IMMUNOCYTOCHEMICAL STUDIES OF THE DEVELOPMENTAL BIOLOGY AND PATHOLOGY OF THE HUMAN KIDNEY.

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SUMMARY

This thesis describes my study of normal and abnormal differentiation in the human kidney. Firstly, I have used immunocytochemical techniques to map the appearance of differentiation markers during renal organogenesis. I have shown that at the stage of tubular induction the induced cells underwent transferrin dependent proliferation. Simultaneously there was a change in intermediate filament phenotype from vimentin to cytokeratin. Tubular epithelium continued to express cytokeratins during development but in the glomerular epithelium cytokeratin expression was transient and was lost during glomerular maturation. As tubular development progressed the segregation of proximal and distal tubular epithelium was marked by the expression of different cell surface antigens, brush border antigen in the proximal tubule and epithelial membrane antigen in the distal tubule. Further developmental progress was accompanied by the appearance of new maturation markers such as ferritin, alpha-1-antitrypsin and carbonic anhydrase C. It is the ureteric bud which induces

tubulogenesis from the metanephric blastema. During this process the ureteric bud expressed a specialised group of cell surface carbohydrates, which have been shown to be important in cell interactions during other embryogenic processes. These molecules were lost as the ureteric bud differentiated to the collecting duct epithelium. Changes in the extracellular matrix, vascular tree and juxta-glomerular apparatus have also been described. I have concluded this first part of the study by formulating a classification of the cell markers according to the developmental stage and site at which they first appeared.

I have then used the same markers to study differentiation in different types of renal dysplasia and renal neoplasms. These are diseases in which differentiation may be abnormal.

In the cases of renal dysplasia I identified two different types of abnormality of differentiation. Firstly, the different components of the nephron showed evidence of developmental arrest. There was morphological immaturity and a failure of all segments of the nephron to express various maturation markers. Secondly, in the mesenchymal compartment heterologous differentiation into non-renal tissues such as cartilage and fat was a frequent occurrence.

The renal neoplasms also showed different forms of abnormal differentiation. The tubular elements of nephroblastoma expressed cytokeratins and either epithelial membrane antigen or brush border antigen but did not express the mature tubular markers such as carbonic anhydrase C and ferritin. The mesenchyme of some cases contained morphologically and immunologically detectable heterologous tissues particularly skeletal muscle. I have provided immunocytochemical evidence that the type B tubules in nephroblastoma are derived from the ureteric bud and grow into the tumours from the adjacent kidney.

It has previously been proposed that renal cell carcinomas arise from the proximal tubule, but I have herein shown that these tumours co-express markers of both proximal and distal tubular epithelium. Similar results have been seen in regenerative epithelium. Neoplastic epithelial cells in the majority of renal cell carcinomas expressed both the vimentin and cytokeratin types of intermediate filament protein. The proliferation of the renal tumours has been studied by the monoclonal antibodies Ki 67 and HB 21.

Renin containing cells have been found in a proportion of both of the main types of renal neoplasm. These were characteristically seen in a perivascular distribution.

During the course of the study a distinct type of renal carcinoma was identified. I have called this tumour collecting duct carcinoma because the tumour cells had the same cytokeratin phenotype and cell surface markers as normal collecting duct epithelium.

Finally I have discussed these various results in the context of our present knowledge of cell commitment and differentiation in the kidney and other tissues. The lack of heterologous epithelial differentiation in renal pathology has suggested that the commitment to renal differentiation persists in disease states. The renal connective tissues showed no such commitment and heterologous differentiation in this cell compartment was relatively common.

CHAPTER 1.

INTRODUCTION.

1.1 General Introduction and Aims of the Study.

In this study I have investigated normal and abnormal cellular differentiation in the kidney using immunocytochemical methods. Differentiation is the process by which cells acquire functional specialisation. The mammalian body is constructed from a limited number of distinguishable cell types, approximately 200 in man (Alberts et al 1983), all of which are derived from a single fertilised ovum. These cells are arranged in complex structural relationships, but the individual cell types are distinguishable because, in addition to "household" proteins required for the survival of all cells, each differentiated cell contains "luxury" proteins, which are essential for the specialised function of that particular cell type. Thus, epidermal cells contain keratins, erythrocytes contain haemoglobin, intestinal epithelium contains digestive enzymes and so on. Differentiated cells

are also morphologically different, but the structural characteristics of a cell are also the result of the expression of the various "luxury" proteins some of which have structural functions.

Therefore, since differentiated cells differ in their content of biologically active molecules, one method of defining, and of measuring differentiation is by the identification of these molecules within the cell. Differentiation has been studied previously by histological, ultrastructural and histochemical techniques. The histological and ultrastructural assessment of differentiation depends on the identificiation of morphological similarities between the tissue being examined and the appropriate fully developed adult tissue. This continues to be a reliable technique in the practice of surgical pathology, but in the study of development and other related processes it is insufficiently sensitive and not specific. Enzyme histochemistry (Braunstein & Adelman 1966) provides a technique for demonstrating the presence of enzymes within cells but has the disadvantage that only some enzymes are suitable for study by this method.

The modern techniques of immunocytochemistry provide a method of accurately identifying numerous differentiation molecules in tissue sections and therefore of measuring cellular differentiation. These techniques have been used extensively in recent years in studies of differentiation in biology and pathology (Polak & Van Noorden 1983).

The first priority of the study was to establish the developmental stage at which the differentiation markers first appeared during normal differentiation. This I have done by performing the immunocytochemical experiments on fetal and adult kidneys. In this way I have classified the different markers according to the stage and site of their expression. I have then used this classification to investigate renal diseases. I have chosen to examine the expression of these differentiation markers in different forms of renal dysplasia and renal neoplasms as examples of diseases in which differentiation may be abnormal. Finally, the relationship between normal and abnormal differentiation has been discussed in the context of our present knowledge of cell commitment and differentiation.

Studies In Embryology and Pathology.

The most immediate relationship between embryology and pathology can be seen in the study of congenital malformations. Since ancient times anatomists, physicians and philosophers have shown great interest in both the description of congenital malformations and the attempt to understand their cause in relation to normal anatomy and embryology (Warkany 1979). Amongst older civilisations the cause of these lesions, as with most disease, was believed to be spiritual. With improved medical understanding an organic basis for congenital anomalies was sought. William Harvey was the first to suggest a pathogenic mechanism when, in 1651, he concluded, from study of the embryology of the facial bones, that the congenital hare lip deformity was due to an arrest of the normal development of the upper jaw and palate. This concept of developmental arrest was largely disregarded for over 150 years until taken up by Meckel (1812) who explained a variety of congenital anomalies as a consequence of arrested embryonic growth.

During the nineteenth century there were numerous isolated descriptions of many congenital lesions but no extensive treatment of the subject

until Ballantyne (1904). His systematic record of congenital malformations implicated the premature cessation of normal development in the genesis of these lesions. Although obviously not the first to suggest this concept, and though he drew heavily on nineteenth century descriptive (Meckel 1812) and experimental work (Dareste, Fere, Cited by Willis 1962), Ballantyne°s is the first textbook of teratology which can be seen as a scientific attempt to understand congenital malformations.

Dereste and Fere had performed the experimental generation of congenital defects during the nineteenth century on their work on malformations in chicks. However, the later work of Spemann & Mangold (1924) is of both major scientific and philosophical significance. They transplanted a small piece of tissue from the dorsal lip of the blastopore of an amphibian embryo at the early gastrulation stage and created an abnormal embryo which formed notochord, endoderm and some mesoderm at the grafted site. This was an extension of earlier blastula constriction experiments of Spemann (1903) and Spemann & Falkenberg (1919). The scientific significance of this work was the identification

of the tissue at the dorsal lip of the blastopore as the site of the primary embryonic organiser, hence initiating the now extensive field of research on inductive tissue interactions in development (Wessels 1977; Sawyer & Fallon 1983; Lash & Saxen 1985). The philosophical significance was that by creating and studying a pathological lesion Spemann & Mangold were able to form conclusions concerning mechanisms in normal embryology. This work is now regarded by many as the start of the discipline of experimental embryology (Slack 1985).

In addition to experimental embryology and teratology, the thorough study of naturally occurring congenital anomalies and other aspects of paediatric pathology has made significant contributions to the understanding of normal embryology and developmental physiology (Lash & Saxen 1985).

Embryological principles have also contributed to other fields of pathology. Cohnheim (1875) proposed that tumours developed from embryonic rests which persisted in adult tissues. Ribbert (1892), following on Cohnheim°s proposals, suggested that embryonic rests did not persist in

adult tissues, but rather that tumours developed from populations of cells which retained embryological properties. These theories were denied by many others. Although these theories are no longer tenable in the original stated form, the relationship between tumour growth and embryonic growth has continued to be studied. These relationships formed the basis of much of Nicholson°s work on tumour biology in the 1920s and 1930s (Nicholson 1922-1935).

This work has been extended recently in many different fields including the discovery of growth factors (Carpenter & Cohen 1979; Levi-Montalcini & Callissino 1979), cell-cell interactions (Spemann 1938; Wessels 1977), the importance of extracellular matrices (Hay 1982) and the regulation of differentiation (Sengel 1976).

One of the recent research interests in this field has been the recognition that many tumours produce proteins and carbohydrates which are not present in adult tissues, but are the form of the related molecule present in fetal tissues (Alpert & Hirai 1983). These oncofetal antigens and oncofetal enzymes have been used as serum and histological tumour markers, and as research tools in the investigation of oncology and embryology (Neville, Heyderman & Grigor 1978; Alpert & Hirai 1983).

There has been interest in the use of embryonic tumours, especially teratomas, as models of the developmental process at the cellular level. Embryonic tumours of neuroblasts (Wright 1910), kidney (Muus 1899; Wilms 1899), liver (Webster 1938), and testes (Geinitz 1862) were all described during the nineteenth or early twentieth centuries, and their relationship to embryonic tissues recognised. Since then histological (Willis 1962), ultrastructural (Graham 1977), tissue culture (Jacob 1978), immunological (Solter et al 1979) and molecular biological techniques (Strickland, Smith & Marotti 1980) have been applied to these tumours in an attempt to learn about their aetiology and clinical behaviour. They have also been used as in vitro models for the study of normal developmental processes (Solter et al 1979; Wartiovaara & Reichert 1984).

The recent expansion of techniques and data on the molecular biology of tumours and congenital defects have shown relationships between these pathological lesions and normal embryology (Lash & Saxen 1985). Many substances identified as tumour growth factors or differentiation factors have been shown to be important during embryogenesis (Marshall 1986; Adamson 1987). There is now a substantial body of literature relating cellular mechanisms in embryology and pathology.

1.2 Embryology of the Kidney.

Descriptive embryology.

During the third week of the development of the human embryo the intra-embryonic mesoderm divides into three separate parts 1) the somitic mesoderm, 2) the lateral plate, which further divides into the somatic and splanchnic mesoderm lining the intra-embryonic coelom, and 3) the intermediate mesoderm (Hamilton & Mossman 1972). The intermediate mesoderm, on either side of midline, loses its contact with the somitic and lateral plate mesoderms and comes to lie anterior and lateral to the notochord. Part of it then forms the nephrogenic ridges. These are paired structures which lie on either side of midline, dorsal to the intra-embryonic coelom extending from the cervical to the sacral regions (Hamilton & Mossman 1972).

It is from these paired nephrogenic ridges that three different, overlapping kidney systems form in the developing mammalian embryo. They develop sequentially both in time and in a cephalo-caudal direction. These kidney systems are

called the pronephros, mesonephros, and metanephros. Felix (1906) was the first to fully describe these three organs, and to appreciate their sequential and overlapping development. He defined these as three stages in the formation of a single urinary system or holonephros.

The first of these three organs is the pronephros. In the human embryo this consists of seven to ten poorly formed tubules which open into the coelomic cavity, and which fuse laterally to form the pronephric duct. The paired pronephric ducts thus formed are simple, tubular structures which run longitudinally in the paraxial mesoderm from the cervical region to the cloaca. The pronephros in man is probably not functional and these tubules regress during the fourth and fifth weeks of intra-uterine life.

Unlike the pronephric tubules, the mesonephric tubules lack a connection with the coelomic cavity. They develop initially as a vesicle. This elongates and twists to form an S-shaped structure. This then fuses laterally with the longitudinal extension of the pronephric duct, which is now called the mesonephric or Wolffian duct. At their medial end the mesonephric tubules

develop a cup-shaped indentation which is vascularised by an outgrowth from the dorsal aorta forming the mesonephric glomerulus (Fraser 1950; Torrey 1954). Boyden (1927), Gerard & Cordier (1934a,b), O°Connor (1938, 1939, 1940), Waddington (1938), and Du Bois (1969) have shown that it is the elongation of the pronephric duct and its growth along the lateral border of the nephrogenic ridge to the cloaca which induces the formation of mesonephric tubules in several different animal species. This shows remarkable similarities to the process of induction of the metanephric tubules.

The elongation of the mesonephric duct, essential for the continued development of the urinary system, occurs by elongation of the nephric duct by cell proliferation and cell migration at the growing tip (Holtfreter 1944; Calame 1962; Croisille, Gumpel-Pinot & Martin 1976). This growth of the mesonephric duct has recently been shown to involve changes in the intercellular adhesive properties of the undifferentiated mesodermal cells of the nephrogenic ridge (Poole & Steinberg 1982). The pronephric duct growth and the guidance of its elongation along the nephrogenic ridge is

controlled by a cranio-caudally travelling intercellular adhesion gradient (Poole & Steinberg 1982; Thiery et al 1984). This change in intercellular adhesion causes the movement and re-orientation of the mesodermal cells which support epithelial differentiation and the elongation of the mesonephric duct.

The mesonephric duct thus formed grows along the length of the nephrogenic ridge and induces the formation of mesonephric tubules. The most caudal mesonephric nephrons are induced by a small unbranched outgrowth of the mesonephric duct which eventually fuses with the distal portion of the mesonephric tubule (Croisille et al 1976).

The mesonephric tubules formed throughout the nephrogenic ridge show striking morphological similarities to the metanephric tubules of the permanent kidney. These similarities were noted by many of the authors who originally described the different kidney systems (Felix 1906; Gerard & Cordier 1934; Fraser 1950). Leeson (1957) working with the rabbit embryo and De Martino & Zamboni (1966) working with the human embryo have performed comparative studies of the mesonephric and metanephric tubules at an ultrastructural

level. They found that the proximal tubular cells of the two kidney types were almost identical but that there were some differences in the distal tubule and glomerulus. The mesonephric distal tubular cells were reported to be less well differentiated, contained fewer cytoplasmic organelles and had a much simpler surface morphology than the metanephric distal tubular cells. Although the podocyte and mesangial cell structure of the mesonephric glomerulus was remarkably similar to that of the metanephric glomerulus, the mesonephric glomerulus had two distinct vascular poles, one for the afferent arteriole and another for the efferent arteriole (De Martino & Zamboni 1966).

In lower animals the biochemical aspects of mesonephric function is similar to that of metanephric function except that the mesonephric nephron, lacking a loop of Henle and an individual collecting duct, has greatly reduced water reabsorption capacity (Du Bois 1969). However, the importance of the excretory function of the mesonephros in the human embryo is still debated (Du Bois 1969).

The metanephros, the third kidney system, develops from the most caudal part of the nephrogenic ridge. Three main components combine during metanephrogenesis. These are the nephrogenic mesoderm, the vascular tree and the ureteric bud.

The ureteric bud appears as a lateral outgrowth of the caudal end of the mesonephric duct (Hamilton & Mossman 1972). It branches out from the mesonephric duct and grows into the nephrogenic mesoderm forming a multiple branching structure. When fully differentiated the ureteric bud will form part of the bladder, the ureter, the pelvicalyceal system, and the collecting ducts of the kidney (Hamilton & Mossman 1972).

Osathanondh & Potter (1963a,b) have subdivided the formation of this ureteric bud derived collecting system into four periods. In the first, the ureteric bud undergoes multiple dichotomous divisions as it grows cranially and laterally. Fusion of the third and fourth generation branches and elongation of the fifth and sixth results in the normal morphology of the pelvicalyceal system and renal pyramids. The further divisions become associated with metanephric nephrons, which form adjacent to, and eventually fuse with each ureteric bud branch.

In the second phase the ureteric bud grows without dividing but continues to induce the formation of metanephric nephrons. At this stage ureteric bud ampullae already carrying a nephron may induce the formation of another. The newly formed metanephric nephrons become attached to the collecting duct of the previous nephron, giving rise to nephron arcades. The third phase is characterised by continued ureteric bud growth, but as each new nephron becomes attached to the ureteric bud the ampulla continues to grow leaving the nephron behind. The ampulla then induces a new nephron. This process repeats itself between four and seven times. These collecting tubules therefore consist of unbranched terminal portions draining the nephrons which form in the outer half of the renal cortex. Finally ampullae disappear and no new nephrons are formed.

The excretory system of the metanephros forms from the most caudal part of the nephrogenic ridge mesoderm, the metanephric blastema. This loose primitive tissue forms a cap over the ingrowing ureteric bud. Under the inductive influence of the ureteric bud ampulla (Saxen & Kohenen 1969), these blastemal cells form a roughly spherical

condensation adjacent to each ureteric bud branch. The condensed cells then adopt an epithelial morphology and the nephrogenic vesicle is formed. The vesicle elongates and twists to form the S-shaped tubule of the primitive metanephric nephron. The upper pole of the S-shaped tubule then fuses with the ureteric bud branch which induced its formation establishing a continuous but primitive nephron. The lower pole of the S-shaped tubule forms the glomerulus and becomes vascularised by an increasingly complex ingrowing capillary tuft. Further maturation and growth of the nephron takes place, during which time the ampulla of the ureteric bud continues to grow cranially and laterally. In this way the most immature nephrons are found at the peripheral, subcapsular, part of the fetal kidney, an area called the nephrogenic zone, with progressively more mature nephrons deeper within the renal cortex (Hamilton & Mossman 1972; Potter 1972).

As the metanephros differentiates there is also relative ascent of the organ from the pelvic region to the posterior abdominal wall. During the ascent of the metanephros the main arterial supply develops from progressively more proximal branches

of the common iliac arteries and aorta. The permanent main renal artery develops from one of the earlier mesonephric arteries which fails to regress (Gruenwald 1943). This ascent of the vascular supply quite commonly gives rise to accessory renal arteries which are found in about 30% of the population (Kissane 1983).

Inductive interactions during renal development.

It is now known that the ureteric bud is important in the induction of the normal differentiation of the metanephros (Saxen et al 1976; Saxen 1987). The intimate association of the ureteric bud, arising from the caudal end of the Wolffian duct, and the developing metanephric nephrons had been described during the nineteenth century (Balfour 1876). The work of Spemann & Mangold (1924) on inductive interactions and tissue organisers during embryogenesis suggested to some authors that the ureteric bud may be the organiser of metanephrogenesis (Boyden 1927; Nicholson 1927). Boyden (1927) reached this conclusion by observing failure of metanephros formation in animals following ligation of the

mesonephric ducts; and Nicholson (1927) because of the constant absence of kidneys in cases of ureteric agenesis which he had observed. Gruenwald (1937, 1942, 1943) followed up these suggestions by inducing metanephros formation from undifferentiated nephrogenic mesoderm *in vitro* by using firstly mesonephric duct and later several other tissues as inducers.

Grobstein (1955, 1956) in a series of papers demonstrated the induction of metanephric tubule formation from undifferentiated mouse metanephric blastema cultured transfilter from the inducer tissues. This work also showed that a wide variety of tissues could function as the inducer in this system, but if induction occurred then the response of the metanephric blastema was always the same, namely the formation of metanephric tubules. This work has been confirmed by several other workers with an increased number of tissues used to induce metanephric tubulogenesis (Saxen *et al* 1968; Unsworth & Grobstein 1970).

In contrast to this specific response of the induced tissue (metanephric blastema) to a wide range of non-specific inducers the ingrowing ureteric bud can only be stimulated to grow and

differentiate by nephrogenic mesoderm (Grobstein 1953, 1955; Le Douarin & Houssant 1967). Kornfeld (1925) was the first to suggest that the metanephric blastema was essential for the branching and growth of the terminal portion of the Wolffian duct, and hence ureteric bud formation. The importance of primitive mesoderm in inducing branching morphogenesis of other tissues, including salivary gland (Grobstein 1953) and pancreas (Grobstein 1967), has been demonstrated and shown to be a property specific to the appropriate mesoderm (Sakakura, Nishizuka & Dawe 1976). Vernier & Smith (1968) have suggested that the metanephric blastema produces a locally acting chemical messenger which induces ureteric bud outgrowth from the mesonephric duct.

It is now apparent that there is a reciprocal relationship between the ureteric bud epithelium and the metanephric blastema (Torrey 1965; Saxen 1987). The relationship is not entirely equal. Whereas the metanephric blastema responds in a specific manner to relatively non-specific inducers, the ureteric bud only grows if specifically induced by metanephric blastema. These aspects of the metanephric blastema-ureteric

bud relationship illustrate two different types of inductive processes involved in organogenesis and differentiation (Holtzer 1968; Wessels 1970; Kratochwil 1972; Wessels 1977).

One of these has been termed instructive induction. An instructive inductive signal is specific for the inducer tissue and it elicits a specific response from the responder tissue. This type of relationship requires that the inducer is at least partially differentiated, either at the structural or at the molecular level, whereas the responder tissue, prior to induction, must be developmentally plastic in order to respond appropriately to one of the variety of possible instructive inductive signals which it may encounter during development. The induction of ureteric bud growth by the metanephric blastema is due to an inductive interaction of this type. Of the various tissues tested only the metanephric blastema induced ureteric bud formation from the mesonephric duct (Grobstein 1953; 1955). During normal embryogenesis parts of the mesonephric duct may be specifically induced to form other structures by other instructive tissue interactions. In this way appropiate parts of the

mesonephric duct can be induced to form ureter, bladder, ductus deferens, seminal vesicle or Gartner°s duct. The epithelia of these structures are morphologically and functionally different, therefore, before induction the mesonephric duct must be developmentally plastic.

The other type of inductive interaction is termed permissive induction. In this type of interaction the inducer tissue supplies a relatively non-specific signal which induces differentiation of the responding tissue along a pathway to which it has previously become committed. Since the inductive signal cannot alter the pathway of differentiation, it can only initiate it, the responding tissue in this case is in a determined state. A useful definition of determination, given by Alberts et al (1983), is that "a cell is determined if it has undergone a self-perpetuating change of internal character that distinguishes it and its progeny from other cells in the embryo, and that commits these progeny to a specialised course of development". The metanephric blastema responds to a variety of tissue inducers by consistently differentiating into metanephric nephrons (Grobstein 1955; Saxen et al 1968; Unsworth & Grobstein 1970). This is therefore an example of permissive induction.

Determination and differentiation.

According to our understanding of permissive induction, the metanephric blastema prior to the induction of differentiation must become determined, but at this stage does not show any morphological evidence of differentiation. When does this determination event occur? Evidence for early commitment to nephric differentiation in amphibia has existed for over fifty years. In 1929 Mackemer showed by the transplantation of segments of intermediate mesoderm to heterotopic sites at different stages of embryogenesis, that the commitment (determination) of nephrogenic mesoderm occurred at some time between the neurula stage and tail bud development in Triton alpestris. He further showed that the process of determination proceeded in a cranio-caudal direction. This commitment to nephric differentiation occurred without any morphological changes in the cells. Determination, therefore, occurred early and without overt differentiation.

The molecular basis of determination has been studied predominantly in simpler amnimals, especially the fruit fly Drosophila melanogaster.

In this species mutations have been described, the biological effect of which is to alter the differentiation pattern of certain body segments (Lewis 1978; Nusslein-Volhard & Wieschaus 1980). The genes in which these so called homeotic mutations (Lawrence & Moratu 1977) occur have been sequenced and show homology to some genes controlling the determination and differentiation of different structures (Kaufman 1980; Bender et al 1983; Garber, Kurowa & Gehring 1983). Similar gene sequences have been identified in the genome of amphibians, reptiles, mice, and man (McGinnis et al 1984; Carrasco et al 1984). The products of these genes show a high degree of amino acid sequence homology to a protein which has been shown to bind to chromosomal DNA in the cell nucleus (Laughton & Scott 1984).

These observations raise the exciting prospect that a group of genes, highly conserved during evolution, regulate determination or differentiation by coding for a group of DNA binding proteins. Thus, the activation of groups of these genes may restrict the genome to allow only specific pathways of differentiation (Slack 1985).

Determination involves commitment to a pathway of differentiation with no overt evidence of such differentiation. The determined cell, by changes in its DNA content or in the structure of its DNA, may be sufficiently specified to enable it to produce tissue specific molecules. In this way determined metanephric blastema may instructively induce ureteric bud outgrowth from the mesonephric duct; this newly formed tissue could then permissively induce the further differentiation of the metanephric blastema (Saxen 1987). This model is at present speculative, but it is a model which explains much of the descriptive and experimental embryology data on nephronogenesis. Evidence is also emerging for such reciprocal inter-relationships between tissues in other organs and in other species (Nieuwkoop 1985).

An understanding of these inductive interactions between different tissues is important in the study of differentiation and especially so when considering abnormalities of differentiation in pathological states.

After induction has occurred differentiation and growth of the metanephric nephron takes place.

Cell differentiation during renal organogenesis.

Until recently the majority of studies on the development of the kidney were of a morphological or descriptive nature. These included descriptive embryology (Hamilton & Mossman 1972), microdissection studies (Potter 1972) or transfilter induction experiments used to study cell interactions during development (Grobstein 1955; 1956).

More recent studies have concentrated on the changes undergone by the cells contributing the development of the nephron. Vernier & Birch-Anderson (1962) and Kazimierczak (1971) have demonstrated the marked changes in the fine structure of the metanephric cells as they differentiate. They have detailed changes occurring during the initial formation of the epithelium and later during the segregation of the tubule and the formation of the glomerulus.

The major contributions to the study of the molecular processes involved in nephronogenesis have come from the Department of Pathology of the University of Helsinki under the direction of Professor L. Saxen. This group have extensively studied the development of the metanephric tubules in vitro using the transfilter induction system

(Saxen et al 1968; Ekblom et al 1981). The cellular alterations which accompany the induction of tubulogenesis in this model have attracted special attention, because of their importance to the study of other cellular interaction in development. They have shown that in these early stages of development there are changes in the extracellular matrix from a mesenchymal to an epithelial type (Ekblom et al 1981; Ekblom, Sariola & Thesleff 1984). The induced tubular cells secrete basement membrane collagens and the non-collagenous basement substance laminin in a polar distribution (Ekblom et al 1980). Ekblom (1981) has suggested that these changes in extra-cellular matrix molecules are important for the orientation, polarity and growth of the developing tubules.

Other early events in the initiation of tubular differentiation include cell proliferation and the synthesis of many new polypeptides (Saxen et al 1968). This proliferative response has been shown to be dependent on transferrin in the culture medium (Ekblom et al 1983; Thesleff & Ekblom 1984). The requirement for transferrin can be by-passed by using iron chelating agents which

cause the internalisation of iron (Thesleff & Ekblom 1984). Although many new proteins are synthesised during early tubular differentiation the identity of many of these proteins is unknown.

These studies have contributed greatly to the understanding of the mechanisms of tubulogenesis, but there have been few studies of cellular differentiation throughout the various different stages in nephron development.

1.3 Renal Dysplasia.

General features.

Renal dysplasia is the abnormal, disorganised development of the renal parenchyma due to anomalous differentiation of metanephric tissue (Risdon 1971). Renal dysplasia is most frequently diagnosed in childhood, but adult cases are described (Pathak & Williamson 1964; Risdon 1971; Fisher & Smith 1975; Kissane 1976). It more commonly affects males (Kissane 1976), and for reasons which remain obscure it is more common in the left than in the right kidney (Kissane 1983).

Accurate figures for the incidence of renal dysplasia have been difficult to obtain because many cases are entirely asymptomatic. Parkkuleinen, Hjelt & Sirola (1959) showed that renal dysplasia occurred in 0.05% of paediatric admissions but in 0.5% of paediatric autopsies in a Swedish Teaching Hospital. Pathak & Williamson (1964) found renal dysplasia in 0.037% of admissions to Great Ormond Street Hospital in London.

It is the most common form of cystic disease of the kidney in childhood (Kissane 1983) and is the most common cause of an abdominal mass in childhood (Pathak & Williamson 1964; Greene, Fanguy & Dahlin 1971).

It can affect the kidney diffusely, segmentally or focally and may be associated with cyst formation (Risdon 1980). It is almost invariably associated with other congenital anomalies of the urinary tract and Risdon (1975) has argued that renal dysplasia should be considered as an abnormality of the development of the whole urinary tract and not simply a renal anomaly.

Most dysplastic kidneys are smaller than normal, and most hypoplastic kidneys also show evidence of dysplasia. Pure renal hypoplasia also exists, the best example being the condition of oligomeganephronie (Royer et al 1962).

The extent of the dysplastic lesion in each case of renal dysplasia is extremely variable ranging from the multicystic kidney, in which the whole kidney is replaced by cystically dilated, dysplastic tubules and ducts, to clinically silent lesions discovered as an incidental finding (Risdon 1971a, b).

Renal dysplasia can only be diagnosed histologically. There is marked parenchymal disorganisation in the affected segment. Immature renal structures are present but without organisation into a complete nephron. There is distortion of the architecture with immature glomeruli (Pasternak 1960), and immature tubules some of which may show cystic dilatation (Rubinstein, Meyer & Bernstein 1961; Kissane 1966). Islands of cartilage are a frequent finding and have been regarded by some authors as definite evidence of dysplasia (Bigler & Killingsworth 1949). The only consistent finding, however, is the presence of immature ducts surrounded by concentric cuffs of mesenchyme (Ericsson & Ivemark 1958), a finding which is not seen in other forms of renal disease (Bernstein 1968; Risdon 1971; Fisher & Smith 1975). These immature ducts are usually lined by a single layer of columnar epithelium resembling the epithelium of collecting ducts and ureteric bud branches, but examples of ducts lined by stratified squamous, mucus secreting, and ciliated epithelium have all been described (Fisher & Smith 1975; Chan, Saw & Myint 1986).

The presence of primitive dilated ducts resembling ureteric bud branches and the frequent association of abnormalities of the ureter and bladder has led to the suggestion that an arrest of ureteric bud growth has occurred to cause renal dysplasia (Osathanondh & Potter 1964a, b; Potter 1972). Based on microdissection studies these authors concluded that cystic renal dysplasia was indeed caused by diminished branching of the ampullary portions of the ureteric bud. This causes a secondary failure of metanephric development because of the role of the ureteric bud in the induction of metanephric tubulogenesis (Grobstein 1955). The cause of this arrested ureteric bud outgrowth is unknown but the association with obstructive lesions of the lower urinary tract may be an important factor (Bialestock 1965; Bernstein 1968). It has been suggested that renal dysplasia is one form of a spectrum of disorders of ureteric bud outgrowth, with renal aplasia (Potter°s syndrome) the most severe form. Interestingly, cases of severe bilateral renal dysplasia have been described, and in affected infants many of the extra-renal features of Potter°s syndrome, such as V-shaped

epicanthic folds, flat nose and low set ears are also seen (Risdon 1980). Mice heterozygous for the Sd mutant develop renal dysplastic abnormalities associated with partial failure of ureteric bud growth, while mice homozygous for the mutant form of the gene suffer from renal agenesis (Gluecksohn-Schoenheimer 1949).

The prognosis and clinical significance of renal dysplasia depends on the extent of renal involvement and on the presence of associated renal, urinary tract and other congenital anomalies.

Related to cystic dysplasia is the segmental renal lesion known as multilocular cystic nephroma (Bennington & Beckwith 1975). There is argument about whether this lesion is a neoplasm or a malformation. It was first described by Edmunds (1892) and over fifty cases have subsequently been recorded in the literature (Aterman, Boustani & Gillis 1973; Bennington & Beckwith 1975). It may be even more common since undoubtedly many cases are mistaken for polycystic disease, multicystic dysplasia or cystic forms of nephroblastoma (Olsen 1984).

Boggs & Kimmelsteil (1956) and Aterman et al (1973) have reviewed the debate on the origin of this lesion. The main hypotheses which have been proposed are that it is a benign form of nephroblastoma, a benign tumour separate from nephroblastoma or a developmental anomaly. The lack of metastases and the segmental nature of the lesion (Potter 1972; Johnson et al 1973) have supported the view that it is a dysplastic lesion.

All the cases described have been unilateral, they are frequently large, and bulge from the lateral surface of the kidney as a lobulated mass. There are numerous cysts of varying sizes which do not communicate with each other. The adjacent kidney is not compressed. The cysts are lined by flattened or cuboidal epithelium, occasionally hobnailed, and the intervening stroma consists predominantly of fibrous tissue (Bennington & Beckwith 1975).

Joshi et al (1979) have described a lesion related to both multilocular cystic nephroma and to nephroblastoma. It has been called cystic, partially differentiated nephroblastoma. It is a multilocular cystic tumour, the stroma of which contains islands of pleomorphic metanephric blastema. Other authors (Olsen 1984) consider this the same lesion as multilocular cystic nephroma.

These various forms of renal dysplasia are abnormalities of the differentiation of the metanephros (Risdon 1971a; Bove & McAdams 1976). Despite the importance of differentiation to this group of diseases there have been few studies of differentiation in renal dysplasia.

The studies of differentiation which have been performed to date have been morphologically based studies. Several authors have provided detailed histological descriptions of the range of appearances seen in renal dysplasia. These studies have emphasised two main features, namely, the immaturity of the nephronic structures in renal dysplasia and the presence of heterologous elements (Ericsson & Ivemark 1958a,b; Bialestock 1965; Bernstein 1968; Risdon 1971a; Kissane 1983). In addition to descriptive histology Risdon (1971a,b) has proposed a grading system based on morphology for the classification of renal dysplasia depending on the extent of involvement of the kidney.

The microdissection studies of Potter (Osathanondh & Potter 1964; Potter 1972) suggested that the primary abnormality in renal dysplasia was an arrest of ureteric bud growth. The metanephric blastema is therefore not induced to differentiate. Earlier, Gruenwald (1939) had observed the absence of both ureteric buds in a 9 mm human embryo in which there was undifferentiated metanephric blastema but no evidence of tubular differentiation.

Ureteric buds are seen in renal dysplasia but they are morphologically abnormal, with dilatation which may be marked (Potter 1972). These cystically dilated structures may also be lined by heterologous epithelium (Ericsson & Ivemark 1958a; Risdon 1971a; Fisher & Smith 1975; Kissane 1983; Chu, Saw & Myint 1986).

A few enzyme histochemical studies have been performed on the kidneys affected by selected dysplastic syndromes (Rapola 1985) but none of these has been sufficiently extensive to provide general conclusions on the differentiation of the dysplastic and immature epithelium of renal dysplasia.

1.4 Renal Tumours.

General Features of Renal Tumours.

The kidney is an uncommon site of neoplasms. In the United Kingdom neoplasms of the renal parenchyma constituted 1.6% of cancer deaths in males and 1.2% in females in 1982 (More 1985). A similar incidence is reported from the United States (Bennington & Beckwith 1975). There is geographical variation in the incidence of primary renal parenchymal neoplasms, countries such as the Republic of Ireland, Italy and Japan have low rates while Scotland and the Scandinavian countries have a high incidence (Muir & Nectoux 1980).

Numerically the most important primary malignant renal neoplasms are renal cell carcinoma, nephroblastoma, and the various types of sarcoma (Lucke & Schlumberger 1957; Bennington & Beckwith 1975; Olsen 1984). The two most common renal tumours are seen at the extremes of life, nephroblastoma in childhood and renal cell carcinoma in late adult life (Bennington & Beckwith 1975).

There has probably been greater debate concerning the histogenesis of renal neoplasms than that of any other group of tumours (Reviewed by Bennington & Beckwith 1975). This has led to a great number of different classifications and of synonyms for the more common renal neoplasms (Bennington & Beckwith 1975; Mostofi 1981; Olsen 1984). The histological classification of renal tumours and associated lesions which I have used in this study is summarised in Tables 1 & 2.

1.4.1 Nephroblastoma and Related Lesions.

Probably the first recorded case of this tumour is that of an encephaloid tumour in a child°s kidney described and collected by John Hunter (cited in Bennington & Beckwith 1975). The first histological description, however, is that of Schuberg (1861), and subsequently numerous cases of embryonal renal tumours of childhood were described during the latter part of the nineteenth century. As a consequence of the recognition of these tumours as a defined entity, there evolved a major debate concerning their histogenesis. Eberth (1872) and Birch-Hirschfeld (1898) believed that

the tumour arose from a persistent mesonephros and Wolffian duct. Although the tumour bears the eponymous title Wilms° tumour, Wilms did not provide the first description of the tumour, nor did he form correct conclusions concerning its histogenesis. Wilms (1899), like Cohnheim (1875), believed the tumour arose from the uncommitted mesoderm of the very early embryo because of the presence of skeletal muscle and fat in many of the cases he described. It was at the end of the nineteenth century that an origin from metanephric tissue was first seriously proposed. Muus (1899) and Busse (1899) independently established, on both embryological and histological evidence, that these tumours arose in, and from, the developing metanephros. Shattock (1894) had suggested, in response to the work of Eberth and Cohnheim, that the tumour was at least as likely to develop from the metanephros as from the mesonephros. Previously Paul (1886) had described a case of an embryonic renal tumour as a "renal monstrosity" and discussed the origin of the tumour from "germinal cells" whose function would normally have been to form the metanephros. He had, therefore, identified two important ideas; that these tumours may be of metanephric origin and that they were, or were related to, malformations or abnormalities of differentiation.

Although Wilms formed the wrong conclusions about the histogenesis of this tumour it was from his and Muus° careful description of the histological features that later writers concluded that the tumours were of metanephric origin (Trappe 1907; Nevinny 1926; Montpellier 1928; Nicholson 1931). The presence of blastema, tubules, stroma, and in some cases, glomeruloid bodies led Nicholson (1931) to conclude that the tumour consisted of abnormal metanephric blastema which proliferated and differentiated in an uncontrolled manner.

Except for the theory of Masson (1938) who identified ganglion cells in some nephroblastomas and suggested a neural crest or neuroepithelial origin of the tumour, most twentieth century workers have accepted that the origin of nephroblastoma is from the metanephric blastema.

Although most authorities now accept this theory of the histogenesis of these tumours there is no conclusive evidence regarding the mechanism by which they arise. Three possible sequences of events have been proposed (Bennington & Beckwith 1975):

1) The development of the tumour prior to birth.

2) De-repression of genetic information returning differentiated cells to cells with an embryonal range of differentiation potentials from which the tumour then develops.

3) Development of the tumour from cells with persistent embryonal differentiation potential.

The first theory is considered unlikely because the peak incidence of nephroblastoma is between 2 and 4 years (Bennington & Beckwith 1975; Olsen 1984) whereas other congenital tumours are most commonly encountered in the first year of life (Willis 1962). Congenital cases of nephroblastoma are known (Bennington & Beckwith 1975), and tumours have been identified in fetuses (Nicholson 1931), but many of the tumours in children under one year have been identified as a histologically and biologically distinct entity, that of congenital mesoblastic nephroma (Bolande, Brough & Izant 1967; Bolande 1973). In addition, cases of nephroblastoma have been described in adults (Olsen & Bischoff 1970; Kilton, Matthews & Cohen 1980), including one in an eighty year old patient (Babaian et al 1980).

Of the remaining possibilities it is difficult to disprove that there is de-repression and a return to an embryonic state. It is the last theory, therefore, which has received most support. It is, of course, a modification of the general theory of Cohnheim (1875) who proposed that all tumours arose from embryonic rests. Although now obsolete as a general theory of oncogenesis it may well be applicable to the special circumstances of the development of embryonic tumours. In the kidney the cells which have such a full range of developmental potential are those of the metanephric blastema.

Persistent renal blastema and the nephroblastomatosis complex.

In the human kidney nodular foci of embryonic renal blastema can persist in post natal life. These frequently show some histological resemblance to nephroblastoma with their mixture of blastemal, stromal, and epithelial elements (Potter 1961; Bove, Koffler & McAdams 1969; Potter 1972; Bennington & Beckwith 1975). Potter (1961) in her original descriptions, and later Shanklin &

Sotela-Avila (1969), considered these lesions to represent incipient or *in situ* nephroblastoma. Bove, Koffler & McAdams (1969) found five cases of persistent renal blastema in 1,895 consecutive autopsies, a higher incidence than one would expect if all of these lesions were nephroblastomas *in situ*. They also found a high incidence (8/46) of persistent renal blastema in kidneys removed for nephroblastoma. Bennington & Beckwith (1975) found an even higher incidence than Bove and his colleagues when examining autopsy material which was restricted to the paediatric age group. Persistent renal blastema is typically multifocal, and frequently bilateral (Bennington & Beckwith 1975; Bove & McAdams 1976).

Foci of persistent renal blastema are also found in association with other diseases particularly congenital malformations. Bove, Koffler & McAdams (1969) found persistent renal blastema in three autopsies from six cases of Trisomy 18, and also noted a high incidence of congenital malformations in other cases in which they had found persistent blastema. Persistent renal blastema has been described in association with Trisomy 13 (Olsen 1984), genitourinary

abnormalities (Bove & McAdams 1976; Chadarevian et al 1977), congenital heart disease (Machin 1980), Beckwith-Wiedemann syndrome (Beckwith 1969, Machin 1980), hemi-hypertrophy, pseudohermaphroditism and familial nephroblastoma (Machin 1980). Many of these conditions associated with persistent renal blastema are also associated with the development of nephroblastoma (Miller, Fraumeni & Manning 1964; Beckwith 1969; Reddy et al 1972; Aron 1974; Bennington & Beckwith 1975; Bove & McAdams 1976).

In addition to persistent blastema, Bove & McAdams (1976) have identified a spectrum of closely related disorders which are associated with the development of nephroblastoma. They have identified five separate lesions, three of which they consider neoplastic (nodular renal blastema, sclerosing metanephric hamartoma, Wilms° tumourlet) and two of which are malformations (glomerular immaturity, cortical cystic lesions).

Nodular renal blastema, synonymous with the persistent renal blastema of Potter (1961) and Bove, Koffler & McAdams (1969), is defined histologically as nodules of immature metanephric tissue consisting of blastema, immature tubules, immature glomeruli and stroma present in the

post-natal kidney. This lesion is usually found in the subcapsular region of the kidney (Potter 1961; Bove, Koffler & McAdams 1969; Bove & McAdams 1976). The histological appearance, especially of immature tubules and glomeruli, suggests some overlap of this lesion with both metanephric hamartoma and glomerular immaturity (Bove & McAdams 1976).

Although most commonly found in the subcapsular region, areas of renal blastema, often multifocal, have been found at the cortico-medullary junction of kidneys from some patients with nephroblastoma (Machin & McGauchey 1984). Histologically this lesion, intralobar nephroblastomatosis, also consists of blastema and immature metanephric components, but shows some differences from the more commonly described superficial nodular renal blastema. The intralobar lesion shows more mitotic activity, is more infiltrative, and shows evidence of mesenchymal heteroplasia with the formation of cartilage and skeletal muscle. It also appears to be associated with a different group of genetic and teratological syndromes, and with a different type of nephroblastoma, namely the fetal

rhabdomyomatous type (Wigger 1976; Gonzalez-Crussi, Hsuch & Ugarte 1981; Mahoney & Saffos 1981). It may also be associated with the subtype of nephroblastoma designated as cystic partially differentiated nephroblastoma (Joshi et al 1979).

In addition to nodular renal blastema and intralobar multifocal nephroblastomatosis, another, probably rarer group of related dysplastic or neoplastic lesions has been described. These lesions are collectively termed nephroblastomatosis (Bove & McAdams 1976). This group of lesions are characterised by renal enlargement, which can be massive, due to metanephric dysgenesis. They are associated with the development of renal neoplasms, especially nephroblastoma. The histological features are the presence of metanephric blastema, abundant immature tubules and glomeruli, without the formation of functioning nephrons (Hou & Holman 1961; Bove & McAdams 1976). There is also nephromegaly due to the nephroblastomatosis.

Bove & McAdams (1976) have divided these lesions into infantile, late infantile, and juvenile, believing each to be a distinct

clinicopathological entity. Others have classified them according to the site and extent of the lesions (Berry 1987). The infantile form, which is the rarest form, is characterised by a pancortical retardation of maturation or dysgenesis affecting most or all of the involved kidney. The kidneys are diffusely enlarged with accentuated fetal lobulation (Hou & Holman 1961). The lesion is associated with extra-renal anomalies (Liban & Kozenitski 1970) but not invariably so (Hou & Holman 1961). A metastasisng nephroblastoma has yet to be described following this form of nephroblastomatosis.

The late infantile form consists of diffuse superficial metanephric dysgenesis, and therefore affects the last generation of metanephric nephrons (Anderson et al 1968). Metastasising nephroblastoma has been described in association with this lesion (Anderson et al 1968).

The juvenile form of nephroblastomatosis is superficial and multifocal. This lesion can present at any age but most children have been over two years at presentation. This form is infrequently associated with other congenital anomalies but the development of a nephroblastoma is common (Bove & McAdams 1976).

Nephroblastomatosis is regarded as a failure of normal maturation but with a proliferative element to the abnormality causing expansion of the immature metanephric tissue resulting in nephromegaly. Although the relationship between dysgenesis and neoplasia in each case is difficult to define, there is a close association between nephroblastomatosis, persistent renal blastema and the subsequent development of a malignant nephroblastoma (Bove & McAdams 1976).

Sclerosing metanephric hamartomas have been described as poorly demarcated lesions, which are extensively collagenised but which may also contain tubular epithelial components. Dilated vessels, presumed to be lymphatics are frequently present. The stromal cells are mostly mature fibrocytes, but plump mesenchymal cells with increased amounts of cytoplasm have also been noted (Liban & Kozenitski 1970; Bove & McAdams 1976; Marsden & Lawler 1983). Smooth muscle cells are sometimes present and the lesion may on occasion be entirely mesenchymal (Marsden & Lawler 1983). In some cases adenomas or lesions described as incipient Wilms' tumours have been described within metanephric hamartomas (Bennington & Beckwith 1975).

Sclerosing metanephric hamartomas are most frequently seen either in the subcapsular area of the kidney or in the junction between the kidney and a nephroblastoma. When present at the kidney-tumour junction they are seen as a poorly demarcated collagenous hamartoma which extends both into the kidney and into the tumour. Marsden & Lawler (1983) recognise intratumour dysplasia of this type as quite a common finding, but stress the difficulty of its recognition at this site.

Metanephric hamartomas are found in the kidneys of up to a third of all cases of nephroblastoma (Bove & McAdams 1976). They are believed to be areas of renal blastema which fail to mature and which subsequently undergo progressive sclerosis. This theory accounts for the presence of tubular components, immature glomeruli and the continuing fibroblastic activity (Liban & Kozenitski 1970; Bove & McAdams 1976; Marsden & Lawler 1983). Although there has been considerable interest in these lesions recently, they were first described by Hexheimer in 1909. He proposed that they were derived from metanephric blastema which, after local failure of normal tubulogenesis had been induced to stromal differentiation. Nicholson (1931) was the first to associate these lesions with the development of a nephroblastoma.

Wilms' tumourlet was also recognised by Bove & McAdams (1976) who arbitrarily defined it as an area of embryonal renal tissue measuring greater than one centimetre in diameter.

The remaining two lesions included in the study by Bove & McAdams are less obviously part of the nephroblastomatosis complex (Marsden & Lawler 1983). These are the glomerular immaturity and the cortical cystic lesions.

Glomerular immaturity is defined as the persistence in post natal life of fetal glomeruli, small glomeruli with prominent fetal type epithelium and inconspicuous capillaries. In the kidneys of most of the 69 cases of nephroblastoma studied by Bove & McAdams (1976) such glomeruli were found, but in 23 cases the lesions were multifocal and in 18 extensive. The fetal glomeruli are surrounded by normal tubules and there is no evidence of an inflammatory lesion, but immaturity is invariably accompanied by glomerulosclerosis. Sclerotic glomeruli alone, without evidence of immaturity, are a frequent finding in the kidneys during childhood (Potter 1972) and should be distinguished from glomerular immaturity.

