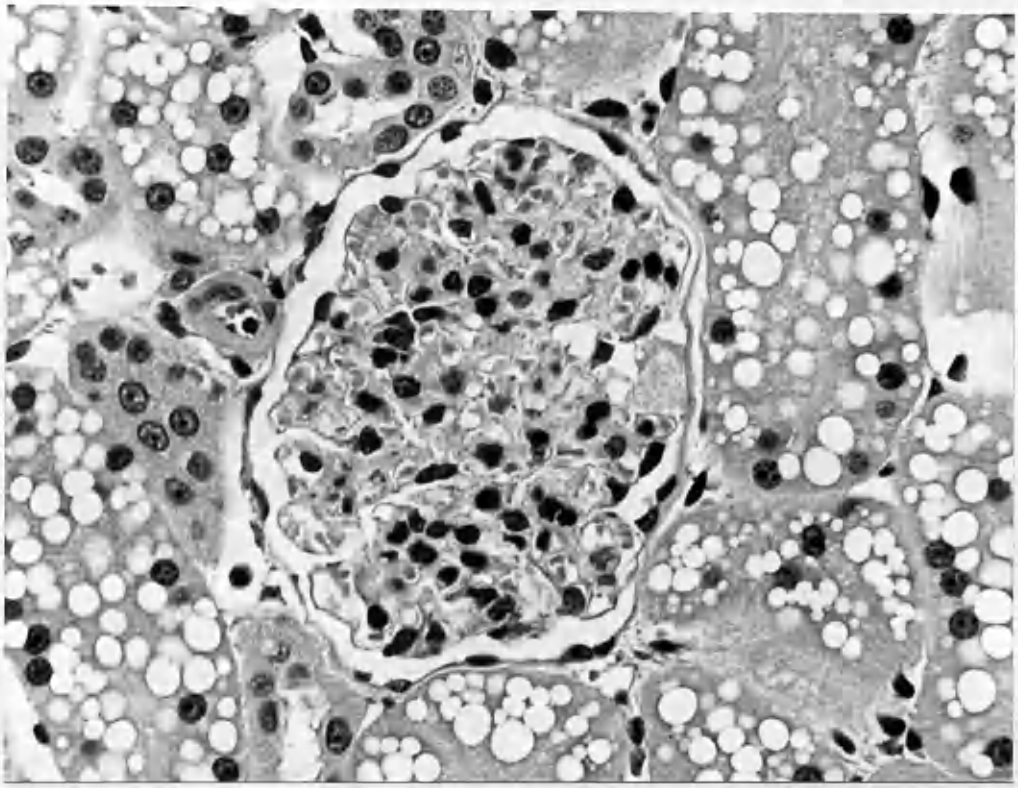


Figure7.4
Lytic lesion
Note the severe glomerular disruption
H & E (x 300)

Figure 7.5
Lytic lesion
Note the large amounts of debris
and extreme disruption
H & E (x 300)