Focal cortical cysts were present in 6 of 69 kidneys removed for nephroblastoma (Bove & McAdams 1976). In three of these the cysts were clearly associated with areas of malformed renal cortex. Cysts have been previously associated with nephroblastoma (Uson, Del Rosario & Melicow 1960; Potter 1961). A cystic partially differentiated form of nephroblastoma has also been recently described (Brown 1975; Joshi et al 1979).

All of these lesions are associated with the development of nephroblastoma has been called the nephroblastomatosis complex by Bove & McAdams (1976) and nephroblastic dysplasia by Marsden & Lawler (1983). That it is associated with nephroblastoma seems conclusive but the precise relationship is less clear. Both groups of authors have addressed this issue. The nephroblastomatosis or dysplasia lesions seem to be a pre-existing abnormality, thus supporting the hypothesis of Cohnheim (1875) and Nicholson (1931) that nephroblastomas develop from embryonic rests. Both the nephroblastomatosis/dysplasia group and a variety of other congenital anomalies may be associated with the subsequent development of a nephroblastoma (Bennington & Beckwith 1975; Marsden & Lawler 1983; Olsen 1984).

Genetic abnormalities and nephroblastoma.

Recent advances in the molecular biology of tumours may help explain the link between the nephroblastomatosis complex and the development of a nephroblastoma. Knudson & Strong (1972) studied the epidemiology of nephroblastoma by comparing data from familial, bilateral, unilateral and from unselected cases. They found, among other things, that familial cases and bilateral cases presented earlier, and that familial cases were more frequently bilateral. The overall epidemiology of familial cases was characteristic of that of an autosomal dominant pattern of inheritance with partial penetrance. The findings suggested, as had been previously proposed for retinoblastoma (Knudson 1971), that a two mutational event was involved. In familial cases the first mutation was present in the germ line in the form of a dominant but abnormal allele; the second mutation occurred in the somatic cells of the kidney. Sporadic cases must develop both mutations in the somatic cells. The association of both familial and sporadic cases with other congenital anomalies, notably aniridia, hemihypertrophy and genito-urinary anomalies, suggests that one of the mutations may affect the development of other organs. In these

cases the mutant gene must be active during embryonic life either as a germ line form or as an early somatic mutation. In cases of nephroblastoma not associated with other anomalies the somatic mutation of this gene must arise later and only in the cells of the metanephric blastema.

Later work using the newly developed techniques of high resolution banding cytogenetics demonstrated the loss of chromosomal fragments during the development of retinoblastoma (Murphee & Benedict 1984). Their cytogenetic and epidemiology data suggested that there existed a pair of suppressor alleles located in chromosome region 13q14 (Murphee & Benedict 1984; Sparkes et al 1983). Loss of this segment from one chromosome and either a germ line or somatic mutation of the other copy was suggested to account for the development of retinoblastoma by a two-mutational model. It was proposed that the normal alleles suppressed the proliferation of the embryonic retinoblasts as differentiation occurred, hence suppressing tumour development.

Cytogenetic studies of familial and sporadic cases of nephroblastoma have also now been performed, including the analysis of associated

anomalies especially aniridia and hemihypertrophy (Riccardi et al 1978; Riccardi et al 1980; Fearon, Vogelstein & Feinberg 1984; Koufos et al 1984; Orkin, Goldina & Sallon 1984; Reeve et al 1984). These studies have shown that in many cases of nephroblastoma there is loss of chromosomal material from the short arm of chromosome 11 in the 11p13 region. This loss may be present in the germ line in cases with multiple anomalies (Riccardi et al 1978; Riccardi et al 1980) or in the tumour cells alone, presumably acquired as a somatic event in sporadic cases (Fearon, Vogelstein & Feinberg 1984; Koufos et al 1984; Orkin, Goldina & Sallon 1984; Reeve et al 1984). This situation has been compared to that described for retinoblastoma and the hypothesis forwarded that suppressor alleles on chromosome region 11p13 contain genes which regulate either differentiation or proliferation. Loss or alteration of both of these alleles causes the development of nephroblastoma. The two step mechanism operates by affecting both alleles in turn either because of germ line or somatic mutations (Klein & Klein 1985).

Histology.

From the original descriptions the complexity of the histological apppearances of this tumour, consisting of blastema, tubules, glomeruli, and mesenchyme including heteroplastic tissue, has been recognised (Cohnheim 1875; Muus 1899; Busse 1899; Wilms 1899). The histological classification and grading of nephroblastoma has therefore presented great difficulties. There have been many attempts at classifications none of which is entirely satisfactory, either as clinically useful or as easily reproducible (Hardwick & Stowens 1961; Perez et al 1973; Lemerle et al 1976; Lawler et al 1977). These classifications have relied on either the degree of differentiation, the type of differentiation or on both these factors. Most authors, using their own classification, have concluded that the better differentiated tumours, regardless of whether epithelial or mesenchymal differentiation was present, have a better prognosis. Lemerle et al (1976), concentrating on the tumour epithelium, demonstrated that well differentiated epithelium within the tumour was of major prognostic importance. This classification was based on the examination of the cytological appearance of the epithelial cells to determine

the degree of differentiation. Evidence from the Manchester Children°s Tumour Registry (Lawler, Marsden and Palmer 1975), confirmed during the larger Medical Research Council Nephroblastoma Trial (Lawler, Marsden & Palmer 1977), showed that the abundance of epithelial tissue in nephroblastoma was one of the most significant features in the histological assessment. Chatten (1976) in a study of 95 cases of nephroblastoma supported this view, and most authorities would now concur (Olsen 1984).

In addition to the assessment of the volume of epithelial tissue present in the tumours several different patterns of epithelial differentiation have been recognised (Lawler et al 1975, 1977; Delemarre et al 1977). Nephroblastoma may contain tubules, pseudoglomeruli, cysts, or papillary structures. The International Society of Paediatric Oncology (SIOP) classification also recognises rosettes as a separate entity (Delemarre et al 1977).

Beckwith & Palmer (1978) have identified a feature, which although uncommon, is associated with a poor prognosis in nephroblastoma. They have called this feature anaplasia and have defined it

histologically according to the nuclear morphology of the tumour cells. Anaplasia may be present in epithelial, stromal or blastemal elements and it may be focal.

Study of the histological differentiation of nephroblastomas and the clinical features of different types has led to the recognition of distinct subtypes of nephroblastoma, some of which are now regarded as separate tumours (Olsen 1984; Gonzalez-Crussi 1984). Beckwith & Palmer (1978) have identified poorly differentiated sarcomatous types of nephroblastoma which have a poor prognosis. These have three different patterns namely rhabdomyomatous, clear cell and hyalinised.

Striated muscle is present in up to 20% of all nephroblastomas (Tremblay 1971; Lemerle et al 1976), but if the major component is otherwise a sarcomatous tumour then Beckwith & Palmer have defined it as a rhabdomyomatous type of nephroblastoma. In some tumours the amount of differentiated skeletal muscle or of rhabdomyoblasts may be so marked that the tumour is called a fetal rhabdomyoblastomatous nephroblastoma (Wigger 1976; Harms et al 1980; Mahoney & Saffos 1981). A separate malignant rhabdoid renal tumour of childhood has also been recognised (Beckwith 1974).

During a review of the metastatic pattern of nephroblastoma Marsden et al (1978) identified a histologically distinct group of tumours which had a marked preponderance to develop bone metastases, a rare event in a typical nephroblastoma. This tumour type has been designated bone metastasising renal tumour of childhood (BMRTC) by Marsden et al (1978), independently called undifferentiated sarcoma of the kidney by Morgan & Kidd (1978) and is probably identical to the clear cell sarcomatous nephroblastoma of Beckwith & Palmer (1978). Clearly there is confusion concerning the terminology of these sarcomatous tumours, which some authorities (Kumar et al 1986) now consider to be distinct tumours with a histogenesis different from that of nephroblastoma.

At the other end of the clinical spectrum, amongst the good prognosis tumours, Bolande, Brough & Izant (1967) identified a distinct childhood renal tumour, usually occurring in the newborn or in the first year of life. This tumour was composed histologically of uniform spindle cells, with minimal pleomorphism, arranged in interlacing bundles. This tumour has been called congenital mesoblastic nephroma (Bolande, Brough &

Izant 1967), but the same lesion is also called the leiomyomatous hamartoma of the kidney (Bogdian et al 1973), fetal mesenchymal hamartoma (Wigger 1969), and fibromyomatous hamartoma (Favara, Johnson & Ito 1968). Again, this tumour is now considered to have a histogenesis different from nephroblastoma (Wigger 1975). Congenital mesoblastic nephroma can metastasize (Fu & Kay 1973; Walker & Richard 1973; Gonzalez-Crussi, Sotelo-Avila & Kidd 1980), but the overall prognosis is good (Beckwith 1974).

Atypical forms of congenital mesoblastic nephroma have been described (Gonzalez-Crussi, Sotela-Avila & Kidd 1980; Joshi, Kasznica & Walters 1986). These have increased cellularity and there is nuclear atypia and a high mitotic rate. Metastases are more commonly seen from this variant.

Joshi et al (1979) have identified a partially differentiated cystic form of nephroblastoma consisting of large cysts with intervening mesenchyme which includes blastematous areas. They have separated this tumour from other nephroblastomas because of its better prognosis but, unlike multilocular cystic nephroma which they believe to be a developmental anomaly, it is nevertheless neoplastic.

There are, therefore, a complex group of interrelated renal neoplasms of childhood which are now recognised. A summary of current classifications is given in Table 1.

Cell differentiation in nephroblastoma.

Nephroblastoma has been shown to resemble the nephrogenic zone of fetal kidney morphologically (Busse 1899; Muus 1899; Wilms 1899), and as discussed in above it has been suggested that the tumour arises from the metanephric blastemal cells (Bennington & Beckwith 1975).

In addition to these and other histological studies there have been studies of differentiation in nephroblastoma using different techniques. Ultrastructural studies, which have concentrated on the epithelial and glomerular elements, have shown that there is a great degree of similarity between the tumour cells and the corresponding cells in the fetal kidney (Ito & Johnson 1969; Tannenbaum 1971).

Enzyme histochemical studies have also emphasised the similarities between nephroblastoma and the nephrogenic zone of fetal kidney (Imbert & Nezelof 1972).

Rousseau-Merck et al (1977) have modified the transfilter induction method of in vitro tissue culture to study tubular differentiation in nephroblastoma. They have shown that it is possible to induce tubular differentiation from nephroblastomas using this method, but the extent to which the cells differentiate is limited. There have been a few other studies using in vitro culture methods to study differentiation in both animal and human nephroblastomas (Ellison, Ambrose & Easty 1969; Rousseau, Nabarra & Nezelof 1974).

Other research on the differentiation of nephroblastoma has utilized animal models of the tumour. Although embryonal nephromas are common tumours of farm animals, particularly the pig, these tumours do not have the same clinical behaviour as human nephroblastomas (Feldman 1928; Anderson & Sandison 1972). Spontaneously appearing related tumours have been described in a variety of animal species (Reviewed by Lucke & Schlumberger 1955). Experimental induction of nephroblastoma has been achieved but the results have been inconsistent (Riopelle & Jasmin 1969; Jasmin & Riopelle 1970). The most succesful agents in this respect have been derivatives of N-nitroso compounds (Riopelle & Jasmin 1969).

There have been a few immunocytochemical studies of differentiation in nephroblastoma including those of Linder (1969), Wallace & Nairn (1972) and Ramaekers et al (1985). As with most other studies in nephroblastoma these have been directed towards studying the epithelial component. Some authors have attempted to identify, with varying degrees of success, molecules which are specific for nephroblastoma and which are not found in fetal or adult kidney (Burtin & Gendron 1973; Wise et al 1975; Burtin 1976; Waghe & Kumar 1977; Kumar, Marsden & Kumar 1980). The significance of these molecules to the different stages in the development of a nephroblastoma is unknown.

1.4.2 Renal Cell Carcinoma.

The histogenesis of renal cell carcinoma.

There has been as much debate concerning the histogenesis of renal cell carcinoma as that of nephroblastoma. Grawitz (1883) following on Cohnheim°s embryonic cell rest theory of the origin of tumours, and having observed cases of intrarenal heterotopic adrenal tissue proposed that this, the most common of malignant adult renal neoplasms was derived from adrenal rests. The histological similarities between the tumours and adrenal tissue gave support to this hypothesis.

In 1893 Sudeck proposed that these tumours developed from the renal tubules. This theory gained support from many pathlogists but the argument continued for seventy years (Bennington & Kradjan 1967). Oberling, Riviere & Haguenau (1960) clearly demonstrated ultrastructural similarities between renal tubular epithelial cells and the cells of renal cell carcinoma. Their ultrastructural observations have been confirmed and extended by others (Seljelid & Ericsson 1965;

Ericsson, Seljelid & Orenius 1966; Fisher & Horvat 1972; Pratt-Thomas et al 1973). Enzyme histochemical studies have shown enzymes common to both renal tubular epithelium and to renal cell carcinoma, and quite different from those seen in the adrenal cortical cells (Braunstein & Adelman 1966). Immunohistological studies performed by Wallace & Nairn (1972) demonstrated renal brush border antigen on the surface of renal cell carcinoma cells.

The weight of evidence has effectively rejected the hypothesis of renal cell carcinoma originating from adrenal rests, but the terms Grawitz tumour and hypernephroma persist in both the literature and in clinical practice. The Armed Forces Institute of Pathology prefer the term renal adenocarcinoma because of the tumour's derivation from the secretory and absorptive epithelium of the renal tubules (Bennington & Beckwith 1975). Many other authors now use the term renal cell carcinoma (Olsen 1984), and it is this term which I will use to designate this tumour in this thesis.

Although the histogenesis of renal cell carcinoma from renal tubular epithelium is now established, the aetiology and pathogenesis of the tumour is not known. A number of factors including chemical carcinogens (Bennington & Kradjan 1967), tobacco smoking (Bennington & Laubschier 1968; Wynder, Kyiohiko & Whitmore 1974), sex hormones (Kirkman 1959; Guerin et al 1969) and viral agents (Guerin et al 1969) have all been shown to cause renal cell carcinomas in experimental animals or to be associated with the development of human cases of renal cell carcinomas.

Related renal lesions.

A relatively frequent finding in kidneys removed for renal cell carcinoma is the presence elsewhere in the kidney of small tumours histologically similar to renal cell carcinoma but measuring less than three centimetres in diameter. These small tumours have created difficulties in definition and terminology (Olsen 1984). Newcomb (1930) and Apitz (1943) found these small tumours in the kidneys of 7% of adult autopsies. Later studies by Reese & Winstanley (1958) and Xipell

(1971) suggested figures of 14% and 22% respectively. To standardise terminology Bell (1950) proposed that tumours of this type which were under three centimetres in diameter were called adenomas, while those which were larger than three centimetres were called carcinomas. This approach provides useful therapeutic and prognostic guidelines for the clinician but has been criticised as arbitrary, and founded on no biological basis (Bennington & Kradjan 1967; Bennington & Beckwith 1975; Bennington 1980). Although these small tumours have a better prognosis than larger ones, metastases from tumours less than three centimetres have been described including two in Bell°s own series (Bell 1950; Talame & Shonnard 1980).

Adenomas occur more frequently in patients with renal cell carcinomas (Cristol, McDonald & Emmett 1946), in elderly patients (Cristol et al 1946; Xipell 1971), in tobacco smokers (Bennington & Laubscher 1968; Bennington, Ferguson & Campbell 1968) and in males (Cristol et al 1946). In short, they seem to exhibit many of the epidemiological features of renal cell carcinoma. This has caused further controversy as to whether these tumours

are small carcinomas or whether they are adenomas, only some of which may become malignant (Stoerk 1908; Nicholson 1909; Newcomb 1936; Kozell & Kirschbaum 1940; De Veer & Haam 1950; Hicks 1954; Long, Utz & Docherty 1966), in a similar manner to the adenoma-carcinoma sequence of carcinogenesis of the colon and rectum (Morson 1978).

As early as 1908 Greene & Brooks suggested that these small tumours should be considered as carcinomas. This view has been supported by many authors for a variety of reasons. Clearly at some point in its development a carcinoma must be less than three centimetres in diameter, and both morphologically (Oberling, Riviere & Haguenau 1960; Seljelid & Ericsson 1965; Fisher & Horvat 1972; Pratt-Thomas et al 1973) and immunocytochemically (Wallace & Nairn 1972) the two lesions are identical. Their frequent co-existence and the finding of common aetiological factors have been forwarded as an argument supporting the belief that they are different stages of the same lesion (Cristol et al 1946; Xipell 1971).

Willis (1968) has suggested that renal cell carcinomas develop from hyperplastic tubules in nephrosclerosis or in renal cystic disease. Renal tumours are found more frequently in end stage chronic renal disease, where cysts and hyperplastic epithelium are also seen (Hughson, Henningar & McManus 1980). This view has been denied by Bennington & Kradjan (1967) because of the lack of any association between tumours and cystic disease or nephrosclerosis in their series. Recent epidemiological and pathological studies have suggested that there is a definite association between renal cystic disease and the subsequent development of a renal cell carcinoma (Hughson, Buchwald & Fox 1986).

Histological classification of renal cell carcinoma.

Histological classifications have proved difficult to formulate and unreliable in clinical usage. Renal cell carcinoma may consist of four different cell types - clear cell, granular cell, spindle cell and basophilic cell (Olsen 1984). Different stromal patterns may be seen in

different cases of renal cell carcinoma. The cells of these tumours may be arranged in several different architectural patterns, namely solid (either alveolar or trabecular), cystic, papillary or tubular. In many cases more than one cell type and more that one architectural pattern are present in the same tumour (Mostofi 1967; Bennington & Beckwith 1975; Olsen 1984). This variation in histological appearances has created the major difficulty in formulating a satisfactory classification.

Several histological grading systems have been developed which are independent of either the cell type or architecture, but rather are based on the degree of cellular differentiation (Hand & Brothers 1932; Arner, Blanek & Van Schreeb 1965) or on nuclear morphology (Skinner et al 1971). Both of these types of grading systems were shown to have prognostic significance, but have not found widespread use in surgical pathology practice, because of the arbitrary nature of the classification criteria and poor reproducibility (Mostofi 1967; Bennington & Beckwith 1975).

Despite the difficulties in formulating a completely satisfactory histological classification, it has been recognised that some types of renal cell carcinoma exhibit different biological behaviour.

Klein & Valensi (1976) reviewed their experience of renal cell carcinomas which were associated with a prolonged disease free survival and identified thirteen cases of a tumour which they believed to be distinct from renal cell carcinoma. These tumours were all stage I at presentation and during follow-up had not developed metastases. Histologically they were composed entirely of uniform cuboidal cells with small regular nuclei, in which pleomorphism and mitoses were not seen. The cytoplasm was strongly eosinophilic and granular. They called these tumours oncocytic adenomas or oncocytoma because of the similarity between these tumours and oncocytic tumours elsewhere in the body (Hamperl 1962).

The first cases of renal oncocytomas described were probably those of Apitz (1943) and of Xipell (1947) who described well differentiated granular cell variants of renal carcinoma. Several series of renal oncocytoma have now been collected (Yu et al 1980; Chaudray et al 1979; Morales et al 1980 ; Harrison et al 1980) the largest of which is that of Lieber, Tomera & Farrow (1981). The great majority have been associated with a good outcome,

although a few cases have developed metastases (Lieber, Tomera & Farrow 1981; Lewi, Alexander & Fleming 1986). Rainwater et al (1986) have performed clinical studies and DNA flow cytometry investigations of renal oncocytomas and have concluded that this tumour should be regarded as a renal neoplasm of low grade malignant potential

There has been a suggestion that renal oncocytoma may have been increasing in incidence since the 1970s although this has yet to be proven (Klein & Valensi 1976; Akhtar & Kott 1979).

Oncocytomas have a characteristic macroscopic appearance. They are circumscribed, a uniform tan colour and frequently have a stellate central scar (Olsen 1984). The granular appearance to the cytoplasm is due to the presence of large numbers of mitochondria which are visible on electron microscopy (Klein & Valensi 1976; Chaudray et al 1979; Johnson et al 1979). It has been argued, because of these appearances, that oncocytoma is derived exclusively from the proximal tubular epithelium (Klein & Valensi 1976; Yu et al 1980) but this has been disputed (Holthofer 1987; Zerban et al 1987). Although containing numerous mitochondria the tumour cells lack some of the

surface specialisations and basal infoldings of the proximal tubular epithelium. Furthermore oncocytic change occurs in other carcinomas, in nephroblastomas and in tumours in other sites (Hamperl 1962; Bennington & Beckwith 1975). It has been argued that the oncocytic appearance does not reflect the histogenesis, but rather a specific functional feature or form of degenerative change (Hamperl 1962; Olsen 1984; Ulrich et al 1985).

In addition to oncocytic tumours being associated with a better prognosis, Mancilla-Jimenez, Stanley & Blath (1976) have shown that renal cell carcinomas which have an exclusively papillary architecture are also associated with a better prognosis, although again metastases may occur.

At the other end of the histological and clinical spectrum Farrow et al (1968a,b,c) described 37 cases, from a series of 2,386 primary renal malignancies seen at the Mayo Clinic over 60 years, which had sarcomatoid features. These tumours had a combined histological appearance with epithelial areas and pleomorphic spindle cell areas. Transitions between the two types were seen. These tumours have been called sarcomatoid renal carcinomas.

Different histological patterns have been seen in the spindle cell areas; rhabdomyoblastic, fibroblastic, fibrohistiocytic or leiomyomatous (Farrow et al 1968c). Tomera et al (1983) have classified sarcomatoid renal carcinomas by separately identifying the epithelial and mesenchymal elements by the morphological criteria used to classify renal cell carcinoma cell types and those used to classify soft tissue tumours elsewhere in the body. Regardless of classification these tumours have invariably behaved in an aggressive manner with survival figures much lower than those of other types of renal cell carcinoma (Farrow et al 1968).

In addition to sarcomatoid renal carcinomas, in which epithelial and spindle cell areas are seen, numerous cases of primary renal sarcomas have been described (Farrow et al 1968; Olsen 1984). These tumours have been classified as for soft tissue tumours in other sites (Enzinger & Weiss 1983). In early series of spindle cell tumours of the kidney fibrosarcoma was reported as the most frequent histological type (Mintz 1937). With the additional aid of electron microscopy it has become clear that leiomyosarcoma is the most common primary malignant soft tissue tumour of the kidney (Tomera, Farrow & Lieber 1983).

Although these malignant mesenchymal tumours remain relatively rare, benign mesenchymal tumours of the kidney are a frequent finding in many large autopsy series (Apitz 1943; Reese & Winstanley 1958; Xipell 1971). Indeed almost the whole spectrum of benign and malignant soft tissue tumours have been described in the kidney, although many are single case reports (Mintz 1937; Farrow et al 1968; Edwards et al 1962; Black & Heinemann 1955; Price & Mostofi 1965; Jardin et al 1980; Alldred, Cathay & McDivitt 1981; Hajdu & Foote 1969; Barrileo 1977; Fein & Harn 1965; Ragharaidh et al 1980; Bennington & Beckwith 1975).

The precise tissue of origin of many of these tumours is not known for certain but, because of the presence of interstitial connective tissue, and vascular components within the kidney it has been assumed that this is the source of the majority of soft tissue tumours (Farrow et al 1968). The biological importance of that assumption is that the interstitial and connective tissue of the kidney is derived from the same tissue as the epithelial component, namely the primitive metanephric blastema. The exception is the vascular endothelium which is now known to be derived from an outgrowth of the embryonic dorsal aorta (Sariola 1985).

Cell differentiation in adult renal tumours.

As discussed above, for many years controversy surrounded the interpretation of the histological assessment of differentiation of renal cell carcinoma. Ultrastructural studies have contributed to the study of renal cell carcinoma, particularly in establishing that the origin of these tumours was indeed renal and not from the adrenal cortex (Oberling et al 1960). Such conclusions were supported by enzyme histochemical studies, which in addition, demonstrated some heterogeneity of differentiation both between different cases and between individual cells in most cases (Braunstein & Adelman 1966). In this study it was suggested that although most of the neoplastic cells showed a proximal tubular type of enzyme profile there were cells which had a distal tubular phenotype.

Experimentally induced tumours in animals have also been used to study differentiation in renal cell carcinomas. N-nitroso compounds have been used to induce nephroblastomas but, in addition, in adult animals these substances can induce epithelial tumours (Hard & Butler 1970, 1971).

These tumours are histologically similar to human renal cell carcinomas, although they metastasise less frequently (McGiven & Ireton 1972). Analysis of differentiation has been based primarily on ultrastructural studies and has emphasised the similarities between the neoplastic cells and proximal tubular epithelium (Hard & Butler 1971; McGiven & Ireton 1972; Dees et al 1976; Bannasch et al 1980). Some groups have also used enzyme histochemistry to study the differentiation of these experimentally induced tumours (Tsuda et al 1985; Tsuda et al 1986).

The use of animal models of this type has also enabled examination of the kidneys at different stages during the development of tumours. In this way dysplastic and preneoplastic lesions have been identified (Bannasch et al 1978, 1980; Tsuda et al 1986).

Immunocytochemistry has been used previously to study differentiation in renal cell carcinoma (Wallace & Nairn 1972; Ueda et al 1981; Holthofer et al 1983; Finstad et al 1985; Vassella et al 1985; Oosterwijk et al 1986a). These studies have particularly investigated the expression of proximal and distal tubular antigens in order to

confirm the proximal tubular differentiation in renal cell carcinoma. There have been some recent studies which have used monoclonal antibodies to study renal cell carcinomas. Some groups have raised monoclonal antibodies specifically to renal cell carcinomas and have identified antigens present in renal cell carcinomas which were not found in normal renal cortex nor in other tumours (Moon et al 1985; Scharfe et al 1985; Oosterwijk et al 1986b). They have suggested that the corresponding molecules may be important in the malignant transformation of the tubular epithelium. Wahlstrom et al (1985) have shown that polyvalent antisera against retroviral products and a monoclonal antibody to HTLV-1 p19 gag react with renal cell carcinoma cells but not with normal adult kidney. Other groups have used monoclonal antibodies raised against other tissues, particularly lymphoid tissue, which fortuitously cross-react with components of the kidney and with renal cell carcinomas (Borowitz et al 1985, 1986). These monoclonal antibody studies are at an early stage since many of the molecules with which they react have not been defined and their functional significance remains unknown.

Many of these immunocytochemical studies have, therefore, used the approach of making monoclonal antibodies, studying the distribution of their binding sites in normal and neoplastic kidney cells and then have analysed the nature and biological signifance of the corresponding antigen. I have used antibodies which react with defined molecules and have studied the distribution of these in renal tumours. The normal function of many of these molecules is known and their distribution in normal and developing kidney is described in Chapter 3.

1.5 Antigens.

The distribution of 23 different antigens has been investigated in this study (Table 1). The antigens have been detected using different types of antibody, monoclonal or polyclonal, and visualised by different immunocytochemical methods.

1.5.1 Intermediate Filament Proteins.

Intermediate filaments are filamentous intracytoplasmic structures composed of a heterogeneous group of proteins and are widely distributed throughout the cells of the body. They probably function as part of the cytoskeleton (Lazarides 1982), and are known to be associated in the cytoplasm with microtubules, the nuclear membrane (Osborn *et al* 1985), desmosomes (Franke *et al* 1982) and contractile proteins (Granger & Lazarides 1978; Wang 1982; Lawson 1983; Price & Lazarides 1983).

Biochemical and immunochemical methods have identified five different families of intermediate filament proteins: (cyto)keratins, vimentin, desmin, neurofilament protein and glial fibrillary acidic protein (Lazarides 1982). Each of these has a characteristic tissue distribution.

In this study I have investigated three of these types of intermediate filament proteins.

Cytokeratins.

Cytokeratins are the intermediate filament protein type characteristic of epithelial cells (Bennett et al 1978; Franke et al 1978). They are insoluble in high salt buffer and in non-ionic detergents (Sun & Green 1978; Fuchs & Green 1978). Extraction of cytokeratins from epithelial cells by these methods, and the subsequent examination of the proteins by 2-dimensional electrophoresis has identified 19 different human cytokeratins each of which shows a characteristic tissue distribution (Moll et al 1982).

Antibodies to different cytokeratins have been raised and their tissue distribution studied using immunocytochemistry (Sun, Shih & Green 1979; Altmannsberger et al 1982; Debus, Weber & Osborn 1982). Early studies on cytokeratin distribution in the kidney, using antibodies to large molecular weight epidermal prekeratins, demonstrated cytokeratin immunoreactivity confined to the epithelial cells of the urothelium and collecting

ducts (Sun, Shih & Green 1979). However, further studies using antibodies to cytokeratin preparations from internal epithelia have demonstrated some cytokeratins in renal tubular epithelial cells (Debus, Weber & Osborn 1982; Holthofer et al 1984). Cytokeratins have not been identified in the glomerular epithelium.

Vimentin.

Vimentin is the intermediate filament protein found in the majority of mesenchymal tissues (Lazarides 1982). It is also found in a variety of embryonic tissues at different stages of their development (Ramaekers et al 1985). It has been described in several cell types in fetal and adult kidney and in renal tumours (Holthofer et al 1984).

Desmin.

Desmin is the intermediate filament protein type found in contractile tissues. It is present in the cells of smooth muscle, cardiac muscle and striated muscle. During embryogenesis it appears at an early stage in the differentiation of muscle cells (Denk et al 1983).

1.5.2 Cell surface antigens.

Epithelial Membrane Antigen.

Epithelial membrane antigen (EMA) is purified from defatted human cream (Ceriami *et al* 1977) and is present on the apical membrane of breast epithelial cells (Heyderman, Steel & Ormerod 1979; Sloane & Ormerod 1981). Using immunocytochemical techniques and an antiserum raised against this antigen the tissue distribution of EMA has been studied (Heyderman *et al* 1979; Sloane & Ormerod 1981). EMA appears to be a differentiation antigen expressed on the membranes of cells which have secretory functions. It is found on a variety of adenocarcinomas and has proved to be a useful marker of epithelial differentiation in surgical pathology practice (Sloane & Ormerod 1981). This diagnostic use of the antigen is enhanced by the fact that it survives routine fixation and embedding procedures.

In the kidney it has been suggested that its expression may be confined to the distal tubule and collecting duct epithelium, while it is absent from glomerular and proximal tubular epithelium (Heyderman *et al* 1979; Sloane & Ormerod 1981).

Brush Border Antigen.

Wallace & Nairn (1962) extracted a crude antigen preparation from the human renal cortex and used this preparation to immunise rabbits. The polyclonal antiserum formed was shown in immunofluorescence studies to identify an antigen on the proximal tubular epithelium apical brush border. The antigen was thereafter designated as brush border antigen (BB). Several other authors have raised anti-BB antisera using similar procedures and have demonstrated a similar tissue distribution (Edgington et al 1967; Linder 1969; Ekblom, Miettinen & Saxen 1980).

Edgington et al (1967) analysed several anti-BB antisera by immunochemical and biochemical methods. They demonstrated several glycoproteins and glycolipids in their antigen extracts which reacted with the antisera. Most of the BB antigens, therefore, are defined by the reaction of polyclonal antisera with crude antigen extracts. However, these antisera have been useful in demonstrating proximal tubular differentiation during embryogenesis (Ekblom et al 1980) and in renal tumours (Wallace & Nairn 1972; Holthofer et al 1983).

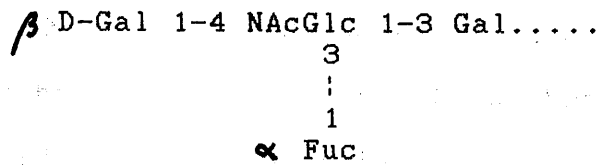
Fucosylated Type II Blood Group Substances.

There has been interest in the expression of the terminal oligosaccharides of cell surface glycoconjugates in embryology and in tumours (Jacob 1979; Solter & Knowles 1979; Wiley 1979; Huang et al 1983; Feizi 1984). Monoclonal antibodies to defined oligosaccharide antigenic determinants have proved particularly useful in these investigations (Feizi 1984).

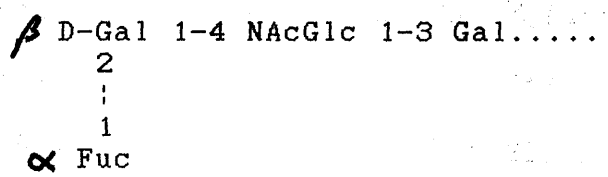
Among the carbohydrates which have been shown to be expressed at defined stages during early embryogenesis are a group of molecules having the common structure of the type II blood group substances (Gooi et al 1981; Kannagi et al 1983; Shevinsky et al 1982). The type II blood group substances are branched or unbranched polymeric forms of N-acetyl lactosamine which have the structure :

$$\beta\text{-D-Gal 1-4 }\beta\text{-D-N Ac Glc - ...}$$

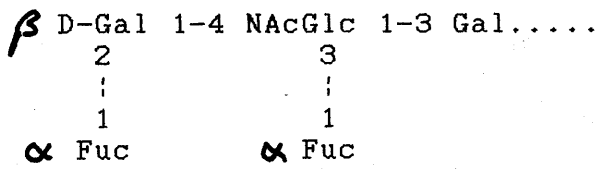
The most important of these carbohydrate antigens, those which have been studied during this project, are fucosylated derivatives of this core molecule. The structures of these various determinants are given in Figure 1.



X-hapten, LeX, SSEA-1



H type II antigen



C 14 antigen

FIGURE 1. STRUCTURES OF THE OLIGOSACCHARIDE ANTIGENS.

In some tissues these epitopes may be masked by terminal sialic acid or α-galactosyl residues (Pennington et al 1986). Exposure of these cryptic sites was achieved by predigestion of the tissue sections by neuraminidase and α-galactosidase.

These α-fucosylated N-acetyl lactosamine epitopes have been found on both membrane glycolipids and glycoproteins in several adult and fetal tissues in a variety of species. Several monoclonal antibodies recognising α-fucosylated N-acetyl lactosamine determinants have been described (Gooi et al 1981; Huang et al 1983). Using different techniques, these determinants have been found on pre-implantation and early post-implantation murine embryos and teratocarcinoma cells in culture (Fox et al 1981; Kapadia et al 1981), human granulocytes and their precursors (Fisher et al 1982; Schienle Stein & Muller-Rucholtz 1982) and in a variety of human and murine normal epithelial and carcinoma cells (Brockhaus et al 1983; Combs et al 1984).

1.5.3 Cytoplasmic proteins.

Proteinase inhibitors.

The proteinase inhibitors alpha-1-antitrypsin and alpha-1-antichymotrypsin are plasma glycoproteins the function of which is to regulate the enzymatic activity of various plasma and tissue proteases (Gans & Tan 1967). They are synthesised by a variety of cells including macrophages (Isaacson et al 1981), tissue histiocytes (Du Boulay 1984) and hepatocytes (Luberman 1983). Deficiency of alpha-1-antitrypsin is associated with pulmonary emphysema and with hepatic cirrhosis (Luberman 1983).

Antibodies have been raised to these proteinase inhibitors and used in immunocytochemical investigations. They have been used as markers in the diagnosis and classification of lymphoreticular neoplasms (Isaacson et al 1981). Proteinase inhibitors have also been described in various non-lymphoid neoplasms including hepatocellular carcinoma (Palmer, Ucci & Wolfe 1980), endodermal sinus tumours (Palmer, Safaii & Wolfe 1976), mixed mesodermal tumours of the ovary (Dictor 1982), gastric carcinoids (Ray et al 1982) and carcinoma of the lung (Silva et al 1984).

They have been detected in connective tissues and soft tissue tumours (Du Boulay 1984). Du Boulay also made the incidental observation that proteinase inhibitors were present in the renal parenchyma.

Carbonic Anhydrase.

Carbonic anhydrase (carbonate dehydratase EC 4.2.1.1) is an enzyme which catalyzes the reaction:

$$H_2O + CO_2 \rightarrow H_2CO_3$$

The enzyme has been identified in erythrocytes, oligodendroglia, and in electrolyte transporting epithelia such as stomach, pancreas, ciliary epithelium and renal tubular epithelium (Maren 1967). Histochemical (Lonnerholm 1973), biochemical (Mattenheimer, Pollak & Muehrcke 1970; Wistrand 1980; Wahlstrand & Wistrand 1980) and immunocytochemical (Spicer, Stoward & Tashian 1979; Brown et al 1982; Brown et al 1983) techniques have demonstrated carbonic anhydrase activity in different parts of the nephron in man

and in rodents. In these various sites the enzyme is thought to be important in the secretion and transport of CO_2, H^+, HCO_3^-, and Cl^- particularly for the efficient function of the urinary acidification process (Warnock & Rector 1981).

Several isoenzymes of carbonic anhydrase have been identified and both membrane bound and cytoplasmic forms exist (Lonnerholm & Wistrand 1984a; Kampulainen 1984). Immunocytochemistry has been shown to provide a more sensitive method of detecting isoenzymes in tissue sections than does enzyme histochemistry (Lonnerholm & Wistrand 1984b).

In this study I have investigated the distribution of carbonic anhydrase isoenzyme C, which is the most important cytoplasmic form found in the human kidney (Kampulainen 1984; Lonnerholm & Wistrand 1984a,b).

S-100 Protein.

S-100 protein was first extracted from nervous tissue (Moore 1965) and was initially thought to be a nervous tissue specific protein (Cicero et al 1970; Dohan et al 1977; Cocchia, Michetti & Donato

1981). It has been shown to be highly conserved in vertebrates (Moore 1972). Its name derives from the observation that it is soluble in 100% ammonium sulphate at neutral pH (Moore 1965). Biochemically S-100 is composed of two similar proteins, S-100a and S-100b, each of which consists of two subunits containing either 93 or 91 amino acids (Isobe & Okuyama 1978; Isobe, Tsuguta & Okuyama 1978; Isobe & Okuyama 1981). Structural studies have shown similarities between S-100 protein and a wide range of calcium binding proteins including calmodulin and troponin-C (Isobe & Okuyama 1978).

Antibodies have been raised to S-100 protein and used in immunocytochemical preparations. Initially these studies were confined to its use in nervous system pathology especially tumours (Dohan et al 1977; Haglid et al 1978; Jacque et al 1979), but later work showed a more widespread distribution of the protein and a greater range of diagnostic uses of its detection in other tissues (Cocchia, Michetti & Donato 1981; Nakajiuma et al 1982a, b, c). Interest has particularly focussed on its use as a marker in the diagnosis or prognosis of melanocytic lesions (Nakajiuma et al

1982a). It has been found in neoplasms of the salivary gland, carcinoid tumours, chordomas and in tumours of Langerhans cells and interdigitating reticulum cells (Nakajiuma 1982c).

Although it has not been found in the kidney it is found in adipose cells and cartilage (Nakajima 1982c). Both of these tissues may be present in renal dysplastic or neoplastic lesions (Ericsson & Ivemark 1958; Risdon 1971). It has been used in this study for this reason.

Myoglobin.

Myoglobin is a single chain polypeptide of molecular weight 16,800 containing a haem group. It is the major oxygen binding protein in the tissues, and its role is the transport and storage of oxygen necessary for cellular aerobic respiration. Large amounts of myoglobin are found in skeletal muscle in all mammals and the protein appears early in development (White, Handler & Smith 1973).

Myoglobin has been identified by both biochemical and by immunocytochemical methods in the cytoplasm of skeletal muscle cells and in

myocardium but not in smooth muscle cells (Kindblom, Seidal & Karlsson 1982; Mukai, Rosai & Halloway 1979). Immunocytochemical techniques using antibodies to myoglobin have been used to study rhabdomyoid differentiation in soft tissue tumours (Kindblom et al 1982; Du Boulay 1985).

Factor VIII Related antigen.

Factor VIII is part of the plasma coagulation cascade and is a complex protein of high molecular weight, possibly greater than 1,000,000, which consists of two separate polypeptide components. These are the clot promoting factor (Factor VIII C) and the Factor VIII related protein (Factor VIII R), which itself can be further separated into the von Willebrand Factor (Factor VIII R:WF) and the precipitating antigen (Factor VIII R antigen) (Austen 1978, 1979).

Factor VIII R antigen, which was studied in this project, was first detected using polyclonal antisera raised in rabbits immunised with human Factor VIII (Bennett, Ratnoff & Levvin 1972). It is biochemically and immunologically distinct from Factor VIII C and from Factor VIII R:WF. (Biggs & Rizza 1984).

Factor VIII R antigen polypeptide has been detected by immunocytochemical methods in endothelial cells and in platelets (Mukai, Rosai & Burgdorf 1980). It is now known that it is synthesised by vascular endothelial cells but not by lymphatic endothelium (Jaffe, Hoyer & Nachman 1973).

Antibodies to Factor VIII R antigen and immunocytochemical techniques have been used to study endothelial cells, both neoplastic and non-neoplastic, in the blood vessels of different tumours (Burgdorf, Mukai & Rosai 1981; Seheter & Hou Jensen 1981; Du Boulay 1985).

Ferritin.

Ferritin is a large iron binding protein with a molecular weight ranging from 430,000 to 900,000 depending on the amount of iron bound (Harrison 1963). It was first isolated from the spleen and liver of a horse and is known to occur in most mammals (Granick 1943). Closely related iron binding molecules have been found throughout the animal kingdom and in some simple plants. The molecule consists of a shell of protein subunits surrounding a core of ferric hydroxyphosphate (Crichton 1971).

Ferritin exists in a variety of molecular forms (isoferritins) which have been isolated from different human organs, each organ having a characteristic isoferritin profile (Powell, Isselbacher & Drysdale 1975). The isoferritins represent populations of hybrid molecules containing dissimilar subunits, tissue specific patterns of isoferritins reflecting the relative properties of different subunit types (Adelman, Arioso & Drysdale 1975).

Although different isoferritins have different molecular weights and isoelectric points most polyclonal antisera react with many different isoferritins (Arioso, Yokerta & Drysdale 1976; Rossiello, Carrero & Giordano 1984).

Characteristic serum ferritin profiles with ferritinaemia have been described in patients with disseminated malignancy (Drysdale et al 1975). These isoferritins are usually acidic and have been identified in several tumour types. Similar isoferritins are found, however, in some normal tissues including heart, liver and kidney (Arioso et al 1976).

Antibodies have been raised to human ferritin and the tissue distribution studied using immunocytochemical techniques (Taylor & Mason 1975). This study (Taylor & Mason 1975) confirmed previous biochemical evidence of the tissue distribution of ferritin (Drysdale et al 1976). In the kidney Taylor & Mason (1975) found ferritin restricted to the proximal convoluted tubules.

1.5.4 Hormones.

Renin.

Renin is a polypeptide hormone (Galen et al 1979a) the mode of action of which is the proteolytic cleavage of a plasma polypeptide substrate (Skeggs et al 1957). It is therefore both a hormone and a proteolytic enzyme. Its major site of synthesis and storage is the granular epithelioid cells of the juxtaglomerular apparatus of the kidney (Goormatigh 1945; Cook 1963; Sutherland 1970; Menard et al 1979; Ryan et al 1982) but it has been identified biochemically and immunocytochemically in other sites (Swales 1979; Thurston et al 1979).

It acts by enzymatic cleavage of its plasma substrate to angiotensin I, this in turn is cleaved by circulating angiotensin converting enzyme to angiotensin II. Angiotensin II is the most potent vasoconstrictor known and one of its main functions is the maintainence of arterial and arteriolar smooth muscle tone and hence of systemic blood pressure (Brown et al 1979; Lindop & Lever 1986). There is some evidence that it may have a role in the local control of circulation, especially in the brain (Swales 1979). The active peptide may also have some effect on the regulation of aldosterone secretion (Brown et al 1979).

There is some evidence that the renin-angiotensin system may function as a locally acting hormone within blood vessel walls in the kidney (Lever & Peart 1962; Cello 1982; Taughner 1982) and in extra renal sites (Malik & Nasjiletti 1976).

Although the precise mechanisms of the regulation of renin synthesis and secretion by the juxtaglomerular apparatus is not known, both the macula densa of the distal tubule and the afferent and efferent arterioles of the glomerulus have

been implicated (Vander 1967; Davis & Freeman 1976). These structures are of course anatomically related to each other and to the epithelioid cells of the juxtaglomerular apparatus.

Antibodies have been raised to renin (Galen et al 1979b; Taughner et al 1982) and the tissue distribution studied by immunocytochemistry (Camilleri et al 1980; Anat et al 1981; Lindop & Downie 1984). These studies have confirmed the presence of renin in the juxtaglomerulaur apparatus and also within the walls of small arteries in the renal cortex (Lindop et al 1983).

Renin secretion by nephroblastomas (Mitchell et al 1970; Masovari, Kontor & Keillay 1972; Ganguy et al 1973; Day & Luetscher 1974) and by renal adenocarcinomas (Lebel et al 1977; Hollifield et al 1975; Leckie et al 1975) has been previously described.

Human Chorionic Gonadotrophin.

Human chorionic gonadotrophin (HCG) is a glycoprotein composed of two non-covalently linked subunits alpha and beta. The alpha subunit shows extensive sequence homology with the alpha subunit

of the posterior pituitary hormones - luteinizing hormone, follicle stimulating hormone and thyroid stimulating hormone (Morgan & Canfield 1971). The beta subunits of these molecules are distinct providing both biological and immunological specificity (Heyderman 1983). HCG is secreted by the normal placenta and can be detected in the urine of pregnant women. Its function during pregnancy is to maintain corpus luteum function until the placenta becomes the major source of steroid hormones, an event which occurs at about 14 weeks gestation.

The monitoring of HCG levels is important in certain diseases of pregnancy, but especially in the monitoring of trophoblastic disease. In addition to being secreted by the placenta, HCG is synthesised by tumour cells in testicular teratoma, especially those containing areas of choriocarcinoma (Parkinson et al 1981).

HCG has been studied in a variety of tumours using immunocytochemistry and this technique has been of use in the classification of testicular tumours (Heyderman 1983). HCG is not found in the normal kidney but has been reported in a renal tumour in which the patient, a 60 year old man, had gynaecomastia and a gastrointestinal upset (Golde et al 1974).

1.5.5 Oncofetal antigens.

Alpha-fetoprotein.

Alpha-fetoprotein (AFP) is a major glycoprotein found in the plasma during embryonic and fetal life, the concentration of which rises progressively during fetal development (Alpert 1976). It is the fetal equivalent of serum albumin, a conclusion which was reached because of structural similarities (Law & Dugaiczyk 1981). Gene sequence homology suggests that evolutionary gene duplication has occurred to produce these two related molecules (Jagodzenski *et al* 1981; Beattie & Dugaiczyk 1982). Detection of serum and amniotic fluid AFP have been used in the antenatal screening and diagnosis of neural tube defects (Brock & Sutcliffe 1972). Serum levels are increased in a variety of diseases including neoplasms of the liver (Alpert, Uriel & De Nechaud 1968; Abelev 1974) neoplasms of germ cells (Alpert 1972; Grigor *et al* 1977), ataxia telangiectasia (Waldmann & McIntyre 1972) and hereditary tyrosinaemia (Belanger 1973).

Carcinoembryonic antigen.

Carcinoembryonic antigen (CEA) is an incompletely defined carbohydrate determinant carried on either glycoproteins or on glycolipids. It was originally described as a specific marker for colorectal carcinoma (Gold & Freedman 1965). The antigen was present in colorectal tumours, demonstrated either biochemically (Gold & Freedman 1965) or immunocytochemically (Heyderman & Neville 1977). CEA is also found in the serum of patients with colorectal carcinoma (Laurence et al 1972). Further work has shown that as a cell marker CEA is less tissue specific than was originally thought, and it has been found in normal tissues including colon, small intestine and breast (Neville & Laurence 1974). It has been identified in carcinomas of the large bowel (Heyderman & Neville 1977), breast (Wahren et al 1978; Walker 1980), testis (Heyderman 1978) and thyroid (Hamada & Hamada 1977).

1.5.6 Antigens associated with cell proliferation.

Nuclear proliferation antigen.

One of the problems associated with the histological assessment of dynamic processes, such as organogenesis, is the static nature of the observations and the difficulties in measuring cell proliferation. Recently an immunocytochemical marker for cell proliferation, the monoclonal antibody Ki 67, has been developed (Gerdes et al 1983). This monoclonal antibody detects a polypeptide antigen which is expressed in cells at all stages of the cell cycle other than G_0 (Gerdes et al 1984). It therefore detects proliferating cells. It has been used previously in immunocytochemical preparations to study the distribution and number of proliferating cells in reactive and in neoplastic processes (Gerdes et al 1983).