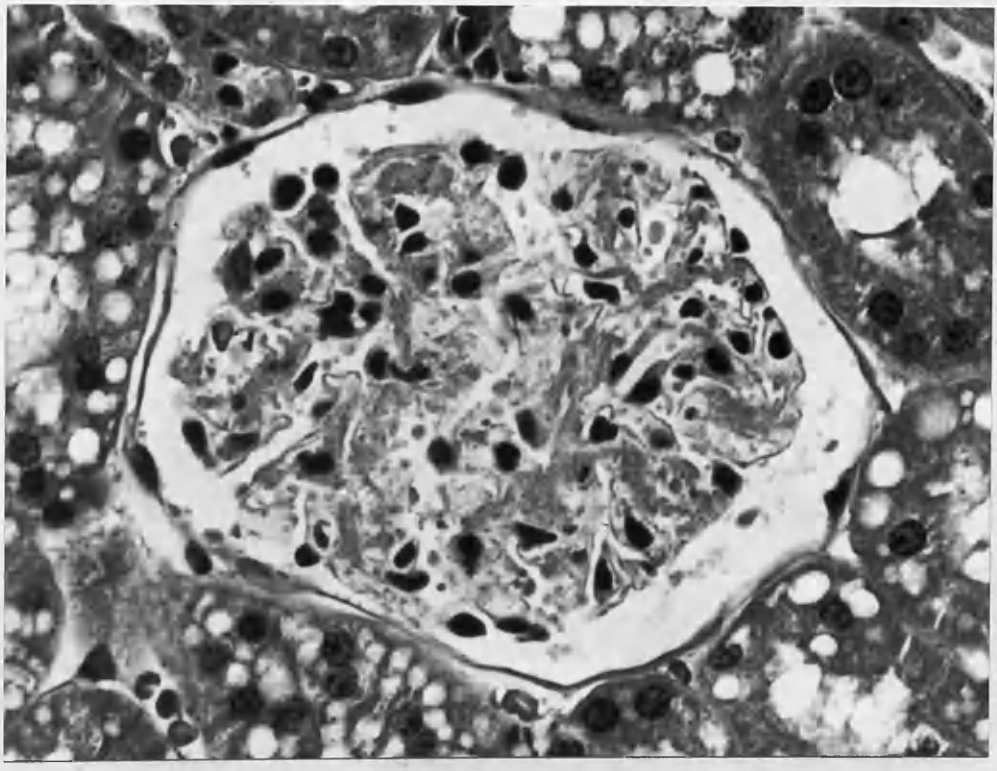


Figure 7.6
'Mild' non-lytic lesion
Note the mesangial processes (arrow)
the intact endothelium emanating from
the endothelial cell (e) and surrounding
the capillary (c)
TEM (x 3000)

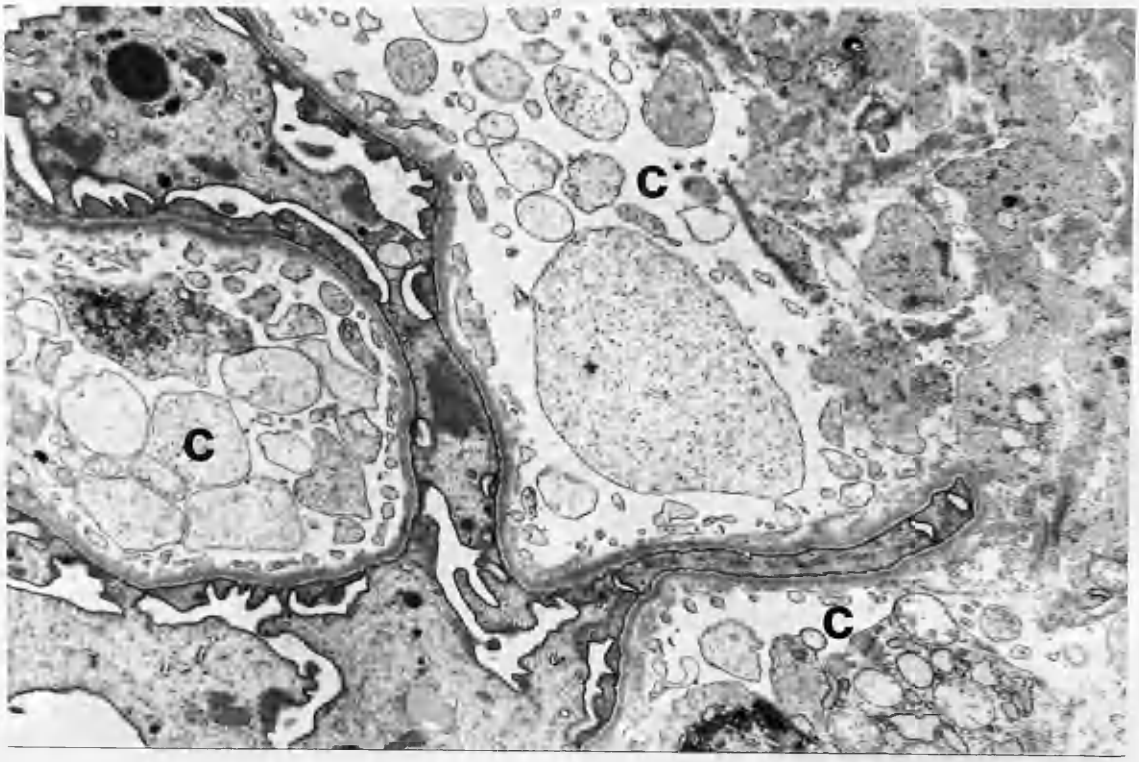
Figure 7.7
'Mild' non-lytic lesion
Note the heavy amounts of
capillary debris (asterisk)
TEM (x 5000)


c
e


*

Figure 7.8
Lytic lesion
Note the endothelial and mesangial destruction
The debris of these cells fill the capillaries (c)
TEM (x 3000)