Transferrin receptor antigen.

Transferrin acts as a mitogen for many different cell populations (Cohen & Fischbach 1977; Broxmeyer et al 1980; Trowbridge & Omary 1981). This activity involves receptor mediated endocytosis of the iron binding polypeptide (Aisen & Listowsky 1980; Hopkins & Trowbridge 1983). The

receptor for transferrin is a membrane bound glycoprotein, the expression of which has been shown to correlate with proliferative activity in a variety of normal tissues and tumours (Sutherland et al 1981; Trowbridge & Omary 1981).

The expression of the cell surface receptor for transferrin in a variety of tissues has been studied by immunocytochemical techniques (Sutherland et al 1981; Trowbridge & Omary 1981).

1.5.7 Extracellular molecules.

Fibronectin.

Fibronectin is a widely distributed glycoprotein present in soluble form in plasma and in insoluble form in tissue matrices (Yamada & Olden 1978). It was first described as cold-insoluble globulin (Morrison, Edsall & Miller 1948). Later work has shown that the same glycoprotein was responsible for a wide range of biological properties and it has been known by a variety of names including large external transformation sensitive protein (LETS) (Hynes & Bye 1974), surface glycoprotein antigen (Vaheri & Ruoslahti 1974) and cell surface protein (Yamada & Weston 1974).

Fibronectins are composed of 200,000 - 250,000 molecular weight monomers. The plasma form is usually a disulphide bonded dimer (Mosher 1975) and the tissue forms are disulphide bonded dimers or multimers (Hynes & Destree 1977; McConnell, Blumberg & Rossow 1978).

Fibronectins interact with a variety of molecules including fibrin, collagen, glycosaminoglycans, DNA and itself (Mosher 1975). It is found in basement membranes and in connective tissue matrices (Stenman & Vaheri 1978). It has been shown to be important in cell adhesion and migration by allowing attachment between different cells and between cells and substrata (Yamada & Olden 1978). Fibronectin appears early in development (Zetter et al 1978) and its distribution changes with differentiation (Linder, Vaheri & Ruoslahti 1975; Critchley et al 1979) although its distribution in the fetal and adult kidney has been a topic of some debate (Weiss et al 1979; Linder, Miettinen & Tornroth 1980; Pettersen & Colvin 1978; Dixon et al 1980).

Its immunocytochemical demonstration has been useful in the some aspects of glomerular pathology (Linder, Miettinen & Tornroth 1979; Dixon et al 1980) and in the diagnosis of non-renal tumours (Du Boulay 1982; Du Boulay 1985).

The murine monoclonal antibody NDOG1 was the product of a hybridoma of cells from a mouse immunised with unsolubilised syncytiotrophoblast microvillous plasma membrane preparations isolated from term human placental villous tissue (Sunderland, Redman and Stirrat 1981). In immunoperoxidase preparations it has been shown to react with an epitope present on villous syncytiotrophoblast of human pregnancies between six weeks and term. The epitope is not expressed on villous cytotrophoblast (Sunderland, Redman & Stirrat 1981; Bulmer, Billington & Johnson 1984). Further studies have shown that the antigen recognised by NDOG1 is present in a specialised connective tissue layer surrounding the acinar epithelium of the normal adult breast, underlying the urothelium and in Wharton°s jelly of the umbilical cord (Stirrat, Sunderland & Redman 1983). Biochemical evidence has shown that the epitope is carried on a complex molecule of a proteoglycan nature containing hyaluronic acid or chondroitin sulphate A,B or C (Stirrat, Sunderland & Redman 1983).

TABLE 1.

Classification of childhood renal tumours and related lesions

Nephroblastoma (Wilms' tumour)

Congenital mesoblastic nephroma

Atypical mesoblastic nephroma

Bone metastasising renal tumour of childhood (BMRTC)

Fetal rhabdomyoid tumour

Cystic partially differentiated nephroblastoma

Multilocular cystic nephroma

Nodular renal blastema

Nephroblastomatosis

Metanephric hamartoma

Glomerular immaturity

TABLE 2.

Classification of adult renal tumours.

Renal cell carcinoma

Renal oncocytoma

Sarcomatoid renal carcinoma

Collecting duct carcinoma

Reno-medullary interstitial cell tumour

Juxtaglomerular cell tumour

Angiomyolipoma

Miscellaneous mesenchymal tumour
(Classified as for non-renal sites, Enzinger & Weiss 1983)

TABLE 3

Antigens included in this study.

Cytokeratins (3 types)

Vimentin

Desmin

Epithelial membrane antigen

Brush border antigen

Fucosylated poly N-acetyl lactosamine

-1 antitrypsin

-1 antichymotrypsin

Carbonic anhydrase C

Ferritin

S-100 protein

Myoglobin

Factor VIII R antigen

Renin

Human chorionic gonadotrophin

Alpha fetoprotein

Carcino-embryonic antigen

Ki 67 nuclear proliferation antigen

Transferrin receptor

Fibronectin

NDOG1 extracellular matrix antigen

CHAPTER 2

MATERIAL AND METHODS.

2.1 Antibodies.

A variety of different types and specificities of antibodies have been used in this study. They have also been visualised by several different techniques.

Anti-cytokeratin antibodies.

I have studied the immunocytochemical distribution of three different types of cytokeratins using three different antisera.

Large molecular weight epidermal prekeratins have been studied using a rabbit polyclonal antiserum raised against human plantar callus. This antiserum binds to antigenic determinants only found on these large molecules.

Two monoclonal antibodies were used to study other cytokeratins. A murine monoclonal antibody (PKK1) to the cytoskeletal peptides of the pig kidney epithelial cell line LLC-PK raised using

the procedure previously described (Holthofer et al 1983) was obtained commercially. This antibody has been shown to recognise epitopes on the 56-, 48-, 45- and 41-kilodalton cytoskeletal polypeptides of the pig kidney cell line LLC-PK1 (Holthofer et al 1983).

The other monoclonal antibody used was the murine monoclonal antibody CAM 5.2 raised against the human colorectal carcinoma cell line HT 29 (Makin, Bobrow & Bodmer 1984). This antibody recognises epitopes on the 50-, 43- and 39-kilodalton cytokeratins of that cell line and, therefore, labels the lowest molecular weight cytokeratins described by Moll et al (1982).

Anti-vimentin antibody.

The distribution of vimentin was studied using a murine monoclonal antibody (IgG) obtained from Amersham, U.K.

Anti-desmin antibody.

Desmin was detected by using a murine monoclonal antibody (IgG) raised against porcine desmin (Amersham, U.K.). In immunochemical and immunocytochemical experiments this antibody has been shown to cross react with human desmin (Du Boulay 1985).

Anti-EMA antibodies.

EMA was detected by a goat polyclonal anti-human EMA antiserum (Seralab) and a murine monoclonal antibody HMFG2 (IgG) (Unipath). In practice the distribution of the antigen detected by these two antibodies was identical.

Anti-BB antiserum.

The anti-brush border (BB antigen) antiserum was a polyclonal rabbit anti-human BB antigen antiserum prepared by a modification (Matthews & MacIver 1982) of the technique of Wallace & Nairn (1962). The kidney of a blood group O rhesus negative patient was obtained fresh at nephrectomy for renal cell carcinoma. The macroscopically normal areas of cortex were dissected from the

remainder and finely chopped. One gram of this tissue was suspended in 10mls of freshly prepared 0.25M sucrose solution buffered at pH 7.3. The solution was homogenised and sonicated in a Kinematica Polytron and then centrifuged at 2000g for 8 minutes. The supernatant was used to prepare the anti-BB antiserum. Two adult male half-lop rabbits, each weighing 4 kg, were immunised with the kidney homogenate. Five mls of the homogenate supernatant was suspended in an equal quantity of Freund°s complete adjuvant. Each animal received 0.5 mls of the emulsion injected intravenously into each hind leg. Two weeks later the rabbits were re-injected with 1ml of antigen and then at weekly intervals for a further six weeks. The rabbits were venesected before the fourth and sixth immunisations and again six weeks after the final immunisation. No ill effects were noted in the rabbits.

The antiserum was tested for the presence of antibodies to the antigen preparation by double gel immunodiffusion in 0.6% agarose in Petri dishes (Matthews & MacIver 1982). The antisera were then tested in immunocytochemical experiments for their reactivity with human kidney (Matthews & MacIver 1982).

Antibodies directed to fucosyl poly-N-acetyl lactosamine derivatives.

The murine monoclonal (IgM) antibodies, AGF 4.48 and AGF 4.36, were originally raised against a human promyeloid cell line HL 60. The production and immunochemical specificity has been described previously (Fisher et al 1982). They recognise epitopes expressed on the surface of granulocytes at different stages during granulopoiesis (Fisher et al 1982). Competitive inhibition studies have shown that these two antibodies recognise 3-fucosyl N-acetyl lactosamine (Figure 1) on myeloid cells (Howie & Brown 1985). They therefore have the same specificity as a variety of other monoclonal antibodies including VEP8, VEP9, 1G10 and SSEA-1 (Andrews et al 1983; Gooi et al 1983). For reasons which are not yet clear this oligosaccharide is the immunodominant group in human lung tumours injected into laboratory mice (Brockhaus et al 1982) and the immunodominant group on the surface of HL 60 cells in mice (Feizi & Childs 1984).

The two antibodies show differences in their tissue distribution (Howie et al 1984) and they have been shown to react with two different determinants on the same oligosaccharide (Howie & Brown 1985). It has been shown by immunocytochemical studies that these epitopes have a widespread tissue distribution including parts of the nephron (Howie et al 1984).

Brown et al (1983) recently reported the production of a monoclonal antibody C14/1/46/10 (designated C14) following immunisation of mice with cell membranes from a rectal adenoma. This antibody reacts with the difucosyl derivative of N-acetyl lactosamine (Figure 1). The antibody is a mouse monoclonal IgM antibody generously donated by Professor R.W. Baldwin in the form of an ascitic fluid.

A murine monoclonal antibody (IgM) directed against the type H blood group substance on a type II precursor chain (Figure 1) was obtained from Dako. This antibody was raised against the synthetic trisaccharide.

Anti-proteinase inhibitor antisera.

Polyclonal rabbit antisera raised against human alpha-1-antitrypsin and alpha-1-antichymotrypsin have been used to detect these proteinase inhibitors (Dako).

Anti-carbonic anhydrase C antiserum.

The anti-carbonic anhydrase C antiserum which I have used was a polyclonal rabbit anti-human carbonic anhydrase C antiserum. It was prepared by immunising a lop-eared rabbit with a commercially available human erythrocyte carbonic anhydrase which had been purified from its ammonium sulphate suspension by dialysis and then incorporated in the water phase of Freund°s complete adjuvant. Four booster doses were given over a six week period. The animal was venesected and serum prepared. During the immunisation procedure the animal showed no ill-effects (Steart 1984).

The anti-carbonic anhydrase activity of the serum was purified by absorption with normal human serum. The resulting antiserum was tested in immunochemical and immunocytochemical experiments for its specificity for carbonic anhydrase C (Weller 1983; Steart 1984).

Anti-S-100 protein antibody.

The antiserum used in this study was a commercially available polyclonal rabbit anti-bovine S-100 protein antiserum (Dako). This antibody cross-reacts with human S-100 protein.

Anti-myoglobin antiserum.

A polyclonal rabbit anti-human skeletal muscle myoglobin antiserum (Behring) was used for the immunocytochemical demonstration of myoglobin.

Anti-Factor VIII related antigen antiserum.

Factor VIII R antigen was detected by use of a polyclonal rabbit anti-human factor VIII antiserum (Behring).

Anti-ferritin antiserum.

The distribution of ferritin was studied using a polyclonal rabbit anti-human ferritin antiserum raised against human hepatic isoferritin (Dako). This antibody has been shown to bind to a broad spectrum of human isoferritins, basic and acidic, in both immunochemical and immunocytochemical experiments (Rossiello, Carrero & Giordano 1984).

Anti-renin antiserum.

Renin was demonstrated by a polyclonal rabbit anti-human renin which was raised against renin purified from a juxtaglomerular cell tumour (Galen et al 1979b). The specificity of the antiserum has been tested in vitro (Camilleri et al 1980) and it has been used previously in immunocytochemical experiments (Amat et al 1981; Lindop, More & Leckie 1983; Lindop, Stewart & Downie 1983).

Anti-HCG antiserum.

HCG was detected by a rabbit polyclonal antiserum (Miles).

Anti-AFP antiserum.

The anti-AFP antiserum used was a polyclonal rabbit antiserum raised against human AFP (Dako).

Anti-CEA antiserum.

Technical difficulties in the interpretation of results using polyclonal antisera against CEA have arisen because of the widespread distribution of an antigen which cross-reacts with CEA. This

antigen is called the normal cross-reacting antigen (NCA or CEX) (Mach & Putztaszeri 1972; Van Kleist, Chavanal & Burtin 1972). The cross reacting antibody to NCA can be absorbed out of anti-CEA antiserum by prior incubation with a spleen extract (Heyderman 1983). In this study CEA was detected using a polyclonal rabbit anti-CEA antiserum absorbed in this way (Dako).

Ki 67.

Ki 67 is a mouse monoclonal antibody which recognises a nuclear protein expressed during the cycle of cell division. It therefore stains cells which are actively involved in cell division (Gerdes et al 1983).

Anti-transferrin receptor molecule.

The cell surface transferrin receptor molecule was identified by using the mouse monoclonal antibody HB 21, which is identical to the 5E9 C11 antibody of Haynes et al (1981).

Anti-fibronectin antiserum.

Fibronectin was identified in tissues using a polyclonal rabbit anti-human fibronectin antiserum (Cappell).

NDOG1.

This is a murine monoclonal antibody raised against a syncytiotrophoblast extract. It has been shown to detect an antigen present on a proteoglycan molecule which is sensitive to hyaluronidase and chondroitin sulphatase (Stirrat, Sunderland & Redman 1983).

2.2 Preparation of Tissues.

2.2.1 Fetal tissues.

Fetal kidneys were processed by two different methods for immunocytochemistry. One method was used in preparation for cryostat sectioning and the other for paraffin embedding of fixed tissue.

For cryostat sectioning kidneys were removed from fetuses obtained during legal terminations of pregnancy. These procedures were performed at the Princess Anne Hospital, Southampton. Ten fetuses of gestational ages from 10 to 18 weeks were obtained. The kidneys were dissected and snap frozen in liquid nitrogen. They were stored until use at -70C.

Sections 6 thick were cut on a cryostat and mounted on glass slides.

For fixation and paraffin embedding fetal kidneys were removed at autopsy from cases of intrauterine deaths or stillbirths. Only well preserved tissue from fetuses with no evidence of maceration were used. The kidneys were bisected longitudinally and both halves fixed in 10% neutral buffered formalin. After fixation for twenty four hours the tissue was embedded in paraffin wax using a standard method.

Sections 4 thick were cut and mounted on glass slides. After dewaxing they were stained by tinctorial methods (Section 2.4). Multiple adjacent sections of selected blocks were cut and stained by immunocytochemical methods.

2.2.2 Pathological material.

Pathological material was identified using the surgical pathology filing systems of the Pathology Departments of the Western Infirmary, Glasgow and Southampton General Hospital, Southampton. Additional material from rare conditions was generously donated by several colleagues. In order to obtain optimal tissue preservation where possible surgical material was used in this study.

The tissue had been fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections 4 thick were cut and mounted on glass slides. After dewaxing they were stained by routine tinctorial methods. The diagnosis was confirmed histologically and multiple adjacent sections from appropriate blocks were cut and stained by immunocytochemical methods.

2.3 Selection of Pathological Material.

2.3.1 Dysplasia.

The pathological material examined in this section is summarised in Table 4. Forty six cases of dysplastic lesions of the kidney were studied. Risdon°s (1971a) definition of renal dysplasia is "the abnormal disorganised development of the renal parenchyma due to anomalous differentiation of metanephric tissue". I have therefore included within this group not only cystic dysplasia but also lesions which belong to the nephroblastic dysplasia (Marsden & Lawler 1983) and the nephroblastomatosis (Bove & McAdams 1976) groups.

Many of the cases of nephroblastic dysplasia and the nephroblastomatosis complex were identified in kidneys which also bore a nephroblastoma.

Cystic renal dysplasia.

The eighteen cases of cystic renal dysplasia were obtained from the files of the Department of Pathology of Southampton General Hospital, Southampton. The original morbid anatomical descriptions and paraffin blocks were available for study.

The diagnosis was confirmed by the examination of tinctorial stained sections using the diagnostic criteria of Risdon (1971a). The presence of separate dysplastic elements was assessed morphologically on these sections. Multiple adjacent sections were cut and stained by immunocytochemical methods.

Multilocular cystic nephroma.

Two cases of this rare lesion were obtained. One from the files of the Department of Pathology of the Western Infirmary, Glasgow and the other was a gift from Dr F Mutch (Royal Infirmary, Glasgow). The paraffin blocks and morbid anatomical specimens were available for study. Both lesions fulfilled the diagnostic criteria for multilocular cystic nephroma (Bennington & Beckwith 1975) on examination of nephrectomy specimens and tinctorial stained tissue sections.

Nodular renal blastema.

One case of nodular renal blastema was obtained from the files of the Department of Pathology, Southampton General Hospital, Southampton. Two further cases were generously referred for study by Dr A Moscosa (King's College Hospital, London).

The morbid anatomical descriptions and paraffin blocks were available for study in all three cases. The diagnosis was confirmed on examination of tinctorial stained sections using the criteria of Bove & McAdams (1976).

Nephroblastomatosis.

One case of this lesion was obtained from the files of the Department of Pathology, Southampton General Hospital. Two cases were generous gifts of Dr F Raafat (Children's Hospital, Birmingham).

The diagnosis was confirmed using the diagnostic criteria of Bove & McAdams (1976) by examination of tinctorial stained sections cut from paraffin blocks and by review of the morbid anatomical descriptions.

Metanephric hamartoma.

These ten lesions were all identified in kidneys which also bore a nephroblastoma. The diagnosis was confirmed histologically using the criteria of Bove & McAdams (1976).

Glomerular immaturity.

These lesions were also identified in kidneys bearing nephroblastomas. The immature glomeruli fulfilled the diagnostic criteria of Bove & McAdams (1976) on histological examination.

Immunocytochemistry.

The distribution of 15 antigens was studied in these dysplastic lesions. These antigens are listed in Table 5.

2.3.2 Renal Tumours.

A total of 98 primary renal tumours were examined. Fixed and paraffin embedded tissue from all 98 tumours was studied. In addition, tissue from 12 of these tumours was received fresh and processed for examination of cryostat sections using those antibodies which could only be examined in tissue processed in this manner.

The tumours were classified histologically (Table 6) by the criteria described by Olsen (1984).

During the course of the study four tumours were recognised which had histological features similar to those of three cases previously described as collecting duct carcinomas or Bellini duct carcinomas (Cromie, Davis & DeTure 1979; O'Brien & Bedard 1980; Hai & Diaz-Perez 1982). These tumours have therefore been classified as collecting duct carcinomas for the purposes of this study. The justification for this classification and the evidence of their cell type is discussed in detail below.

Morphological assessment.

In addition to classifying the tumours by morphological criteria the presence of each different cell and tissue type present was assessed by histological examination.

Nephroblastoma.

The tumours were classified according to the amount of epithelial differentiation present as described by Lawler et al (1977). The type of epithelial differentiation which was seen was recorded by the criteria of the SIOP (Delemarre et al 1982). This classification recognises blastema, rosettes, tubules, papillary structures, microcysts and pseudoglomeruli. The tubules were further subdivided into Type A or Type B as described by Hou & Azzopardi (1967).

Different types of mesenchymal tissue were also identified on tinctorial stained sections.

The presence of anaplasia as defined by Beckwith (Beckwith & Palmer 1978) was recorded.

Renal cell carcinomas.

The renal cell carcinomas were classified according by cell type and tissue architecture according to Olsen (1984) and by nuclear grading according to Skinner et al (1971). The spindle cell component of the sarcomatoid renal cell carcinomas were classified separately from the epithelium by the criteria of Tomera et al (1984), a classification which is largely based on the classification of soft tissue tumours (Enzinger & Weiss 1983).

The renal sarcomas were classified according to the classification for soft tissue tumours in other sites (Enzinger & Weiss 1983).

Immunocytochemistry.

The antigens studied in these renal tumours are summarised in Table 7.

2.4 Staining Procedures.

Sections were stained by standard procedures for haematoxylin and eosin, reticulin, periodic acid-Schiff (PAS) and Van Gieson°s trichrome.

2.5 Immunocytochemical Procedures.

Three different immunocytochemical methods were used in this study. Table 8 describes which primary antibodies were visualised by each of these three methods and which type of tissue was used.

2.5.1 Immunoperoxidase anti-peroxidase method.

The staining used was a modification of that described by MacIver & Mepham (1982).

(1) Sections were de-waxed in 2 changes of xylene (10 minutes each), and transferred to 70% alcohol through absolute alcohol.
(2) Endogenous peroxidase was blocked by treating the sections with freshly prepared 0.5% hydrogen peroxide in methanol for 10 minutes, followed by washing in tap water.

(3) Prior to enzymatic digestion (if used) sections were placed in glass distilled water for 10 minutes in a water bath to warm to 37 C.

(4) Sections were then incubated with the appropiate enzyme (See Section 2.6).

(5) Sections were then washed in cold tap water to stop enzymatic digestion and washed twice by Tris-HCl buffer pH 7.6 for 10 minutes each in a moist chamber.

(6) After draining off the excess buffer, the primary antibody was applied (See Appendix 1 for the details of incubation times and antibody dilutions).

(7) Sections were washed by Tris-HCl buffer pH 7.6 three times for 10 minutes each and drained.

(8) The second layer was applied for 30 minutes at the dilutions given in Appendix 2.

(9) Sections were washed 3 times in Tris-HCl buffer pH 7.6 three times for 10 minutes each and drained.

(10) The peroxidase anti-peroxidase complex was applied for 30 minutes.

(11) Sections were washed and drained in Tris-HCl buffer pH 7.6 three times for 10 minutes each.

(12) Freshly prepared diamino-benzidine (DAB) was applied for 10 minutes. The sections were then washed in Tris-HCl buffer pH 7.6 and then in tap water for 5 minutes. Sections were countered by Harris° haematoxylin, differentiated by 2% acid alcohol and blued in tap water. They were then dehydrated, cleared in xylol and mounted in DPX.

2.5.2 Avidin-biotin peroxidase method.

This method is a modification of the technique of Hsu, Raine & Fanger (1981).

(1) The technique is the same as that used for the PAP method until the stage of incubation with the second antibody.
(2) The sections were incubated with the biotinylated goat anti-mouse IgM antiserum for 1/2 hour.
(3) Sections were then washed in Tris-HCl buffer pH 7.6 three times for 5 minutes each.
(4) The sections were then incubated with the avidin-biotin peroxidase complex for 1/2 hour.
(5) Sections were then washed and drained in Tris-HCl buffer three times for 10 minutes each.

(6) Freshly prepared DAB was applied for 10 minutes. The setions were washed in Tris-HCl and then in tap water for 5 minutes. They were then counterstained and mounted as before.

2.5.3 Indirect peroxidase method.

This method was used to visualise the binding of monoclonal antibodies to cryostat sections.

(1) Frozen sections were cut and mounted on slides coated with polylysine. The sections were then fixed in cold acetone for 3 minutes.
(2) Sections were incubated with 2% bovine serum albumin (BSA) in phosphate buffered saline (PBS) for 1 hour.
(3) Sections were washed three times in PBS for 5 minutes each.
(4) Sections were incubated for 1 hour with the appropiate primary antibody (Details of dilution factors are given in Appendix 1).
(5) Sections were washed three times in PBS for 5 minutes each.
(6) They were then incubated for 1 hour with peroxidase conjugated rabbit anti mouse immunoglobulin.
(7) The sections were then washed three times in PBS for 5 minutes each.

(8) Freshly prepared DAB was applied for 10 minutes. Sections were washed, counterstained and mounted.

2.6 Enzymatic digestion.

Trypsin.

The visualisation of many antigens in formalin fixed paraffin embedded tissue is greatly enhanced by pretreating the sections by tryptic digestion before the application of the immunocytochemical methods. The method used was a modification of the method of MacIver & Mepham (1982).

Enzymatic digestion was carried out using trypsin Grade II (Sigma) prepared as a 0.1% solution (w/v) in distilled water, containing 0.1% calcium chloride and adjusted to pH 7.8 using N/10 sodium hydroxide, maintaining a temperature of 37 C. The optimal time of the digestion was determined by titration.

Enzyme digestion was terminated by washing (x3) in PBS.

Glycosyl hydrolases.

Cell surface carbohydrates may be masked by the addition of terminal sugars, particularly sialic acids. The cryptic antigenic determinants

can be exposed to antibody by prior digestion of the tissue by specific glycosyl hydrolases. The specificity of these enzymes provides data on the nature of the terminal or masking sugars. In this study two glycosyl hydrolases have been used to expose cryptic carbohydrate antigenic sites. These were neuraminidase which digests sialic acids from terminal positions, and α-galactosidase which cleaves α galactosyl residues from terminal sites.

Neuraminidase (EC 3.2.1.18) from Clostridium perfringens was incubated with tissue sections for two hours at room temperature and pH 4.6. α-galactosidase (EC 3.2.1.22) from green coffee beans was incubated with tissue sections overnight at 37 C and at pH 6.0.

2.6 Control Experiments.

The control experiments described by MacIver & Mepham (1982) have been performed. Antibodies were titrated against a known positive tissue and the maximum dilution giving a positive reaction were used in the experiments. Normal serum controls in which appropiate normal serum replaced specific antibody in the immunocytochemical experiments. A positive control experiment was run at the time of all immunocytochemical tests. The tissues used for these controls have been detailed previously (Du Boulay 1985). The specificity of the antibodies used had been confirmed by absorption studies where the purified antigen was available.

TABLE 4

Renal dysplasia; Cases studied

Classification.	Number studied
Cystic renal dysplasia	18
Multilocular cystic nephroma	2
Nodular renal blastema	3
Nephroblastomatosis	3
Metanephric hamartoma	10
Glomerular immaturity	6

TABLE 5

Antigens studied in renal dysplasia.

Cytokeratins
- CAM 5.2
- PKK1
- Epidermal prekeratin

Epithelial membrane antigen

Brush border antigen

α-1 antitrypsin

α-1 antichymotrypsin

Carbonic anhydrase C

Fibronectin

S-100 protein

Desmin

Myoglobin

Factor VIII R antigen

Alpha fetoprotein

Carcino-embryonic antigen

TABLE 6

Classification of renal tumours studied.

Tumour type	Number of cases
Nephroblastoma	36
Cystic partially differentiated nephroblastoma	1
Renal cell carcinoma	44
Renal oncocytoma	8
Collecting duct carcinoma	4
Renal sarcomas	5

TABLE 7

Antigens studied in renal tumours.

Cytokeratins
- CAM 5.2
- PKK1
- Epidermal prekeratin

Vimentin

Desmin

Epithelial membrane antigen

Brush border antigen

Fucosylated poly N-acetyl lactosamine

α-1 antitrypsin

α-1 antichymotrypsin

Carbonic anhydrase C

Ferritin

S-100 protein

TABLE 8

Immunocytochemical methods.

Indirect immunoperoxidase

HB 21*

Ki 67*

NDOG1*

Vimentin

Desmin

Avidin-biotin peroxidase

AGF 4.48

AGF 4.36

C14

CAM 5.2

PKK1

TABLE 8

Immunocytochemical methods.

Indirect immunoperoxidase

HB 21*

Ki 67*

NDOG1*

Vimentin

Desmin

Avidin-biotin peroxidase

AGF 4.48

AGF 4.36

C14

CAM 5.2

PKK1

Peroxidase anti-peroxidase

Epidermal prekeratin

EMA, HMFG2

BB

Carbonic anhydrase C

Ferritin

A1AT

A1ACT

S-100 protein

Factor VIII R antigen

HCG

CEA

AFP

Renin

Fibronectin

Myoglobin

CHAPTER 3.

CELL DIFFERENTIATION DURING RENAL ORGANOGENESIS.

3.1 Results.

3.1.1 Morphology.

Microscopical examination of tissue sections stained by haematoxylin and eosin showed that all the fetal kidneys studied were normal for the recorded gestational ages. There was an outer capsule, a subcapsular nephrogenic zone, a cortex, and a medulla. As the gestational age of the fetuses increased the nephrogenic zone became less prominent while the cortex became more prominent. In each kidney succeeding generations of nephrons were seen, the most primitive in the nephrogenic zone and progressively more mature nephrons deeper in the cortex and medulla. The development of nephrons could be thus studied sequentially in each fetal kidney.

Stage 1: Metanephric blastema.

In the outermost nephrogenic zone the ureteric bud branches were seen growing into the undifferentiated metanephric blastema (Figure 2). The ureteric bud branch was a blind ending tubular structure lined by a single layer of epithelial cells. The terminal portion of the ureteric bud was dilated; this portion being called the ampulla. The remainder of the ureteric bud branch to the last point of division was called the interstitial portion. The more proximal parts of the ureteric bud formed the collecting duct of the more deeply placed earlier formed nephrons. The cells of the ureteric bud branch were cuboidal with clear cytoplasm, and a centrally placed nucleus which contained finely granular chromatin. At the ampulla the cells had less cytoplasm, the nuclei were smaller, stained more darkly and were closer together. A basement membrane, demonstrated by the periodic acid-Schiff and reticulin stains, was seen surrounding the ureteric bud epithelium.

The undifferentiated metanephric blastema consisted of tightly compressed ovoid cells with round dark staining nuclei and scant cytoplasm. Underlying the blastema, around the interstitial portions of the ureteric bud, the stromal cells were more elongated. The nuclear chromatin in these spindle cells was finely granular.

Stage 2: Nephrogenic rosette and vesicle.

At the next developmental stage a condensation of blastema which formed a rosette was seen adjacent to some ureteric bud branches. The rosette consisted of a single or double layer of columnar cells, with elongated, dark staining nuclei, and a small amount of cytoplasm. The long axes of the nuclei were arranged radially. No basement membrane was seen.

The nephrogenic rosette then formed a vesicle with a central lumen (Figure 3). A basement membrane, incomplete in places, was seen around the nephrogenic vesicle. Mitotic figures were seen in nephrogenic rosettes and vesicles.

Stage 3: S-shaped tubule.

In nephrons at the next developmental stage the vesicle had lengthened and twisted to form an S-shaped tubule, the upper pole of which approximated to the ureteric bud branch (Figure 4). The cells of the S-shaped tubule remained columnar with oval, dark staining nuclei. The nuclei were basally situated and there was a

moderate amount of apical eosinophilic cytoplasm. Occasional mitotic figures were seen in the S-shaped tubule. A PAS and silver staining basement membrane was seen underlying the epithelium at this stage. The S-shaped tubule can be subdivided according to the known developmental fate of each segment. Thus, the lower limb of the S-shaped tubule is the glomerular pole and the remainder is the tubular pole. The tubular pole can be further subdivided into the distal tubular segment at the upper limb and the proximal tubular segment at the middle limb.

Stage 4: Early maturation stage.

The upper pole of the S-shaped tubule was then seen fused with the ampulla of the ureteric bud branch forming a single primitive nephron structure with a continuous lumen (Figure 5). The parietal epithelium at the glomerular pole of the tubule had flattened, but the visceral epithelium had remained columnar. The tubule had acquired convolutions at right angles to the original twist of the S. The tubular epithelial cells were now cuboidal with round nuclei, but the proximal and

distal tubular epithelium remained cytologically indistinguishable. The ureteric bud had become the collecting duct. In the glomerular tuft at this stage of nephronogenesis mesangial cells and endothelial cells could be separately identified. The mesangial cells were irregular plump cells with pale staining cytoplasm and a round vesicular nucleus. The endothelial cells were spindle shaped with an elongated dark staining nucleus and scant cytoplasm.

Stage 5: Late maturation stage.

In nephrons in deeper parts of the renal cortex the cells of the visceral glomerular epithelium had flattened and the lobular architecture of the glomerular tuft was becoming apparent (Figure 6). In addition, by this stage the cells lining the proximal and distal tubule were morphologically distinct. Thus, the proximal tubular cells were cuboidal with granular eosinophilic cytoplasm and an apical brush border. Their nuclei were small, round and haematoxyphilic. The distal tubular epithelial cells were smaller, with clear cytoplasm. A brush

border was not apparent in the distal tubular cells. The nuclei of these cells were also small, round and dark staining. Early descent of the loop of Henle was seen at this stage. The cells of this structure were still cuboidal.

Stage 6: Mature nephrons.

Finally fully mature nephrons with all the adult structures present were seen in the deep cortex of kidneys from fetuses of greater than twenty weeks gestation.

In fetuses of earlier than eighteen weeks gestation the large collecting ducts in the medulla were seen to be lined by a multiple layered cuboidal epithelium. These structures in older fetuses and in adults were lined by a single layer of cuboidal epithelium.

The renal interstitium.

The interstitium of the kidney also develops from the metanephric blastema and matures in parallel with the adjacent nephron. In the nephrogenic zone underlying the metanephric

blastema there were large spindle cells with a moderate amount of cytoplasm lying in a pale staining extracellular matrix (Figure 3). Deeper within the renal cortex the interstitial cells were much smaller and more elongated, finally becoming inconspicuous in the mature areas of the renal cortex and medulla. In the mature areas of the renal cortex no extracellular matrix was seen.

Blood vessels in the nephrogenic zone were sparse. Only a few capillaries were seen adjacent to the interstitial portion of the ureteric bud branch and in the interstitium between pairs of adjacent nephrogenic vesicles. At the S-shaped tubule stage capillaries were seen around the ureteric bud branch with additional small blood vessels seen in the concavity of the lower pole of the S-shaped tubule (Figure 4). In more developed nephrons increasingly complex capillary plexuses were seen both in the glomerular tuft and around the maturing tubules.

In this way nephronogenesis, although a continuous process, can be histologically subdivided into developmental stages within which the cellular distribution of differentiation markers may be examined.

3.1.2 Immunocytochemistry.

The distribution of intermediate filament antigens in the developing kidney.

Vimentin was seen in the cytoplasm of the cells of the metanephric blastema. In the epithelium of the ampulla of the ingrowing ureteric bud there was focal expression of vimentin. Vimentin was present in the basal cytoplasm of the cells of the tubular rosette and of the renal vesicle (Figure 7). By the S-shaped tubule stage of development vimentin was absent from the epithelium of the tubular pole and from the adjacent ureteric bud. At this stage, however, this antigen was seen in the cytoplasm of the cells of both the parietal and visceral epithelium of the glomerulus (Figure 8). As the parietal glomerular epithelium flattened vimentin expression was lost, but the antigen persisted in the visceral glomerular epithelium. Hereafter in the epithelium of the developing and fully formed adult nephron vimentin expression was confined to the visceral glomerular epithelium.

Vimentin was seen in the cytoplasm of the interstitial cells at all stages of their development. Vimentin was also seen in the mesangial cell cytoplasm throughout glomerular development. The vascular endothelium and smooth muscle contained vimentin antigen at all stages of development.

At the earliest stage of nephron development, strongly positive staining with CAM 5.2 was seen in the cytoplasm of the epithelium of the ampullae of the ingrowing and branching ureteric buds (Figure 9). PKK1 staining, although not seen in the ampullary portion of the ureteric bud branch, was present in the cytoplasm of the cells of the deeper portions of the ureteric bud, where this structure constituted the collecting ductule for the more deeply placed nephrons. CAM 5.2 staining could also be seen in the deeper portions of the ureteric bud. The large collecting ducts in the medulla of the kidney stained with all three anti-cytokeratin antibodies (Figure 10) as did the urothelium of the renal pelvis.

No cytokeratins were seen in the undifferentiated metanephric blastema. In the renal vesicles which form from the blastema, focal

positive staining with CAM 5.2 was seen in some of the primitive epithelial cells (Figure 9). In nephrons at the S-shaped tubule stage of development CAM 5.2 antigen expression was seen in the cells at the tubular pole and in those cells of the glomerular pole which were the precursors of the visceral glomerular epithelium. No CAM 5.2 staining was seen in the cells of the parietal glomerular epithelium at this develpmental stage. The other cytokeratin antigens were not seen in the S-shaped tubule.

In the maturing nephrons, in which fusion of the S-shaped tubule and the ureteric bud branch had occurred, both CAM 5.2 and PKK1 positive staining were observed in the tubules. The antigens were present in both proximal and distal tubules. At the early maturation stage of development, in glomeruli in which the parietal epithelium had flattened but the visceral epithelium was still columnar, CAM 5.2 positive staining was seen in the cytoplasm at the base of the visceral glomerular epithelial cells, but was absent from the cells of the parietal epithelium (Figure 11). CAM 5.2 antigen was lost from the visceral glomerular epithelium as it flattened. In

mature nephrons, in fetal or in adult kidney, the visceral glomerular epithelium showed no cytokeratin staining, however, the parietal glomerular epithelium, tubular epithelium, and loop of Henle stained with CAM 5.2 (Figure 12) and with PKK1, while the collecting ductules, collecting ducts, and urothelium stained with CAM 5.2, PKK1, and the antibody to epidermal prekeratin.

Staining for desmin was only seen in the smooth muscle cells of the large blood vessels of the late fetal and adult kidney.

The distribution of cell surface antigens.

At the earliest stage of development of the nephrom EMA was found on the apical cell membrane of the epithelium of the ingrowing ureteric bud, but was not detected on the cells of the metamephric blastema. EMA was not seen in the renal vesicle. However, in those nephrons at the S-shaped tubule stage of development EMA was found on the luminal surface of the epithelium at the distal tubular pole. The ureteric bud adjacent to the S-shaped tubule continued to express EMA

(Figure 13). After fusion of the ureteric bud with the upper pole of the S-shaped tubule, EMA could be seen in both of these structures but on neither the proximal tubule nor glomerular epithelium. At later stages of development EMA continued to be expressed by distal tubular and collecting duct epithelium. At no time was it found on proximal tubule, or glomerular epithelium. It was not present on the mesangium nor on any of the interstitial mesenchymal cells.

In the adult kidney EMA was demonstrated on the apical membrane of the distal tubule, thick ascending limbs of the loop of Henle, and collecting duct epithelium. It was present on the luminal surface of the cells of the macula densa (Figure 14).

BB antigen was first expressed in nephrons after the stage of fusion of the ureteric bud and the S-shaped tubule when it was seen on the luminal surface membrane and apical cytoplasm of the epithelial cells of the proximal tubule. The glomerulus, distal tubule, and collecting duct epithelium showed no BB antigen expression. Throughout nephronogenesis BB antigen expression persisted on, and was restricted to, the proximal

tubular epithelium. It was expressed throughout the length of the proximal tubule. In the adult kidney the antigen was found only on the apical membrane and cytoplasm of the proximal tubule epithelium (Figure 15).

The AGF 4.48, AGF 4.36 and C14 monoclonal antibodies all stained the surface membrane of the epithelial cells of the ampulla of the ureteric bud branch. The remainder of the ureteric bud and the metanephric blastema did not express the AGF 4.48, the AGF 4.36 nor the C14 determinants (Figure 16). The renal vesicle and S-shaped tubule did not stain with these antibodies. The AGF 4.48, AGF 4.36 and C14 staining on the ureteric bud ampulla persisted until after fusion of the ureteric bud with the distal tubular pole of the S-shaped tubule (Figure 17), but was then lost from this site.

During the late maturation stage of nephronogenesis AGF 4.48, AGF4.36 and C14 all stained the luminal surface of the maturing proximal tubule epithelium (Figure 18). Initially this staining was present in all segments of the proximal tubule but was gradually lost during maturation until, in the majority of mature

nephrons in the late fetal and adult kidneys, staining with these antibodies was seen only on the pars recta of the proximal tubule and the thin limb of the loop of Henle (Figure 19). In the juxtamedullary nephrons in the adult kidneys AGF 4.48, AGF 4.36 and C14 staining was present throughout the length of the proximal tubule.

Following digestion of tissue sections with neuraminidase staining with AGF 4.48, AGF 4.36 and C14 was seen throughout the proximal tubule, but was not seen in the collecting ductules and ducts, nor on the distal tubular epithelium. -galactosidase digestion had no effect on the binding of these antibodies.

H Type II blood group substance was only detected on the differentiated collecting duct epithelium of mature nephrons in fetal or adult kidney.

No staining was seen in the collecting ducts, distal tubule, or glomerulus with these antibodies. Occasional scattered white blood cells in the interstitium stained with AGF 4.48 but the other mesenchymal tissues in the kidney showed no reactivity.

The distribution of cytoplasmic protein antigens.

Alpha-1-antitrypsin and alpha-1-antichymotrypsin showed the same cellular distribution. The ingrowing ureteric bud branches contained no stainable proteinase inhibitors. The cytoplasm of some of the cells of the metanephric blastema was found to contain proteinase inhibitors (Figure 20). The antigens were also seen in the large, spindle-shaped cells of the primitive mesenchyme underlying the blastema between ingrowing ureteric buds. No staining with these antibodies was observed in the cytologically mature interstitial cells in fetal or adult kidneys.

Proteinase inhibitors were not identified in the tubular component of the kidney at the renal vesicle or S-shaped tubule stages of development. The antigens were observed in the tubular component of the those nephrons which were at the early maturation stage. Here granular cytoplasmic staining was observed in the proximal tubular epithelium (Figure 21). This staining in the proximal tubule was present throughout its length. No staining was seen in the distal tubular or glomerular epithelium, although an occasional proteinase inhibitor containing cell was seen

within the glomerular tuft. The proteinase inhibitor containing cells in the glomerulus were observed in those glomeruli which were vascularised but not at more primitive stages.

In the adult kidney proteinase inhibitors were seen in the proximal tubular epithelial cells. Their distribution was patchy but showed no segment specific pattern within the proximal tubule. Some proteinase inhibitor containing cells were seen within vascularised glomerular tufts and an occasional positive monocyte was seen in blood vessel lumina. Distal tubules, loops of Henle, collecting ducts, and interstitial cells stained with neither antibody.

Ferritin was not found in the ureteric bud branches nor in the metanephric blastema. In the epithelial part of the nephron it was first seen diffusely staining the cytoplasm of the cells of the proximal tubule at the late maturation stage of development (Figure 22). Throughout development it persisted at this site, although in the adult kidney the staining was patchy, again no segment specific pattern was apparent in the proximal tubule. Ferritin was not seen in other parts of the nephron, but was seen in macrophages in the interstitium and in vascular smooth muscle cells.

Carbonic anhydrase C was not seen in the early stages of nephronogenesis. It was first seen in the tubular component of the kidney at the early maturation stage of development. There was diffuse staining of the cytoplasm of the epithelial cells throughout the tubular part of the nephron and in the primitive collecting ductule (Figure 23). The staining was considerably more intense in the distal tubular epithelium than in other parts of the nephron. Staining was seen in the tubular epithelium at all developmental stages in the fetal kidney, although in the large medullary collecting ducts the staining had an irregular distribution such that cells staining intensely were seen adjacent to cells which showed no staining (Figure 24). In the adult kidney carbonic anhydrase C was found in the distal tubule, and the collecting ducts, where again staining was patchy, but was not seen in the proximal tubular epithelium. The antigen was also absent from the cells of the macula densa of the distal tubule. It was not found in the glomerulus nor on any of the interstitial and vascular components of the kidney. Red blood cells, seen within vessels, contained carbonic anhydrase C.

No staining for myoglobin was seen in either fetal or adult kidneys.

Factor VIII R antigen was only found in the cytoplasm of vascular endothelial cells. In the fetal kidney it was seen in the endothelium of thin walled vessels underlying the metanephric blastema, in the endothelium of larger vessels deeper in the kidney, and in the endothelium of the developing glomerular tuft (Figure 25). In the adult kidney it was present in the endothelium of all blood vessels except the capillaries of the glomerular tuft.

The distribution of hormones.

Within the fetal kidney immunoreactive renin was identified in the cytoplasm of small spindle cells intimately associated with, but lying outside the vascular endothelium. In the thin walled capillaries underlying the metanephric blastema an occasional cell containing renin was seen. These vessels were apparently growing into the blastema between pairs of developing nephrons.

Renin was seen associated with the vessels adjacent to the glomerular pole of the S-shaped tubule. In nephrons at the early maturation stage of development renin containing cells were

localised to the vascular pole of the glomerulus. In the more mature glomeruli renin containing cells were seen in the walls of the afferent arteriole (Figure 26). No renin containing cells were seen associated with the peritubular capillaries, or the vessels of the vasa recta in the fetal or adult kidneys.

No reactivtity was seen in fetal or adult kidneys stained with anti-human chorionic gonadotrophin antiserum.

The distribution of oncofetal antigens.

Neither CEA nor AFP were identified in the fetal or adult kidneys.

The distribution of proliferation associated antigens.

HB21

HB21 immunoreactivity was not seen on the epithelial cells lining the ureteric bud, nor on the collecting ducts at any developmental stage. No HB21 immunoreactivity was found in the

metanephric blastema until 16 weeks of gestation, after which an occasional isolated cell in the blastema stained. Almost all of the cells of the renal vesicle and the S-shaped tubule showed intense staining with this antibody (Figure 27). At the late S-shaped developmental stage the proportion of cells which were HB21 positive was reduced at the glomerular pole but remained high at the tubular pole. At the early maturation stage of development the expression of the transferrin receptor antigen was lost from both the flattened parietal and columnar visceral glomerular epithelium (Figure 28). During the maturation stages there was progressive loss of transferrin receptor antigen expression by the tubular cells until in the mature fetal and adult nephron the antigen was entirely absent from the tubular epithelium.

The mesangial cells of the developing glomerular tuft stained with the antibody. At the early stage of glomerular development most of the mesangium stained (Figure 28), but in glomeruli at later stages only those mesangial cells at the periphery of the glomerular tuft expressed the antigen.

The endothelium of blood vessels lying under the metanephric blastema stained with this antibody. The endothelium of the developing glomerular tuft also expressed the antigen but with maturation the HB21 positive endothelial cells were confined to the sub-epithelial part of the glomerulus. In the fully formed glomeruli in both fetal and adult kidney only an occasional cell expressed the antigen.

The interstitial cells did not express the transferrin receptor antigen, although an occasional positive cell with the morphology of a macrophage was seen in the interstitium.

Ki 67

When viewed under a low power objective, sections of fetal kidney stained with the Ki 67 antibody showed a striking zone of positive cells at the inner surface of the metanephric blastema. When examined in detail it was seen that in all the cells which expressed the Ki 67 antigen the reactivity was granular and was localised to the cell nucleus.

A few cells in the ingrowing ureteric bud branch expressed the Ki 67 antigen. These cells were found in the ampulla of the ureteric bud, while the interstitial portion showed no reactivity (Figure 29). Cells expressing the Ki 67 antigen were seen in the metanephric blastema although the majority of blastemal cells were negative. At the rosette, renal vesicle, and S-shaped tubule stages of nephronogenesis all the tubular cells strongly expressed the Ki 67 antigen (Figure 29). The ureteric bud adjacent to the S-shaped tubule still contained Ki 67 positive cells. At the early maturation stage of nephronogenesis the Ki 67 antigen was no longer seen on either the parietal or the visceral glomerular epithelium (Figure 30). The antigen was still seen on tubular cells at this stage but as nephron maturation progressed there was a gradual reduction in the proportion of tubular cells which stained . By the late maturation stage, and in the adult kidney, only a very occasional tubular cell stained with the Ki 67 antibody.

In the mesangium of the early developing glomerulus the majority of cells showed staining with the Ki 67 antibody. As the glomerulus matured

the Ki 67 positive cells were seen to lie at the peripheral part of the glomerular tuft (Figure 30). In the fully formed glomerulus in fetal and adult kidney no Ki 67 staining was seen.

In the immature mesenchyme underlying the metanephric blastema there were a few cells which expressed the Ki 67 antigen. Some interstitial cells in the deeper part of the cortex were also Ki 67 positive.

The endothelium of blood vessels underlying the blastema and in the developing glomerulus showed Ki 67 expression, but endothelium in the mature parts of the kidney did not stain.

The distribution of extracellular antigens.

Fibronectin immunoreactivity was found in the metanephric blastema where there was a delicate intercellular and pericellular distribution of staining. The ingrowing ureteric bud showed a strikingly different staining pattern. The epithelial cells did not contain fibronectin but strong staining was present at the base of the cells, sharply delineating the ureteric bud from

the surrounding blastema (Figure 31). The cells of the nephrogenic rosette and the vesicle showed a pattern of staining similar to the ureteric bud, a strong but narrow rim of peritubular staining sharply demarcated the boundary between the tubule and the metanephric blastema (Figure 31). Within the tubular component of the kidney this peritubular pattern of fibronectin staining persisted and was seen in the adult nephron.

In glomeruli there was similar sub-epithelial staining both under the parietal and the visceral epithelium at all stages (Figure 32). Within the glomerular tuft fibronectin could be identified around or possibly within mesangial cells. This staining was observed in both immature and mature glomeruli. Fibronectin was also seen delicately outlining the basement membrane of glomerular capillaries. It was not possible to determine whether this staining was related to the epithelium or to the endothelium.

The primitive mesenchymal cells of the developing renal interstitium showed some intercellular fibronectin but in the mature connective tissue this was sparse.

Fibronectin was found underlying the endothelium of blood vessels growing into the metanephric blastema, and in larger renal blood vessels. The antigen was also seen lying between the cells of vascular smooth muscle.

In sections of fetal kidney stained with the NDOG1 monoclonal antibody no reactivity was seen in the ureteric bud branches, nor in the metanephric blastema. In nephrons at the S-shaped tubule and early maturation stages of development NDOG1 immunoreactivity was detected. Staining was not seen in the epithelium but rather in the mesenchymal cells immediately surrounding the tubules and glomeruli of nephrons at this developmental stage. Diffuse cytoplasmic staining was seen in the plump mesenchymal cells at this site (Figure 33). No staining was seen in the more primitive mesenchymal cells. In the mesenchyme surrounding more mature nephrons there was no staining with the NDOG1 antibody. Some NDOG1 positive mesenchymal cells were seen immediately adjacent to large collecting ducts in the medulla of fetal kidney. The mesangium showed no reactivity with the NDOG1 antibody.

No NDOG1 reactivity was seen in the adult kidneys.

3.2 Discussion.

Three separate tissues combine to form the mammalian kidney. These are the metanephric blastema, the ureteric bud and a vascular bud from the dorsal aorta. Several different tissue interactions and pathways of differentiation are seen during renal organogenesis.

3.2.1 The growth and differentiation of the ureteric bud.

The relationship between the ureteric bud and the metanephric blastema has been studied by Grobstein (1955, 1956, 1967). In a transfilter induction system, of a variety of tissues tested, only the undifferentiated metanephric blastema was capable of inducing the growth, branching, and differentiation of the ureteric bud. The metanephric blastema was also capable of inducing ureteric bud-like outgrowth of other primitive epithelia (Bishop-Calame 1966). In other tissues which exhibit branching patterns of morphogenesis it has been shown that the mesenchyme is responsible for directing the structure and type

of branching of epithelial bud growth (Sakakura, Nishizuka & Dawe 1976). These results suggests that the intercellular signal initiating ureteric bud outgrowth from the mesonephric duct is of the "instructive" type. Thus, a highly tissue specific signal induces the differentiation or a particular growth pattern of the responding tissue. The responding tissue, until it receives the instructive signal is not committed to that pathway of differentiation (Holtzer 1968; Wessels 1970; Kratochwil 1972; Wessels 1977). Indeed because of the range of possible instructive signals which it may encounter, and to which it may respond, it is likely that the mesonephric duct retains considerable developmental plasticity (Bishop-Calame 1966). Once a signal has been encountered, differentiation occurs and the developmental options become restricted. This suggestion is very reasonable since it is known that different parts of the mesonephric duct must differentiate into collecting duct epithelium, urothelium, the ciliated epithelium of the ductus deferens, the secretory epithelium of the seminal vesicle, or the cuboidal epithelium of Gartner°s duct depending on their anatomical position and

the sex of the embryo (Hamilton & Mossman 1972). In addition to these normal differentiation options of the mesonephric duct, experimental manipulations can induce differentiation into other types of tissues. If fetal bladder epithelium, derived from the caudal part of the mesonephric duct, is implanted into the hormone dependent mesenchyme of the prostate the instructive mesenchymal-epithelial interaction characteristic of that tissue induces the mesonephric duct epithelium to differentiate into prostatic acini, including the synthesis of prostatic acid phophatase (Cunha and Lung 1980).

In this study, the growth of the ureteric bud branches has been studied by examining the distribution of proliferation associated antigens in the ureteric bud epithelium. The cells which expressed the Ki 67 nuclear proliferation antigen, and which by implication were the actively dividing cells, were located in the ampulla of the ureteric bud branch. These data suggest that the ureteric bud grows by the mitotic division of its own epithelial cells rather than by recruitment of cells from the adjacent mesoderm. It also shows that the ampulla, which is important in the

induction of metanephric blastema (Wolff 1968; Saxen & Kohenen 1969), is mitotically active. Potter (1972) has previously suggested that ureteric bud growth probably occurred by mitotic activity in the ampulla. Epithelial cell proliferation, as opposed to cell recruitment, has been shown to occur during the growth of other budding epithelia during embryogenesis such as the tracheal bud (Alescio & Cassini 1962) and the pancreatic bud (Ronzio & Latter 1973).

The proliferating cells in the ureteric bud branch did not express the cell membrane transferrin receptor antigen. In in vitro studies of transfilter induction of the metanephric nephron Ekblom et al (1983) and Thesleff & Ekblom (1984) have shown that although transferrin acts as a mitogen for the responding tissue, the inducing tissues were unresponsive to transferrin in the culture medium. Growth of the ureteric bud would seem to occur by the transferrin-independent proliferation of ampullary cells which pushes the ampulla into the overlying metanephric blastema.

In addition to growth and branching, the peripheral ureteric bud branches differentiate into the collecting duct epithelium of the adult kidney. This process involves the synthesis of specialised molecules essential for the secretory and absorptive functions of the collecting ducts.

Some antigens, for example EMA and low molecular weight cytokeratins, were expressed by the ureteric bud and collecting duct epithelial cells at all developmental stages. The expression of some other antigens, however, was found to change during differentiation. The earliest immunocytochemical studies on prekeratin antigens in tissues demonstrated that within the kidney these substances were present only in collecting duct epithelium and urothelium (Sun, Shih & Green 1978). This study has confirmed that in the kidney antigens derived from epidermal prekeratins are found only in the collecting ducts and urothelium. These two epithelia develop from the ingrowing ureteric bud, but during the development of the kidney epidermal prekeratins were not found in the peripheral branches of the ureteric bud. Only the smaller molecular weight cytokeratins detected by the antibodies CAM 5.2 and PKK1 were seen in the ureteric bud branches growing into the metanephric blastema. Indeed, the ampulla of the ureteric bud only expressed CAM 5.2 antigens. Using an immunofluorescence technique Holthofer et al (1983) also found PKK1 immunoreactivity in the ureteric buds in human fetal kidney. These

authors, in the same experiments, demonstrated the coexpression of PKK1 antigen and vimentin in the epithelium of the ureteric bud but not in the adult collecting ducts. In this study using the immunoperoxidase technique vimentin antigen was found in the epithelium of the ureteric buds, albeit sparsely, but not in fetal nor in adult collecting duct epithelium. The expression of large molecular weight cytokeratins and the loss of vimentin are features of the collecting duct differentiation of ureteric bud epithelium.

Further differences between the ampulla of the ingrowing ureteric bud and the differentiated epithelium were seen in their expression of fucosylated-N-acetyllactosamine determinants. The antigens detected by AGF 4.48, AGF 4.36 and C14 were found on the surface of the cells of the ampulla of the ureteric bud branch but not on the cells of the remainder of the ureteric bud or differentiated collecting ducts. The expression of these antigenic determinants persisted at this site until after fusion of the ureteric bud with the S-shaped tubule. It has been shown that the cells of the ampulla initiate tubulogenesis of the metanephric blastema (Wolff 1968; Saxen & Kohenen

1969). The chemical nature of the signal is not known but, although initially it was suggested that the message was a diffusible mediator, evidence obtained by the examination of the filter pores in transfilter induction experiments have demonstrated a requirement for cell-to-cell contact for induction (Saxen et al 1965; Waartiovaara 1974).

The 1-3 fucosylated-N-acetyllactosamine epitopes recognised by the monoclonal antibodies AGF 4.48 and AGF 4.36 have been found on other tissues (Howie et al 1984). Several different antibodies recognising these determinants have been described and the epitopes demonstrated on both membrane glycolipids and glycoproteins (Lloyd, Kabat & Licerio 1968; Gooi et al 1981; Hakomori et al 1981; Huang 1983). By using various different techniques these determinants have been found on early pre-implantation murine embryos and teratocarcinoma cells (Fox et al 1981; Kapadia, Feizi & Evans 1981), human granulocytes and their precursors (Fisher et al 1982), and in a variety of human and murine normal epithelial and carcinoma cells (Brockhaus et al 1982; Huang et al 1983; Combs et al 1984).

Fucosylated-N-acetyllactosamine on cell membranes is chemically related to type II blood group substances, Forsmann antigen, and other similar carbohydrate determinants which have been shown to be developmental stage specific antigens occurring in early embryos (Willison & Stern 1978; Kapadia, Feizi & Evans 1981; Shevinsky et al 1982).

The AGF 4.48, AGF 4.36 and C14 determinants were present on those cells which initiate tubulogenesis and which subsequently fuse with the S-shaped tubule. By contrast, the blood group H (Type II) molecules were not found on the ureteric bud ampulla but were seen on collecting duct epithelium. The signifance of these developmentally regulated changes in these and related carbohydrate antigens remains unknown. An endogenous mammalian lectin which binds to 3-fucosyl-N-acetyllactosamine has been described on hepatocytes (Prieels et al 1975). It is possible that lectin-sugar interactions at the cell surface may function as intercellular signals in the induction of renal tubular differentiation.

An alternative hypothesis is suggested by the work of Pennington et al (1986). By dissociating the cells of early mouse embryos and reaggregating

them in the presence of monoclonal antibodies against several oligosaccharides these authors have shown that fucosyl-N-acetyllactosamine is important in the cell-to-cell adhesion of the early embryo. Thiery et al (1984) demonstrated that the protein molecules L-CAM and N-CAM, which are associated with increased cell-to-cell adhesion, are essential for normal morphogenesis, although their precise mechanism of action is not known.

Ozawa et al (1985) have shown that fucosyl-N-acetyllactosamine, SSEA-1 in their terminology, is carried on a large molecular weight membrane bound glycoprotein called embryoglycan.

Intercellular adhesion, mediated by the fucosyl-N-acetyllactosamine oligosaccharides, may be important in establishing the cell-to-cell contact required for the induction of tubular differentiation. It is not possible, using immunocytochemistry, to determine which of these functions the ampullary expression of 1-3 fucosyl-N-acetyllactosamine fulfills.

Cell adhesion may also be important in the growth of the ureteric bud. The cellular form of fibronectin has been shown to have sites which bind to collagen and to cell membrane glycoproteins (Yamada 1978). This polypeptide is known to increase adhesion of epithelial cells to basement membrane substances and to mesenchymal extracellular matrices. This is thought to assist in the migration of epithelial cell sheets on acellular substrata (Yamada & Olden 1978; Kleinman, Klebe & Martin 1981). In this study I have demonstrated a thin layer of immunoreactive fibronectin at the junction of the ureteric bud epithelium and the undifferentiated metanephric blastema in fetal kidney. Fibronectin has also been found at such epithelial-mesenchymal interfaces of other budding epithelia during embryogenesis (Linder et al 1975; Zetter et al 1978; Critchley et al 1979).

Other molecules involved in cell-cell and cell-substratum adhesion have been found on ingrowing epithelial buds, including the ureteric bud, in vertebrate embryos. Thiery et al (1984) and Poole & Steinberg (1982) have shown that the growth of the pronephric and mesonephric ducts in

mice and in chickens is associated with changes in adhesion between the epithelial cells and their substrata. They have also shown that the cells of the ingrowing ureteric bud in mice express the calcium independent cell adhesion molecule L-CAM.

The adhesive properties of the epithelial cells of the ureteric bud, determined by the expression of certain polypeptides and oligosaccharides, which have been demonstrated immunocytochemically in this study, may be essential for the growth and normal morphology of the ureteric bud.

There was some heterogeneity of the phenotype of the differentiated collecting duct epithelium. Heterogeneity of collecting duct epithelium has been previously reported in several animal species. The majority of the collecting duct epithelial cells in rodents° kidneys are of a "light" type, but there is an additional population of "dark" or intercalated cells (Rhodin 1962; Ericsson et al 1969). The cytoplasm of these dark cells is more intensely eosinophilic, is more electron dense and contains increased levels of DPNH-tetrazolium reductase demonstrable by enzyme histochemistry (Novikoff 1960). Detailed

ultrastructural examinations have shown differences in the endoplasmic reticulum profiles and in mitochondrial morphology between light and dark cells (Novikoff 1960). The number of dark cells is increased in hypokalaemic states (Ericsson et al 1969). The kidney responds to hypokalaemia by increasing the urinary secretion of acid, a function which is dependent on carbonic anhydrase C in the nephron. These observations have led to the suggestion that the dark cells of the collecting ducts contain carbonic anhydrase C. The characteristic morphology and numbers of the carbonic anhydrase C containing cells demonstrated in the collecting ducts in this study lends some support to this theory. If this is true then the separation of the light and dark cells occurs during the maturation phase of development, when the distribution of carbonic anhydrase C changes from being present throughout the lenght of the collecting duct epithelium to being restricted to a subset of individual cells in the mature fetal and adult collecting duct. This evidence also suggests that at certain stages of development the collecting duct epithelial cells can all synthesise carbonic anhydrase C. It has previously

been suggested that the distinction between light and dark cells is a functional one and that individual cells can switch from one to the other depending on functional demands (Ericsson et al 1969).

Therefore, changes can be described during the growth and maturation of the ureteric bud and the structures derived from it.

3.2.2 The growth and differentiation of the metanephric tubules.

The cellular changes which occur during the induction of tubulogenesis and early tubule differentiation in the mouse kidney have been studied in vitro using a transfilter induction model (Grobstein 1955, Saxen et al 1968; Ekblom, Sariola & Thesleff 1984; Thesleff & Ekblom 1984). Thesleff & Ekblom (1984) have shown the first event in the induction of tubulogenesis in this model is that the cells of the metanephric blastema acquire responsiveness to a serum growth factor. By analysing the capacity of different serum constituents to stimulate blastemal cells it has been shown that the serum growth factor

required for *in vitro* tubulogenesis is transferrin. Transferrin when added to transferrin-free serum in the culture medium induces the blastema to undergo a rapid burst of proliferative activity which was measured by the uptake of tritiated thymidine (Ekblom, Sariola & Thesleff 1984; Thesleff & Ekblom 1984). This altered responsiveness occurs before any morphological changes were detected in the blastemal cells. The development of transferrin responsiveness occurs independently of specific serum factors but tubular differentiation requires both the presence in serum of the appropriate growth factor, and that the blastemal cells respond to it by cell division.

Transferrin acts as a mitogen for many different cell populations (Cohen & Fischbach 1977; Ekblom *et al* 1981; Broxmeyer *et al* 1980; Pelus *et al* 1981; Trowbridge & Lopez 1982; Gentile & Broxmeyer 1983; Partanen, Theseleff & Ekblom 1984). Cell membrane-bound receptors for transferrin have been shown to have a developmental stage specific pattern of expression during the differentiation of some tissues (Cohen & Fischbach 1977; Podlewski *et al* 1978; Kuromi,

Goneri & Hasegawa 1981; Markelonis 1982; Newman et al 1982; Stomatos et al 1983). The differentiation of skeletal muscle has been shown to require a proliferative response to a serum growth factor called myogenesis factor. Ii, Kimura & Ozawa (1982) have shown that myogenesis factor is transferrin.

Proliferation of uninduced blastema grown in transferrin-free serum can be induced by the addition of iron-chelating agents to the culture medium (Ekblom 1985). These iron binding molecules increase the intracellular concentration of iron. Transferrin also increases the intracellular iron concentration by receptor mediated endocytosis (Octave et al 1983).

In the metanephric blastema following the transferrin induced proliferation a change in the type of collagen synthesised by the blastemal cells occurs, from a type III to a type IV collagen (Ekblom 1981; Ekblom et al 1981). This switch from a connective tissue type of collagen to a basement membrane type is the first detectable expression of an epithelial phenotype in the induced metanephric blastema. Synthesis of the basement membrane collagen and also the

non-collagenous basement membrane glycoprotein laminin, which is another early event in tubulogenesis, have been suggested to be essential for the correct polarisation of epithelial cells and hence the morphogenesis of the nephrogenic vesicle (Ekblom et al 1980; Ekblom et al 1981).

In this study I have shown that at the first stage of tubulogenesis in the human fetal kidney, in vivo, the blastemal cells which had condensed adjacent to the ureteric bud branch expressed the cell membrane transferrin receptor antigen. The cells of the metanephric blastema did not express this antigen. Accompanying the expression of the transferrin receptor was the expression of the nuclear proliferation antigen Ki 67. Since this antigen is expressed by cells at all stages of the cell cycle other than G_0, Ki 67 provides a sensitive assay for proliferating cells in tissue sections (Gerdes et al 1984). These observations provide immunocytochemical evidence which supports the theory that in vivo the initiation of tubulogenesis from the metanephric blastema involves transferrin-dependent proliferation of the induced cells, and that the action of transferrin is mediated through a membrane-bound receptor.

Tubulogenesis was also accompanied by a change in the intermediate filament phenotype. The cells of the metanephric blastema, like most mesodermal cells, expressed the vimentin type of intermediate filament protein and did not express cytokeratin antigens. Although vimentin continued to be expressed by the cells of the nephrogenic rosette and vesicle these cells also expressed the CAM 5.2 cytokeratin antigen.

By the S-shaped tubule stage of development most of the tubular cells no longer expressed vimentin and only expressed the CAM 5.2 intermediate filament antigen. No PKK1 antigen was detected at the S-shaped tubule stage but was seen at later stages of nephronogenesis. Holthofer et al (1983) found vimentin immunoreactivity in the cells of the metanephric blastema but no such reactivity in the nephrogenic vesicle or tubule. Using immunofluorescence methods they found PKK1 immunoreactivity in the tubular pole of the S-shaped tubule. These differences between the observations recorded in this study and those of Holthofer et al (1983) may have occurred because of different fixation, processing, and immunocytochemistry techniques used. All of these

factors have been shown previously to affect the immunocytochemical detection of intermediate filament antigens (Altmannsberger et al 1982; Osborn & Weber 1983; Holthofer et al 1983).

However, a change in the intermediate filament phenotype has occurred during tubulogenesis and it can now be seen that by the nephrogenic vesicle stage the tubular cells may be architecturally, cytologically, and immunocytochemically defined as epithelial cells. They undergo further differentiation to form the fully functional nephron.

3.2.3 Segregation of the metanephric nephron.

By the S-shaped tubule stage of nephronogenesis the developmental fate of the tubular cells depends on their position within the S-shaped tubule. The lower limb will become the glomerulus, the middle limb will become the proximal tubule, and the upper limb will become the distal tubule and loop of Henle (Hamilton & Mossman 1972; Potter 1972). Study of the expression of tubular antigens, especially brush border antigen and epithelial membrane antigen,

has shown that immunocytochemically detectable segregation has also occured by this stage. The cells of the upper limb of the S-shaped tubule expressed EMA on their luminal surface while the cells of the middle limb expressed BB antigen. The cells of the developing glomerulus expressed neither of these tubular antigens. *In vitro* segregation of the metanephric nephron in transfilter induced mouse metanephros occurs at the equivalent stage (Ekblom *et al* 1981). These authors demonstrated segregation by the immunofluorescence staining of the tubules using BB antigen to identify proximal tubule, and Tamm-Horsfall glycoprotein to identify distal tubule.

Tamm-Horsfall glycoprotein is found in the cytoplasm of the distal tubular epithelial cells (Lewis, Schwartz & Schenk 1972; Hoyer *et al* 1974). It, therefore, has a different cellular distribution from EMA. Heyderman *et al* (1981) have demonstrated that these two antigens are on different molecules. In the *in vitro* segregation of the murine metanephric nephron the expression of brush border antigen preceded the expression of Tamm-Horsfall glycoprotein by 24 hours (Ekblom *et*

al 1981). It has been suggested, therefore, that proximal tubular differentiation occurs not only in a different part of the tubule from distal tubular differentiation but at a different rate. However, some work has suggested that in in vivo nephronogenesis Tamm-Horsfall glycoprotein is not synthesised by the distal tubular cells until even later in development (Lewis, Schwartz & Schenk 1972; Hoyer et al 1974). Using immunoperoxidase staining of fixed fetal kidney it is not possible to determine whether there is a time interval between brush border antigen expression by the proximal tubule and EMA expression by the distal portion of the S-shaped tubule.

The tubular cells at the S-shaped tubular stage of nephronogenesis no longer expressed the vimentin intermediate filament antigen which had been present in the cytoplasm of these cells earlier in tubule formation. The staining with CAM 5.2 was by this stage much more diffusely present within the cytoplasm of the cells of the tubular pole. At the glomerular pole, however, these changes in intermediate filament antigen expression did not occur. The parietal glomerular epithelium continued to express vimentin. The

visceral epithelium expressed vimentin, but at later stages of development of the S-shaped tubule this epithelium had also started to show some CAM 5.2 staining in the cytoplasm.

3.2.4 The growth and maturation of the metanephric tubule.

After fusion of the S-shaped tubule with the ureteric bud branch the different segments of the nephron are in continuity. The nephron, however, continues to grow. The tubule lengthens considerably and acquires many convolutions. Growth of the tubule, especially the elongation and descent into the medulla of the loop of Henle, continues throughout fetal and into post-natal life (Potter 1972). In this study the maturing tubular epithelium continued to express the transferrin receptor antigen and the Ki 67 nuclear proliferation antigen. At the later stages of nephronogenesis progressively fewer tubular epithelial cells expressed these antigens. Tubular growth would therefore seem to continue by the transferrin dependent mitotic division of tubular epithelial cells, with a progressive reduction in

the proportion of actively dividing cells after an initial phase during which growth is very active. There have been few studies on the kinetics of tubular growth during nephronogenesis. Those which have been performed are limited to studies of the increase in size of the whole kidney or to measuring the increase in tubular length during post-natal growth (Fetterman et al 1965).

Although initially the tubular segments are morphologically similar, they can be defined by their relative position. The tubular epithelial cells continued to express the segregation antigens EMA and brush border antigen throughout development. Further maturation of the epithelium from the different tubular segments results in morphological changes and in the expression of molecules associated with specific tubular functions.

During the maturation of the tubule carbonic anhydrase C was expressed in the cytoplasm of the tubular epithelial cells. The enzyme was initially found in the epithelium lining the proximal tubule, distal tubule and the collecting ducts. With maturation staining for carbonic anhydrase C became less strong in the proximal tubule but

remained strong in the distal tubular epithelium. In the post-natal kidney carbonic anhydrase C was barely detectable in the proximal tubule but was seen in the distal tubule.

A similar distribution of this enzyme was found in the adult human kidney using immunocytochemical techniques by (Kumpulainen 1984). Enzyme histochemical techniques have revealed enzyme activity in the cytoplasm of the distal tubule, but also on the luminal border of the proximal tubular epithelium (Lannerholm & Wistrand 1983; Lannerholm 1984). These authors have argued that proximal tubular membrane-bound enzyme is immunologically, and possibly biochemically, different from the cytoplasmic form of the enzyme.

Carbonic anhydrase C catalyses the hydration of metabolically produced carbon dioxide to carbonic acid (White, Handler & Smith 1970). In the renal tubular epithelium this acid dissociates, the hydrogen ion is secreted, and the bicarbonate reabsorbed to regulate blood pH (Pitts 1950). This acidification of urine occurs throughout the length of the tubule and is one of the essential functions of the nephron. Although

in this study carbonic anhydrase C has been demonstrated by immunocytochemical techniques at the early maturation stage enzyme activity cannot be confirmed by these methods.

The proximal tubular epithelium also showed the expression of new tubular antigens as it matured. The fucosylated-N-acetyl lactosamine determinants detected by AGF4.48, AGF4.36 and C14, which were demonstrated on the ampulla of the ureteric bud, were also found on the proximal tubular epithelium. These epitopes were seen on the luminal surface of the tubular cells at the early maturation stage. They were initially present in all subsegments (Jacobson & Kokker 1976) of the proximal tubule but as maturation progressed their distribution became more restricted. In the adult they were only seen on the cells of the pars recta of the proximal tubule, except in some juxtamedullary nephrons where they persisted throughout the proximal tubular length. Pretreatment of sections with neuraminidase exposed binding sites for these antibodies throughout the length of the proximal tubule.

Howie and Brown (1985) also using the immunoperoxidase method and the AGF 4.48 and AGF 4.36 antibodies found a similar distribution of these epitopes in the adult kidney. They also showed that pretreatment with neuraminidase unmasked antibody binding sites throughout the length of the proximal tubule. Similar results using other monoclonal antibodies to these oligosaccharides have been obtained in the adult kidney (Fox et al 1981; Schiemle et al 1982; McCarthy et al 1985). It would seem that these forms of fucosylated-N-acetyl lactosamine determinants are present throughout the proximal tubule but that on the majority of cells the oligosaccharides have a terminal sialic acid which masks the epitope. The loss of immunoreactivity to these antibodies seen during development is probably due to terminal sialylation occuring at the maturation stage.

Rauvala (1976) described a fucoganglioside extracted from human renal cortex whose glycosyl side chain had the structure:

$$\overset{\diagdown}{\times}\text{Neu 2-3 Gal 1-4 (Fuc 1-3) GlcNac 1-3 Gal}$$

...

The terminal of this oligosaccharide is clearly identical to a sialylated AGF 4.48 and AGF 4.36 determinant. It seems reasonable to suggest the unusual, possibly unique, renal fucoganglioside isolated by Rauvala (1976) is identical to the AGF 4.48 and AGF 4.36 binding antigen on the proximal tubular epithelium. The precise function of such a molecule at this site is unknown. The fucosylated-N-acetyl lactosamine determinants identified in the ureteric bud epithelium may not be carried on the same type of molecule. Gooi et al (1985) have identified the SSEA-1 carrying molecule on some embryonic tissue as a 35,000 M.W. glycoprotein called embryoglycan, but they have found SSEA-1 reactivity on a glycolipid in adult tissues. Similar glycolipids have been shown to function as cofactors for membrane bound enzymes in other sites (Hakomori 1981). The activity of the cofactor can be modulated by alterations in their terminal glycosylation.

The immunological determinant is a carbohydrate and is therefore synthesised by the action of glycosyl transferases. So the genetic control of the expression of the determinant is probably the same for the glycolipid and the

glycoprotein forms of the antigen although synthesis of the core molecule, either protein or lipid, will be under separate genetic control (Ginsburg 1972).

Alpha-1-antitrypsin and alpha-1-antichymotrypsin are lysosomal proteinase inhibitors whose function in plasma is to regulate the enzymic activity of many serum proteases (Gans & Tan 1967). They were both found in the cytoplasm of proximal tubular epithelial cells. The staining pattern was granular, as in many other tissues, reflecting their lysosomal localisation within the cytoplasm (Isaacson et al 1981). Ultrastructural examination of the kidney has shown that lysosomes are abundant in the tubular epithelial cell cytoplasm, being more numerous in the proximal tubule than the distal tubule (Miller & Palade 1964; Christensen 1980). Using electron microscopy and enzyme histochemistry lysosomes and some lysosomal enzymes have been identified in the maturing proximal tubular epithelium of the mammalian fetal kidney (Pugh & Walker 1965; Wachstein & Bradshaw 1965; Waartivaara 1966; Zeller 1973).

The proteinase inhibitors regulate the activity of some lysosomal enzymes. It is known that small protein molecules and oligopeptides, which pass through the glomerular basement membranes into the urinary filtrate, are absorbed by proximal tubular epithelial cells in endocytotic vesicles, and digested by fusion of these with lysosomes (Pugh & Walker 1965). The free amino acids which are produced are passed back into the circulation via the peritubular capillaries. The significance of proteinase inhibitor regulation of this activity in the proximal tubule is not known. Although some defects of renal tubular transport of certain amino acids have been described in cases of alpha-1-antitrypsin deficiency a consistent renal lesion is not a feature of that clinical syndrome.

These two proteins were seen in the cells of the metanephric blastema and in the immature renal interstitial cells, but were not seen in the S-shaped tubule, so changes in the expression of the alpha-1-antitrypsin and alpha-1-antichymotrypsin genes must occur during the early stages of tubulogenesis.

The iron-binding protein ferritin was first localised in the proximal tubule of the human kidney usng immunocytochemical techniques by Mason & Taylor (1975). The ferritin which has been extracted from the kidney and analysed biochemically and electrophoretically was an acidic ferritin. This differed from the basic ferritin present in large amounts in the liver and reticulo-endothelial system (Powell et al 1975). However, it has been shown that although electrophoretically and biochemically different these ferritins have extensive immunological cross-reactivity (Arora et al 1970; Powell et al 1975; Jacobs & Worwood 1975; Rossiello et al 1984).

In this study ferritin was first seen in the developing nephron at the late maturation stage of nephronogenesis where it was confined to the proximal tubule. It is known that in adult tissues ferritin synthesis is stimulated by iron but it is not known whether the same regulatory mechanisms control ferritin synthesis de novo in fetal tissues.

3.2.5 The maturation of the glomerulus.

In the S-shaped tubule phenotypic differences between the glomerular and tubular poles were already apparent. In this study the change in intermediate filament phenotype which was a feature of tubulogenesis did not occur in the visceral glomerular epithelium. The visceral glomerular epithelium still showed vimentin immunoreactivity. However, this epithelium also expressed the cytokeratin antigen CAM 5.2. The parietal glomerular epithelium expressed neither of these antigens at this developmental stage. Neither the visceral nor the parietal glomerular epithelium expressed the tubular antigens EMA or BB antigen. The glomerular epithelium is not only phenotypically different from the tubular epithelium at the S-shaped tubule stage but there are also differences between the visceral and parietal glomerular epithelia.

With maturation the CAM 5.2 cytokeratin antigen, which at first had a focal granular distribution within the visceral glomerular epithelial cells, became localised to the basal cytoplasm of these cells at the early maturation stage of development. Ultrastructural studies on the developing mammalian glomerulus have

demonstrated a condensation of filamentous or fibrillary material at the base of the columnar visceral glomerular epithelial cells while the glomerular tuft was being vascularised (Vernier & Birch-Andersen 1962; Mounier et al 1986). Connections between these filamentous structures and the underlying basement membrane, through the cell membrane, have been seen. Although the CAM 5.2 antigen has been extensively used as an immunohistochemical marker of epithelial differentiation in the practice of surgical pathology, the determinant is carried on the low molecular weight cytokeratin filaments which form the cytoskeleton of epithelial cells. The cytoskeleton and its attachments to the extracellular matrices are important in morphogenesis (Edelman 1982). herefore, it may be that during the ingrowth of the blood vessels which form the glomerular tuft the visceral epithelial cells depend upon a keratinous cytoskeleton to maintain cell structure.

Later during nephronogenesis the visceral glomerular epithelium lost its CAM 5.2 reactivity, but continued to express vimentin. It was at this stage, after the loss of cytokeratin expression by

the visceral epithelium, that the parietal glomerular epithelium started to express CAM 5.2 antigen. Parietal glomerular epithelium did not express vimentin.

Holthofer et al (1983) found vimentin expression by the fetal visceral glomerular epithelium, but it was not detected until the early maturation stage in their experiments. In the adult kidney they also found vimentin in the visceral glomerular epithelium. Frank, Tuzynski & Warren (1981) found vimentin only in the mesangium and endothelial cells of the glomerulus of hamster kidneys. Studying both rat and bovine kidney Bachmann et al (1983) found vimentin in both epithelial layers of the glomerulus.

There have been no previous reports of the expression of cytokeratin antigens by visceral glomerular epithelium, but Holthofer et al (1983) found low molecular weight cytokeratin antigens detected by the monoclonal antibody PKK2 in human parietal glomerular epithelium. Bachmann et al (1983) did not find cytokeratin antigens in rat or bovine visceral glomerular epithelium, but did find some in the parietal epithelium in these species. Whether these various differences in intermediate filament expression represent a true species difference or a difference of technique remains to be elucidated.

Franke et al (1982) have defined true epithelia as tissue in which both desmosomal junctions and cytokeratins are found. In glomeruli desmosomes are sparse in the parietal epithelium and absent from the visceral epithelium. The differentiated visceral epithelium pnly expressed the vimentin type of intermediate filament, although parietal epithelium expressed cytokeratin. The only other epithelium which expresses vimentin and not cytokeratin is eye lens epithelium (Ramaekers et al 1980). This epithelium also lacks desmosomes (Ramaekers et al 1980). These findings create new difficulties in defining epithelia and epithelial-like tissues.

The nuclear proliferation antigen Ki 67 has been used to study the role of cell division in the growth and development of the glomerulus. The co-expression of the transferrin receptor antigen and the Ki 67 antigen seen during tubulogenesis persisted in the S-shaped tubule. In the glomerular epithelium, however, the expression of these two antigens was lost by the early maturation stage. At this stage the parietal epithelium was beginning to flatten but the visceral epithelium was still columnar and the

cell nuclei close together. These results suggest that proliferative activity in the glomerular epithelium ceases at this early stage of development. The glomerulus still grows to reach adult size and as it does so the visceral epithelium covers an increasingly complex and lobulated glomerular tuft. The glomerular epithelium also undergoes morphological changes. The crowded columnar epithelium of the early maturation stage can be seen, firstly, becoming less closely packed, and then flattening considerably so that in the adult kidney the epithelial cell cytoplasm covering the surface of the glomerular tuft is inconspicuous in histological sections. In the light of this evidence, it seems reasonable to suggest that the cell number in the glomerular epithelium reaches its adult total early in development, proliferation ceases, and the growth of the glomerular tuft continues by the flattening and extension of the epithelial cell cytoplasm during podocyte formation.

Although the glomerular epithelium did not express the transferrin receptor antigen or the Ki 67 antigen after the early maturation stage, the

mesangial cells and the endothelial cells of the developing glomerular tuft continued to express both antigens. At the early maturation stage both of these cell types expressed the proliferation associated antigens but as maturation proceeded reactivity was confined to the endothelial cells at the periphery of the developing glomerular tuft. Therefore, the mesangium and endothelium of the glomerulus would seem to grow, like other parts of the nephron, by transferrin-dependent proliferation and division *in situ* rather than by recruitment from the blastema. The localisation of the proliferating cells at the peripheral parts of the glomerulus suggest that the endothelium, in effect, pushes the overlying glomerular epithelium out into the glomerular space. This will give rise to the lobulated tuft covered by the flat visceral epithelium of the adult kidney.

The mesangial cells and the endothelial cells both expressed intracytoplasmic vimentin. Holthofer *et al* (1983) and Bachmann *et al* (1983) in their respective experiments both found vimentin in the mesangium of the developing glomerular tuft.

The mesangial cells and endothelial cells differed in their pattern of fibronectin expression. Mesangial cells, like many mesenchymal cells, showed an intracellular expression of fibronectin, but in the glomerular endothelial cells, as in endothelium elsewhere, fibronectin was found associated with the basement membranes. This combined endothelial basement membrane and mesangial cell pattern of fibronectin expression within the developing glomerulus is similar to that obtained in the adult glomerulus by Dixon et al 1980. However, in other immunocytochemical experiments Stenman & Vaheri (1978) and Linder, Miettinen & Tornroth (1980) could only find this antigen in the mesangium.

Interspecies chimaeric studies in which mouse metanephros was grown on chick chorio-allantoic membrane have shown that the ingrowing endothelial cells of the developing kidney synthesize their own basement membrane collagens, laminin, and fibronectin (Ekblom et al 1982; Sariola et al 1983; Ekblom, Sariola & Thesleff 1984). In these experiments the mesangium synthesised a mouse specific fibronectin. Fibronectin possesses binding sites for basement membrane collagens

(Yamada 1982), and for cell membrane glycosaminoglycans and proteoglycans. These properties enable the molecule to cause adhesions between the cells and the substrata (Yamada & Olden 1978; Vaheri et al 1978). Such cell-substratum adhesions may be important for the development of the endothelium, mesangium, and the glomerular basement membranes during glomerular growth and development. Dixon et al (1980) have demonstrated changes in fibronectin reactivity in a variety of glomerular diseases and have proposed a role for fibronectin in normal and abnormal glomerular function.

Thus, the glomerulus develops by changes in structure and function of three cell types - epithelium, endothelium and mesangium.

3.2.6 The growth and differentiation of the renal interstitium.

The renal interstitium, like the nephron, originates from the metanephric blastema. It is the supporting tissue of the developing and adult nephron. The small, round cells of the blastema elongate, becoming progressively less conspicuous

with differentiation until, in the mature parts of the late fetal and adult kidney, these cells are identified only with difficulty under light microscopy, lying between the endothelium of the peritubular capillaries and the tubular epithelium. The renal interstitial cells are known to have specific biosynthetic pathways and specific functions (Lerman et al 1972), but little has been written about the immunocytochemistry or embryology of these cells.

The interstitial cells expressed vimentin antigens in their cytoplasm at all stages of development but did not express cytokeratin antigens. Therefore, they may be defined according to their intermediate filament phenotype as mesenchymal cells (Lazarides 1982; Osborn et al 1982).

In this study I have found that initially the spindle shaped cells differentiating from the metanephric blastema contained the lysosomal proteinase inhibitors alpha-1-antitrypsin and alpha-1-antichymotrypsin, but these antigens were lost during differentiation. Some of the cells of the blastema also expressed these antigens. Ultrastructural studies have shown lysosomes in

the cytoplasm of the blastemal cells and the primitive mesenchymal cells of fetal kidney. In the adult kidney the cortical interstitial cells did not contain lysosomes (Lerman et al 1972; Bulger & Nagle 1973).

The interstitial cells within the active nephrogenic zone, like the developing nephron, expressed both the transferrin receptor antigen and the Ki 67 nuclear proliferation antigen. It would seem that like the nephron the interstitial cells also undergo transferrin-dependent proliferation during early differentiation. Why this event accompanies epithelial differentiation for one group of cells and interstitial differentiation for another is not known. However, the interstitium seems to grow with the associated nephron and with maturation the expression of the proliferation antigens is lost.

The interstitial cells surrounding the S-shaped tubule and early maturation stage nephrons expressed the NDOG1 antigen in their cytoplasm. This antigen is recognised by a monoclonal antibody raised against trophoblastic tissue (Sunderland, Redman & Stirrat 1981) which in tissue sections stains only the

syncytiotrophoblast (Bulmer, Billington & Johnson 1984). The antigen is found on a few specialised adult tissues including the connective tissue surrounding breast acini and underlying the urothelium (Sunderland, Stirrat & Redman 1981). The determinant is thought to be part of the hyaluronic acid or chondroitin sulphate molecules (Sunderland, Stirrat & Redman 1981). The reason for the expression of this trophoblastic antigen by the interstitial cells which surround the primitive nephron is not clear. In transfilter induction experiments it was shown that the developing tubule required the presence of the adjacent mesenchymal cells for normal growth, elongation and S-shaped tubule formation (Gossens & Unsworth 1972). It was also demonstrated that there was no uptake of mesenchymal cells into the tubule once induction had occurred and that the requirement for the mesenchymal cells seems to be a secondary, possibly supporting function (Saxen & Sakselsa 1971; Gossens & Unsworth 1972). The mesenchyme surrounding the nephron at this stage may provide an as yet unknown but nevertheless essential factor for tubular morphogenesis. Alteration in the expression of hyaluronic

acid-like molecules has been shown to occur during the morphogenesis of other tissues (Oster, Murray & Maini 1985). The evidence suggests that these changes in the extracellular matrix may be important in inducing cell compaction and cell-cell interactions during organogenesis. The signifance of the expression of the NDOG1 antigen by the renal interstitial cells at this early stage in nephronogenesis is unknown.

In this study fibronectin was seen lying between the cells of the metanephric blastema. As the interstitial cells differentiated fibronectin was initially seen in a similar distribution but the mature interstitial cells, usually lying singly between tubules and capillaries, did not contain fibronectin. Fibronectin was seen in the adjacent tubular and endothelial basement membranes. This confirms the distribution of fibronectin in the renal cortex described by Dixon et al (1980). In the interstitium of the medulla fibronectin was seen within the collagenous extra-cellular matrix lying between the collecting ducts and the thin limbs of the loops of Henle.

This lack of fibronectin associated with the cortical interstitial cells in immunocytochemical experiments supports previous ultrastructural studies which have shown that the mature cortical interstitial cells are a specialised type of mesenchymal cell which does not synthesise significant amounts of extra-cellular martrix substances (Maunsbach & Christensen 1980).

3.2.7 The development of the juxtaglomerular apparatus.

The juxtaglomerular apparatus consists of the vascular pole of the glomerulus, the specialised renin-secreting cells in the walls of the afferent arteriole, and the macula densa of the distal tubule. The histology of the fetal kidney demonstrates how these structures come together to form the adult juxtaglomerular apparatus. At the S-shaped tubule stage, blood vessels grow into the concavity of the lower pole of the S to vascularise the developing glomerulus. The first part of the developing distal tubule is adjacent to these vessels at this stage. This portion of the distal tubule remains at this vascular pole while the remainder of the distal tubule elongates and becomes convoluted.

The renin containing cells were first seen adjacent to blood vessels growing into the metanephric blastema. Renin was seen in similar perivascular cells in the nephrogenic zone of the fetal kidney using immunofluorescence microscopy by Phat et al (1982).

As the S-shaped tubule formed these cells lay at the lower pole of the tubule. Therefore, during glomerular development the renin containing cells were already part of the juxtaglomerular apparatus. The first part of the distal tubule lies at the vascular pole of the developing glomerulus. The cells on the glomerular side of this portion of the tubule adopt a characteristic morphology and are known collectively as the macula densa (Barajas 1970; Barajas 1971). They have receptors on their luminal surface which detect changes in the sodium concentration of the tubular filtrate and these changes result in the modulation of renin secretion by the granular epithelioid cells of the juxtaglomerular apparatus (Lindop & Lever 1986).

The cells of the macula densa have antigenic differences from the rest of the distal tubule. Although they expressed EMA on their luminal

surface they did not contain carbonic anhydrase C. During development, at the early maturation stage, when carbonic anhydrase C was first seen in the nephron, the cells of the portion of the distal tubule adjacent to the vascular pole of the glomerulus did express the antigen. The enzyme was lost, however, during maturation. This suggests that the macula densa develops from appropriately placed distal tubular cells during tubular maturation.

Matthews & MacIver (1982) have shown a further antigenic difference between the macula densa and the remainder of the distal tubule. They found Tamm-Horsfall glycoprotein throughout the length of the distal tubule except in the cells of the macula densa.

Thus, there is asynchrony in the development of the juxtaglomerular apparatus, the renin containing cells appearing before the formation of the vascular pole of the glomerulus and of the macula densa.

3.2.8 Summary.

In summary, analysis of the developmental stage and site specific pattern of expression of different antigens has shown that some antigens are important in growth and early nephron differentiation, some during tubular segregation and some appear as markers of cytoplasmic and functional maturation. These antigens as developmental markers may be considered within the broad classification of a) proliferation markers, b) markers of tubulogenesis, c) segregation markers, d) maturation markers. The groups into which each marker is placed is given in Table 9.

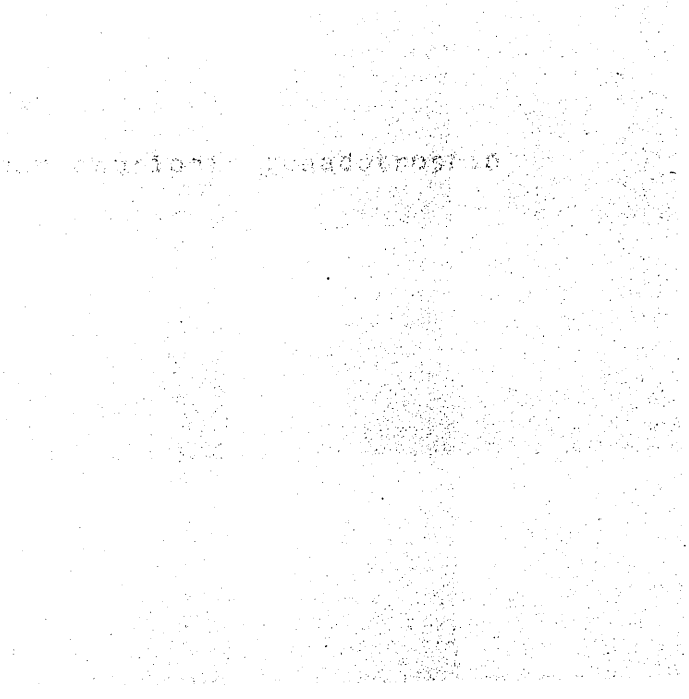


TABLE 9

Classification of renal differentiation markers and developmental stage.

Proliferation markers

Ki 67

Transferrin receptor

Tubulogenesis markers (Stage 2)

Cytokeratins, CAM 5.2 & PKK1

Segregation markers (Stage 3)

Epithelial membrane antigen

Brush border antigen

Tubular maturation markers (Stages 4&5)

-1 antitrypsin

-1 antichymotrypsin

Ferritin

Carbonic anhydrase C

Fucosylated poly N-acetyl lactosamine

Hormones

Renin

Human chorionic gonadotrophin

Oncofetal antigens

- Alpha fetoprotein
- Carcinoembryonic antigen

Extracellular matrix molecules

- Fibronectin
- NDOG1

Markers of mesenchymal cells

- Factor VIII R antigen
- S-100 protein
- Desmin
- Myoglobin

CHAPTER 4.

DIFFERENTIATION IN RENAL DYSPLASIA

4.1 Results

4.1.1 Cystic renal dysplasia.

Morphology.

The different tissues which were recognised during morphological part of the study are listed in Table 10.

The most consistent feature was the finding of primitive ducts. These were large ducts lined by columnar epithelium which showed minimal pleomorphism. Mitoses were not seen in these structures. These ducts were surrounded by a concentric cuff of small spindle shaped mesenchymal cells (Figure 34). A thick PAS positive basement membrane was usually present. In some cases these ducts showed cystic dilatation and in two cases this change was marked, the cysts occupying most of the renal substance with only a minimal amount of intervening fibrous tissue. In

two cases a mucus secreting epithelium was seen lining some of the dysplastic ducts. In a further two cases the epithelium lining the ducts was double layered. The inner layer was columnar and the outer layer cuboidal. In one case the columnar epithelium was ciliated. Frequently the dysplastic ducts aggregated to form a nodular collection surrounded by cuffs of immature mesenchymal cells. The primitive ducts in some cases showed continuity with the renal pelvis.

The glomeruli, when present, showed poorly developed capillary tufts and were lined by a hyperchromatic cuboidal epithelium. Cystic dilatation of the glomerular space was frequently seen. Periglomerular fibrosis and sclerosis of the glomerular tufts were features in six cases, but there was no inflammatory infiltrate surrounding these glomeruli. In many cases no obvious tubular pole could be seen in these immature glomeruli even after examination of multiple adjacent sections. The tubules seen within dysplastic areas of these kidneys had a wide range of morphological appearances. In some cases well differentiated tubules, indistiguishable from normal tubules were seen but in these areas there was a marked

increase in interstitial mesenchyme. The majority of the tubules were more immature. They were lined by a flattened epithelium in which mitoses were seen. Many were cystically dilated and the lumen contained homogeneous eosinophilic material which was PAS positive. When numerous these tubules gave the so-called "thyroidisation" appearance to the renal cortex.

In all cases there was an increase in mesenchymal tissue. This usually consisted of dense fibrous tissue surrounding the primitive ducts and tubules (Figure 34). In some cases the mesenchyme was more cellular, the cells having a plump, spindle shaped or stellate morphology. This type of mesenchyme was usually seen between aggregates of tubules. Mitoses were seen in these cellular areas of mesenchyme. At the edges of the dysplastic areas increased interstitial mesenchyme was noted between the tubules in the adjacent, non-dysplastic, renal cortical tissue, thus blurring the distinction between the normal and abnormal segments.