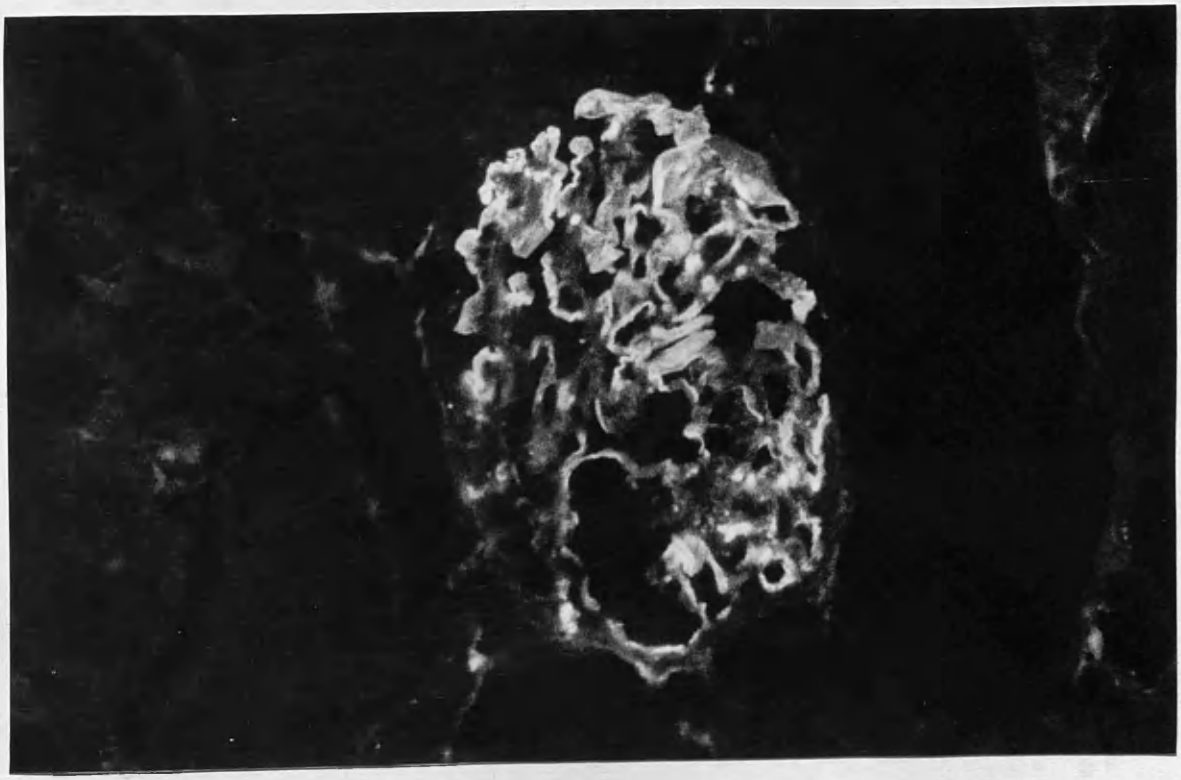
Figure 7.9
Lytic lesion
Note the heavy capillary (c) debris
(asterisk) together with the partial
destruction of the epithelial cell (e)(arrow)
TEM (x 4000)


C
C
C


C
*

Figure 7.10
IgG deposition
Note the granularity of deposits
Immunofluorescence (x 250)

Figure 7.11
C_3 deposition
Note the heavy granular
deposition appears almost linear
Immunofluorescence (x 250)



DISCUSSION

The aim of this experiment was to introduce the pre-formed combination of the two drugs into the general circulation with the object of simulating the development of the spontaneously induced lesion with separate injections of K HCl and Na P.

Of the three methods of administration of the precipitates employed in this chapter the first (direct intravenous inoculation, Group 1) proved to be the least successful in terms of reproducing the severe necrotising lesion. It is possible that the lack of success was due to a dilution effect of the precipitates which was such that the amount of the precipitate actually reaching the glomerular capillaries was insufficient to initiate damage within the glomeruli.

Nevertheless, in two of the cats examined from this group the lesion was evident although in one only a focal form of the glomerulopathy was observed. Overall, however, Group 1 showed a lower incidence of capillary debris deposition than that described for the spontaneous cases in Chapter 4.

In the second group, on the assumption that most of the precipitates might well be filtered out of the circulation by the mononuclear system prior to reaching the glomerular capillaries, the injection of

precipitates was given intracardially into the left ventricle thus ensuring that the maximum amount of precipitates should reach the aorta and hence the renal arteries.

This regimen proved more successful than Group 1 as five of the six animals used showed heavy deposits of debris lying within the glomeruli, with two of these five cats exhibiting a diffuse lytic glomerulopathy.

The third method of administration of precipitates i.e. the cannulation of an individual kidney, proved to be the most effective in recreating the severe necrotising glomerulopathy.

In each of the animals examined from this group the kidney which received the combination of the two drugs showed severe lysis of the endothelial and mesangial cell types of each glomerulus examined. There were, therefore, large amounts of glomerular capillary debris. Nevertheless, despite the necrosis of endothelial and mesangial cells the visceral epithelial podocytes appeared largely unchanged with the TEM, although, on occasion, the amount of damage was so severe that the visceral epithelium began to be affected. With the SEM only occasional areas of fusion and / or swelling of processes to be seen.

In only one of the contralateral kidneys from these animals in Group 3 was there any evidence of

glomerulopathy. In this animal a severe necrotising lesion associated with heavy capillary cytoplasmic debris was found. As the renal vein of the cannulated kidney was clamped up to the time of exsanguination it can only be assumed that the lytic lesion in this kidney was a spontaneous event.

Thus, in general, the intravascular combination of the two anaesthetic agents K HCl and Na P was clearly seen to be the initiating factor behind the occurrence of the spontaneous lesion first described in Chapter 4 and tended to support the premise that an intravascular reaction between K HCl and Na P occurs with subsequent deposition of this cytotoxic combination in the glomerular capillaries.

The experiments described in Groups 4 and 5 of this chapter have indicated that anaesthesia with Halothane alone or in combination with Na P did not appear to have any injurious effects on the renal glomeruli of the cat. However, for the routine veterinary examination of cats the use of Halothane requires a great deal of care to ensure its safe and efficient administration. This is particularly so in the fractious animal.

Furthermore, the assessment of any glomerular lesion induced by the use of pre-formed K HCl and Na P precipitates (Group 6) could be correctly assumed to be due to this drug combination and not to the Halothane

alone. It was shown that only when precipitates of K HCl and Na P were given intracardially to Halothane anaesthetised cats that a mild glomerulopathy was produced. However, unlike Group 2, there was no evidence of the severe necrotising lesion occurring in any of the animals within this group.

Thus if Groups 2 and 6 are compared i.e. comparing the use of K HCl, as the primary sedative, administered in combination with the precipitate formed by the addition of K HCl and Na P (Group 2) to Halothane followed by similarly formed precipitates (Group 6), and injected into the cat by the same route, it can be observed that in each and every case the former group of animals exhibited more severe luminal debris than those found in the cats of the latter group. This was reflected in the higher occurrence of the necrotising lesion in the former group.

Thus there would seem to be an indication that even when using the artificial situation whereby the combination of the two drugs in the form of precipitates is given to the animal in a pre-formed state the effect upon the glomeruli appears to be enhanced if the animal was already anaesthetised with K HCl.

This hypothesis was also tested using Na P alone in place of the pre-formed precipitates (Group 5) i.e. Halothane followed by Na P. It can be seen from the

results section that this regime did not produce any evidence of the necrotising lesion or of the presence of the luminal debris as seen elsewhere.

In the non-experimental state two separate phenomena may be occurring in that a) the initial dose of K HCl, used as a sedative, may be circulating in the vasculature and there combines with the subsequently administered Na P. With a certain proportion of the precipitates formed as a result of this combination being filtered out in the glomeruli; and / or b) that the K HCl attaches itself either passively or actively to the glomerular endothelium and following injection of Na P causes the combination of the two drugs *in situ* on the glomerular endothelium.

Both of these situations, or a combination of the two, leading to the formation of precipitates *in vivo* and hence to the possible consequence of glomerular endothelial and glomerular mesangial destruction.

However, as has previously been examined this would seem to be a completely random event depending entirely on the individual case.