Some differentiated mesenchymal tissues were also seen. Myxoid tissue, consisting of small stellate cells lying in a loose myxoid

extracellular stroma was seen in three cases. Islands of mature adipose tissue lying in a dense fibrous background were seen in five cases. Cartilage was identified in twelve cases. Bars or islands of cartilage lay within areas of fibrous tissue but in some cases were surrounded by tubular and glomerular structures.

The blood vessels within areas of cystic dysplasia were also markedly abnormal. Two distinct vascular patterns were seen, frequently co-existing in individual cases. One type of abnormal vessel was a large artery with medial thickening, but more strikingly with very marked intimal proliferation and fibrosis. In one case disruption of the internal elastic lamina and focal calcification was seen in the walls of these vessels. The other type of abnormal vasculature was a plexiform structure composed of dilated, thin walled endothelial lined channels. The mesenchyme lying between these dilated vessels was a mixture of smooth muscle and fibrous tissue.

Immunocytochemistry.

Cytokeratin antigens were found in the epithelial cells in renal dysplasia. These antigens were not found in the mesenchymal tissues. The primitive ducts expressed antigens detected by all three anti-cytokeratin antibodies. Staining was seen around the peripheral parts of the cytoplasm (Figure 35). In those cases in which double layered epithelium was seen, only the inner columnar layer stained with these antibodies. The tubules in renal dysplasia expressed only the antigens detected by CAM 5.2 and PKK1. The immature glomeruli did not stain with these antibodies.

The tubular segregation antigens EMA and BB antigen were also seen in the epithelial cells in renal dysplasia. The primitive ducts expressed EMA on their luminal surface (Figure 36) but not BB antigen. Within the tubular components both EMA and BB antigen were seen. There were many more EMA positive tubules than BB antigen positive tubules in all cases. Cuboidal epithelium expressed both antigens but the flattened regenerative type of tubular epithelium only expressed EMA. Examination of serial sections suggested that although many EMA positive tubules were BB antigen negative and

vice versa, there were a few tubules in which both antigens were expressed. Visceral glomerular epithelium expressed neither antigen, but in two cases both EMA and BB antigen were detected on parietal glomerular epithelium which showed morphological evidence of tubular metaplasia (Figure 37).

Alpha-1-antitrypsin and alpha-1-antichymotrypsin were seen in small well differentiated tubules. The antigens were detected in the cytoplasm of the cuboidal cells with a proximal tubular morphology. In addition to this expression of alpha-1-antitrypsin and alpha-1-antichymotrypsin by dysplastic tubular epithelial cells immunoreactive proteinase inhibitors were seen in some mesenchymal cells. These antigens were found in a few plump spindle cells within myxoid areas of the dysplastic mesenchyme in three cases. The antigens were also found within cells which had the morphology of macrophages. These macrophages were seen as part of an inflammatory infiltrate in the medulla of the dysplastic areas of four cases. Proteinase inhibitors were not found in other mesenchymal tissues.

Carbonic anhydrase C was not identified in the dysplastic areas of these kidneys, but it was seen in the adjacent kidney. The mucus secreting and bronchial type of epithelia in cystic dysplasia stained for CEA but not for AFP.

Factor VIII R antigen stained the endothelium of the large abnormal arteries in cystic renal dysplasia, but did not stain the endothelium of the dilated plexiform vessels. The endothelium of the glomerular capillaries did not contain Factor VIII R antigen.

S-100 protein was seen in the adipose cells and in the chondrocytes seen in cystic renal dysplasia. The outer layer of the double layered ductal epithelium expressed S-100 protein (Figure 38).

Fibronectin was seen in the fibrous mesenchyme and associated with the basement membranes of tubular epithelium and vascular endothelium. Sclerotic glomeruli showed intense staining for fibronectin in the sclerotic areas, but in other glomeruli fibronectin was associated with the basement membranes.

No staining for myoglobin nor for desmin was observed.

4.1.2 Multilocular cystic nephroma.

Morphology.

Two multilocular cystic nephromas were studied. These were large segmental abnormalities consisting of numerous large dilated cysts which did not communicate with each other. Despite the great size of these lesions the adjacent renal cortex was not compressed.

The cysts were lined by flattened cuboidal or hobnailed type of epithelium (Figure 39). The hobnailed epithelium in both cases showed a mild degree of cytological atypia. The intervening stroma consisted of variably cellular fibrous tissue with focal myxoid areas. In both cases some hyalinised collagenous tissue was present. No heterologous tissue was seen but mast cells were prominent.

Immunocytochemistry.

The epithelium lining the cysts in multilocular cystic nephroma stained with all three anti-cytokeratin antibodies (Figure 40). Most of this epithelium also expressed EMA on the apical cell surface, and in a few smaller cysts

staining with AGF 4.48 and AGF 4.36 was observed. No staining for BB antigen, nor for any of the cytoplasmic proteins was observed in the epithelium.

The fibrous connective tissue showed intercellular staining for fibronectin. Some intracellular fibronectin and A1AT were seen in the more cellular areas of mesenchyme and in the myxoid areas.

Factor VIII R antigen was seen in the endothelium of blood vessels within the mesenchyme of these multilocular cystic nephromas.

No staining for desmin, myoglobin nor S-100 protein was observed.

The kidney adjacent to the multilocular cystic nephromas was morphologically and immunocytochemically normal.

4.1.3 The nephroblastomatosis complex.

Morphology.

Nodular renal blastema.

Three cases of nodular renal blastema were studied. In all three cases the lesion had a subcapsular distribution (Figure 41). The nodules of persistent blastema were well circumscribed, but not encapsulated. The morphology of all three cases was similar.

Most of the abnormal tissue was blastematous, consisting of islands of dark staining oval cells with ill defined cell borders but minimal pleomorphism. Mitoses were not seen. There was some tubular differentiation in two cases. This consisted of circular aggregates of columnar cells surrounding a small central lumen (Figure 41). Glomerular bodies were not seen. Small thin walled capillaries entered the nodules from the adjacent renal cortex. No other mesenchymal component was noted.

In one case glomerular immaturity was seen in the adjacent renal cortex.

Nephroblastomatosis.

Three cases of nephroblastomatosis were available for study. Morphologically these showed some similarities to nodular renal blastema, but

the lesions were much more extensive. There were areas of blastema consisting of ovoid hyperchromatic cells arranged in sheets. In all three cases tubular epithelial differentiation was prominent. The epithelium was cuboidal with mostly small central nuclei. Pseudoglomeruli were numerous in two cases. These consisted of vascularised glomerular tufts but with a hyperchromatic cuboidal epithelium (Figure 42). Mesenchymal tissue was inconspicuous. A few well differentiated interstitial cells lay between tubules and some capillaries were also seen. There was no heterologous mesenchymal tissue.

In two cases the areas of nephroblastomatosis were demarcated from the adjacent renal cortex by a band of dense fibrous tissue. The adjacent cortex in one of these cases contained immature glomeruli. In these two cases islands of malignant nephroblastoma were seen arising within the nephroblastomatosis (Figure 43). These were both classical triphasic nephroblastomas with abundant epithelial differentiation.

The third case was unusual in many respects. In this case the nephroblastomatosis lesion occupied the entire bulk of a lower duplex kidney.

It had developed in a child with a caudal dysplasia syndrome which included spina bifida, split notochord, lower limb anomalies, duplication of the hind gut and persistent cloacal fusion.

The nephroblastomatosis lesion showed other features. There was an area of cystic renal dysplasia with primitive ducts, tubules, sclerosis and bars of cartilage. There were also two areas, within the nephroblastomatosis, of tissue which morphologically resembled fetal lung. Bronchi, lined by pseudostratified ciliated epithelium were seen growing into lung parenchyma with alveolar structures and a rich capillary network (Figure 44). The bronchi were in continuity with collecting duct like structures in the adjacent nephroblastomatosis.

Metanephric hamartoma.

These lesions were identified in ten cases of nephroblastoma. They varied in size from 1mm to 2cms in approximate diameter. They were seen in three sites. In two cases hamartomas were seen in the subcapsular part of the kidney, in nine cases they were seen at the interface between the tumour and the adjacent kidney and in three cases they were identified within fibrous septae which ran between islands of neoplastic tissue.

The morphology of the lesions was similar in the three different sites. The main bulk of tissue consisted of collagenous fibrous tissue. Occasional tubules, collecting ducts or immature glomeruli were identifiable. Although immature these structures were cytologically well differentiated, lacked pleomorphism and mitoses were not seen. In six cases ectatic endothelial lined spaces were present. These did not contain red blood cells.

When present at the tumour-kidney interface or within tumours these lesions were ill-defined and in some cases difficult to distinguish from well-differentiated components of the nephroblastoma.

Glomerular immaturity.

Glomerular immaturity was seen in ten cases. These glomeruli had poorly developed vascular tufts containing slightly dilated capillaries. The glomerular epithelium, on both surfaces, consisted of hyperchromatic cuboidal cells with scanty cytoplasm.

These glomeruli were not demarcated from the adjacent renal cortex. The surrounding tubules and interstitium were normal. Although sclerosis was seen in some immature glomeruli an inflammatory infiltrate was not seen. Mild cystic dilatation of Bowman's space was seen in two cases.

Immunocytochemistry.

Nodular renal blastema.

The blastema of these lesions did not stain with any of the antisera tested.

Staining of the epithelial cells was seen with CAM 5.2 but not with PKK1 or epidermal prekeratin. CAM 5.2 staining was focal within the epithelial cell cytoplasm. In both cases a few well differentiated tubules expressed EMA on their apical surface (Figure 45). BB antigen, again located on the epithelial cell surface membrane, was seen in one of the cases. The epithelial cells did not express proteinase inhibitors, fucosylated poly-N-acetyllactosamine determinants nor carbonic anhydrase C.

The inconspicuous interstitial cells of these lesions failed to express any mesenchymal antigens but fibronectin was detected associated with the tubular basement membrane.

Factor VIII R antigen was seen in the endothelium of the small blood vessels in these lesions.

Nephroblastomatosis.

A similar distribution of antigens was seen in all three cases. The blastemal areas of nephroblastomatosis did not express any of the antigens tested.

Low molecular weight cytokeratins were demonstrated in the cytoplasm of the tubular epithelial cells by CAM 5.2. The majority of the epithelial cells stained with CAM 5.2 but no staining was observed with either the PKK1 antibody or the antiserum to epidermal prekeratins. Many of the tubules expressed EMA on their apical surface. Some tubules, negative for EMA expressed BB antigen, but there were a few tubules which expressed neither antigen. No immunoreactivity for alpha-1-antitrypsin, fucosylated poly-N-acetyllactosamine determinants nor for carbonic anhydrase C was seen. The interstitial cells failed to express any of the mesenchymal antigens. Fibronectin was seen

associated with capillary and tubular basement membranes. Peritubular capillary endothelium contained immunoreactive Factor VIII R antigen. Staining was seen in the cytoplasm of the long thin endothelial cells.

In two cases of nephroblastomatosis tubular metaplasia of the parietal glomerular epithelium of immature glomeruli was seen. These metaplastic cells expressed low molecular weight cytokeratins in the cytoplasm and BB antigen and EMA on the apical surface membrane.

Metanephric hamartoma.

There was a delicate intercellular staining for fibronectin seen in the mesenchymal areas of these lesions. In the majority of cases the spindle cells did not express any of the mesenchymal antigens. The exception to this was two cases in which foci of cells expressing desmin were seen. In these cells there was a diffuse cytoplasmic pattern of staining. In one case a few adipose cells showed reactivity to S-100 protein in the peripheral rim of cytoplasm. The endothelial cells lining blood vessels contained Factor VIII R antigen but the endothelium lining the dilated vascular channels did not contain this antigen.

Low molecular weight cytokeratins detected by CAM 5.2 and intermediate molecular weight cytokeratins detected by PKK1 were seen in the tubules of the metanephric hamartomas. There was diffuse staining of the cytoplasm of the epithelial cells with both antibodies. These tubules also expressed on their apical cell surface either EMA or BB antigen.

Glomerular immaturity.

The epithelium of immature glomeruli expressed none of the antigens tested. The endothelium did not contain Factor VIII R antigen but the basement membranes showed reactivity with the antibody to fibronectin.

4.2 Discussion.

4.2.1 Cystic renal dysplasia.

Renal dysplasia is an abnormality of the differentiation of the metanephros (Risdon 1971a). It is frequently associated with abnormalities, especially obstructive lesions, of the lower urinary tract (Bernstein 1968; Risdon, Young & Chrispin 1975). It has been suggested that these associated lesions are important in the pathogenesis of renal dysplasia (Bialestock 1965). Using microdissection techniques Osathanondh & Potter (1964) demonstrated that in dysplastic kidneys there is reduced branching of the ureteric bud with cystic dilatation of the ampullae. This results in a failure to induce metanephric differentiation from the blastema.

These microdissection studies have shown that the cysts within the dysplastic areas were dilated portions of the ureteric bud (Osathanondh & Potter 1964; Potter 1972). In this study the epithelium lining the dysplastic cysts expressed all three types of cytokeratin antigen. Since the large molecular weight epidermal prekeratins have been

found only on the ureteric bud derived epithelium in the fetal and adult kidney (Chapter 3), this observation provides immunocytochemical evidence which supports the conclusions derived from the microdissection studies.

Not only the cysts, but the primitive ducts, characteristic features of dysplastic kidneys (Ericsson & Ivemark 1958; Risdon 1971a), also had the cytokeratin phenotype of ureteric bud derived epithelium. These structures, like the ureteric bud, expressed EMA, but did not express carbonic anhydrase C. During normal nephronogenesis as the ureteric bud differentiates into collecting duct epithelium it expresses the epidermal prekeratins, then later, carbonic anhydrase C (Chapter 3). The immunocytochemistry data on the ductal epithelium in renal cystic dysplasia suggests that this epithelium has incompletely differentiated into its normal tissue, namely, collecting duct epithelium.

Heterologous epithelial differentiation was seen in the primitive ducts. Glandular, ciliated, and in one extreme case, bronchial type epithelium was seen. These heterologous elements also expressed antigens, for example CEA, not normally

found in the kidney. Heterologous epithelium of this type and squamous epithelium has been described previously (Fisher & Smith 1975; Chu & Myint 1986). An interesting, and previously undescribed, feature was the presence of a double layered epithelium lining some primitive ducts in two cases. Only the inner layer was of an epithelial phenotype, while the outer layer had the morphology and expressed antigens previously described in the myoepithelium of the breast and salivary gland (Nakajima et al 1982).

The tubular component of dysplastic segments showed no evidence of heterologous differentiation, but these tubules did show evidence of immaturity of differentiation. The cells of the tubular epithelium were morphologically immature, confirming previous descriptions (Ericsson & Ivemark 1958; Risdon 1971a; Fisher & Smith 1975).

In the immunocytochemical experiments these cells also exhibited relative immaturity. Although they expressed some early tubular differentiation markers such as EMA, BB antigen and A1AT, these tubules failed to express carbonic anhydrase C.

The dysplastic glomeruli have also been described as immature with poorly developed capillary tufts (Pasternak 1960). The glomerular capillary endothelium did not express Factor VIII R antigen. In the developing glomeruli of fetal kidneys Factor VIII R antigen was found in this site, but not in adult glomeruli (Chapter 3). The detection of fibronectin within sclerotic areas of a few glomeruli is similar to findings observed in areas of glomerular scarring caused by other glomerular diseases (Dixon et al 1980).

Some tubular metaplasia of the parietal glomerular epithelium was seen. Tubular metaplasia of parietal glomerular epithelium has been described in several other pathological conditions (Ward 1970). The metaplastic tubular epithelium expressed both the distal tubular antigen EMA and the proximal tubular antigen BB antigen. Similar co-expression of these tubular antigens has been found in regenerative tubular epithelium in different renal lesions including glomerulonephritis, interstitial nephritis and acute tubular necrosis (Fleming & Matthews 1987). However, the pathogenesis of tubular metaplasia of glomerular epithelium in cystic renal dysplasia is unknown.

Immunocytochemical studies of the distribution of renin containing cells in dysplastic kidneys have shown that there is also immaturity of the juxtaglomerular apparatus. The renin containing cells in dysplastic kidneys are found in a perivascular distribution similar to that observed in the nephrogenic zone of fetal kidney (Amat et al 1981).

Within the mesenchymal component of dysplastic kidneys there was morphological evidence of heterologous differentiation. Several types of mesenchymal tissue were seen including myxoid tissue, fibrous tissue and cartilage. Previous authors have also emphasised the wide range of connective tissue elements seen in renal cystic dysplasia (Ericsson & Ivemark 1958; Risdon 1971a; Fisher & Smith 1975; Kissane 1984). The cells within the immature myxoid tissue expressed cytoplasmic proteinase inhibitors in a distribution similar to that observed in the mesenchyme of the nephrogenic zone of fetal kidney and in the immature mesenchyme of nephroblastomas (Chapter 5).

The fibrous tissue contained intercellular fibronectin and both the adipose cells and the chondrocytes expressed S-100 protein. Both of these antigens are found in similar tissues in other sites (Du Boulay 1985a; Roholl, de Jong & Ramaekers 1985).

Several previous authors have described the heterologous differentiation of the mesenchyme in cystic renal dysplasia as metaplastic transformation. Metaplasia means the change from one type of differentiated tissue to another type of differentiated tissue. Since the abnormality in cystic renal dysplasia is a failure of the nephrogenic mesenchyme to differentiate, heterologous differentiation in renal dysplasia is an example of heteroplasia. Willis (1962) has emphasised the importance in distinguishing these two phenomena, since the genetic and cellular processes involved may be entirely different, particularly in the requirement for initial de-differentiation during metaplasia.

I have previously discussed the reciprocal nature of the tissue interaction during development between the ureteric bud and the metanephric blastema (Chapter 1). In renal

dysplasia the failure of the ureteric bud to adequately branch causes a secondary failure of initiation of tubular differentiation of the metanephric blastema (Potter 1972). Since the ingrowing ureteric bud is equally dependent on the differentiating metanephros to regulate normal differentiation of collecting duct epithelium (Grobstein 1956; Vernier & Smith 1969), the ureteric bud derived epithelium in renal dysplasia would be expected to show abnormalities. As we have seen this is indeed the case. The epithelium of the primitive ducts showed morphological and immunocytochemical evidence of immaturity, and also showed heteroplastic differentiation to several different epithelial cell types.

The blood vessels within dysplastic segments are morphologically abnormal. Both pathologically thickened arteries and plexiform vessels can be seen. The endothelium of the latter type of vessel in this study failed to express Factor VIII R antigen. Previous authors, studying other diseases, have suggested that this implies that such plexiform vessels are probably lymphatics, since lymphatic endothelium does not express Factor VIII R antigen (Mukai et al 1980). However,

in the kidney I have shown that changes in the phenotype of the vascular endothelium occur during development, and that some vascular endothelium, for example glomerular capillary endothelium in the adult kidney, does not express Factor VIII R antigen (Chapter 3). It remains possible, therefore, that the plexifrom vessels seen in cystic renal dysplasia and in metanephric hamartomas are vascular and not lymphatic.

The changes in the other type of abnormal vessel, the thickened artery may be secondary to altered blood flow through the diseased segment. Similar abnormalities in arteries feeding vascular malformations have been described (Catto 1985).

The significance of the abnormal vasculature of the dysplastic kidney, and hence of ischaemia, in the causation or extent of some of the abnormalities of the dysplastic kidneys is unknown.

4.2.2 Multilocular cystic nephroma.

The precise nature of this lesion has been debated (Boggs & Kimmelstiel 1956; Fowler 1971; Aterman et al 1973; Johson et al 1973). The major

issue is concerned with deciding whether multilocular cystic nephroma is a developmental anomaly or a neoplasm. As with other previously reported cases of multilocular cystic nephroma, both lesions studied here were segmental and consisted of large multilocular cysts with narrow intervening fibrous septa. The epithelium lining the cysts showed some morphological variation, but in the immunocytochemistry experiments it had the cytokeratin phenotype of ureteric bud derived epithelium. It also expressed EMA and the epithelium of some cysts expressed the fucosylated poly-N-acetyllactosamine determinants. No proximal tubular epithelium was identified by these techniques.

The mesenchyme lying between the cysts was mostly fibrous tissue with a few more cellular areas. In the immunocytochemical experiments this tissue expressed the antigens found in fibrous tissue elsewhere, including that seen in renal cystic dysplasia.

In this study, however, there is insufficient evidence to conclusively answer the debate regarding the nature of multilocular cystic nephroma. However, there was evidence of only mild

atypia, in the epithelial component, and no mitotic activity was seen. The immunocytochemical data suggests that the cysts are derived from the ureteric bud as are the cysts in renal dysplasia. I have therefore been unable to find any evidence in support of the hypothesis that multilocular cystic nephroma is neoplastic and have found some evidence relating it to renal cystic dysplasia as a developmental anomaly.

4.2.3 The nephroblastomatosis complex.

This group of related lesions are characterised by structural disorganization, the presence of immature elements and an association with nephroblastoma. The most obvious of these features is the immaturity of all elements of the nephron (Bove & McAdams 1976).

In nodular renal blastema there is blastema and morphologically immature tubular epithelium. In this study the tubular epithelium only expressed the early differentiation antigens such as low molecular weight cytokeratins detected by CAM 5.2, and the cell surface antigen EMA. This epithelium did not express the maturation markers.

This immunocytochemical data supports the morphological evidence for arrested tubular epithelial differentiation in nodular renal blastema.

It has been suggested that this lesion arises because of failure of ureteric bud ampullae to grow into the subcapsular portion of the metanephric blastema, and therefore fails to induce tubulogenesis (Bove & McAdams 1976). No ureteric bud ampullae were detected by immunocytochemical methods in nodular renal blastema.

The blastema and epithelium in cases of nephroblastomatosis is also morphologically immature (Bove & McAdams 1976). Like the epithelium in nodular renal blastema the epithelium in nephroblastomatosis only expressed early tubular differentiation markers.

Nephroblastomatosis is a more extensive lesion than nodular renal blastema, frequently causing nephromegaly which can be massive (Hou & Holman 1961; Bove & McAdams 1976). It has been suggested that there is a proliferative element to this lesion causing an expansion in the volume of immature renal tissue (Bove & McAdams 1976). I

have shown that there is a close relationship between proliferation and differentiation during nephronogenesis (Chapter 3), and that the immature tubular epithelium proliferates rapidly following the induction of differentiation. It is possible that in nephroblastomatosis there is an uncoupling of the close relationship between proliferation and differentiation so that the affected tissue can proliferate but fails to differentiate normally. These possible abnormalities in the relationship between proliferation and differentiation may also occur in embryonal tumours and are discussed more fully below (Chapter 5).

4.2.4 The inter-relationships of dysplastic lesions.

The morphological and immunocytochemical studies suggest relationships amongst these renal dysplastic lesions. Previous studies have discussed such relationships (Potter 1972; Bove & McAdams 1976; Marsden & Lawler 1983).

All of these lesions are abnormalities of differentiation of the metanephric blastema (Risdon 1971a; Potter 1972; Bove & McAdams 1976). There are differences, obviously in structure and in anatomical distribution within the kidney, but also in their relationship with other pathological conditions such as the association of some renal dysplastic conditions with nephroblastoma (Bove & McAdams 1976; Marsden & Lawler 1983).

Cystic renal dysplasia occurs when there is a segmental (or a complete) failure of ureteric bud branching (Potter 1972) and the pyramidal shape of these lesions has been emphasised previously (Marshall 1953). Within the affected segment the blastema differentiates, incompletely and abnormally, but persisting blastema, that is entirely undifferentiated tissue, is unusual. There seems to be no increased risk of the subsequent development of a nephroblastoma in patients suffering from this form of renal dysplasia.

In the nephroblastomatosis complex the abnormality of differentiation occurs when the subcapsular blastema is not induced by ureteric bud branches (Bove & McAdams 1976; Marsden &

TABLE 10

Tissue morphology in renal dysplasia.

Primitive ducts	18
Cysts	17
Immature glomeruli	16
Cartilage	12
Adipose tissue	5
Myxoid tissue	3

Lawler 1983). Uninduced blastema seems to be capable of persisting at this site. Differentiation is incomplete but rarely heterologous. These lesions are associated with subsequent development of a nephroblastoma (Berry 1987).

If differentiation eventually occurs in an area of nodular renal blastema the resulting lesion is the sclerosing metanephric hamartoma (Marsden & Lawler 1983). Uninduced blastema *in vitro* eventually differentiates into fibroblasts and synthesises collagen (Saxen *et al* 1968). It seems that a similar event probably happens *in vivo* with the formation of a fibrous or sclerosing lesion. The immature tubules of nodular renal blastema may then differentiate further, but still incompletely, to give rise to the type of tubule seen in metanephric hamartomas.

The nature, fate and risks of development of a nephroblastoma from these lesions therefore, seems to be related to the type of ureteric bud abnormality which has caused the lesion initially, and more importantly on the presence of surviving undifferentiated blastema. In some ways this conclusion repeats the hypothesis forwarded by

Cohnheim in the middle of the nineteenth century, namely the proposals that tumours, especially embryonal tumours, develop from tissue which has retained an embryonic developmental potential (Chapter 1).

CHAPTER 5

5.1 Results.

5.1.1 Nephroblastoma.

Morphology.

The nephroblastomas in the series of 36 cases showed a wide range of histological appearances in keeping with the varied morphology of that tumour.

Blastema was seen in 30 cases. The proportion of blastema within each tumour ranged from cases in which only a few islands were seen to other cases in which most of the tumour was blastematous. The blastemal component consisted of islands or sheets of closely packed uniform, undifferentiated, ovoid cells with poorly demarcated cytoplasm. The nuclei were round or ovoid, strongly haematoxyphilic, had coarsely clumped chromatin, and in some cells one or two small basophilic nucleoli. In all cases mitoses were frequent in blastemal areas. In two cases there was anaplasia in the blastemal areas. Necrosis with an associated acute inflammatory cell infiltrate was seen in the blastemal areas of 24 cases.

Gradual transitions of blastema into other types of tissue, either epithelial or mesenchymal, were seen.

Tubules (Type A) were present in 31 cases. The results of grading the tumours from 0 to +++ on the proportion of the tumour which was epithelial are shown in Table 11. The tubules showed a continuous spectrum of differentiation and in individual tumours a range of histological appearances were seen. The most poorly differentiated tubules consisted of tall columnar cells with a large densely staining elongated nucleus and a small amount of pale eosinophilic cytoplasm. These epithelial cells were arranged in ill-defined tubular structures (Figure 46). Well differentiated tubules were those in which there was a well defined circular or ovoid aggregate of cuboidal cells with a round nucleus and a moderate amount of eosinophilic cytoplasm. The majority of tubules in the tumours was at a point between these two extremes of histological appearances. Mitotic figures were seen in tubular epithelium. One of the cases in which blastemal anaplasia was seen also showed anaplasia of the epithelial component.

Tubules of Type B morphology were seen in 12/36 cases. These tubules were well differentiated, circular and lined by a low cuboidal epithelium. The epithelium showed minimal pleomorphism, the nuclei were small and round. There was a moderate amount of eosinophilic cytoplasm. These tubules were most frequently seen in mesenchymal areas of the tumours and were surrounded by a concentric cuff of mesenchyme (Figure 47). They ranged in size from tiny terminal branches, containing only a few cells, to large tubules containing more than twenty cells when seen in cross section. The larger tubules showed some cystic dilatation. In one case numerous small terminal branches were seen surrounding a larger central tubule (Figure 47).

Ten nephroblastomas contained glomeruloid bodies. In three cases these were very numerous and consisted of well formed structures with a vascular glomerular tuft lined by a cuboidal epithelium. In the remaining cases glomeruloid bodies were sparse and poorly formed. In these seven tumours the pseudoglomeruli consisted of a small epithelial outgrowth lying within a circular or crescent shaped space, also lined by

epithelium. Both epithelial surfaces consisted of small dark staining cells with scanty cytoplasm. A vascular component was not seen in most of the glomeruloid bodies in the tinctorial stained sections, but in three cases red blood cells lying within an endothelial lined structure were present.

Microcysts were seen in 10/36 cases of nephroblastoma. These were rarely numerous in individual tumours. They consisted of cystically dilated structures lined by a cuboidal or columnar epithelium.

Papillary structures were seen in two cases.

Heterologous epithelium was seen in three cases. In one of these there were areas of differentiated tubular structures lined by mucin secreting columnar epithelium or by stratified squamous epithelium. In another only mucin secreting epithelium was present. In the third case there were numerous branching tubules some of which were seen in continuity with the overlying pelvic urothelium. These tubules were lined by a double layered epithelium. There was an internal columnar layer and an external cuboidal layer. Both cell types had clear cytoplasm which did not

stain with the periodic acid Schiff method. The tubules were surrounded by a PAS positive basement membrane. In places a mucin secreting epithelium was also seen.

The heterologous epithelium in these three cases showed minimal nuclear pleomorphism and mitotic figures were very infrequent. In all three cases, elsewhere in the tumours there were typical Type A and Type B epithelium.

Different types of mesenchymal tissues were seen in 31/36 cases. In five of the tumours the mesenchymal tissue constituted almost all of the tumour bulk. In all cases most of the mesenchymal tissue consisted of immature spindle cells with oval nuclei and poorly defined cytoplasm lying in a pale staining matrix. Mitotic figures and nuclear pleomorphism were seen. In some tumours a little intercellular collagen was present. In addition to this poorly differentiated mesenchyme, areas of differentiated mesenchymal tissue were recognised in tinctorial stained sections. The distribution of these tissues is summarised in Table 12.

Adipose tissue consisted of well differentiated clear cells with a compressed rim of cytoplasm and an inconspicuous nucleus. These cells were arranged in small clumps often with intervening collagen. Skeletal muscle cells could be seen on haematoxylin and eosin stained sections but in all cases cross striations became more apparent by the use of PTAH staining. Large skeletal muscle cells were present but less well differentiated rhabdomyoblasts adjacent to the differentiated cells were also seen.Only a few smooth muscle fibres were seen in the two tumours containing this tissue. Myxoid tissue consisted of a pale staining loose mesenchyme with pleomorphic spindle or stellate cells. The intercellular matrix stained with alcian blue. In one tumour densely hyalinised and hypocellular connective tissue was seen. In the differentiated mesenchymal areas mitotic figures were very sparse. The blood vessels in the nephroblastomas ranged from large dilated vessels with surrounding smooth muscle to small capillaries.

Immunocytochemistry.

Markers of tubulogenesis.

No cytokeratin antigens were seen in the cells of the undifferentiated blastema nor of the mesenchymal component of the nephroblastomas.

In the tubules of nephroblastoma cytokeratin antigens were detected. In all cases in which tubules were seen staining of the cytoplasm of the tubular epithelial cells with CAM 5.2 was present (Figure 48). The pattern of staining varied with the morphology of the tubules. In those tubules which were morphologically least well differentiated no CAM 5.2 staining was seen, but as the degree of tubular differentiation progressed initially some granular cytoplasmic staining was observed and in the well differentiated tubules there was a more diffuse staining pattern, with reactivity distributed round the cell periphery (Figure 48). Staining with the PKK1 antibody was seen in only a few tubules in four cases. Three of these tumours had been graded as +++ on the amount of tubular epithelium present. PKK1 staining was also localised to the peripheral parts of the cytoplasm of the tubular epithelial cells. By staining serial sections with CAM 5.2 and PKK1 it was seen that the cells which were PKK1 positive were also CAM 5.2 positive.

Epidermal prekeratins were seen in small well differentiated Type B tubules which were present in 12 cases (Figure 49). They also stained with PKK1 and CAM 5.2, the staining with all three antibodies being localised to the peripheral parts of the cytoplasm. The small terminal branches of the Type B tubules only stained with CAM 5.2. In three tumours the heterologous stratified squamous and mucinous epithelium which was seen as part of the Type B tubules stained with all three anti-cytokeratin antibodies.

The flattened epithelium of the microcysts showed variable staining. These epithelial cells stained with PKK1 and with CAM 5.2, but in two cases also stained with the antibody to epidermal prekeratin.

Pseudoglomeruli and papillary structures failed to stain with any of the cytokeratin antibodies.

The segregation markers EMA and BB antigen.

In the nephroblastomas EMA was only present on the apical surface of the epithelium of some well differentiated tubules (Figure 50), although there

were other well differentiated tubules which did not contain EMA. This restriction of EMA expression to well differentiated tubules was independent of the histological pattern of the tumour as a whole.

Some but not all microcysts stained with the antibodies to EMA. Pseudoglomeruli and papillary structures did not show EMA staining. EMA was seen on the apical surface of the Type B tubules when these were present.

BB antigen expression was also restricted to some well differentiated tubules (Figure 51), while others failed to express the antigen. The anti-BB antibody failed to stain pseudoglomeruli, papillary structures or microcysts. By contrast to EMA, BB antigen was not found on the Type B tubules.

Neither antibody stained the blastemal nor the mesenchymal components of the tumours. No staining was seen on endothelial cells.

Maturation markers.

Alpha-1-antitrypsin and alpha-1-antichymotrypsin showed the same distribution in nephroblastoma. The cytoplasm of some of the plump hyperchromatic cells of the undifferentiated metanephric blastema contained these proteinase inhibitors, although the majority of these cells were negative. The cytoplasm of the spindle cells of the loose primitive mesenchyme seen between islands of blastema in many nephroblastomas showed strong granular and diffuse staining with antibodies to the proteinase inhibitors (Figure 52). Positive staining was also seen within the cells of three tumours showing areas of rhabdomyoid differentiation within the mesenchyme. The proteinase inhibitors were not seen in myxoid areas, adipose cells nor in endothelial cells. The tubular and glomeruloid components of the nephroblastomas were also negative. Both proteinase inhibitors were present in the mononuclear cell component of the inflammatory infiltrate frequently seen in the necrotic areas of many of these tumours.

No staining for carbonic anhydrase C was seen in nephroblastoma.

No staining for ferritin was seen in the neoplastic component of nephroblastoma but ferritin was seen in the cytoplasm of macrophages within necrotic areas of the tumours.

Staining of the nephroblastomas with the monoclonal antibodies AGF 4.48 and AGF 4.36 was seen in two different sites. In six cases both AGF 4.48 and AGF 4.36 stained the luminal surface of Type B tubules. The staining was not present on all such tubules, but rather was restricted to the small terminal branches (Figure 53). In three cases aggregates of these tubules staining with these antibodies were seen surrounding a larger unstained Type B tubule (Figure 53).

The other site of expression of the fucosylated N-acetyl lactosamine epitopes was only seen in one case. Two neoplastic tubules (Type A) consisting of columnar cells expressed these determinants on the luminal surface (Figure 54). Review of the same area of this tumour in the sections stained for EMA and BB antigen showed that the AGF 4.48 and AGF 4.36 positive tubules had also been positive for BB antigen but not EMA. The tumour in which these tubules were identified was unusual in other respects. Within the mesenchymal component there were large amounts of mature adipose tissue and skeletal muscle cells. The tumour had been removed from a child with a family history of nephroblastoma.

No staining was observed with the antibodies to alpha-fetoprotein nor to human chorionic gonadotrophin. In a single case a focus of blastemal cells stained intensely with the antibody to CEA. Staining was present throughout the cell cytoplasm.

Mesenchymal antigens.

Staining for vimentin was seen in the blastema, immature mesenchyme and the differentiated mesenchymal components. Some staining was also seen in the primitive tubular epithelium in 15 cases. Vimentin was not seen in well differentiated tubular epithelium.

In nephroblastoma Factor VIII R antigen was only seen in vascular endothelial cell cytoplasm, but the various histologically different areas in nephroblastomas showed a different pattern. In blastemal areas two appearances were seen. In the majority of tumours (21 cases) there was a branching and anastomosing pattern of capillaries within the blastema. The endothelium lining these vessels contained Factor VIII R antigen. In the blastemal areas of 9 cases blood vessels were very

scanty and when present did not consistently stain for Factor VIII R antigen. The mesenchymal areas of nephroblastoma contained blood vessels of various sizes from small arteries or veins to capillaries. The endothelium of all these vessels contained Factor VIII R antigen.

The vascular pattern was variable in the epithelial areas of nephroblastoma, and depended on the amount of epithelium present in the tumour. In tumours of grades + or ++ of epithelial content no particular relationship between Factor VIII R antigen positive endothelium and the neoplastic tubules was seen. Capillaries were seen adjacent to some tubules but not to others. However, in all tumours with grade +++ epithelial content a striking peritubular capillary network was seen. All of the endothelium lining this type of blood vessel contained Factor VIII R antigen. The positive cells were seen closely applied to the epithelial basement membrane (Figure 55). Pseudoglomeruli, when seen, did not contain Factor VIII R antigen.

There was S-100 protein in the thin peripheral rim of cytoplasm in the mature adipose cells seen in 4 nephroblastomas (Figure 56). In one case a focus of blastemal cells showed S-100 protein in their cytoplasm.

In three nephroblastomas some small thin spindle shaped cells which stained for S-100 protein were seen. These cells were sparse, but were seen in a characteristic position. They were intimately associated with thin walled blood vessels, lying just outside the endothelium (Figure 57).

No other cell types stained with the antibody to S-100 protein.

In 5 tumours strong cytoplasmic staining for myoglobin was seen in large rhabdmyoid cells. Some of these cells contained cross-striations visible in the immunocytochemical preparations but others showed diffuse staining of the cytoplasm for myoglobin (Figure 58). In addition, some myoglobin containing cells with a moderate amount of cytoplasm and a round nucleus were identified in the blastema of three cases of triphasic nephroblastoma (Figure 59). These cells had not been identified as rhabdomyoid in the adjacent tinctorial stained sections.

Staining for desmin was seen in the striated muscle cells in 5 cases of nephroblastoma. In addition, a few polygonal or round cells in the blastema also contained desmin. The smooth muscle cells around arteries in these tumours contained desmin. In 2 cases islands of smooth muscle cells contained desmin.

Extracellular molecules.

There was a delicate reticular pattern of fibronectin distribution in the blastemal areas of nephroblastoma. This interstitial staining pattern was also seen in immature mesenchyme, but at this site some intracellular staining was also seen. In areas of epithelial differentiation fibronectin was seen associated with the epithelial basement membrane (Figure 60). Fibronectin was also associated with the endothelial basement membranes in the various types of blood vessels seen in nephroblastoma.

In pseudoglomeruli fibronectin was also associated with the basement membranes.

The sarcomatoid nephroblastomas showed only weak interstitial staining for fibronectin.

No staining for NDOG1 was seen in the 5 nephroblastomas studied.

Proliferation associated molecules.

The nuclear proliferation molecule, detected by Ki 67, was seen in some cells of the undifferentiated blastema in all five cases studied.

Staining for this antigen was, however, seen in almost all of the tubular epithelial cells (Figure 61). Mesenchymal cells also expressed this molecule at about the same level as the blastema.

In one case Type B tubules were seen. Some staining for the nuclear proliferation antigen was seen in cells lining this structure (Figure 62).

The blastemal cells did not express the cell surface transferrin receptor detected by HB 21. By contrast the tubular epithelial cells stained strongly for this antigen (Figure 63). There was no staining with this antibody in the type B tubules. Only weak staining of a few cells in the mesenchymal areas was seen using HB 21.

Endothelial cells stained with HB 21 and a few showed Ki 67 immunoreactivity.

Hormones.

Nineteen nephroblastomas were tested for the presence of renin.

Cells containing immunoreactive renin were found in ten cases. The staining for renin consisted of cytoplasmic granules. The cells containing renin were variable in morphology. In

the majority of cases they were long thin spindle shaped cells with oval nuclei and long cytoplasmic processes (Figure 64). In a few cases the renin containing cells were more plump with round nuclei. In some areas these more round cells were morphologically indistinguishable from adjacent tumour cells.

Although the morphology of renin containing cells was variable they were remarkably consistent in their position within the tumours. They were intimately associated with blood vessels within the tumours, lying immediately outside the vascular endothelium (Figure 64). The vessels ranged in size from small thin walled capillaries to dilated arterioles. The renin containing cells were most frequently seen at the junction between the blastema and mesenchymal areas within the tumours.

Renin containing cells were not seen associated with pseudoglomeruli.

5.1.2 Cystic Partially Differentiated Nephroblastoma.

Morphology.

One case of this rare childhood renal tumour was available for study. The tumour consisted of numerous large cysts with intervening tissue. The cysts were lined by a cuboidal or a flattened simple squamous epithelium. In one cyst a hob nailed type of epithelium was seen. The tissue lying between the cysts contained a mixture of blastemal, mesenchymal and epithelila elements. The predominant tissue type was mature fibrous connective tissue in which were trapped some islands of adipose tissue, smooth muscle and striated muscle.

The blastema had the same morphology as the blastema of the other nephroblastomas but pleomorphism was minimal and mitotic figures were not seen.

The epithelium in the intervening connective tissue consisted of immature tubular epithelium similar to that observed in classical nephroblastoma.

Immunocytochemistry.

The reaction of most of this tumour was similar to that observed in the corresponding elements in the other nephroblastomas.

The feature which distinguishes this tumour from other nephroblastomas is the presence of numerous large cysts. The cyst epithelium stained for all three cytokeratin antigens, for EMA but not for BB antigen. Some cyst lining cells expressed the fucosylated N-acetyllactosamine epitopes detected by AGF 4.48 and AGF 4.36 on the luminal surface.

5.1.3 Renal cell carcinoma.

Morphology.

A variety of histological appearances were seen within the series of renal cell carcinomas. The classification of tumours according to cell type, architectural pattern and nuclear morphological grade is summarised in Table 13.

Twenty eight tumours consisted of predominantly clear cells. These cells were large cuboidal or columnar cells with most of the cytoplasm unstained in the tinctorial stained preparations, although a thin peripheral rim of cytoplasm and a distinct cell border were seen (Figure 65). In the majority of cases the nucleus was uniform, small and mitotic figures were infrequent. This is reflected by the low nuclear grading of the clear cell tumours.

The predominant cell type in 7 tumours was the granular cell. These cells had a finely granular eosinophilic cytoplasm. Granular cells showed considerable cytological and nuclear pleomorphism. Cells of this type were frequently seen within clear cell carcinomas and conversely clear cells were a common finding in some areas of granular cell tumours.

Only one tumour was of basophilic cell type. These cells were small cuboidal cells with a round dark nucleus and a small amount of cytoplasm.

Eight tumours contained sarcomatoid tissue. The predominant cell type in these areas was a long thin spindle cell with marked variation in cell size and marked nuclear pleomorphism (Figure 66). Occasional multinucleate cells were seen in these tumours. A high mitotic rate was seen in the sarcomatoid areas. Elsewhere these tumours had either clear cell areas (6 cases) or granular cell areas (2 cases).

The general architecture of the renal carcinomas was variable. The predominant architectural appearance in each tumour is given in Table 13. Solid tumours had either a trabecular or more commonly an alveolar architecture. These

tumours also possesed a prominent delicate anastomosing capillary network. Papillary tumours were those in which frond-like processes of tumours cells were supported by a fibrovascular core. In these papillary tumours massive necrosis, haemorrhage, neutrophil infiltrates and cholesterol clefts were all prominent. In 7 tumours there was a predominantly tubular architecture but there were occasional alveolar or papillary areas in all of these case. Only one cystic tumour was seen. Papillary outgrowths of tumours grew into the cystic spaces of this tumour.

The architecture of the sarcomatoid areas was variable. In 3 cases there was a distinct storiform architecture, multinucleate cells and a high mitotic rate resembling the appearances seen in malignant fibrous histiocytoma. In 3 cases the spindle shaped tumour cells were arranged in interlacing sheets and bundles resembling fibrosarcoma. In 2 cases hyalinised areas of sarcomatoid tissue were seen and in one of these there were islands of bone and pleomorphic cartilage.

The stromal components of the carcinomas were also examined. In all cases this consisted of fibroblasts within collagenous septae. In 6 cases the stroma was hyalinised and in 2 there was abundant mucopolysaccharide demonstrated by staining with alcian blue. Lipid and iron laden macrophages were seen in large numbers in 2 clear cell tumours and in one granular cell tumour, all of which had a papillary architecture.

In general clear cell carcinomas were a lower grade than the granular cell tumours and the sarcomatoid carcinomas were all high grade.

Examination of the invasive portion of the tumours within the adjacent renal cortex showed that the low grade tumours grew en masse with a thick fibrous septum separating tumour from renal cortical tissue. By contrast the high grade tumours, especially the spindle cell component of sarcomatoid carcinomas, infiltrated between tubules, glomeruli and blood vessels with entrapped tubules and glomeruli showing degenerative changes or sclerosis.

Immunocytochemistry.

The markers of tubulogenesis.

In all of the clear cell carcinomas CAM 5.2 positivity was seen. The staining was more marked at the periphery of the cytoplasm (Figure 67) and the great majority of the cells in each tumour stained, although an occasional unstained cell was seen. Five tumours contained cells which expressed antigens detected by the PKK1 monoclonal antibody and in one tumour foci of tumour cells which contained epidermal prekeratin were seen.

All of the granular cell carcinomas expressed CAM 5.2. Staining was seen diffusely throughout the cytoplasm (Figure 68). Foci of tumour cells in two tumours stained with the antibody to epidermal prekeratin and in one case with the PKK1 antibody.

CAM 5.2 staining was seen in the basophilic carcinoma, and in the epithelial component of the sarcomatoid carcinomas. PKK1 positivity was only demonstrated in one of the sarcomatoid carcinomas. The spindle cell component of the sarcomatoid carcinomas showed no reactivity for the anti-cytokeratin antibodies. Trapped tubules were seen within the sarcomatoid carcinomas and these tubules stained with CAM 5.2 and PKK1.

Segregation markers.

EMA was present in all of the renal cell carcinomas studied. In these tumours EMA was expressed predominantly on the tumour cell surface (Figure 69). In those tumours with a papillary or tubular architecture the antigen was confined to the apical surface of the tumour cells (Figure 69), however, in those tumours with a more solid architecture the staining for EMA was present on the whole cell surface (Figure 70). Intracellular lumina lined by EMA positive cell membranes were seen in some tumours. There was heterogeneity of EMA expression by cells within individual tumours, with EMA negative cells seen in some tumours which elsewhere contained EMA positive cells; indeed EMA negative cells could be seen adjacent to EMA positive cells in some cases.

In three cases of granular cell tumours the staining for EMA was seen in a granular pattern within the cytoplasm.

The epithelial component of the sarcomatoid renal cell carcinomas expressed EMA but staining was not seen in the spindle cell component. Most of the tubules trapped within the sarcomatoid carcinomas expressed EMA.

Of the 44 renal cell carcinomas studied 41 expressed BB antigen. This antigen was seen on the cell surface membrane of the epithelial tumour cells. In those tumours which showed some tubule formation the antigen was found on the apical surface of the tumour cells (Figure 71), but in tumours with a solid architectural pattern the antigen could be seen on all of the cell surfaces. Within individual tumours there was some heterogeneity of staining, so that tumour cells expressing BB antigen could be seen adjacent to negative cells.

In five of the granular cell tumours, in addition to the membrane bound antigen, there was granular staining of the cytoplasm of some tumour cells.

The spindle cell component of the sarcomatoid renal cell carcinomas did not express BB antigen although the epithelial component of the same tumours did show BB antigen expression.

Maturation markers.

Binding of AGF 4.48 and AGF 4.36 was seen in 12 cases. Staining was seen on the surface membrane of the epithelial cells. In well differentiated tumours with a papillary or tubular architecture staining was seen on the luminal border (Figure 72), but in less well differentiated tumours staining was found on all surfaces of the tumour cells. There was marked heterogeneity of staining within positive tumours, cells binding the antibodies could be seen adjacent to areas in which there was no antibody binding.

The spindle cell areas of the sarcomatoid renal cell carcinomas failed to stain with any of the antibodies.

Proteinase inhibitors were found in 18 of the 44 renal cell carcinomas studied, but in one case only alpha-1-antitrypsin was present. The presence of the proteinase inhibitors did not correlate with the overall histological appearance of the tumours. The antigens were found in the cytoplasm of the tumour epithelial cells. The staining was usually present diffusely throughout the cytoplasm, however, in some clear cells the staining was restricted to the compressed

peripheral rim of cytoplasm. In a single case a PAS positive hyaline intracytoplasmic globule within a tumour cell stained for alpha-1-antitrypsin.

There was marked heterogeneity of expression of these antigens within the cells in individual tumours, a single cell or group of cells reacting strongly with the appropiate antibody were seen adjacent to cells which showed no reactivity (Figure 73). On the serial tinctorial stained sections no morphological difference between these cells was seen.