GENERAL SUMMARY

AND CONCLUSIONS

The recent surge of interest into spontaneous glomerular disease in the cat (Osborne and Vernier, 1973; Nash et.al., 1979; Wright et.al., 1981; Lucke, 1982) has highlighted the importance of a real understanding of normal glomerular structure and the need to establish how the cat glomerulus reacts to autolytic as well as pathological change.

Chapter 1 of this work sought to describe the normal morphology of the cat glomerulus and thus expanded the only other known work of its kind (Zimmerman, 1933 (cited by Mueller, 1958)). This section of the work also added information concerning fixation methods, thickness of section and variation of glomerular morphology in different regions of the renal cortex.

The comparison of fixation methods employed showed that, although both immersion and perfusion methods are adequate for both conventional light and electron microscopy, perfusion fixation appeared to be superior to immersion fixation with respect to improved differentiation of constituent glomerular cells and associated glomerular capillary patency.

Several disadvantages of perfusion fixation were, however, apparent; these included the artificial

widening of capillary lumina and urinary spaces together with poor perfusion of a small, but significant, proportion of outer cortical glomeruli. In general, however, most glomeruli appeared well-perfused.

In the present study the high osmolarity fixative employed (Karnovsky's) was introduced into the kidney under simple manual pressure, although at a controlled flow rate. There is some evidence in the literature that this method may have a number of disadvantages. These include a) the use of excess manipulation of the kidney which may affect subsequent histological and ultrastructural detail (Cook et.al., 1965), b) the need to ensure a fixative of suitable osmolarity to prevent the phenomenon known as "Osmolarity Shock" (Yun and Kenny, 1976) and c) the need for a constant supply pressure approaching that of the blood supply entering the kidneys under normal conditions.

To counter these points a controlled pressure system, designed to give a more uniform glomerular perfusion, might well be devised and a compatible fixative of appropriate osmolarity employed. Both of these innovations have been found to be successful by Griffith et.al. (1967) and Elling et.al. (1977) in their studies of the rat and pig kidney. The routine use of controlled perfusion techniques during post-mortem procedures would, however, not normally be a practical

proposition.

The crucial role of section thickness in the study of the glomerulus was also highlighted in this study. In thick paraffin wax sections (i.e. 10um.), glomeruli appeared hypercellular and it was not possible to identify with any certainty the various glomerular cell types. The use of 6um. sections, which are routinely used in many instances reported in the literature (e.g. McManus, 1948; Bulger et.al., 1979) also proved unacceptable in the present study. Again this was due to the poor differentiation of glomerular cell types and tended to negate the benefits of good fixation. The use of thin paraffin wax sections of 2-3um., however, overcame the problems of visualisation and differentiation of cell types.

The present study also provided the first detailed TEM study of the normal cat glomerulus. As such it provided essential base-line parameters for any future study concerned with the evaluation of morphological changes accompanying various glomerular disorders.

A comparison was made with previous TEM studies in other species in order to examine to what extent the cat glomerulus exhibited similar morphological structure to those species. Thus the observations of Andrews and Porter (1974) on the similarity of gap size between adjacent tertiary podocytic processes on the GBM were

not confirmed in this study. The existence of a single cilium issuing from the cells of the parietal epithelium of Bowman's capsule also failed to be confirmed and as such adds to the major work on the subject, that of Latta et.al. (1961), who neglected to examine the cat. Likewise, the presence of direct shunts by-passing the glomeruli, as described by Ljunqvist (1964, 1975) was not found in the present work.

The present study also provided the first SEM views of the normal cat glomerulus. Once again, as with the TEM, it provided essential base-line parameters for future SEM studies involving pathological alteration to the glomerulus. Similarities as well as differences between the cat and other mammals were described and discussed. For example, the diverse observations of Buss and Kronert (1969) in the rat and Fujita et.al. (1970) in the rat and rabbit, concerning the arrangement of tertiary processes on the visceral epithelium. In the present study, the evidence supported the observations of the latter workers. The presence of microvilli on the cell body and also on the primary and secondary processes also confirmed the findings of Fujita et.al. (1970).

In Chapter 2 of the present work, only the second account of the post-natal development of the feline kidney was recorded. This part of the work confirmed that the process of nephrogenesis in the cat was similar to the general mammalian pattern; differences were perceived only in the overall timing of events. For example, new glomeruli were formed from the nephrogenic zone until well into the second month of life and these glomeruli only attained full functional maturity towards the end of the third month of life. This is in contrast to the dog where new glomerular formation finishes during the first month of life and functional maturity is attained by the sixth week of life (Horster et.al., 1971). The time scale for the cat was also much longer than present studies would suggest for the rat where glomerular nephrogenesis is concluded within a few days of birth (Kazimierczak, 1978; 1980). However, according to MacDonald and Emery (1959) the time scale in the human is much longer than that of the cat with the time taken to attain the full complement of glomeruli lasting until perhaps 11 months after birth. These glomeruli may remain immature until the fifth year of life with there then being a steady increase in the proportion of fully mature glomeruli until full functionality is attained at approximately 12 years of age (MacDonald and Emery, 1959).

Although there is growing interest in spontaneous glomerulonephritis in the cat, only a small number of studies have been carried out on the autolytic changes occurring in the cat glomerulus. To date, no detailed sequential histological and ultrastructural study has been undertaken. Yet, as previously discussed, renal tissue cannot always be obtained immediately after an animal dies or is destroyed. This is especially important where pathological changes involving the kidney are under investigation.

Initially the main feature evident in autolytic glomeruli studied in Chapter 3, both by light and electron microscopy, was tubular intra-glomerular epithelial reflux. This phenomenon has previously been observed in the normal dog and cat (Mullink and Feron, 1967), the normal rat (Cook et.al., 1965) and in pathological conditions in man (Dixon et.al., 1971). The main reason for this reflux has been suggested to be over-handling of the kidneys prior to fixation (Cook et.al., 1965).

In this part of the work other phenomena observed included necrosis of both endothelial cells and visceral epithelial cells. These are features which are sometimes associated with pathological changes such as glomerulonephritis (Movat et.al., 1962; Strunk et.al.,

1964), diabetes (Farquhar et.al., 1959; Suzuki et.al., 1966), and toxaemia of pregnancy (Pollack and Nettles, 1960; Wakamori et.al., 1962).

In the present study the main feature of interest observed in autolytic kidneys with the TEM was the remarkable preservation of the GBM and the attached foot processes. This was despite the disintegration of their parent cells. The tertiary processes remained morphologically distinct until approximately 60 hours after death.

The first SEM study of the autolytic cat kidney was also reported in this dissertation. The assessment of autolytic glomerular changes was diminished due to the SEM's inability to fully examine the glomerular endothelium and mesangium within the glomerulus itself. Although most of the visceral epithelium was seen to fuse into a roughened cytoplasmic sheet as early as 24 hours after death there were areas, which were also seen by the TEM, of morphologically recognisable foot processes as late as 60 hours post-mortem. Surface microvilli and 'blebs' were also evident on the visceral epithelium from 20 minutes onwards. These remained in evidence for some time until a general loss of preservation took place.

In the light of the results described, several important factors have emerged. First, there is a

variation at which autolytic degeneration takes place and, although morphologic alterations were observed as early as five minutes post-mortem with the TEM and ten minutes with the light microscope, areas of normal glomerular morphology remained distinct under TEM examination until as late as 24 hours post-mortem. Second, although the GBM was somewhat swollen as observed with the TEM it remained intact with a proportion of its apposed foot processes remaining in position and distinctively so until at least 60 hours after death.

In Chapter 4 of this work, a severe necrotising lesion primarily of the glomerular endothelium and, to a lesser extent, of the glomerular mesangium was identified for the first time. The information gained from the study of autolytic kidneys left no doubt as to the recent and severe nature of the lesion. Indeed, the severity of the pathology observed indicated that, had the lesion occurred some time prior to death, then tubular epithelial damage must surely have resulted. This is especially true of the proximal tubules which are much more sensitive to anoxia than are the glomeruli (Latta et.al., 1965), yet no damage was observed.

The only feature common to all the animals used in this chapter, and incidentally the animals used in the

previous three chapters, was the fact that they had received both K HCl and Na P, the latter no more than three minutes prior to the removal and fixation of the kidneys. These findings tended to suggest an intravascular interaction between the two drugs with a resultant cytotoxicity for glomerular endothelial and mesangial cells.

The deposition of C_3 and IgG as detected by immunofluorescence, within the glomerular capillaries, lent support to the immediacy of the lesion.

The effect of such a lesion being present in an experimental study of the cat glomerulus would surely negate or render inconsequential any other morphological event under study.