In addition to the staining of the epithelial cells in renal cell carcinomas the spindle cell component of three of the sarcomatoid renal cell carcinomas expressed the proteinase inhibitor antigens (Figure 74).

Sixteen renal cell carcinomas stained with the antibody to ferritin. Staining was present diffusely throughout the cytoplasm of the epithelial cells (Figure 75). There was heterogeneity of ferritin expression by the tumour cells. The spindle cells of the sarcomatoid carcinomas did not contain ferritin, but elsewhere in the epithelial portion of these tumours staining for ferritin was seen.

In addition to the staining of neoplastic cells with the anti-ferritin antibody some spindle shaped cells within the inflammatory infiltrate in many of these tumours expressed ferritin (Figure 76).

No staining for carbonic anhydrase C was seen in the renal cell carcinomas. No staining for CEA or AFP was seen in these renal cell carcinomas.

Mesenchymal antigens.

Vimentin was found in the tumour cells in 18 renal cell carcinomas. The staining was seen in the cytoplasm of the epithelial cells (Figure 77). The stromal cells also stained for vimentin.

Factor VIII R antigen was detected in the cytoplasm of the endothelial cells lining the blood vessels of all the renal cell carcinomas. In the majority of cases these vessels were thin walled sinusoids or capillaries separated from the epithelial tumour cells by a narrow basement membrane (Figure 78). Occasional larger blood vessels were seen in some tumours, the endothelium of these vessels also stained for Factor VIII R antigen.

The tumour cells of the renal cell carcinomas did not stain for S-100 protein but a stromal component did stain. These cells were a sparse population of small spindle cells closely associated with blood vessels. These cells were seen in only five renal cell carcinomas.

Myoglobin and desmin were not found in the renal cell carcinomas.

Extracellular molecules.

Fibronectin was seen associated with the basement membranes surrounding the epithelial tumour cells in the renal cell carcinomas with a tubular, papillary or cystic architecture (Figure 79). In the tumours with a solid architecture the fibronectin was seen surrounding aggregates of tumour cells but not associated with individual tumours cells. No intracellular fibronectin was seen in the epithelial cells.

In the sarcomatoid renal cell carcinomas of the fibrosarcoma type there was a delicate pericellular and an intracellular distribution of fibronectin. The malignant fibrous histiocytoma type of sarcomatoid renal cell carcinomas intracellular staining for fibronectin was seen.

Fibronectin was also associated with the basement membranes of endothelial cells in the renal cell carcinomas.

The renal cell carcinomas were not studied with NDOG1.

Proliferation associated molecules.

Very few cells, less than 2%, in the renal cell carcinomas expressed the nuclear proliferation marker, Ki 67 (Figure 80). In all cases studied, however, there was strong expression of the transferrin receptor molecule by most of the tumour cells (Figure 81).

Endothelial cells expressed the transferrin receptor molecule. In addition, some endothelial cells showed Ki 67 immunoreactivity.

Hormones.

Renin was found in 13 of 44 renal cell carcinomas studied. The distribution amongst the different types of tumour is given in Table 14. The majority of tumours which contained immunostainable renin were clear cell carcinomas.

In the renin containing tumours the positive cells were small perivascular cells located immediately external to the endothelium of blood vessels in the tumours. Granular cytoplasmic staining was seen in these cells (Figure 82). These cells were sometimes very scanty in number and usually present singly at any one site. They were constant in position but variable in morphology. Their nuclei were most commonly elongated and smaller than the adjacent tumour cells. They had inconspicuous nucleoli. When closely applied to a blood vessel the cells often had long cytoplasmic processes which contained the renin positive granules. When the renin containing cells were in a more loosely formed stroma and not compressed by surrounding tumour cells they were larger and polygonal, occasionally approaching the size of the adjacent epithelial tumour cells. It could not be established with certainty on histological criteria whether these cells were tumour cells or not. The renin cells did not contain alpha-1-antitrypsin nor alpha-1-antichymotrypsin.

In 2 cases cells which had the histological appearance of epithelial tumour cells contained granular deposits of immunoreactive renin. These cells were otherwise indistinguishable from the remainder of the tumour cells and were present focally throughout the tumours.

No staining for HCG was seen in the renal cell carcinomas.

5.1.4 Oncocytoma.

Morphology.

Eight oncocytomas were studied. There were well circumscribed tumours consisting entirely of uniform granular cells. The cells were cuboidal with intensely eosinophilic, granular cytoplasm and small round nuclei. Mitotic figures were very infrequent. Grading based on nuclear morphology showed that five were grade I and three were grade II.

The tumour cells were arranged in an alveolar (six cases) or tubular (two cases) architecture. In four cases the stroma showed an acellular eosinophilic component which also stained with alcian blue. This appearance did not correlate with nuclear grade.

Immunocytochemistry.

The oncocytomas all expressed the cytokeratin antigens. In all cases there was diffuse cytoplasmic staining for CAM 5.2 (Figure 83). Three cases also expressed PKK1 and in one case a small group of cells showed weak staining for epidermal prekeratin.

All cases showed reactivity for EMA and 7/8 stained for BB antigen. The staining pattern, however, was different from that observed in the renal cell carcinomas. In addition to surface staining for these antigens granular cytoplasmic staining was also seen (Figure 84).

Three oncocytomas showed focal staining for the fucosylated N-acetyllactosamine determinants detected by AGF 4.48 and AGF 4.36. As in the renal cell carcinomas the staining with these antibodies was seen on the cell surface.

There was granular cytoplasmic for alpha-1-antitrypsin and alpha-1-antichymotrypsin in 5/8 oncocytomas. The staining reaction was markedly heterogeneous within individual tumours.

Ferritin was identified in four cases, the staining being seen in the tumour cell cytoplasm.

No staining was observed for carbonic anhydrase C, CEA, HCG, AFP or prostatic acid phosphatase.

Factor VIII R antigen stained the capillary endothelial cells in the oncocytomas. These preparations demonstrated the rich capillary blood supply of these tumours, although hypovascular areas were noted in the centre of some tumours.

In three cases small spindle shaped renin containing cells were seen immediately adjacent to the capillaries. In one of these cases there were cells with the morphology of oncocytic tumour cells in which there was granular staining for renin.

Fibronectin staining ws seen associated with epithelial basement membranes in the oncocytomas.

No staining was seen for S-100, desmin nor myoglobin.

5.3.5 Collecting duct carcinoma.

Morphology.

Several features were common to all four cases of these rare renal tumours. The main tumour mass, in the renal medulla, consisted of a papillary adenocarcinoma. A single layer of uniform cuboidal cells with a clear or faintly granular cytoplasm

was seen arranged in a papillary architecture supported by a delicate fibrovascular stroma (Figure 85). The nuclei were round frequently vesicular with a single nucleolus. Nuclear pleomorphism was minimal and mitoses were infrequent. Attentuated urothelium, showing no cytological atypia, was seen overlying this medullary portion of the tumour mass.

The tumours extended into the renal parenchyma in a characteristic manner. Anastomosing tubules lined by cuboidal or columnar cells, showing considerable pleomorphism and a high mitotic rate, were seen infiltrating the renal parenchyma and eliciting a marked desmoplastic reaction (Figure 86). The tumour cells in this infiltrative portion were highly atypical, cuboidal with indistinct cell borders and granular pale eosinophilic cytoplasm. The nuclei were vesicular with finely granular chromatin and frequently possessed a prominent central eosinophilic nucleolus (Figure 87).

In three cases atypical epithelial cells were seen in the collecting ducts of the adjacent kidney.

Immunocytochemistry.

The epithelial cells of these four tumours stained with all three anti-cytokeratin antibodies (Figure 88). They also expressed, on the luminal surface of the cells, EMA but did not show staining for BB antigen nor for the fucosylated N-acetyllactosamine determinants.

No staining for ferritin, carbonic anhydrase C, alpha-1-antitrypsin, CEA, AFP, HCG or prostatic acid phosphatase was seen.

There was staining for fibronectin in the basement membranes of the tumour epithelial cells and in the dense fibrous tissue surrounding the infiltrating tumours. Factor VIII R antigen stained the capillary endothelium of the blood vessels in these tumours, but they were less well vascularised than the other forms of renal cell carcinomas studied. No renin was seen in these tumours.

There was no staining for S-100 protein, myoglobin nor desmin in the collecting duct carcinomas.

5.3.6 Renal Sarcoma.

Morphology.

The histological type of the five renal sarcomas studied is given in Table 15.

The two renal fibrosarcomas were high grade pleomorphic spindle cell tumours. Mitoses were frequent. The cells were long, thin, and spindle shaped with indistinct cytoplasm and an elongated nucleus. There was pericellular staining seen in the reticulum stained sections. Both tumours infiltrated between the tubules and glomeruli of the adjacent renal cortex.

Of the two leiomyosarcomas, one was a low grade tumour and one a high grade. The low grade tumour was well differentiated consisting of spindle cells arranged in interlacing bundles. The cells had eosinophilic cytoplasm and oblong, cigar shaped nuclei. A clear perinuclear halo was seen in many cells. There was a moderate number of mitoses. The high grade tumour had a similar appearance but with a higher mitotic rate and a greater degree of pleomorphism.

The malignant fibrous histiocytoma was a highly pleomorphic tumour with numerous mitoses. There were irregular fibroblasts, epithelioid cells and multinucleate giant cells. In places there was a marked storiform architecture to the tumour. Areas of necrosis and haemorrhage were prominent.

Immunocytochemistry.

These tumours did not express the cytokeratin antigens, EMA, BB antigen, carbonic anhydrase C, ferritin nor fucosylated N-acetyllactosamine.

Granular cytoplasmic staining for alpha-1-antitrypsin was seen in the malignant fibrous histiocytoma.

Delicate interstitial staining for fibronectin was seen in all sarcomas but in the fibrosarcomas cytoplasmic staining for fibronectin was also seen.

Factor VIII R antigen staining the capillary endothelium showed that these tumours were of low vascularity. No renin was seen in these tumours.

Cytoplasmic staining for desmin, but not for myoglobin, was seen in the leiomyosarcomas. This was not seen in the other sarcomas.

No staining for S-100 protein was seen in these tumours.

5.2 Discussion.

5.2.1 Cellular Differentiation in Nephroblastoma.

The classical form of nephroblastoma is a triphasic tumour in which there are areas of undifferentiated blastema, areas of epithelial differentiation and a variety of mesenchymal tissues (Busse 1899; Muus 1899; Wilms 1899; Bennington & Beckwith 1975). The relative proportion of these three tissues varies greatly from case to case and monotypic variants consisting of only one of these tissue types are common (Bennington & Beckwith 1975). The tumour therefore shows a similar pattern of differentiation as the blastema of fetal kidney. This has suggested that these tumours originate from metanephric blastema which has undergone malignant transformation (Muus 1899; Busse 1899; Bennington & Beckwith 1975)

Early attempts to correlate histological appearance with clinical presentation or outcome were inconclusive (McCurdy 1934; Weisel, Docherty & Priestly 1943; Evans 1966). It has recently been shown that epithelial differentiation in

nephroblastoma is of prognostic significance (Lawler et al 1975; Chatten 1976; Delemarre et al 1982). The morphology of epithelial differentiation has consequently attracted attention and different epithelial structures have been identified (Lawler et al 1977; Delemarre et al 1982).

The different epithelial tissues which have been described previously in nephroblastoma are rosettes, tubules, pseudoglomeruli, cysts and papillary structures (Lawler et al 1977; Delemarre et al 1982).

Tubules are the epithelial structures most frequently encountered in nephroblastoma, the reported frequency ranging from approximately 70% to 80% (Lawler et al 1977; Delemarre et al 1982). In this study tubules were present in 86% of the 36 nephroblastomas. The results of grading the tumours according to the amount of tubular epithelium present in each case are comparable to the previously reported figures (Lawler et al 1977; Delamarre et al 1982).

The proportion of the tumours containing cysts, pseudoglomeruli, and papillary structures are also comparable to the figures previously reported (Lawler et al 1977; Delemarre et al 1982).

The proportion of different mesenchymal tissues found by histological methods in this series of nephroblastomas is comparable to that reported elsewhere (Tremblay 1971).

The relationship between epithelial differentiation and clinical behaviour has concentrated on the volume of tumour which can be histologically defined as tubular (Lawler et al 1977). The biology of tubular differentiation in nephroblastoma has also attracted some attention recently (Sariola et al 1985; Kumar et al 1986). Studies of differentiation in other embryonal tumours, including neuroblastoma and teratoma, have been used as models of normal developmental processes (Solter et al 1979; Robertson 1981).

The biology of tubular differentiation from the neoplastic blastema has been studied by adapting the transfilter induction system of experimental embryology to the culture and induction of nephroblastoma. Rousseau, Nabbarra & Nezelof (1974) demonstrated the applicability of combining human nephroblastoma cultured in vitro with heterologous tissues (chick ureteric bud or mouse mesencephalon) to induce differentiation of the blastema. Of the fifteen nephroblastomas

studied in this way three tumours showed an increase in the number of tubules formed, and in six cases prolonged survival of the tumour explant without further differentiation was acheived (Rousseau-Merck et al 1977). Tubular differentiation in experimental and spontaneously occuring renal tumours of animals has also been observed in transfilter induction experiments (Seilern, Aspany & Kratochwil 1962; Ellison, Ambrose & Easty 1969).

In all of these experiments tubular differentiation has been noted, but, in addition, prolonged growth of the tumours has been observed. In this study (Chapter 3), and in previous work elsewhere, it has been shown that one of the essential stages of tubular induction in normal nephronogenesis is enhanced proliferative responses, particularly to transferrin (Ekblom et al 1984). It may be that transfilter induction of tubular differentiation from nephroblastomas also enhances tumour growth responses to transferrin or other growth factors in the tissue culture medium and thus prolongs the survival of these tumours in in vitro culture systems.

I have shown that epithelial differentiation in the 5 nephroblastomas studied showed expression of the transferrin receptor molecule and the nuclear proliferation marker Ki 67. These data would support the suggestion that epithelial differentiation in nephroblastoma, like the equivalent stage in normal development, is accompanied by proliferative activity.

This observation may have consequences for the therapy and prognosis of nephroblastoma. It is known that predominately epithelial tumours respond optimally to anti-tumour chemotherapy (Delemarre et al 1982). Gonzalez-Crussi (1984) has studied the effects of therapy on the histological pattern of nephroblastomas and has found that there appears to be a selective loss of epithelial elements and survival of the mesenchyme. ince this type of therapy is directed at proliferating populations these results also support the hypothesis that in nephroblastomas, as in fetal kidney, epithelial differentiation is accompanied by transferrin dependent cell division.

Epithelial differentiation of nephroblastoma has been studied by both light (Lawler, Marsden & Palmer 1977; Delemarre et al 1982) and electron

microscopy (Balsaver, Gibley & Tessner 1968; Ito & Johnson 1969; Tannenbaum 1971). I have used morphological and immunocytochemical techniques to investigate epithelial differentiation in nephroblastomas.

Until recently there had been few studies of the expression of renal tubular antigens in nephroblastoma and those which have been performed have used ill defined polyclonal antisera against crude renal antigen preparations (Linder 1969; Wallace & Nairn 1972). These two studies showed, however, that the epithelial component of nephroblastoma did express some renal tubular antigens, including brush border antigen and Tamm-Horsfall glycoprotein.

I have studied the renal tubular antigens in fetal kidney and in nephroblastoma. The earliest specific manifestation of epithelial differentiation which was observed in fetal kidney was the expression of low molecular weight cytokeratin antigens detected by CAM 5.2. This was followed in sequence by the expression of the larger molecular weight cytokeratin antigens detected by the PKK1 antibody.

I have shown tha the epithelial components of nephroblastoma express appropiate cytokeratin antigens. Poorly differentiated tubular epithelium does not express cytokeratin antigens. Low molecular weight cytokeratins, detected by CAM 5.2, are seen in tubular epithelium of a cytologically moderate degree of differentiation, but the larger molecular weight cytokeratins demonstrated by the PKK1 monoclonal antibody are only seen in well differentiated tubules which also expressed the CAM 5.2 antigens. This apparent sequence of cytokeratin antigen expression follows that observed in tubules during the normal development of the nephron. The neoplastic metanephric tubules, like the normal renal tubules, fail to express the very large molecular weight epidermal prekeratins.

Altmannsberger et al (1984) and Ramaekers et al (1985) have studied the expression of intermediate filament antigens in nephroblastoma. Both groups have reported that the blastema of the tumour, like its normal counterpart, expressed vimentin antigens, while the neoplastic epithelial cells expressed cytokeratins. Both groups have also described some tumour cells which expressed

both types of intermediate filament antigens. Both types of intermediate filament are transiently co-expressed by tubular epithelial cells during the early stages of tubulogenesis.

Intermediate filament expression in nephroblastoma therefore seems to mirror that seen during normal renal embryogenesis. Similar findings have been recorded in the epithelial areas of embryonic lung tumours where the pattern also mimicks normal epithelial differentiation during lung development and in adult lung (Ramaekers et al 1985).

In addition to expressing these markers of tubulogenesis, the tubules in nephroblastoma can express tubular segeregation markers. Wallace & Nairn (1972) denmonstrated brush border antigen and Tamm-Horsfall glycoprotein expression by tubular epithelial cells in two cases of nephroblastoma by immunofluorescence. Studying the distribution of the two antigens in serial sections they concluded that they were expressed by different tubules. Linder (1969) also demonstrated the expression of renal tubular antigens by the tubules of nephroblastoma, but the failure of these same tubules to express other tubular antigens. The antisera used by Linder were, however, poorly characterised.

I have shown that neoplastic tubules in nephroblastoma can express the tubular segregation markers EMA and Brush Border antigen. Only tubules which were morphologically well differentiated expressed these antigens. EMA and BB antigen were expressed on the apical surface of tubular epithelial cells. The two antigens seemed to be expressed by different cells as had been the case with BB antigen and Tamm-Horsfall glycoprotein in the study by Wallace & Nairn (1972).

In contrast to the apical localisation of these cell surface antigens fibronectin was found at the base of the tubular cells. The tubular epithelial cells therefore demonstrated the normal polarity of antigen expression seen in the metanephric tubules. Sariola et al (1985) and Kumar et al (1986) have shown that the distribution of the basement membrane related proteins fibronectin, laminin, and type IV collagen correlate with the degree of epithelial cell differentiation in nephroblastoma and that the site of expression of these antigenas is similar to that observed in the normal metanephric tubules. Ultrastructural studies have also demonstrated normal epithelial cell polarity with

tubular basement membranes, lumina, brush borders and typical apical cytoplasm in the epithlelial cells in nephroblastoma (Ito & Johnson 1969; Tannenbaum 1971).

Specific alterations in the distribution of basement membrane collagens, laminin and fibronectin are early events during tubulogenesis from normal metanephric blastema (Ekblom et al 1980; Ekblom et al 1981; Lash et al 1983; Theseleff & Ekblom 1984). Ekblom and his colleagues have argued that alterations in cell adhesion and the secretion of basement membrane substances are essential steps in the initiation of tubulogenesis by establishing the polarity of the presumptive epithelial cells. The development of a lumen and of specialised apical structures are secondary, but nevertheless essential, in defining cell polarity and, hence, in the continued epithelial differentiation of tubules in the metanephros. My results and those of others (Ito & Johnson 1969; Tannenbaum 1971; Sariola et al 1984; Kumar et al 1986) support the view that cell polarity is maintained during tubule formation in nephroblastoma.

More advanced degrees of tubular differentiation in nephroblastoma have been tested by studying the distribution of the cytoplasmic proteins alpha-1-antitrypsin, alpha-1-antichymotrypsin, ferritin and carbonic anhydrase C. These antigens all appeared in different segments of the developing nephron after fusion of the ureteric bud and the S-shaped tubule. These are therefore markers of tubular maturation. The epithelial components of nephroblastoma failed to express any of these proteins. Linder (1969) also failed to detect in nephroblastoma kidney antigens which appeared at late stages of nephronogenesis.

These results suggest that the neoplastic metanephric blastema of nephroblastoma is capable of a normal progression of epithelial differentiation but that such differentiation becomes arrested at a relatively immature stage. The majority of the tubules in nephroblastoma are morphologically immature; even the well differentiated neoplastic tubules never reach a degree of differentiation morphologically comparable to the early maturation stage of renal embryogenesis. The immunocytochemical studies performed during this investigation, therefore, suggest that there is an arrest of tubular maturation before the early maturation stage.

The exception to the rule was a single nephroblastoma in which a few tubules expressed the 1-3 fucosyl N acetyl lactosamine determinants detected by the monoclonal antibodies AGF 4.48 and AGF 4.36, epitopes which do not appear in metanephric tubules until the early maturation stage of nephrogenesis. This tumour was exceptional in other ways. It showed extensive adipose tissue and skeletal muscle in the mesenchymal component and had been removed from a child with multiple congenital anomalies and a strong family history of nephroblastoma. It is possible that some cases of familial nephroblastoma have a different level of maturation arrest from that seen in the sporadic cases. Certainly they do show other clinical and epidemiological differences from the sporadic cases (Knudson & Strong 1972).

Arrest of differentiation has been shown to occur in a variety of other tumours including chronic granulocytic leukaemia (Greaves 1982), cerebral astrocytoma (Thomas & Graham 1980), retinoblastoma and neuroblastoma (Murphree & Benedict 1984; Robertson 1984; Klein & Klein 1985).

There is evidence of maturation arrest in other components of nephroblastoma. Pseudoglomeruli are found in between 10% and 20% of nephroblastomas (Lawler et al 1977; Delemarre et al 1982). Bennington & Beckwith (1975) commenting on the light microscopical appearances of pseudoglomeruli suggested that vascularisation of these glomeruli was rare. This observation was confirmed on ultrastructural examination by Ito & Johnson (1969). They found that the epithelial component of pseudoglomeruli was well differentiated, to the extent of forming foot processes, but that vascular endothelium and mesangium were not seen. Abundant basement menbrane substances were produced by the glomerular epithelial cells.

In this study the great majority of the pseudoglomeruli lacked immunoreactivity for Factor VIII R antigen. This antigen was found in the endothelial cells during the vascularisation of the early glomeruli in the fetal kidney. Fibronectin was demonstrated in the pseudoglomeruli of nephroblastoma thus supporting the ultrastructural observation of the production of basement membrane related substances by the neoplastic glomerular epithelial cells.

Interestingly, *in vivo* during glomerulogenesis, the glomeruli are vascularised and mesangial cells appear before epithelial cells develop foot processes (Vernier & Birch-Andersen 1962). In nephroblastoma there would therfore appear to be asynchrony of development of glomeruli, because of the failure to vascularise the tufts of the neoplastic glomeruli.

Probably associated with the incomplete vascularisation of the glomerular tufts in nephroblastoma is the failure to form a juxtaglomerular apparatus. The renin containing cells in nephroblastoma are associated with blood vessels lying within the tumour. The renin containing cells of the juxtaglomerular aapparatus are thought to be derived from undifferentiated mesodermal cells adjacent to the blood vessels in the fetal renal cortex (Phat *et al* 1981). It is now known that these blood vessels grow into the metanephros from an outgrowth of the dorsal aorta (Sariola *et al* 1984). At the moment, it is not known whether the renin containing cells grow in with these blood vessels or whether they are induced to differentiate *in situ* under the influence of the developing vascular bud. The same problem applies to the development of the renin containing cells in nephroblastoma.

Do these cells develop from tumour cells induced to synthesised renin under an inductive influence from the adjacent blood vessels, or have they grown in from the adjacent kidney with the blood vessels? There is considerable evidence that tumours stimulate the ingrowth of blood vessels from adjacent tissues (Folkman et al 1971) and it seems likely that the blood vessels in nephroblastoma have grown in from the adjacent kidney (Shahabidin et al 1986).

Two pieces of evidence argue against renin containing cells being carried in with these blood vessels. Firstly, metastatic tumours growing within the kidney are vascularised but these tumours were shown not to contain immunoreactive renin (Lindop & Fleming 1984). Secondly, immunoreactive renin has been identified in several metastatic nephroblastomas growing in organs other than the kidney (Lindop, Fleming & Gibson 1984).

Since nephroblastoma is derived from the undifferentiated metanephric blastema it is possible that one of the differentiation options for the blastemal cells would be to form juxtaglomerular renin containing cells.

Examination of the immunocytochemical preparations shows that although in the majority of nephroblastomas these were small thin spindle cells, in some cases the cells were more plump, and in adjacent tinctorial stained sections were indistinguishable from other tumour cells. In the normal fetal kidney the renin containg cells first appear as thin spindle cells adjacent to the ingrowing vascular bud (Phat et al 1971). This evidence suggests that the renin containing cells in nephroblastoma are possibly tumour cells induced to produce renin by the adjacent blood vessels. Clearly further experimentation is required to confirm this.

However, a normal juxtaglomerular apparatus is not formed in nephroblastoma. This may be because of the failure to fully vascularise glomeruli but the renin secreting system in nephroblastoma is immature in other ways. There are several cases of renin secreting nephroblastomas on record (Mitchell et al 1970; Masovari, Kontor & Kallay 1972; Gangulay et al 1973; Luciani et al 1979; Sheth et al 1978; Spahr, Demers & Shochat 1981). The evidence of renin secretion in these cases is based on high concentrations of renin in the

preoperative plasma and in the tumour, with a fall to normal plasma renin concentrations following nephrectomy. In two of these cases (Luciani et al 1979; Sheth et al 1978) ultrastructural examination of the tumours demonstrated polypeptide storage granules with the morphology of renin storage granules within tumour cells.

Patients with renin secreting tumours often have clinical and biochenmical evidence of increased renin activity such as hypertension and hypokalaemia (Mitchell et al 1970; Brown et al 1973; Schambelen et al 1973). These abnormalities are also cured by nephrectomy. Analysis of the case records of nineteen of the cases of nephroblastoma in the present study showed no significant difference in the blood pressure or serum potassium values between those patients whose tumour contained immunoreactive renin and those whose tumour did not (Lindop et al 1984). Furthermore these variables were not influenced by nephrectomy in either group of patients. Thus, there was no clinical evidence of increased renin activity in this series of patients.

It is possible that the renin containing cells in these tumours were unable to secrete renin, or that the renin secreted was biologically inactive. Day & Luetscer (1974) analysed the renin in the plasma and in the tumour of the case described by Gangulay et al (1973). The renin was of a larger molecular weight and was biologically less active in an in vitro testing system than normal human plasma renin. It may have been a prohormone, which was incompletely activated.

Renin is synthesised from a single strand of messenger RNA on the endoplasmic reticulum as a large preprohormone (Panthier et al 1982). This translation product requires a programmed enzymatic cleavage, to firstly a prohormone, then finally the active form of the enzyme, which is released into the circulation under appropiate regulation (Galen et al 1984). The antibody used in this study has been shown to bind to both active and inactive forms of renin in vitro. The immunocytochemical, clinical and biochemical evidence suggests that nephroblastomas contain cells which synthesise renin, incompletely process the molecule and release a biologically inactive form. This suggests that renin synthesis by

nephroblastomas may be more common than is clinically apparent. There is evidence that this also occurs in renal carcinomas. A similar phenomenon has been described in the secretion of other biologically inactive prohormones by a variety of tumours (Odell & Wolfson 1978). There is therefore both morphological and biochemical immaturity of the juxtaglomerular apparatus in nephroblastoma.

In addition to differentiation towards different components of the nephron in nephroblastoma there are frequently areas of mesenchymal differentiation (Muus 1899; Busse 1899; Wilms 1899; Bennington & Beckwith 1975). The mesenchyme, like the epithelium, differentiates from the neoplastic metanephric blastema (Bennington & Beckwith 1975). Morphologically the mesenchyme may be fibroblastic or myxoid, but adipose cells and smooth muscle may also be seen. An occasional finding is the presence of tissues which are not part of the normal metanephros such as neural tissue, striated muscle, cartilage and bone (Busse 1899; Wilms 1899; Masson 1938; De Muylder 1947; Bodian & Rigby 1964; Bannayan, Huvos & D°Angio 1971; Bennington & Beckwith 1975).

The most commonly seen mesenchymal tissue consists of broad, solid sheets of irregular spindle cells lying in a loose extracellular matrix. In places collagen deposition may be conspicuous. Myxoid tissue may be prominent. In these areas the cells show no specific morphological features of differentiation. Electron miscroscopical studies of these areas have shown the cells to have some of the features of fibroblasts or fibrohistiocytic cells but variations between cells within each tumour are seen (Balsaver, Gibley & Tessner 1968; Ito & Johnson 1969; Tannenbaum 1971; Tremblay 1971; Rousseau & Nabarra 1974; Olsen 1984). The collagen associated with these cells has been described as having a periodicity typical of embryonic tissues (Balsaver, Gibley & Tessmer 1968)

Some of these immature mesenchymal cells contain lysosomes as demonstrated in electron microscopic studies. I have found proteinase inhibitors in the primitive mesenchyme of nephroblastoma. The primitive interstitial cells within the nephrogenic zone of fetal kidney also expressed the proteinase inhibitor antigens. It seems reasonable to suggest that the primitive mesenchymal cells in nephroblastoma are the neoplastic equivalent of the early renal interstitial cells.

The presence of tubules derived from the ureteric bud within nephroblastoma was noted in the original descriptions of the tumour (Muus 1899; Busse 1899). In his description of an embryonic renal tumour in a stillborn fetus, Nicholson (1931) also described these tubules and by cutting serial sections of the tumour showed that they were in continuity with either the collecting ducts or the calyceal system of the kidney in which the tumour was growing. Hou & Azzopardi (1967) described similar tubules in a series of nephroblastomas. They called them type B tubules and showed that they were morphologically different from the typical neoplastic tubules of nephroblastoma. Their studies also demonstrated their continuity with adjacent collecting ducts.

These tubules were frequently surrounded by a concentric cuff of small spindle cells similar to those seen in the medulla of dysplastic kidneys. The primitive ducts in renal dysplasia are also derived from the ureteric bud epithelium. In this study I have shown that type B tubules, present in 33% of cases of nephroblastoma, expressed epidermal prekeratin antigens. These cytokeratins in the normal and developing kidney are restricted

to the collecting duct epithelium. The terminal branches of the type B tubules did not express large molecular weight cytokeratins but, like the ureteric bud ampullae, only expressed the CAM 5.2 antigens. These terminal branches also expressed the carbohydrate determinants recognised by the monoclonal antibodies AGF 4.48 and AGF 4.36. During nephrogenesis these epitopes were expressed on the surface of the epithelium of the ureteric bud ampullae. As the ureteric bud differentiates into collecting duct epithelium this determinant is lost.

This data provides morphological and immunocytochemical support for the hypothesis that the type B tubules are derived from the ureteric bud and are not a neoplastic component of nephroblastoma. These immunocytochemical studies not only support the existence of ureteric bud derived structures within some nephroblastomas but argues that the terminals of the tubules have the phenotype of ureteric bud ampullae. The expression of Ki 67 reactivity in the nuclei of Type B tubules suggests that these tubules are actively growing into the nephroblastomas.

The ampulla of the ureteric bud acts as the inducer for normal tubulogenesis in fetal kidney (Wolff 1970; Saxen & Kohenen 1969). Nicholson (1931) previously suggested that the ureteric bud derived tubules may influence epithelial differentiation and possibly clinical behaviour in nephroblastoma. A similar view was expressed by Hou & Azzopardi (1967) and certainly *in vitro* heterozygous ureteric bud has been shown to induce partial tubular differentiation in human (Rousseau-Merck *et al* 1977) and animal nephroblastomas (Seilern *et al* 1962; Ellison *et al* 1969).

This present study suggests that ureteric bud like structures may be stimulated to grow into nephroblastomas from the adjacent kidney. The mechanism by which this may occur is not known, but during normal embryogenesis the metanephric blastema has a trophic effect on ureteric bud growth, a property which may be dependent on a chemical mediator released by the metanephric blastema (Vernier & Smith 1969). If this same mechanism is indeed operating in the induction of ureteric bud growth in nephroblastoma then there are similarities between this and tumour induced angiogenesis (Folkman *et al* 1971).

5.2.2 Differentiation of renal cell carcinoma

For many years there was considerable debate concerning the histogenesis and epithelial differentiation of renal cell carcinoma (Reviewed by Bennington & Kradjan 1967). The ultrastructural studies by Oberling and others (Oberling *et al* 1960; Seljelid & Ericsson 1965; Ericsson *et al* 1966; Fisher & Horvat 1972; Pratt-Thomas *et al* 1973) have conclusively shown that these tumours differentiate towards and are probably derived from the renal tubular epithelium.

Electron microscopic studies have also shown that the epithelial cells are often well differentiated and show several features which are also seen in normal proximal tubular epithelium (Tannenbaum 1971). The majority of the clear cells, granular cells or basophilic cells of renal cell carcinomas have surface specialisations including the presence of microvilli (Oberling *et al* 1960; Seljelid & Ericsson 1965; Ericsson *et al* 1966; Okada *et al* 1969). In the apical cytoplasm and surface membrane numerous membrane bound vesicles and a brush border glycocalix can be seen (Tannenbaum 1971). These various authors have also

noted the deep basilar infoldings of the plasma membrane, a feature of proximal tubular epithelium (Tisher 1966). These infoldings occur in association with the basement membrane supporting the tumour cells in the majority of cases (Tannenbaum 1971). The tumour cells also possessed intercellular junctions (Oberling et al 1960; Ericsson et al 1966), and Tannenbaum (1971) noted a moderately dense condensation of fibrillary material around the basolateral parts of the cells. Electron microscopy reveals the differences between the main types of tumour cells. Clear cells contain abundant fat droplets and glycogen (Seljelid & Ericsson 1965a) while the granularity of the granular cell cytoplasm is seen to be due to a very large of mitochondria (Seljelid & Ericsson 1965b; Tannenbaum 1971).

These different groups of electron microscopists have concluded that the cells of renal cell carcinoma are probably derived from the proximal tubular epithelium. This view has in part been supported by other authors citing histochemical and immunocytochemical evidence.

Braunstein & Adelman (1966) studied 12 renal cell carcinomas by enzyme histochemistry. They concluded that the most significant result was the distribution of the alkaline phosphatase reaction demonstrated by the technique of Burstone (1952). This enzyme is present in the brush border of the proximal tubular epithelium. The majority of their cases of renal cell carcinomas showed reactivity for this enzyme. A few cases were, however, negative. The alkaline phosphatase positive cases also showed reactivity for other proximal tubular enzymes such as 5° nucleotidase and glucose-6-phospatase.

Wallace & Nairn (1972) studied the cells of 11 adeonocarcinomas of the renal cortex by immunofluorescence using antibodies to human renal brush border, to identify proximal tubular cells, and Tamm-Horsfall glycoprotein, to identify distal tubular cells. They found that 10/11 tumours expressed the brush border antigen, the only negative tumour being one which was histologically anaplastic. Only two tumours contained immunodetectable Tamm-Horsfall glycoprotein. They supported the concept of a proximal tubular phenotype of renal cell carcinoma cells. Several

subsequent studies have used immunocytochemistry to confirm the presence of different brush border antigens, detected by several different antisera, on the surface of the epithelial cells of renal cell carcinomas (Linder 1969; Holthofer et al 1983).

I have confirmed the presence of a brush border antigen on the cells of the majority of renal cell carcinomas. Interestingly, although the BB antigen studied here appears later during renal embryogenesis than most of the other brush border antigens studied it is present on a comparable proportion of these tumours.

There is other data, overlooked or ignored by the majority of authors, which demonstrates that other types of differentiation are present in renal cell carcinoma. In their enzyme histochemical study Braunstein & Adelman (1966) concluded that the majority of the tumours which they had had an oppurtunity to study showed evidence of proximal tubular differentiation. They also pointed out that a significant minority of renal carcinomas may be considered as distal tubular on enzyme histochemical evidence. Tannenbaum (1971) showed that the ultrastructural

features of many clear cell carcinomas were more typical of distal tubular cells than of proximal tubular cells. The early immunocytochemcal study of Wallace & Nairn (1972) showed two carcinomas which expressed the distal tubular marker Tamm-Horsfall glycoprotein.

These studies were all small studies consisting of between ten and twelve cases of carcinomas. Larger, more detailed studies have been performed recently, although many of the conclusions have been based on a simplified interpretation of the results (Holthofer et al 1983).

The initial studies of the distribution of EMA in various human tissues and tumours showed that it was expressed on the small number of renal cell carcinomas studied (Heyderman et al 1979; Sloane & Ormerod 1980). In this study I have shown that during embryogenesis and in the adult kidney EMA is confined to the distal tubule and collecting duct, but that it was expressed by the cells of all the renal cell carcinomas studied. This suggests that renal cell carcinomas do not exclusively differentiate towards proximal tubular epithelium.

This is further supported by separate observations on the distribution of some blood group antigens in these tumours. A, B and H blood group substances, detected by mixed cell agglutination testing, were found on the majority of renal carcinoma cells, but in the normal kidney were found in the distal tubular and collecting duct epithelium only (Sarosdy & Lamm 1982). Likewise, the Thomsen-Friedenreich antigen, found in the distal tubule is also present in the majority of renal cell carcinomas (Ghazizadeh, Kagawa & Kurokawa 1985).

Some of the blood group and related cell surface carbohydrates can be detected in tissue sections by lectin histochemistry. These techniques have been applied to renal histopathology and a nephron segment specific pattern of the binding sites of some lectins found (Vierbuchen et al 1980; Holthofer et al 1981; Farraggiana et al 1982). The alpha fucose binding lectin of Lotus tetragonolobus (LTA) binds to the proximal tubule, soybean agglutinin (SBA) which is specific for N-acetyl D-galactosamine or D-galactose, the agglutinin from Dolichos biflorus (DBA) specific for N-acetyl D-galactosamine, and

Peanut agglutinin (PNA) specific for D-galactose all bind to the distal tubule and collecting duct epithelium in tissue sections. Other lectins have been studied but have been found to bind to other cell types in the kidney, for example Ulex europeus agglutinin binds to endothelium (Holthofer et al 1981; Farraggiana et al 1982).

The binding of these lectins to renal cell carcinomas has been studied using lectin histochemical preparations most commonly by using fluorochrome labelled lectins (Holthofer et al 1983) but more recently with biotin labelled lectins and avidin-biotinylated peroxidase reagents (Ulrich, Horvat & Krisch 1985).

Holthofer et al (1983) found that of 30 renal cell carcinomas studied LTA bound to none, but SBA and PNA bound to 70% and 20% respectively. Using lectin peroxidase preparations and subclassifying the 22 renal cell carcinomas which they studied, Ulrich, Horvat & Krisch (1985) found that although LTA bound to 9/10 low grade carcinomas it bound to none of the 12 higher grade tumours. SBA (12/22), DBA (8/22) and PNA 18/22) all bound to the cells of renal carcinomas, including some of the LTA positive cases.

I have investigated the distribution of defined carbohydrates on the surface of renal tumour cells by using immunocytochemistry and the monoclonal antibodies AGF 4.48 and AGF 4.36. These two antibodies recognise the trisaccharide β-D-galactose 1-4 (α-L-fucose 1-3) N-acetyl-β-D-glucosamine. This oligosaccharide is expressed by different cells and at different sites during renal embryogenesis. The epitopes were expressed on the ampulla of the ureteric bud. Later in development they were expressed on the proximal tubular epithelium, but with maturation their expression became restricted to the pars recta of the proximal tubule and the loop of Henle (Chapter 3).

I have shown that the AGF 4.48 and AGF 4.36 antibodies reacted with only a proportion of renal cell carcinomas. The tumours showed other evidence of proximal tubular differentiation by the expression of brush border antigen, proteinase inhibitors, and ferritin, and showed no evidence of ureteric bud differentiation. It seems reasonable to assume that the AGF 4.48 and AGF 4.36 binding molecules in some renal cell carcinomas are the same as the immunoreactive molecules in the proximal tubular epithelium.

The AGF 4.48 and AGF 4.36 epitopes possess terminal β-D-galactose and α-L-fucose. The oligosaccharide therefore possesses binding sites for the carbohydrate binding lectins LTA (fucose), PNA and RCA (galactose). All of these lectins show a similar distribution in the kidney as AGF 4.48 and AGF 4.36 binding sites. The variability of the binding of these lectins in renal cell carcinoma (Holthofer et al 1983; Ulrich et al 1985) and the variability of binding of the AGF 4.48 and AGF 4.36 monoclonal antibodies observed in this study are comparable. It would seem that the expression of cell surface glycoconjugates is independent of the histological type or nuclear grade of renal cell carcinomas (Ulrich et al 1985).

The AGF 4.48 and AGF 4.36 monoclonal antibodies were raised against the human promyeloid cell line HL 60. They recognise determinants on the granulocyte series (Fisher et al 1982). These antibodies, therefore, demonstrate cross reactivity between leukocyte antigens and renal tubular antigens. Similar cross reactivity with renal tubular epithelium has been observed using several monoclonal antibodies raised against other human white cell types (Metzgar et al 1981;

Jeres, Borowitz & Metzgar 1982; McKolanis, Finn & Metzgar 1983; Platt, Le Bien & Michael 1983). These antibodies have also been shown to bind to the cells of renal cell carcinomas in immunocytochemical investigations.

Borowitz et al (1986) studied the expression of four leukocyte differentiation antigens by 21 renal cell carcinomas. The antigens studied were those defined by the monoclonal antibodies CALLA, p24, DU-HL60-4, and DU-HL60-3. Although CALLA, DU-HL60-4 and DU-HL60-3 all bound to normal proximal tubules the p24 antibody does not and in the kidney is restricted to the distal tubule and some blood vessels. All of these antibodies bound to a variable proportion of renal carcinomas.

Therefore, there are a great variety of molecules which in the normal kidney are confined to the distal tubule or collecting duct but which are also expressed by renal cell carcinoma cells. However, the function of many of these molecules in the kidney are unknown. The distribution in renal tumours of some functionally defined molecules has also been investigated in this study.

Cytokeratin molecules are the intermediate filament type of protein which are found in epithelial cells (Bennett et al 1978; Franke et al 1978; Moll et al 1982). These intermediate filament proteins are important as part of the cytoskeleton of epithelial cells (Lazarides 1982). Large molecular weight cytokeratins are found in the collecting duct epithelial cells but not in tubular epithelium, but smaller molecular weight cytokeratins which are detected by the monoclonal antibodies PKK1 (Holthofer et al 1983) and CAM 5.2 (Makin et al 1984) are found in the tubular epithelium and in the collecting duct epithelium (Chapter 3). I have studied the distribution of these molecules in renal carcinomas. The cells of all 44 renal cell carcinomas expressed the CAM 5.2 antigen, and 5/44 expressed the PKK1 antigen. The four tumours designated on histological grounds as collecting duct carcinomas all additionally expressed large molecular weight epidermal prekeratins. Only 3 of the other renal carcinomas expressed these molecules and in each case only a very few cells stained positively.

My findings are similar to those of Schlegel et al (1980) and of Espinoza & Azar (1982). Espinoza & Azar (1982) failed to identify cytokeratin filaments in five renal carcinomas by studying both immunocytochemistry, using a polyclonal antiserum against epidermal prekeratin, and electron microscopy. Schlegel et al (1980) used a rabbit polyclonal anti-keratin antibody and the immunoperoxidase technique to study the distribution of these antigens in human tumours, but found no reactivity in either of the two renal carcinomas they studied.

Carbonic anhydrase is a functionally important enzyme found in the renal tubular epithelium. Immunocytochemical and enzyme histochemical studies have shown that at least two of the carbonic anhydrase isoenzymes are present in the kidney (Lonnerholm & Wistrand 1984). In this study I have used an antiserum against the carbonic anhydrase C isozyme. This antigen was found in the cytoplasm of the distal tubular epithelium and in some cells of the collecting ducts. However, carbonic anhydrase was not found in the series of renal carcinomas. There is evidence that the synthesis and activity of carbonic anhydrase in

the distal parts of the nephron may be in part controlled by serum or urinary filtrate potassium concentration (Heptinstall 1983). The failure of tumour cells to synthesise the enzyme may be in part because of a loss of cell sensitivity to these changes or to a restricted differentiation of tumour cells such that they are unable to synthesise this polypeptide.

Some proteins which may be important in proximal tubular function are expressed in renal carcinomas. Thus, the cells of the majority of renal carcinomas contained the proteinase inhibitors alpha-1-antitrypsin and alpha-1-antichymotrypsin. These molecules first appeared in the epithelial component of the kidney at the late maturation stage of development.

The majority of renal carcinomas also contained immunoreactive ferritin. The polyclonal antiserum used in this study does not distinguish between acidic and basic forms of this large molecular weight protein (Rossiello et al 1985). Ferritin has been identified in the kidney by biochemical (Harrison et al 1974) and immunocytochemical methods (Taylor & Mason 1975). The isoferritin found in the normal kidney is the

acidic type (Harrison et al 1974). Acidic ferritins have also been found in the plasma of patients with disseminated malignancy of various types (Marcus & Zinburg 1975). Clearly biochemical, especially electrophoretic, analysis of ferritin in renal carcinomas will be required to determine whether the ferritin produced by these tumours is identical to that found in the normal human kidney.

Other functionally important proteins such as angiotensin converting enzyme have been shown to be synthesised by renal tumours (Takada et al 1985).

Tamm-Horsfall glycoprotein is a cytoplasmic molecule found in the distal tubular epithelium of the kidney (Tamm & Horsfall 1952) and it has been used as a marker for distal tubular epithelium in immunocytochemical studies (Wallace & Nairn 1972). It is found only infrequently in renal carcinomas (Wallace & Nairn 1972).

Thus, there seems to be an extremely complex mixture of renal tubular antigen expression in renal carcinoma cells. A similarly mixed pattern of antigen expression was found in regenerative epithelium in several inflammatory diseases of the

kidney (Fleming & Matthews 1987). These authors have argued that a certain degree of developmental plasticity of the renal tubular epithelium is essential for complete functional regeneration.

Willis (1962) noted the increased risk of development of renal cell carcinomas in patients with increasing age, ischaemic nephrosclerosis, some forms of renal cystic disease and in patients with long standing renal failure. He suggested that renal carcinomas may therefore develop from regenerative or hyperplastic epithelium in the kidney. More recent epidemiological and pathological studies have supported Willis' conclusions (Hughson et al 1986).

There is evidence that the majority of human and animal tumours develop from the proliferating cell compartment of the relevant tissue (Farber 1970) and that the effects of many carcinogenic agents require cycles of mitotic division to establish the malignant change in affected cells (Farber 1981). The dividing cell populations in many tissues are self renewing stem cells (Leblond 1964). The kidney, however, is classified as a stable tissue, that is one in which the rate of cell division in the adult is low but in which the

majority of cells retain the capacity to divide under an appropiate stimulus (Leblond 1964). It has been argued that the renal tubular epithelium retains a degree of developmental plasticity and the capacity for re-differentiation to different renal tubular phenotypes during regeneration following tubular injury (Fleming & Matthews 1987).

Similar cell division and variability in differentiation pathways can be seen in renal carcinoma cells. In this study 41/44 renal cell carcinomas simultaneously expressed the proximal tubular marker brush border antigen and the distal tubular marker EMA. As far as can be determined by the examination of serial sections, single cells expressed both antigens. In studies of lectin binding sites in renal tumours Ulrich et al (1985) found that the majority of renal cell carcinomas simultaneously expressed terminal glycosyl residues typical of both the proximal and distal tubular epithelium.

Not only may renal cell carcinomas simultaneously express renal tubular markers characteristic of different parts of the nephron, but also may express different intermediate

filament proteins. Hermans et al (1983) and Holthofer et al (1984) have both shown that the cells in the majority of renal cell carcinomas express vimentin and cytokeratin types of intermediate filaments. In the current study vimentin was also found in a significant proportion of renal cell carcinomas. Vimentin is the intermediate filament protein characteristic of mesenchymal tissues, and it is found during renal embryogenesis in the metanephric blastema. These findings suggest that in renal cell carcinoma the developmental options may include extreme de-differentiation to a primitive phenotype.

Such de-differentiation is also illustrated by the existence of sarcomatoid renal carcinoma. Sarcomatoid renal carcinomas are tumours in which there is both an epithelial and a spindle cell component (Farrow et al 1968). The epithelial component can show a similar range of histological appearances as "pure" renal cell carcinomas, and in this study both clear cell and granular cell types were seen. The spindle cell components can also show a variety of histological appearances, resembling fibrosarcoma, malignant fibrous

histiocytoma or leiomyosarcoma (Farrow et al 1968; Tomera et al 1983). In addition, neoplastic bone and cartilage can be seen as in one case in this series. More detailed investigations of the nature of the spindle cell component are lacking. The electron microscopical appearances of the spindle cell component of one of these tumours was studied by Deitchman & Sidhu (1980). They found tight intercellular junctions between tumour cells and concluded that the spindle cells of all sarcomatoid renal carcinomas are epithelial spindle cells.