In Chapter 5, any possible effect either of the two above mentioned drugs had on its own was examined. In both cases no effect was detected either by light or electron microscopy or by immunofluorescence.

Thus the conclusion was reached that the combination of the two drugs *in vivo*, which was seen to form a white precipitate *in vitro*, was responsible for the initiation of the lesion.

In Chapter 6, an attempt was made to define the physical parameters which may have influenced the development of the lesion. The results indicated that there was no correlation between the times of administration of the two drugs and the development of the glomerular lytic lesion. There would appear, however, to be a relationship between the relative concentrations of the two drugs in so far as increasing relative concentrations of the two drugs increased the likelyhood of the development of the lesion.

In addition to the random appearance of the lytic lesion, a less severe or "mild" lesion not previously described was also observed. On histological examination, this lesion was characterised by the presence of large amounts of cytoplasmic debris in the capillary lumina together with alterations to the glomerular endothelium itself. When viewed by the TEM and SEM occlusion of the glomerular capillaries was seen to be caused by swelling of the endothelial cytoplasm together with some mesangial swelling. However, unlike the severe lytic lesion there was no detachment of the endothelial cytoplasm from the GBM.

As in the lytic lesion there was, however, deposition of both C_3 and IgG; however, this was seen in only a proportion of those animals exhibiting the mild lesion and then only of diminished intensity.

Nevertheless, despite attempts to elucidate the mechanism of the lesion by varying the concentrations of the two drugs and the time interval between their administration both forms of this lesion would appear to occur in an entirely random fashion and be entirely variable in its effect in any particular animal.

In the final chapter (Chapter 7), use was made of pre-formed combinations of the two drugs to mimic the proposed reaction taking place in the general circulation and / or the renal vasculature. This was effected using either K HCl as the primary sedative or Halothane to act as a negative control.

When using K HCl, the direct administration of these 'precipitates' into the renal artery resulted in the production of a lesion identical to that first described in Chapter 4 i.e. glomerular endothelial lysis. The administration of these 'precipitates' intravenously or intracardially was also successful in producing the lytic lesion, however the latter was only present in four out of 12 cats used. A further five animals out of the group of 12 exhibited the mild form of the lesion with the remaining three animals showing no signs of endothelial damage.

However, when using Halothane as the primary dose instead of K HCl and then administering the precipitates

the most that was observed was the mild form of the lesion.

This tended to suggest that the primary dose of K HCl may adhere temporarily to the glomerular endothelium whereupon a more violent reaction takes place in situ on the arrival of Na P into the glomerular capillaries.

Nevertheless, it would appear that both the mild and severe lytic lesions are nothing other than completely random events with the conditions required for the lesion's induction in any particular animal being quite unpredictable.

Thus the theory generated in the present study would tend to suggest that these two chemicals are reacting, producing a precipitate, in vivo in the same manner as they do in vitro.

The effect of this precipitate generated by the in vivo reaction would surely negate or at least invalidate any morphological event under study in the renal glomerulus. The effect on physiological studies after the primary sedation of the animal of the animal with K HCl and then further maintenence of sedation by barbiturate must also raise questions as to the validity of any results obtained.

Nevertheless this combination of chemicals is used in the clinical situation both in the cat and in

sub-human primates.

It is also evident from the present work that there is a need for a much longer term study into the effect(s) generated in cats by the use of these drugs. However, with only a relatively small proportion of animals being affected, even under the artificial conditions employed in the present study, the number of animals presented for clinical examination subsequent to a clinical situation where the two chemicals were used in combination at normal levels might be very low.

Despite this need, a longer term study was not possible in the present work as the animals involved were, in the main, presented expressly for the purpose of humane destruction.

In conclusion, a long term follow-up study entailing the collection of a record of each animal undergoing clinical procedures which involved the use of these two drugs and the animals subsequent medical history, especially involving any renal disease.

The following papers were directly associated with the work carried out in this thesis:

Necrotising glomerulopathy in cats euthanased with ketamine hydrochloride and sodium pentobarbitone.
N.G. Wright, A.W. Minto, A.S. Nash.
Veterinary Record (1982) 111, pp.127-128.

Renal biopsy in the normal cat: an examination of the effects of a single needle biopsy.
A.S. Nash, J.S. Boyd, A.W. Minto, N.G. Wright.
Research in Veterinary Science (1983) 34, pp.347-356.

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