The spindle cells of these tumours expressed some mesenchymal antigens, but not epithelial antigens. Thus, although the epithelial areas could express low molecular weight cytokeratin antigens, EMA, and brush border antigen these antigens were not found in the spindle cell component.

The 3 tumours in which the spindle cell component was similar to malignant fibrous histiocytoma all expressed the proteinase inhibitor antigens, alpha-1-antitrypsin and alpha-1-antichymotrypsin in their cytoplasm. Malignant fibrous histiocytomas also express these

molecules, indeed this has been used as a diagnostic test for such tumours (Du Boulay 1985). Although proteinase inhibitors are found in the proximal tubular epithelium the failure of the spindle cells to express BB antigen, which is developmentally a more primitive proximal tubular marker than the proteinase inhibitors, makes it unlikely that the spindle cells are exhibiting proximal tubular differentiation. Proteinase inhibitors are also found in the cells of the undifferentiated metanephric blastema and of the primitive interstitial mesenchyme of the nephrogenic zone of fetal kidney. These antigens were also found in the equivalent neoplastic cells in nephroblastoma. The combination of vimentin expression, proteinase inhibitor expression and the failure of expression of epithelial antigens suggests that this may be a true mesenchymal component of sarcomatoid renal carcinoma. The pattern of expression of fibronectin, and the presence of collagen, seen in the tinctorial stained sections, suggests that the fibrosarcoma type of spindle cell component is also mesenchymal. Clearly fully differentiated mesenchymal tissues can also be present in these tumours.

These data suggest that sarcomatoid renal carcinoma are true carcinosarcomas with both neoplastic epithelial and mesenchymal components. How do such tumours develop? It has been suggested that renal carcinomas may develop from proliferating hyperplastic or regenerative epithelium, and that regenerative epithelium is developmentally plastic showing a capacity for de-differentiation and re-differentiation along different pathways (Fleming & Matthews 1987). The kidney is, of course, a mesodermal organ and both the epithelial and the mesenchymal component are derived from the same undifferentiated tissue (Hamilton & Mossman 1972). The development of carcinosarcomas may be an extreme form of the developmental plasticity of the neoplastic renal tissue.

In the renal cell carcinomas there was evidence of renal hormone activity. The secretion of erythropoietin and consequent polycythaemia has been previously described in renal cell carcinoma. Renal cell carcinomas have also been associated with the inappropriate secretion of other hormones (Sibley & Rosai 1981). There have been occasional cases of renal cell carcinoma in which there was

evidence of renin secretion (Hollifield et al 1975; Lebel et al 1977; Leckie et al 1978). Hollifield et al were able to demonstrate cytoplasmic granules with the morphology of renin granules. Lindop, More & Leckie (1983) had the oppurtunity to study the case of Leckie et al by electron microscopy and immunocytochemistry. They were able to confirm the presence of renin within this tumour by these techniques. In the present study immunostainable renin was found in a proportion of renal cell carcinomas. As was seen in the nephroblastomas the renin containing cells had a characteristic spindle shaped morphology and perivascular distribution.

It is not possible to decide with certainty whether these cells were tumour cells or cells growing into the tumour with the supporting vascular stroma. Lindop & Fleming (1984) have shown that metastatic tumours in the kidney do not contain immunoreactive renin. They have argued that the presence of renin containing cells is a specific feature of primary renal tumours. Further experiments will be necessary to investigate the capacity of renal carcinoma cells to produce and secrete renin.

The renal cell carcinomas which were available for study with the proliferation associated molecules showed expression of both of the antigens. Almost all cells in the tumours expressed the transferrin receptor molecule, but there was only a very low level of expression of the Ki 67 marker. Renal cell carcinomas generally have a low growth rate so the low level of Ki 67 expression may have been expected. The expression of the transferrin receptor molecule at a high level is seen in regenerating tissues, in which not all cells which express the molecule are in cycle (Haynes et al 1981).

5.2.3 Renal oncocytoma.

Klein & Valensi (1976) originally recognised renal oncocytoma by studying the renal cell carcinomas in their centre which had been either Grade I or II and in which subsequent metastases were not recorded. They then argued that these tumours were distinct from the other types of renal cell carcinoma and that they should be called renal oncocytoma or proximal tubular adenoma. Since then several series of renal oncocytoma have been reported (Bonavita, Pollack & Banner 1981; Lieber, Tomera & Farrow 1981). Although the findings of most of these series have confirmed the benignity of renal oncocytoma, metastases have been recorded in the large series from the Mayo Clinic (Lieber, Tomera & Farrow 1981).

The Mayo Clinic group have also studied these tumours by DNA flow cytometry (Rainwater et al 1986). They found that most of the tumours were entirely diploid, but in some cases polyploidy and aneuploidy were found. Their conclusion was that renal oncocytoma should be regarded as a tumour with low rather than zero metastatic potential or malignancy.

It has been argued that renal oncocytomas are derived from the proximal convoluted tubule (Klein & Valensi 1976). This argument has been based on the light microscopical similarity between renal oncocytes and the proximal tubular epithelium; and on the ultrastructural appearances of the tumour particularly the presence of large numbers of mitochondria (Lieber, Tomera & Farrow 1981).

In the present study the immunocytochemical reactions of the oncocytomas were very similar to other forms of renal cell carcinoma. Of particular importance to a discussion of the differentiation pattern of renal oncocytoma is the observation that these tumours expressed the distal tubular marker EMA. The pattern of staining showed some differences from the pattern seen in renal cell carcinomas, namely cytoplasmic staining in addition to surface staining for EMA. Nevertheless the expression of this molecule suggests that oncocytomas do not exclusively show proximal tubular differentiation. This view is further supported by the lectin histochemistry data of Ulrich et al (1985). The oncocytomas which they studied expressed some cell surface glycoconjugates which were found in distal tubular epithelium.

Although this study may raise doubts about the precise nature of renal oncocytomas, their separate identification from other forms of renal cell carcinomas is valuable because of their better prognosis (Lieber, Tomera & Farrow 1981; Lewi, Alexander & Fleming 1986).

5.2.4 Collecting duct carcinoma.

The possibility that some renal carcinomas may arise from the collecting ducts was first raised by Mancilla-Jimenez *et al* (1976) who noted atypical hyperplasia of the collecting duct epithelium in three of the cases of papillary carcinoma which they reviewed. They did not discuss any other histological, radiological or clinical differences which distinguished these three cases from the remainder of their series.

More complete clinical and pathological data on possible collecting duct carcinomas have been presented in three cases subsequently recorded (Cromie *et al* 1979; O'Brien & Bedard 1980; Hai & Diaz-Perez 1982). The case recorded by Cromie and his colleagues was histologically a mixed tumour with both papillary urothelial and tubular clear cell components. Similar mixed tumours have been reported previously (Graves & Templeton 1921; Patch & Rea 1926; Balch 1935; MacAlpine 1947; Rupel & Sutton 1950; Gillis, Finnerty & Maxted 1971; Fallon & Schellhammer 1975; Vonenschenbach, Johnson & Ayala 1977) and the classification of these tumours remains debatable.

O'Brien & Bedard (1980) described a case of a well differentiated papillary, non-mucin secreting adenocarcinoma growing into the renal pelvis in a young girl. In places there were tubular areas with invasion of the renal parenchyma. Their ultrastructural studies suggested the tumour was of collecting duct origin. The case reported by Hai & Diaz-Perez (1982) was in a 22-year-old male. Histologically the tumour consisted of areas of papillary adenocarcinoma and areas in which infiltrating tubules were surrounded by a brisk desmoplastic reaction. The tumour cells were pleomorphic, with vesicular nuclei, prominent nucleoli, a high mitotic rate and a rim of faintly eosinophilic cytoplasm. They identified glomeruloid bodies within the tumour. Several duct-like structures, resembling collecting ducts, in which there was cellular atypia were seen. These two cases show striking similarities to the cases identified in the present study.

A desmoplastic reaction around infiltrating tubules, as seen in the present series of collecting duct carcinoma, is unusual in renal cell carcinoma. Periductal fibrosis is seen around the primitive collecting ducts in renal dysplasia (Ericsson & Ivemark 1958), so it may be that abnormal collecting duct epithelium induces fibroblast proliferation within the kidney.

The immunocytochemical studies provide further evidence of collecting duct differentiation in these tumours. Although CAM 5.2 and PKK1 were found to bind to both collecting duct and renal tubular epithelium, the large molecular weight epidermal prekeratins have only been found in collecting duct cells. These large molecular weight cytokeratins have been demonstrated in this series of collecting duct carcinomas. These antigens were only found in a very few cells in a small minority of the renal cell carcinomas. Collecting duct epithelium expressed surface EMA but not BB antigen, and the collecting duct carcinomas showed the same antigen expression pattern. Although the primitive ureteric bud expressed the fucosylated N-acetyllactosamine antigens neither the differentiated collecting duct epithelium nor the collecting duct carcinomas showed reactivity with AGF 4.48 or AGF 4.36.

The separate identification of these tumours is important in studying the epidemiology, aetiology and biology of urinary tract neoplasms. The collecting duct, along with the ureter, renal pelves and calyces, develops from the ingrowing ureteric bud, which is itself derived from the

mesonephric duct (Hamilton & Mossman 1972). The collecting ducts may be susceptible to carcinogenic stimuli which are different from those which affect the metanephric portion of the kidney, from which the majority of renal cell carcinomas probably arise.

Fleming & Lewi (1986) included the present cases in their study of six cases of collecting duct carcinoma. Four of these six cases were identified in a series of 225 nephrectomies performed for renal tumour. Mancilla-Jimenez et al (1976) described three possible cases of collecting duct carcinoma in a series of 221 renal tumours. This tumour would, therefore, seem to constitute between 1 and 2% of renal tumours. At least some of these tumours, particularly those showing infiltration of the renal parenchyma, behave in an aggressive manner, with short patient survival.

In summary, the use of immunocytochemical markers has helped to confirm the distinction of a group of renal tumours which show collecting duct differentiation.

5.2.5 Renal sarcoma.

The five renal sarcomas studied expressed the antigens previously described for these various tumours in extra-renal sites (Du Boulay 1985; Roholl et al 1985). The leiomyosarcomas expressed the intermediate filament protein desmin. The case of malignant fibrous histiocytoma contained proteinase inhibitors. The fibrosarcomas expressed the vimentin type of intermediate filament protein.

5.2.6 Vascularisation of renal tumours.

The study of the vascularisation of renal tumours was not a major aim of this study and immunocytochemistry is not a particularly good technique for studying the process of vascularisation. Nevertheless some observations made during the course of this study deserve comment.

I have shown that during the vascularisation of the fetal kidney there is endothelial cell proliferation and the expression by the endothelial cells of the cell surface receptor for transferrin (Chapter 3). This transferrin dependent proliferative activity was lost as development progressed. In the renal tumours studied using Ki 67 and HB 21 the endothelium also showed evidence of transferrin dependent proliferation. The endothelial cells of the tumour blood vessels expressed the transferrin receptor antigen and some also expressed the nuclear proliferation marker.

This evidence suggests that the blood vessels actively grow into renal tumours from the adjacent kidney. This process of tumour angiogenesis has

been demonstrated previously in a variety of tumours (Folkman et al 1971). The phenomenon is thought to depend on tumour angiogenesis factors, molecules which are synthesised by tumour cells which stimulate the mitotic division of vascular endothelial cells (Folkman et al 1971). Angiogenesis factors have been isolated from some renal tumours (Shahabidin et al 1986).

The role of the renin-angiotensin system during renal angiogenesis has attracted attention recently (Lindop & Lever 1986). It has been suggested that angiotensin II may act as a mitogen for endothelium (Campbell 1985). In the present study it has been shown that there are renin containing cells intimately associated with blood vessels growing into renal tumours. Renal tumours have also been shown to contain angiotensin converting enzyme and to be capable of generating angiotensin II (Takada et al 1985). The role of this cascade in tumour angiogenesis in the kidney remains to be explored.

Not only do blood vessels grow into renal tumours from the adjacent kidney, but they also show specialised morphological features. In the nephroblastomas which showed extensive tubular

differentiation the blood vessels formed a dense peritubular capillary network. In renal cell carcinomas the blood vessels also form a dense capillary network closely apposed to tumour epithelial cells. Although there is evidence for angiogenesis factors in renal organogenesis and in renal tumours (Shahabidin et al 1986), the mechanisms which result in the development of the specialised renal microcirculation remain unknown.

The vascular endothelium of the blood vessels in the renal tumours stained with the antibody to Factor VIII R antigen. This was the same reactivity as that seen in the normal renal peritubular capillary network but different from that observed in the glomerular endothelium. However, during the vascularisation of the kidney both of these types of endothelium expressed Factor VIII R antigen. It is not possible to utilise this antigenic heterogeneity of renal endothelium to investigate endothelial cell differentiation.

TABLE 11

Epithelial grading of the nephroblastomas.

Epithelial grade	Number of cases
0	5
+	15
++	10
+++	6

TABLE 12

Tissue morphology in nephroblastoma.

Tissue	Number of cases
Blastema	30
Tubules (Type A)	31
Type B tubules	12
Pseudoglomeruli	10
Microcysts	10
Papillary structures	2
Heterologous epithelium	3
Mesenchyme	31
Myxoid tissue	12
Adipose tissue	4
Skeletal muscle	5
Smooth muscle	2
Hyalinised connective tissue	1

TABLE 13

Table 13a

Histological classification of renal cell carcinomas

Architecture:	Cell type		
	Clear cell.	Granular cell.	Basophil.
Solid	11	1	-
Papillary	7	1	1
Tubular	4	3	-
Sarcomatoid (Epithelial cell type)	6	2	-

TABLE 13

Table 13b

Nuclear grade of renal cell carcinoma.

Cell type:	Nuclear grade			
	I	II	III	IV
Clear cell	16	6	6	-
Granular cell	2	2	2	1
Basophil	1	-	-	-
Sarcomatoid	-	-	3	5

TABLE 14

The classification of the renin containing tumours

Tumour type	Number containing renin
Renal cell carcinomas	
Clear cell	10
Granular cell	3
Basophil cell	-
Sarcomatoid	-
Collecting duct carcinoma	-
Oncocytoma	3
Renal sarcoma	-

TABLE 15

Histological classification of the renal sarcomas.

Leiomyosarcomas	2
Fibrosarcomas	2
Malignant fibrous histiocytoma	1

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS.

This thesis describes an investigation of the differentiation of different components of the human kidney. Using immunocytochemistry I have mapped the sequence of appearance of a series of differentiation markers during embryonic development allowing them to be classified according to the developmental stage at which they first appear within the separate cell lineages. These data have then been applied to the study of abnormal differentiation in renal pathology.

Some aspects of this study of molecular markers in renal pathology raise questions of the use of the concepts of histogenesis and differentiation in the classification of renal and other neoplasms. The problems of the use of these concepts in tumour pathology has recently been discussed by Gould (1986).

These two terms are derived from embryological studies. Histogenesis is the process during which cells acquire functional features, whereas differentiation is the acquisition of these

features irrespective of the way in which they have been acquired. These embryological definitions have been modified slightly for the needs of the tumour pathologist. In that special context histogenesis refers to the cell(s) from which the tumour has developed. This classification is histogenetic or ontogenetic. Differentiation refers to the degree of resemblance of the tumour cells to the corresponding mature tissue cells. It therefore generates a phenotypic classification.

There are clearly some difficulties in applying these concepts to accurate tumour classification. The major source of these difficulties is a lack of knowledge of the processes of tissue development, the rigidity of commitment to cell lineages, the control of terminal differentiation and the molecular and genetic basis of the abnormalities arising during tumour development.

There are clear differences in the way that these two schools of thought would classify tumours and difficulties in defining and applying criteria upon which each classification is based.

Superficially a phenotypic classification appears to be the easier to apply. The phenotype of the tumour cells would be assessed by various criteria, (biochemical, morphological or immunological) the resemblance to normal cell types measured and the tumour classified as a tumour of that particular cell type. There are difficulties in this type of approach. The different features of a cell are so numerous (although at present we can only measure a tiny proportion of these) that we must select the relevant criteria or ultimately have an almost infinite number of tumour classes. At present we do not know which features of a tumour cell are biologically important and therefore cannot decide which have the most valuable discriminatory potential.

Some tumours present extreme difficulties to a phenotypic classification. For example tumours may show more than one pattern of differentiation and therefore more more than one phenotype. This particularly applies to embryonic tumours. Nephroblastoma contains epithelial cells and mesenchymal cells each of which expresses differentiation markers appropiate to these different cell types. Tumours of this type are difficult, if not impossible, to classify in a rigourously applied phenotypic classification.

A phenotypic classification assumes that the tumour phenotype remains fixed during the clinical course of the tumour. Several workers have shown that tumours change their behaviour, including the expression of of differentiation markers as they progress. This behaviour has been termed tumour evolution (Klein & Klein 1985). It is obviously unsatisfactory that the classification of a tumour may change during the course of its development and progress. An example to illustrate this problem is haematological malignancies in which cells found during blast crisis may have an entirely different phenotype from those present in the early stages of the disease (Greaves 1982). Similar phenotypic changes are seen in the nephroblastomas recurring after chemotherapy, when mesenchymal components tend to predominate regardless of the original histology of the tumour (Gonzalez-Crussi 1984).

The final difficulty of a phenotypic classification is the problem of convergent development of tumour cells. This phenomenon is seen in metaplasia and metaplastic tumours, that is cells showing a phenotype different from that seen in the tissue in which they arise. The

majority of tumours of the renal pelvis, ureters and bladder are urothelial and are therefore of the same phenotype as the normal epithelium of these tissues. A small proportion of tumours from this site are of a squamous cell type. A rigourously applied phenotypic classification will not distinguish this tumour type from squamous cell carcinoma of the skin or bronchus. By restricting the examination of the tumour to establishing its phenotype this classification does not recognise differences in the developmental history of two tumours which at presentation show the same phenotype. Phenotypic classifications do not allow the different clinical behaviour, aetiological factors and biology important in the development of phenotypically similar tumours to be distinguished. This difficulty arises because in pathological states epithelia, or other tissue types, showing metaplasia come to resemble cells from other tissues which obviously have different developmental histories. These metaplastic tissues are showing convergent development by moving closer together in phenotype as terminal differentiation, normal or abnormal, occurs.

These various difficulties in applying a tumour classification based solely on the tumour phenotype make such a classification impractical and ultimately subjective.

A classification based entirely on histogenetic principles also presents difficulties. One of the main difficulties is the identification of the charcteristics of a tumour which reveal its histogenesis. In other words in many cases, it is difficult to examine a tumour and be certain from which cell type it developed.

A histogenetic classification will fail to recognise some of the complexities of tumours which are known to occur. If all lung tumours are derived from bronchial stem cells, despite their differing appearances and behaviour, they would all be classified as of one type. Willis (1962) argued that this was the case but most contemporary tumour pathologists would disagree with what is an oversimplified view of this particular group of tumours.

Metaplastic tumours again give rise to problems of classification. Assuming that urothelial (transitional cell) carcinoma and squamous cell carcinoma of the renal pelvis both

develop from urothelial stem cells then a histogenetic classification would fail to recognise the divergent differentiation which is seen in these two tumours. Metaplasia is therefore divergent differentiation within a histogenetic classification, but is convergent in a phenotypic classification. Metaplasia and metaplastic tumours present difficulties to both classifications.

There are limitations to the validity and value of classifications based exclusively on either phenotypic or histogenetic principles. The optimal classification may be one in which both sets of principles are combined. Such a classification would identify the tissue of origin of tumour cells and describe the pattern of differentiation seen within the tumour.

Amongst several difficulties a very major one is our present lack of knowledge of the types of cells from which tumours arise. This type of knowledge would ideally combine the mechanism of tumour formation, the developmental stage of the cell from which the tumour develops and the normal sequence of events during the differentiation of the cell from which the tumour develops.

In this study I have related data on tumour cell phenotype to the sequence of appearance of cell markers during normal kidney development. Combining this with other data on the cell biology of renal development provides a basis for the formulation of a classification of renal tumours.

The immediate histogenesis of renal tumours is the metanephric blastema. In the case of some renal tumours the cells may have progressed through several normal developmental stages before the tumour developed. Renal cell carcinoma probably develops from a cell which has already become a renal tubular epithelial cell. One of the difficulties is the identification of the precise developmental stage of the cell at which neoplastic transformation occurs. Although elucidation of this point requires much more experimentation some important points can be made.

The application of histogenetic principles to a classification of renal tumours implies that renal cell carcinoma is more closely related to nephroblastoma (because of their common histogenesis from the metanephric blastema) than to other non-renal carcinomas. In some respects this is true. Cytogenetic investigations have

shown that familial and some non-familial nephroblastomas and familial renal cell carcinomas share abnormalities, particularly deletions, of the short arm of chromosome 11 (Orkin 1984). Some chemical carcinogens when administered to young animals cause embryonal tumours but administered to adult animals cause renal cell carcinomas (Guerin et al 1969).

A major biological implication of a histogenetic classification is the need to understand the degree of commitment to differentiation along a certain lineage which the cells at any point on that lineage possess. I have discussed some aspects of commitment and differentiation in renal development above, and my observations on renal tumour cell differentiation have some bearing on this issue.

Studies of renal tubulogenesis using different inducers in the transfilter induction system (Grobstein 1956; Saxen 1987) have shown that the induction of tubular differentiation from the metanephric blastema is of the permissive type. The metanephric blastema has already been committed to one line of differentiation, that is it is in a determined state. The validity of

applying histogenetic principles to tumour classification can be tested by asking whether tumours arising from this determined tissue show the same restricted developmental options as the metanephric blastema does during renal development.

In this investigation I have studied epithelial differentiation in a number of different renal tumours by morphological and immunocytochemical methods. Other studies using similar (Holthofer et al 1983) or other methods (Tannenbaum 1971) have shown similar results, namely heterologous differentiation of neoplastic renal epithelium is an extremely rare event. This would suggest that even in neoplasms in which polyploidy, aneuploidy and specific chromosomal defects are seen the commitment to the specific renal developmental lineage remains.

The biological significance of the lack of heterologous epithelium can only be interpreted within the framework of a classification which includes histogenetic principles. Further I have tested the validity of histogenesis in renal tumours by studying the range of differentiation options seen in these tumours

In contrast to the lack of heterologous epithelia, heterologous mesenchyme can be seen in renal pathology, especially in nephroblastomas. This observation can also be explained by understanding the tumour histogenesis and the nature of the commitment of the metanephric blastema. The metanephric blastema is in a determined state with respect to its potential for epithelial differentiation. If it is not induced to form epithelium, either in a normal or a neoplastic state, it remains mesenchymal, eventually becoming refractory to induction (Saxen 1987). Persistence of the metanephric blastema in undifferentiated or mesenchymal states gives rise to the range of lesions forming the nephroblastomatosis complex. If the metanephric blastema persists as mesenchyme it can then show the differentiation options of embryonic mesenchyme. Therefore, metanephric blastema, which in the pathological state, has not been induced to form epithelium, can form a variety of mesenchymal tissues. This can be seen experimentally (Saxen 1987) and in the dysplastic and neoplastic lesions described in this thesis.

Understanding the histogenesis of renal tumours and the nature of the determined state of the blastema has accounted for the patterns of homologous and heterologous differentiation seen in developmental renal pathology.

Another factor important to histogenetic classification is the related topic of the reversibility of progress along a developmental lineage.

For many cell lineages progress along a developmental pathway is irreversible, a mature polymorph does not de-differentiate into a myeloblast, a keratinocyte cannot become an epidermal basal cell (Leblond 1964). The renal epithelium does not show a similar stepwise irreversible progress to the mature state except to the extent to which they are committed to renal development.

The differentiated renal epithelium expresses a variety of specific functions, and therefore specific molecules, appropiate to the particular tubular segment in which the cell lies (Heptinstall 1983). In both human and experimental renal tubular lesions, such as ischaemic or toxic damage, the affected epithelial cells shed their

specific functional component, namely the apical membrane and cytoplasm, and adopt a more simplified form (Bucher & Malt 1967; Solez 1983). In other words, the renal tubular epithelial cells can "de-differentiate" to a simpler phenotype. During the recovery phase following such injury the cells can again acheive structural, biochemical and functional maturity (Bergsma & Pound 1980; Solez 1983).

Having defined differentiation as the fully functional state it then follows that in tubular regeneration following acute renal epithelial injury the tubular epithelium shows that developmental movement (de-differentiation and re-differentiation) within the cell lineage is possible. I will call this phenomenon vertical developmental plasticity within a cell lineage.

This form of developmental plasticity is seen in those tissues which have the form of tissue kinetics described by Leblond (1964) as stable tissues. These tissues, such as the liver and kidney, are characterised by the adult parenchymal cells retaining the capacity for mitotic division in appropriate circumstances, particularly regeneration following tissue injury.

Developmental plasticity is essential for this type of regeneration but equally important is the ability to replace dead cells which may be functionally distinct from the regenerating population, that is they may be of a different phenotype. This phenomenon is seen in renal tubular regeneration following certain toxic insults particularly mercuric chloride induced renal tubular necrosis. In this experimental model the toxin causes frank necrosis of the cells of the pars recta of the proximal tubule but the basement membrane remains intact. Regeneration and functional recovery occurs by proliferation of the tubular epithelial cells adjacent to the damaged areas. The daughter cells then migrate on the intact basement membrane to replace the lost cells of the pars recta. For functional recovery, however, these daughter cells must now behave as pars recta cells rather than as the cells of the parts of the nephron from which they were derived. That means that they must alter their phenotype, although remaining within the range of nephron developmental options (Bergsma & Pound 1980).

Fleming & Matthews (1987) have provided immunocytochemical evidence for a similar developmental plasticity in human renal disease.

This phenomenon of alteration of phenotype within one defined set of developmental lineages I will describe as horizontal developmental plasticity. I wish to distinguish it from metaplasia which I define as heterologous differentiation occuring in a differentiated tissue.

By studying the differentiation of the renal tubular epithelial cells in disease these two forms of developmental plasticity have been observed in a cell lineage which has become committed and which apparently does not show heterologous differentiation.

In the present study both forms of developmental plasticity have been observed.

Since Leblond (1964) showed that renal epithelial cells retain the capacity for cell division, it seems reasonable, at present, to assume that renal cell carcinoma develops from a parenchymal epithelial cell, which at one stage was a differentiated cell. Yet there are poorly differentiated tumour cells in renal cell carcinoma which are defined as such by morphology or by the lack of expression of tubular differentiation markers and expression of immature

markers such as vimentin. Therefore, the cells in renal cell carcinoma are showing vertical developmental plasticity or de-differentiation. This loss of maturation markers and re-expression of immature markers was a common occurrence in the present series of renal cell carcinomas.

Horizontal developmental plasticity was also seen in these tumours in which mixed phenotypes, such as the co-expression of EMA and BB antigen, were seen in the immunocytochemical experiments. Similar mixed phenotypes were seen in renal biopsy material also using the differentiation markers EMA and BB antigen (Fleming & Matthews 1987). In renal dysplasia mixed tubular epithelial phenotypes and tubularisation of glomerular epithelium show that this phenomenon is not restricted to neoplastic differentiation.

By studying the differentiation of cells within a defined group of lineages, that is a population of defined histogenesis, the two related but separate phenomena of horizontal and vertical developmental plasticity have been observed. By retaining evidence of epithelial differentiation such as the expression of CAM 5.2 reactivity, and by the failure to exhibit

heterologous differentiation these cells have shown fidelity to their histogenesis while retaining flexibility of developmental response in pathological processes.

So far I have distinguished horizontal developmental plasticity from metaplasia. For the purposes of this discussion I have considered metaplasia to imply heterologous differentiation. From the preceding discussion, particularly the significance of the determined state of the metanephric blastema with respect to epithelial differentiation options, this distinction can be seen to be biologically important.

Metaplasia and heteroplasia can be seen in renal pathology, but the cells exhibiting these phenomena have a different histogenesis. The heterologous epithelial differentiation seen in this study was found in cells derived from the ureteric bud epithelium. The different developmental history of the ureteric bud illustrates why heterologous differentiation can occur in this cell lineage.

The ureteric bud is an epithelial outgrowth of the mesonephric duct. The differentiation of the cells of the different portions of the ureteric

bud into collecting duct epithelium, urothelium etc. is controlled by inductive interactions of the instructive type between the different mesenchymes and the epithelium of the ureteric bud (Saxen 1987). The ureteric bud epithelium remains plastic but is directed along the appropriate developmental lineages by the instructive signals which it receives.

In certain pathological circumstances, such as renal dysplasia or nephroblastoma in which the metanephric blastema is abnormal the correct instructive signal may not be given to the ureteric bud derivatives growing into the abnormal tissue. In the present study the resulting abnormalities of ureteric bud differentiation were manifest by continuing ureteric bud growth and immaturity accompanying nephroblastoma or ureteric bud heteroplasia with squamous, mucinous or other types of epithelial differentiation.

Because of the plasticity of the ureteric bud epithelium when neoplasms of this tissue develop heterologous differentiation may occur. The most common neoplasm of the ureteric bud shows urothelial differentiation, but squamous carcinoma, adenocarcinoma and other rare forms of

epithelial differentiation may occur (Olsen 1984). Similar changes in phenotype of the ureteric bud derived tissues are seen in chronic inflammation in which squamous metaplasia is a particularly common finding (Olsen 1984).

Ureteric bud epithelium remains developmentally plastic, is not in a determined state and its differentiation is controlled by intercellular signals of the instructive type. It is therefore, an epithelium which, unlike renal parenchymal epithelium, readily shows both heteroplasia and metaplasia.

The collecting ducts are derived from the ureteric bud and from this knowledge of their histogenesis one may expect heterologous epithelial differentiation to occur in this epithelium. Metaplasia of the collecting ducts in chronic inflammatory lesions has been described (Willis 1962). The collecting duct carcinomas have only recently been recognised, and are, at present, defined by their phenotype, that is by differentiation criteria. It may be expected that examples of metaplastic neoplasms arising in the collecting ducts will be seen. Further experience of these tumours will be necessary to confirm this.

In conclusion, there has been debate concerning the concepts of histogenesis and differentiation as they are applied to tumour classification (Gould 1986). I have argued that restriction to the use of one of these concepts would result in difficulties in accurate and complete classification of tumours and in the failure to recognise and explain biologically interesting and important phenomena such as metaplasia (divergent or convergent development). A combined use of both sets of ideas would seem to be optimal. One of the difficulties with such an approach is our present lack of data on cell developmental lineages, the restriction of developmental options (commitment) and the reversibility of developmental progress. This lack of knowledge is expressed in the present difficulty in distinguishing molecules which are of histogenetic significance from those which merely represent a feature of differentiation regardless of the developmental pathway by which it has been expressed.

I have studied these problems in the kidney because some of these facts have been established by several years of experimental embryology work

in this field (Saxen 1987) I have mapped the expression of various cell markers during renal development in order to study the differentiation in pathological states.

I have then shown that the use of both histogenetic and differentiation criteria to classify these diseases reveals the complexity of developmental pathology of the kidney. Understanding the developmental pathway and degree of commitment to renal epithelial differentiation of the metanephric blastema resolves the apparent paradox of lineage fidelity (the restriction of developmental options which allows only renal epithelial differentiation) and the persistence of both vertical and horizontal developmental plasticity within that group of options. I have discussed the range of lesions in which the developmental plasticity is overtly expressed and have shown that it is an essential part of the regeneration of the stable populations of Leblond (1964). The distinction between the above concepts of developmental plasticity and the forms of heterologous differentiation, heteroplasia and metaplasia, has been emphasised and its biological significance expressed in relation to the nature of the tissue interactions which govern terminal differentiation.

More detailed analysis of histogenesis and differentiation in renal and other branches of pathology are a major field of research which will involve examining the molecular somatic cell genetics and biochemistry of neoplasia, determination and differentiation.

IMMUNOCYTOCHEMICAL STUDIES OF THE DEVELOPMENTAL BIOLOGY AND PATHOLOGY OF THE HUMAN KIDNEY.

Submitted by

STEWART FLEMING

for the Degree of Doctor of Medicine

to the University of Glasgow

Volume 2

The Departments of Pathology of the Universities of Glasgow and Southampton.

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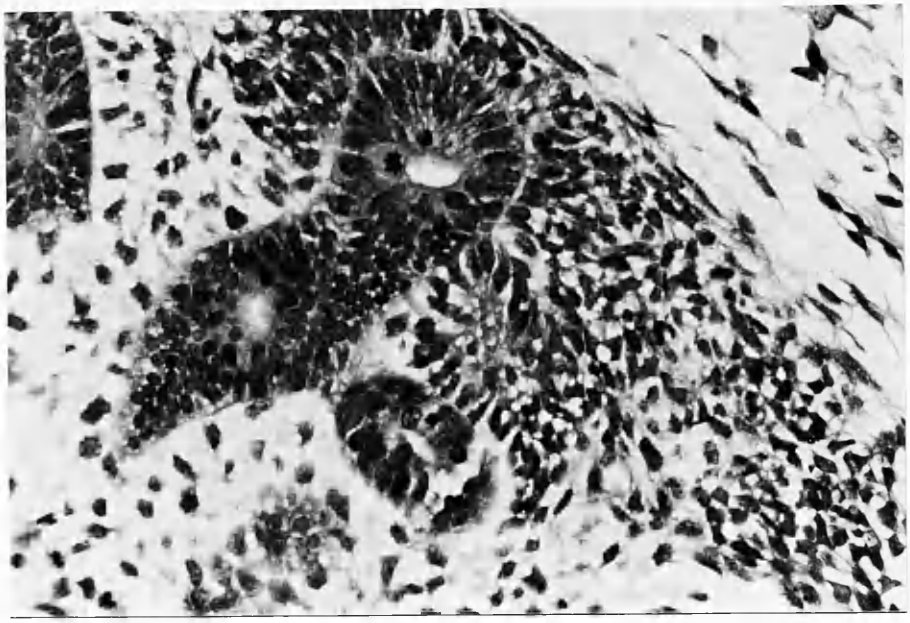
FIGURE 3. A nephrogenic vesicle has formed from the metanephric blastema adjacent to the ureteric bud branch. Fetal kidney. H&E.



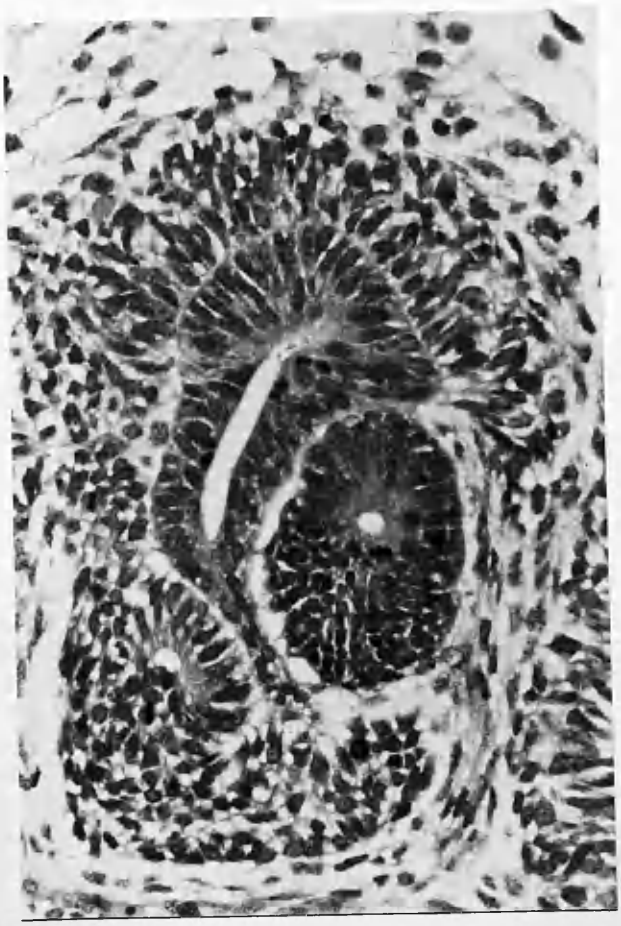
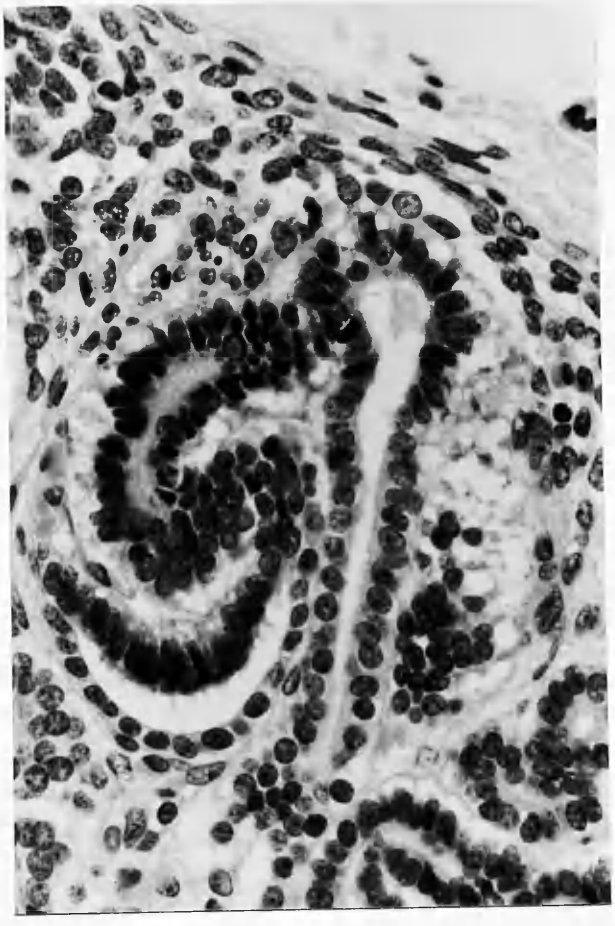


FIGURE 4. At this stage the S-shaped tubule has formed. The upper pole of the tubule approximates to the ureteric bud ampulla. Fetal kidney. H&E.

FIGURE 5. After fusion of the S-shaped tubule and the ureteric bud a primitive nephron with a continuous lumen has been formed. Fetal kidney. H&E.



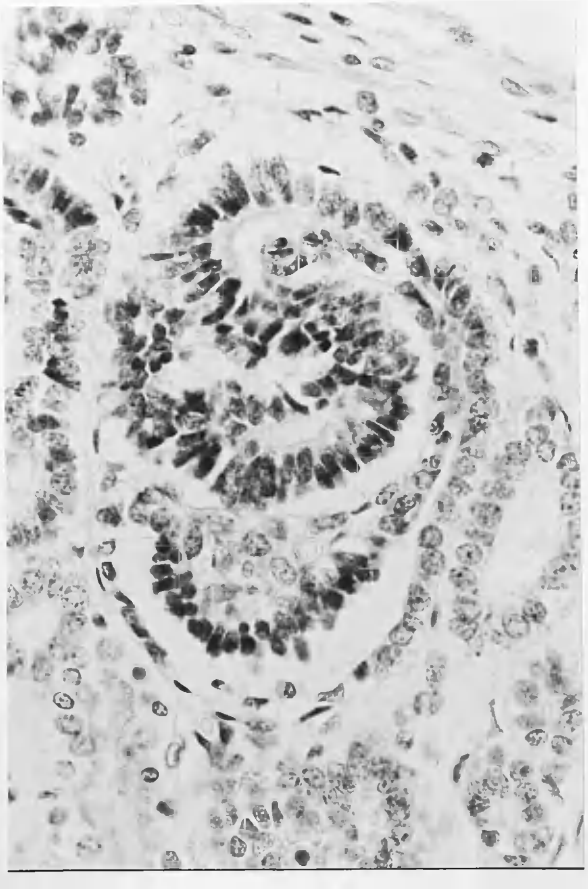


FIGURE 6. The glomerular tuft by this stage is showing more complete vascularisation and a lobulated architecture. Fetal kidney. H&E.

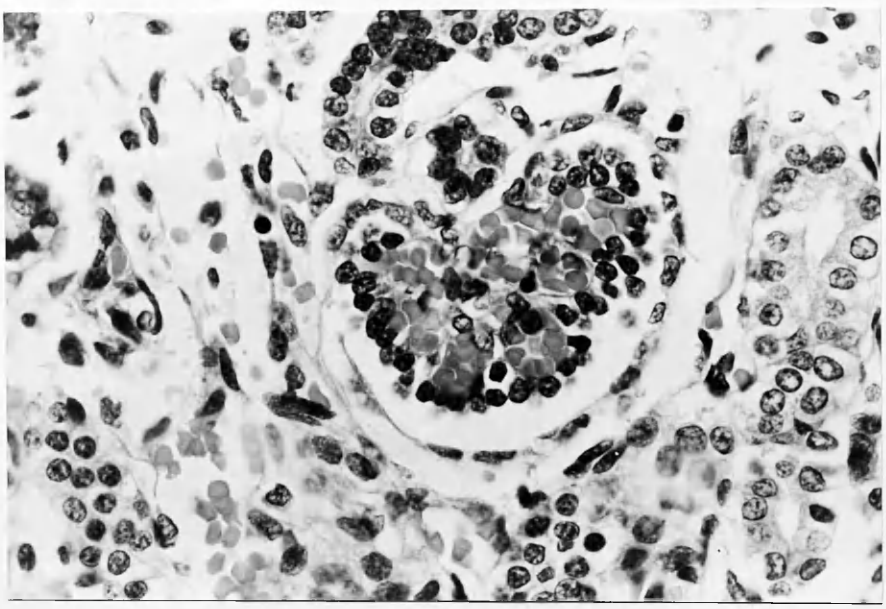
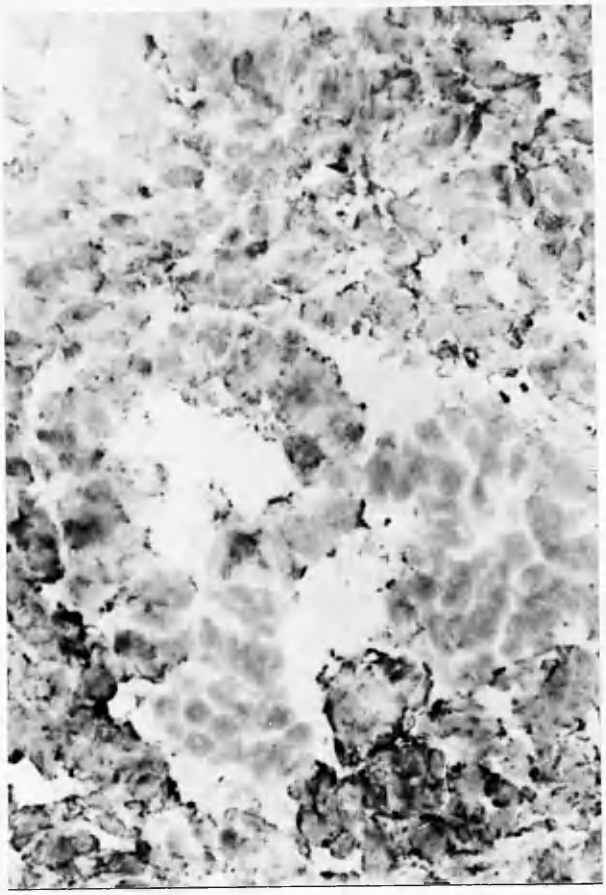


FIGURE 7. The metanephric blastema shows the expression of vimentin. Some focal positivity is also present in the ureteric bud epithelium. Fetal kidney. Immunoperoxidase.

FIGURE 8. At the S-shaped tubule stage vimentin is no longer seen in tubular epithelium but its expression persists in the glomerular epithelium. Fetal kidney. Immunoperoxidase.



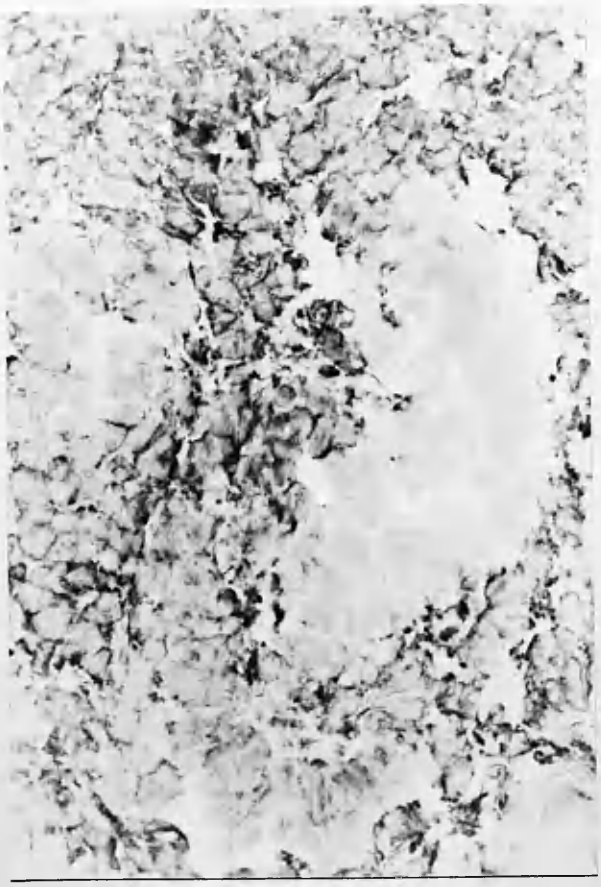
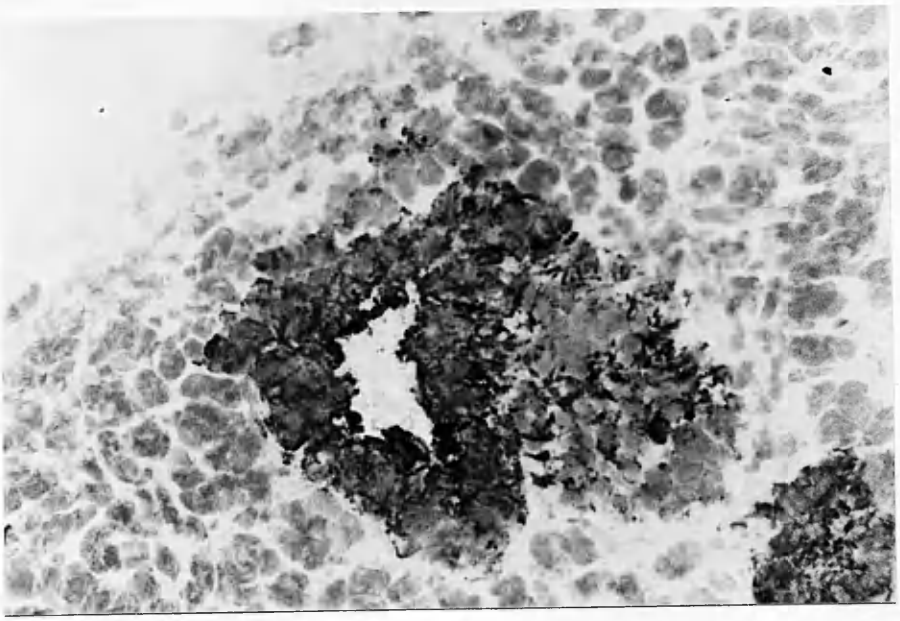


FIGURE 9. The epithelium of the ureteric bud stains with CAM 5.2. Focal positivity is present in the renal vesicle but not in the undifferentiated blastema. Fetal kidney. Immunoperoxidase.

FIGURE 10. The epithelium of the medullary collecting ducts stain with the antibody to epidermal prekeratin. Fetal kidney. Immunoperoxidase.



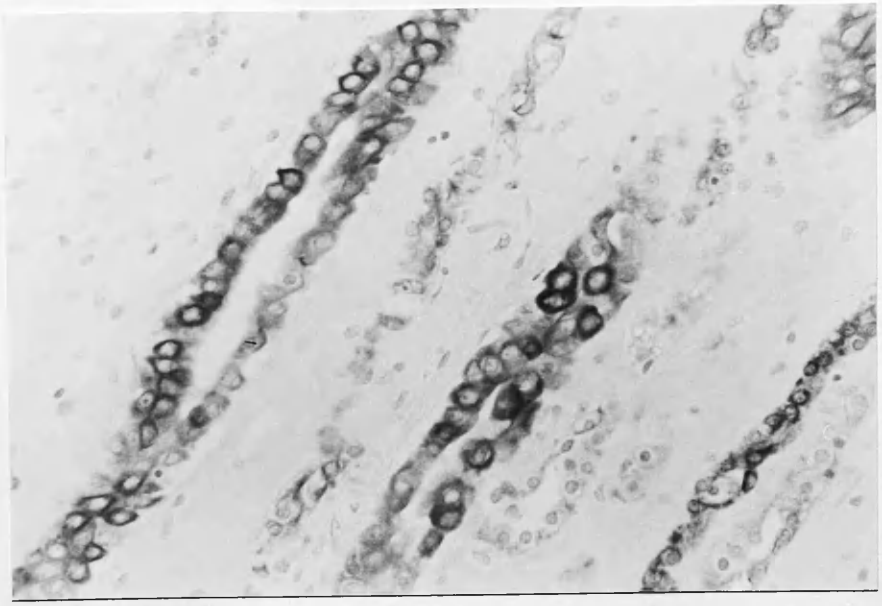
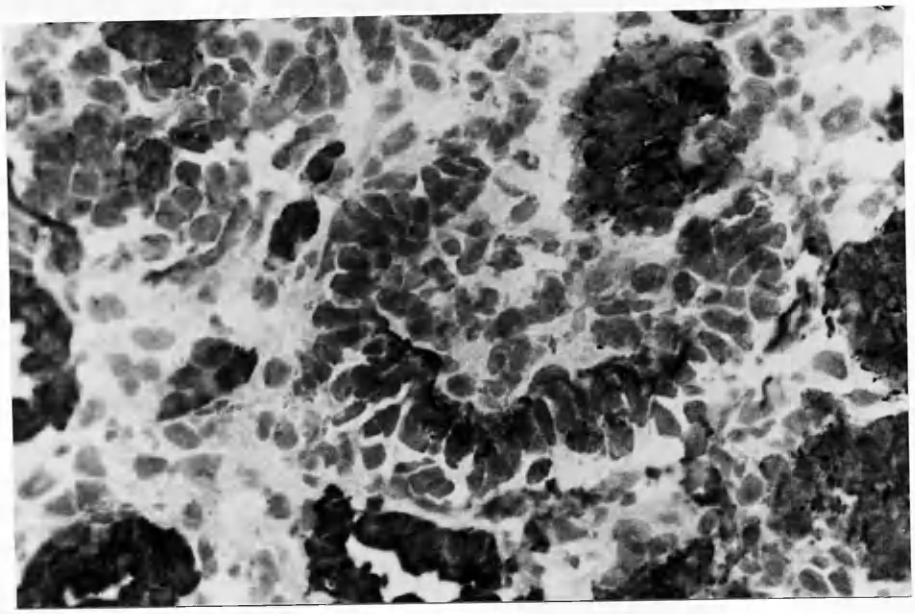


FIGURE 11. CAM 5.2 stains the base of the columnar cells of the visceral glomerular epithelium. The parietal epithelium is negative. Fetal kidney. Immunoperoxidase.

FIGURE 12. CAM 5.2 stains the tubules and parietal glomerular epithelium in the renal cortex. The visceral glomerular epithelium is negative. Adult kidney. Immunoperoxidase.



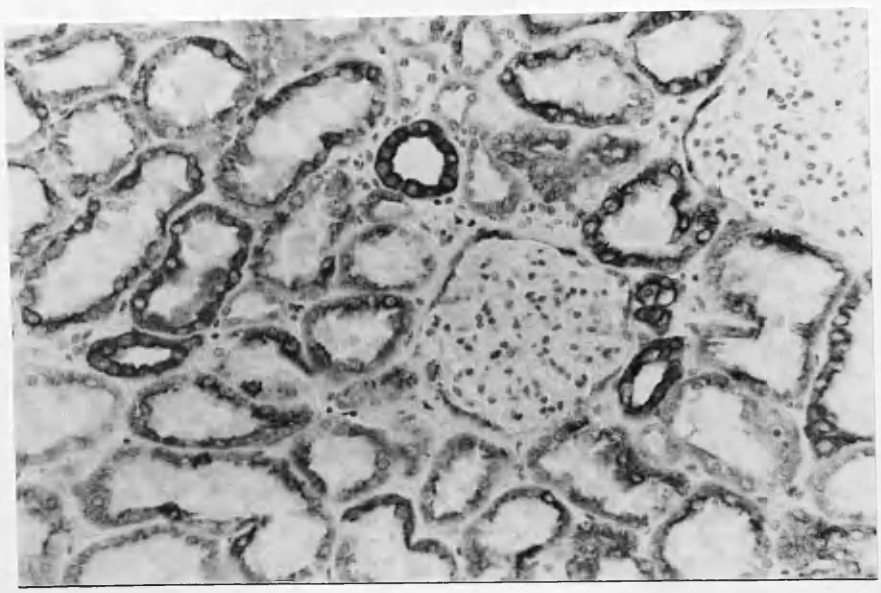
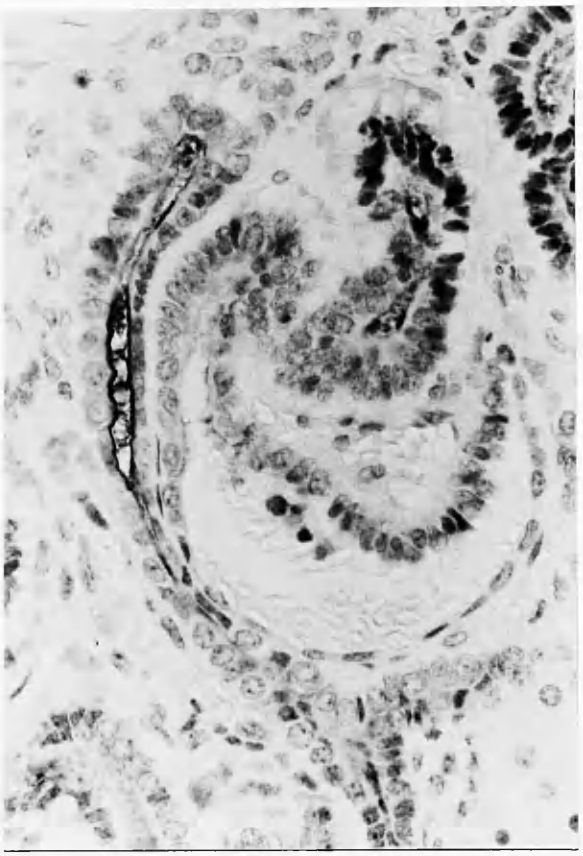


FIGURE 13. EMA is seen on the surface of the cells of the ureteric bud and those at the upper pole of the S-shaped tubule. Fetal kidney. Immunoperoxidase.

FIGURE 14. In the adult kidney EMA staining is present on the cells of the distal tubule but not proximal tubule. Adult kidney. Immunoperoxidase.



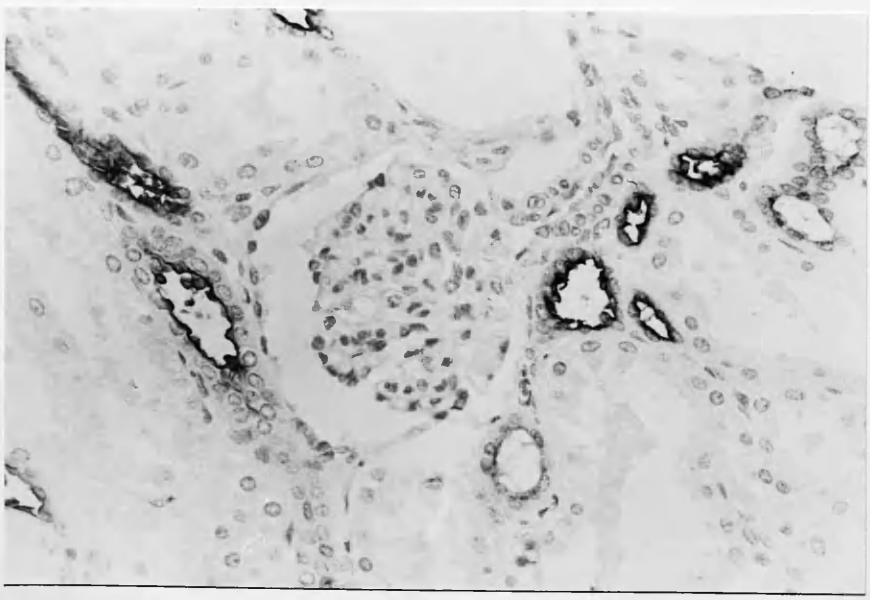


FIGURE 15. The apical border of the proximal tubular epithelium stains with the brush border antibody. Adult kidney. Immunoperoxidase.

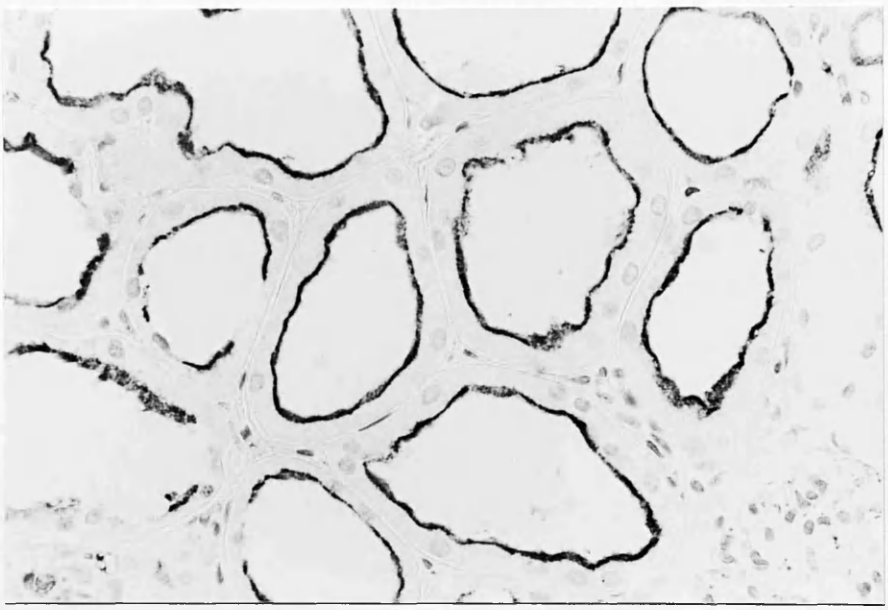
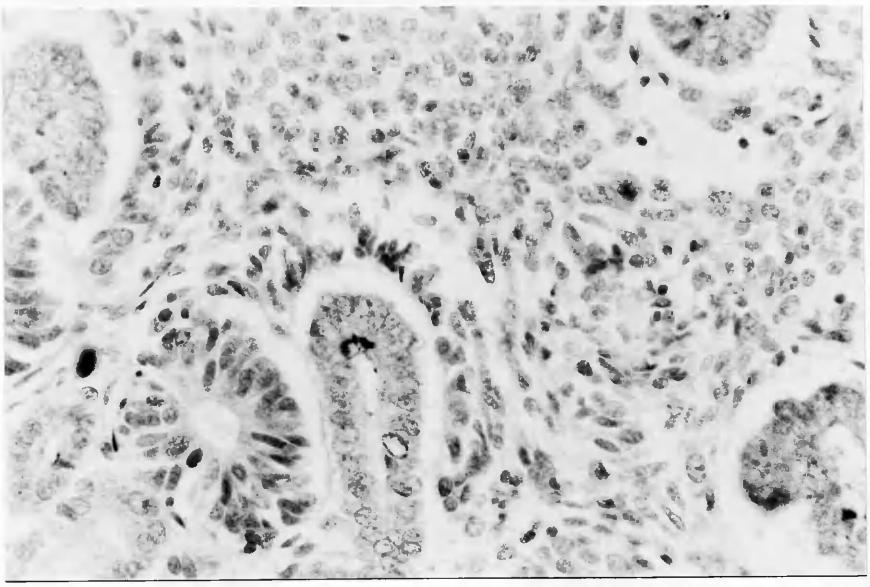


FIGURE 16. The ureteric bud is seen growing into the metanephric blastema. Staining with AGF 4.36 can be seen on the surface of the epithelial cells of the ampulla. Fetal kidney. Immunoperoxidase.

FIGURE 17. The ureteric bud has fused with the S-shaped tubule. Staining with AGF 4.36 is present on the cells at the junction between these structures (arrow). Fetal kidney. Immunoperoxidase.



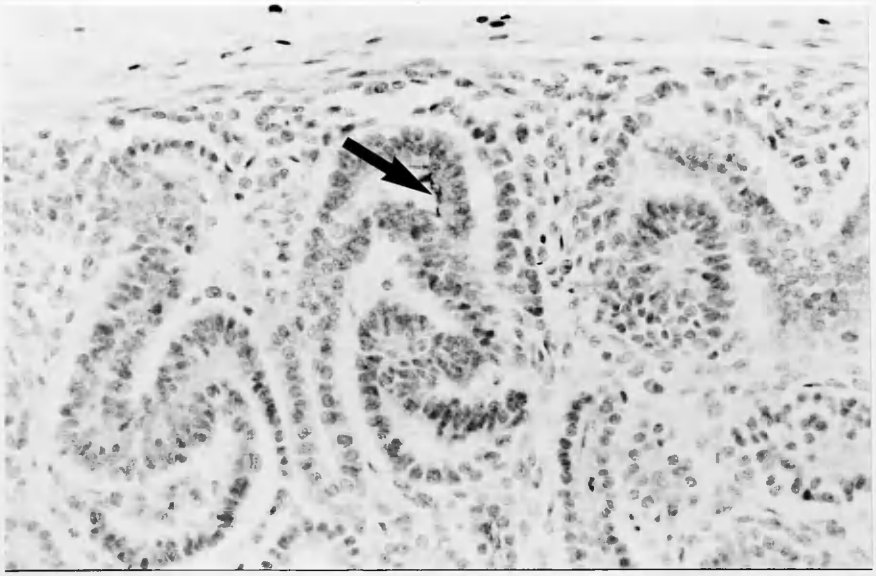
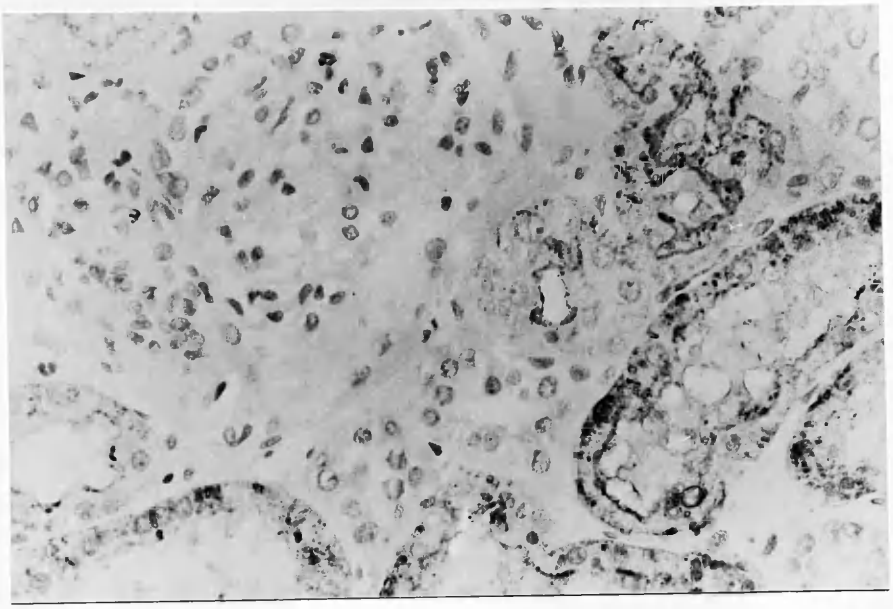


FIGURE 18. Reactivity for AGF 4.48 demonstrated on the maturing proximal tubular epithelium. Fetal kidney. Immunoperoxidase.

FIGURE 19. In the adult kidney reactivity for AGF 4.48 is restricted to the pars recta and loop of Henle. Adult kidney. Immunoperoxidase.



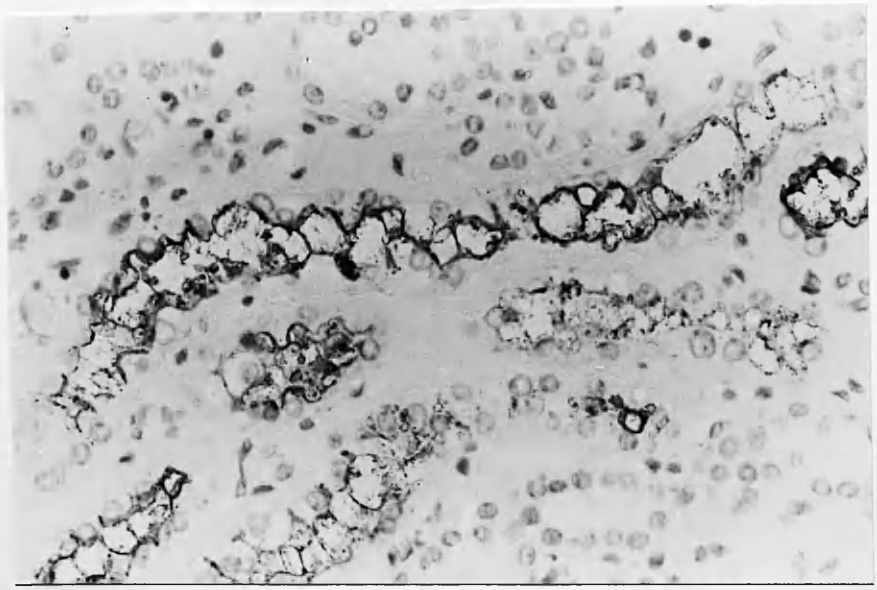
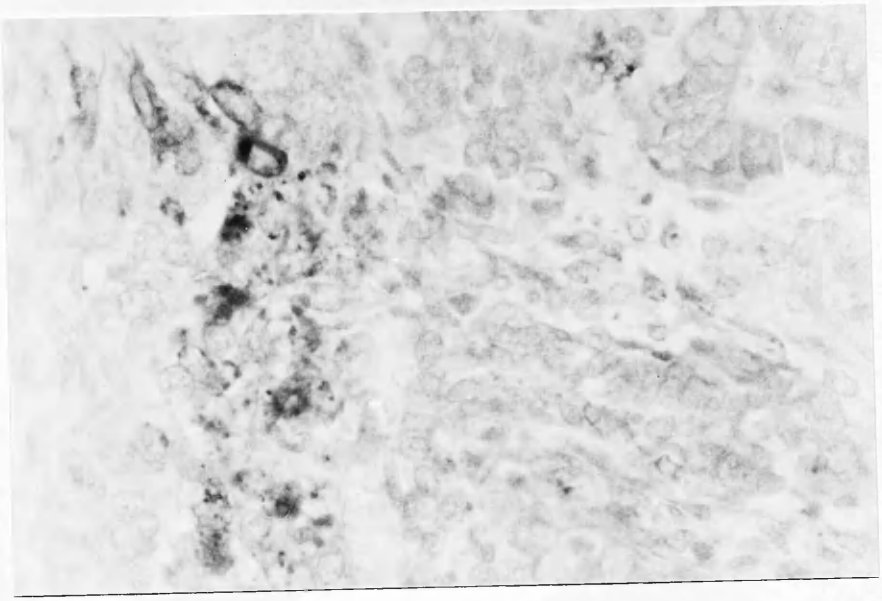


FIGURE 20. The cytoplasm of some of the cells of the metanephric blastema contain alpha-1-antitrypsin. Fetal kidney. Immunoperoxidase.

FIGURE 21. In the maturing proximal tubular epithelium there is staining for alpha-1-antitrypsin. Fetal kidney. Immunoperoxidase.



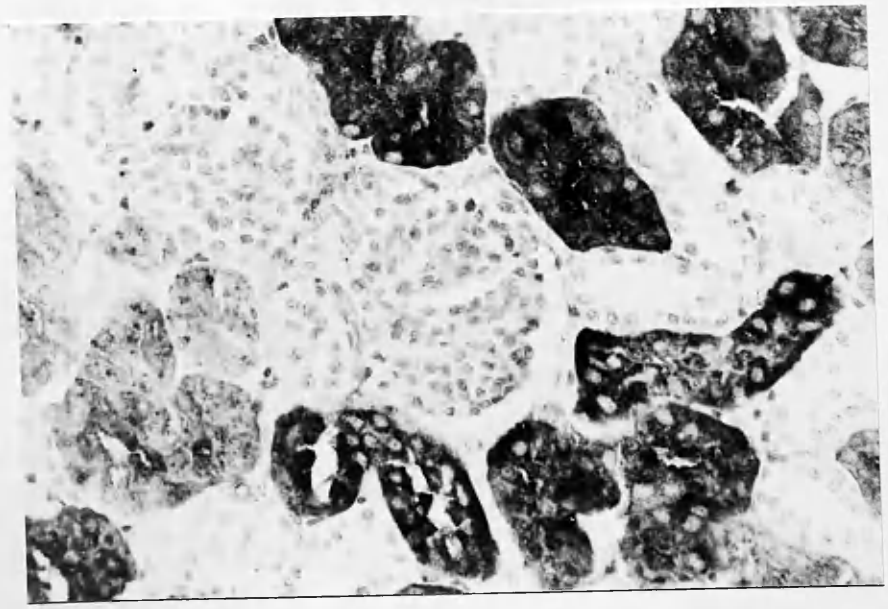


FIGURE 22. The cytoplasm of the proximal tubular epithelium is seen to contain ferritin at the late maturation stage. Fetal kidney. Immunoperoxidase.

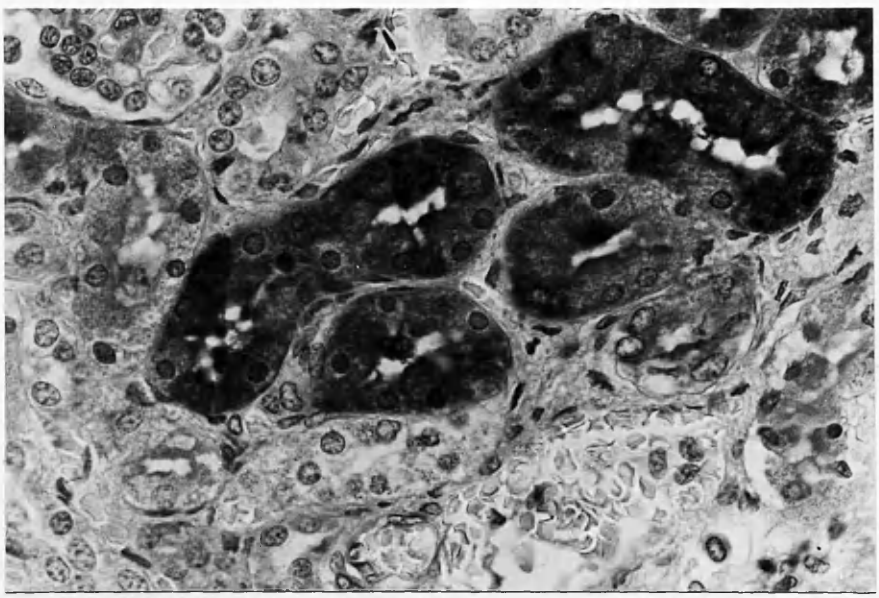


FIGURE 23. Carbonic anhydrase C can be found in tubular epithelium throughout the nephron at this stage of development. Fetal kidney. Immunoperoxidase.

FIGURE 24. The adult collecting ducts contain two populations of cells depending on their reactivity for carbonic anhydrase C. Adult kidney. Immunoperoxidase.

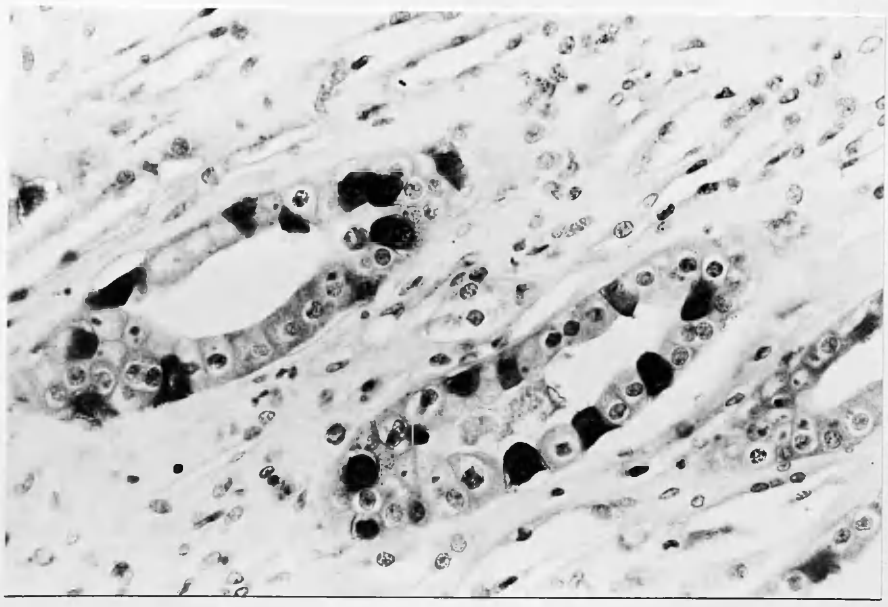


FIGURE 25. The endothelium of the developing glomerular tuft contains factor VIII R antigen. Fetal kidney. Immunoperoxidase.

FIGURE 26. In the more mature glomeruli renin containing cells are found in the walls of the afferent arteriole (arrow). Fetal kidney. Immunoperoxidase.

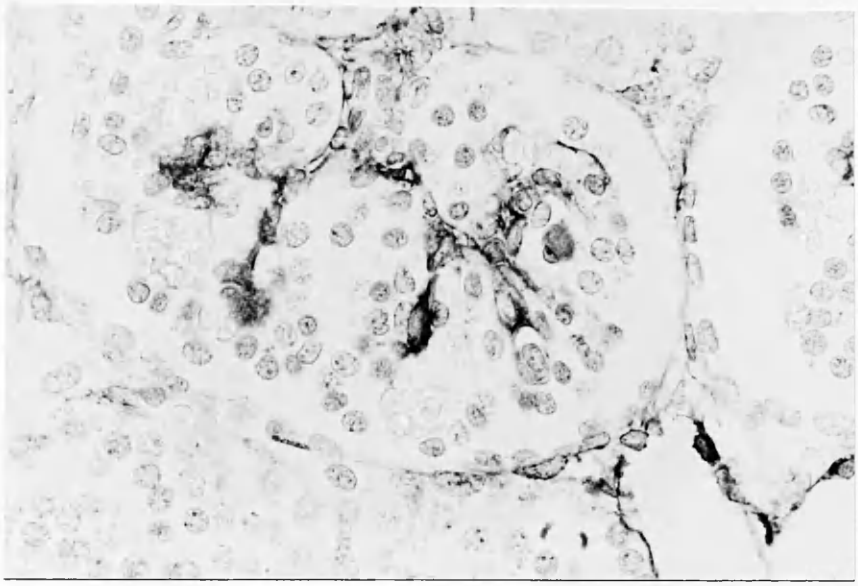
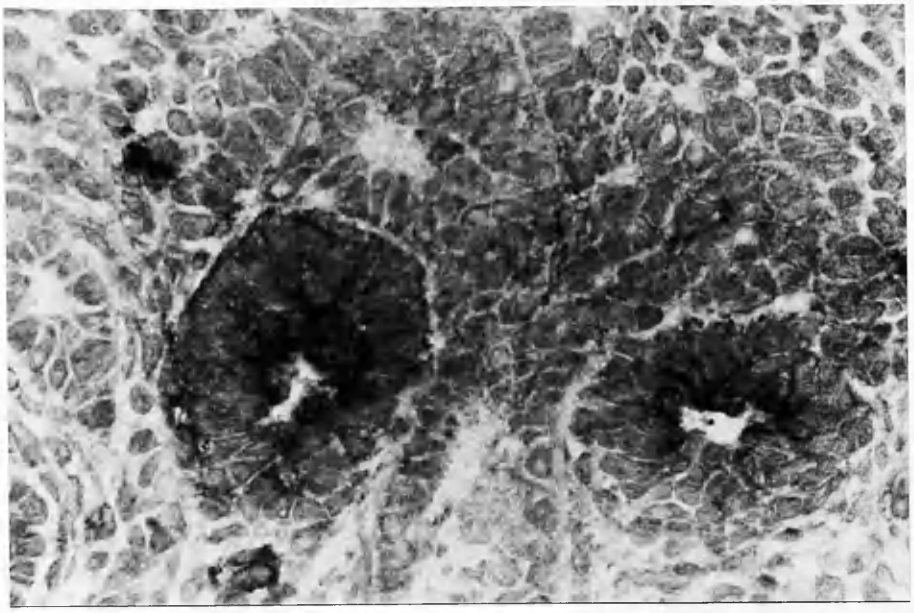


FIGURE 27. The cells of the renal vesicle show expression of the transferrin receptor. There is no reactivity in the ureteric bud epithelium. Fetal kidney. Immunoperoxidase.

FIGURE 28. HB 21 reactivity is seen on the cells of the tubular epithelium but has been lost from the glomerular epithelium. Fetal kidney. Immunoperoxidase.



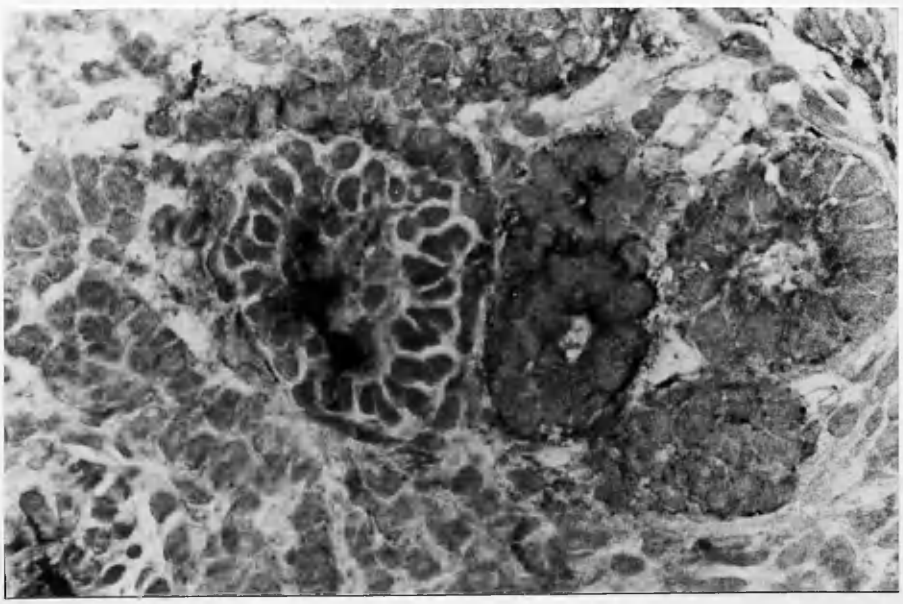
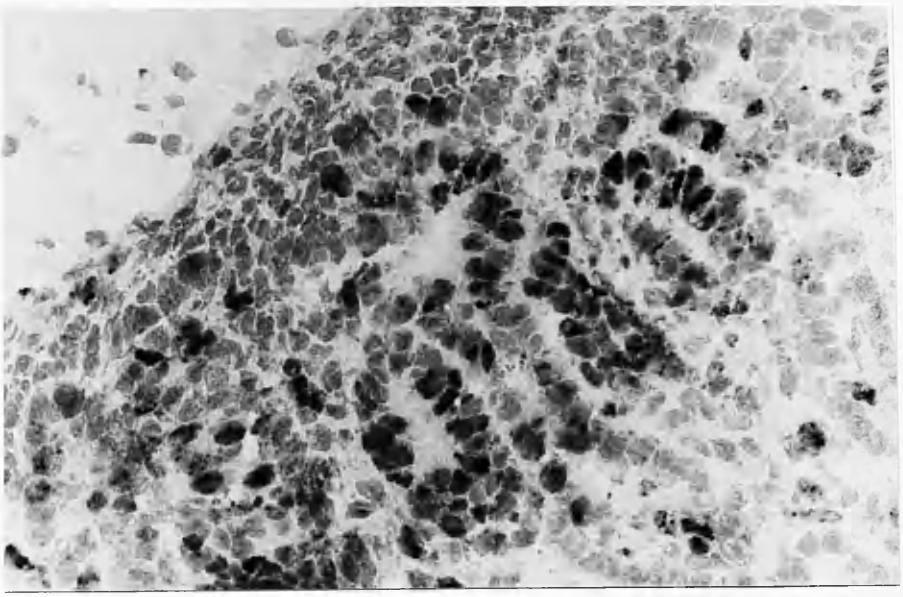


FIGURE 29. The nuclei of most of the cells of the renal vesicle bind Ki 67. Some reactivity is seen in the condensed blastema and in the ureteric bud epithelium. Fetal kidney. Immunoperoxidase.

FIGURE 30. In the maturing glomerulus Ki 67 reactivity has been lost from the glomerular epithelium although the endothelium continues to show reactivity. Fetal kidney. Immunoperoxidase.



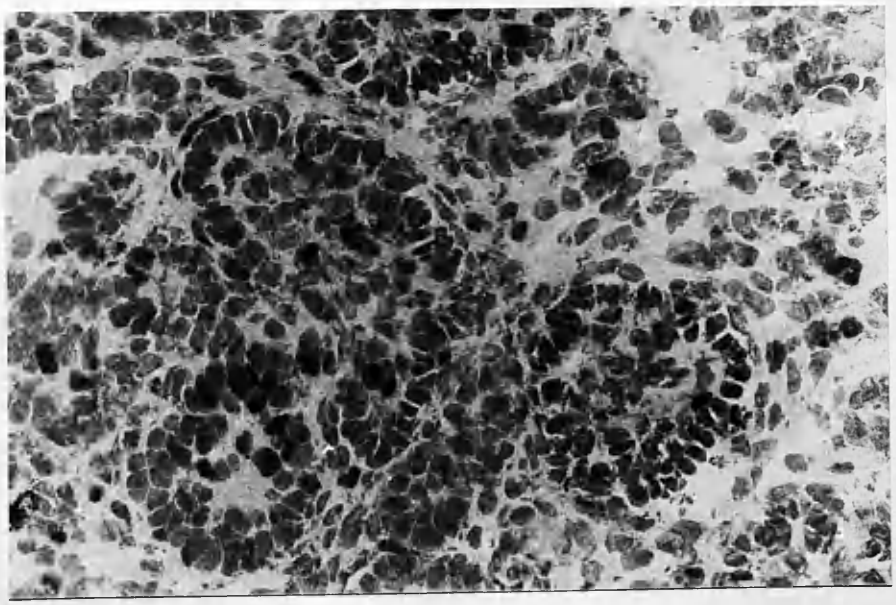
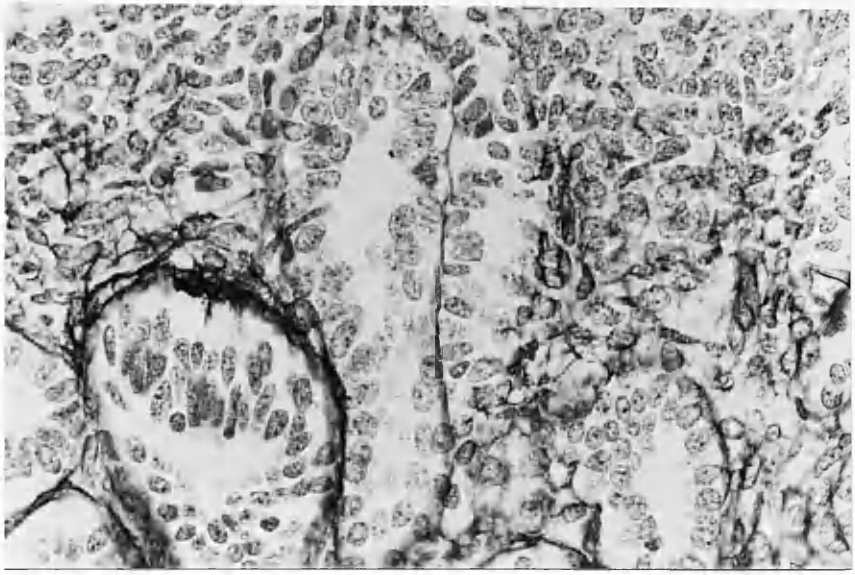


FIGURE 31. Staining for fibronectin delineates the basal margin of the epithelium of the uerteric bud and renal vesicle. Fetal kidney. Immunoperoxidase.

FIGURE 32. In the developing glomerulus fibronectin staining was seen in the glomerular tuft. Fetal kidney. Immunoperoxidase.



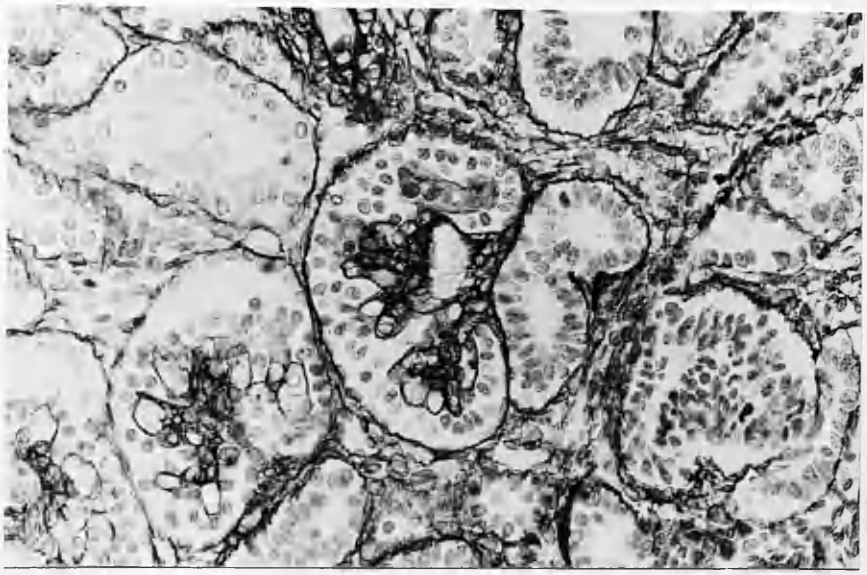


FIGURE 33. Diffuse staining for NDOG1 is seen in the mesenchyme surrounding the developing nephrons. Fetal kidney. Immunoperoxidase.

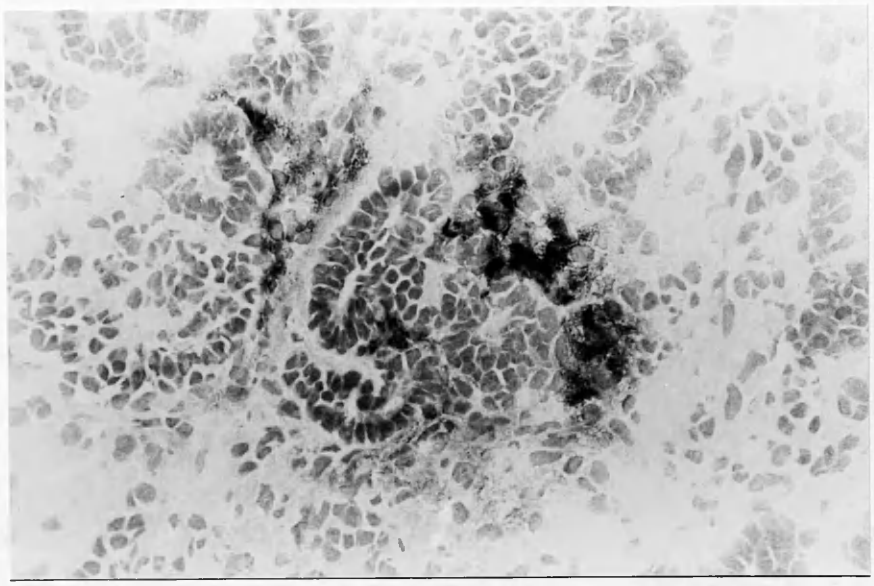


FIGURE 34. The primitive ducts of renal dysplasia are lined by a columnar epithelium and are surrounded by a concentric cuff of mesenchyme. Renal dysplasia. H&E.

FIGURE 35. The cytoplasm of the primitive ductal epithelium shows staining for epidermal prekeratins. Renal dysplasia. Immunoperoxidase.

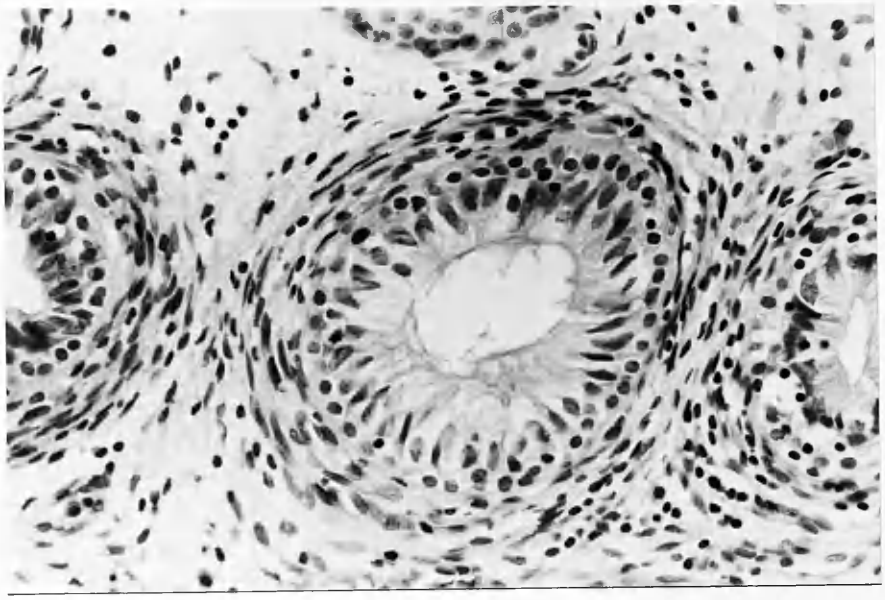
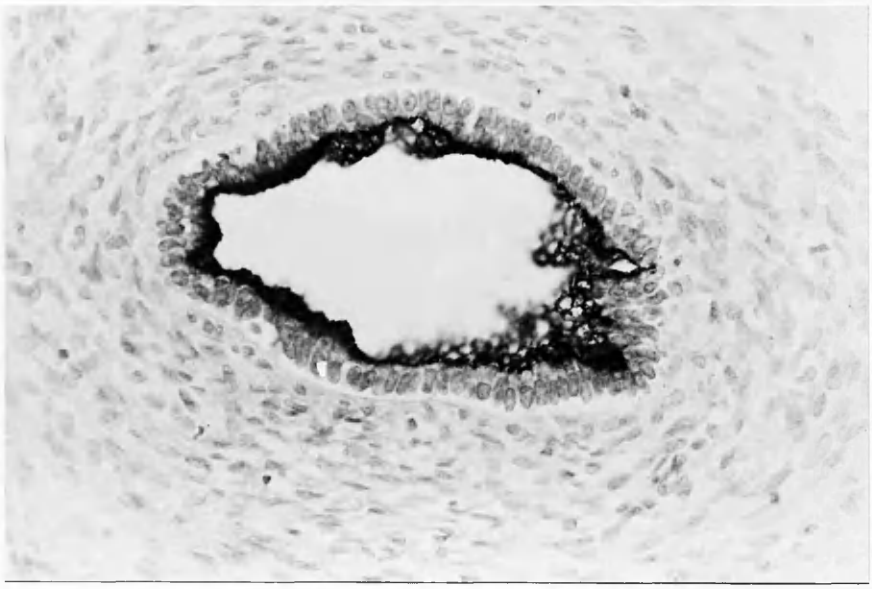


FIGURE 36. The primitive ducts in renal dysplasia express EMA on the luminal surface. Renal dysplasia. Immunoperoxidase.

FIGURE 37. In this example of tubular metaplasia of glomerular epithelium the parietal glomerular epithelium expresses surface EMA. Renal dysplasia. Immunoperoxidase.



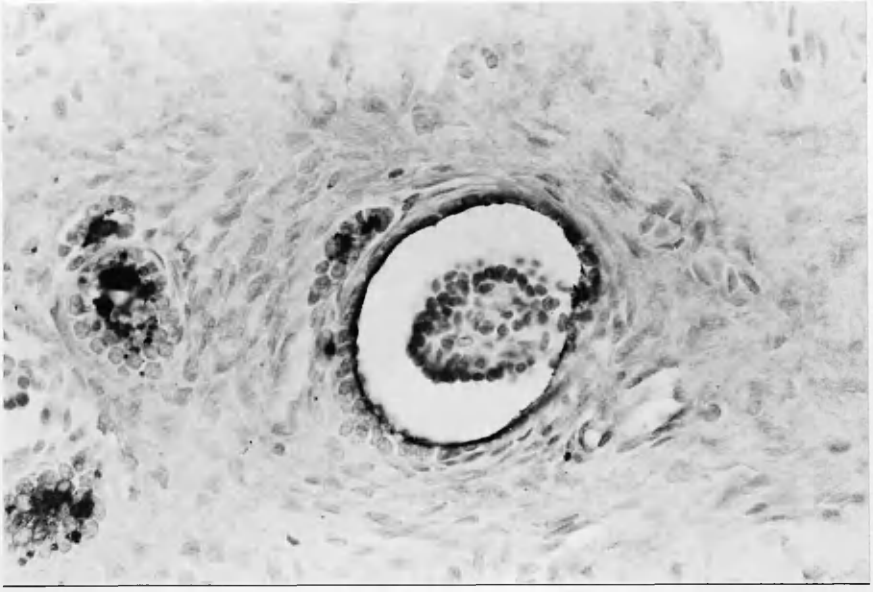


FIGURE 38. S-100 protein can be seen in the outer layer of those primitive ducts which had a double layered epithelium. Renal dysplasia. Immunoperoxidase.

FIGURE 39. Some of the epithelial cells lining cystic and ductular structures in multilocular cystic nephroma have a hob nailed morphology. Multilocular cystic nephroma. H&E.

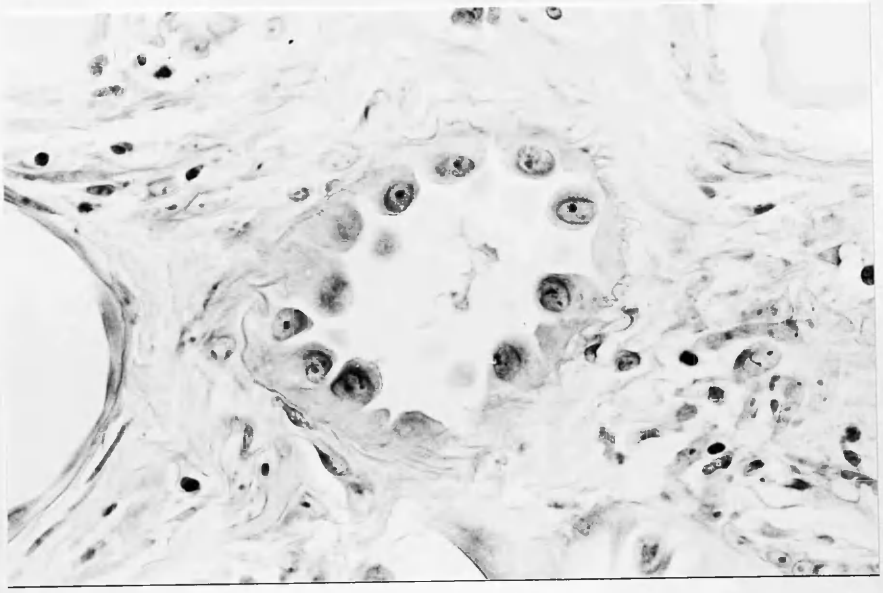


FIGURE 40. Staining for epidermal prekeratin can be seen in the cystic epithelium in multilocular cystic nephroma. Multilocular cystic nephroma. Immunoperoxidase.

FIGURE 41. The persistent blastema in nodular renal blastema is seen underlying the renal capsule. The lesion consists of primitive blastema and some immature tubular structures. Nodular renal blastema. H&E.

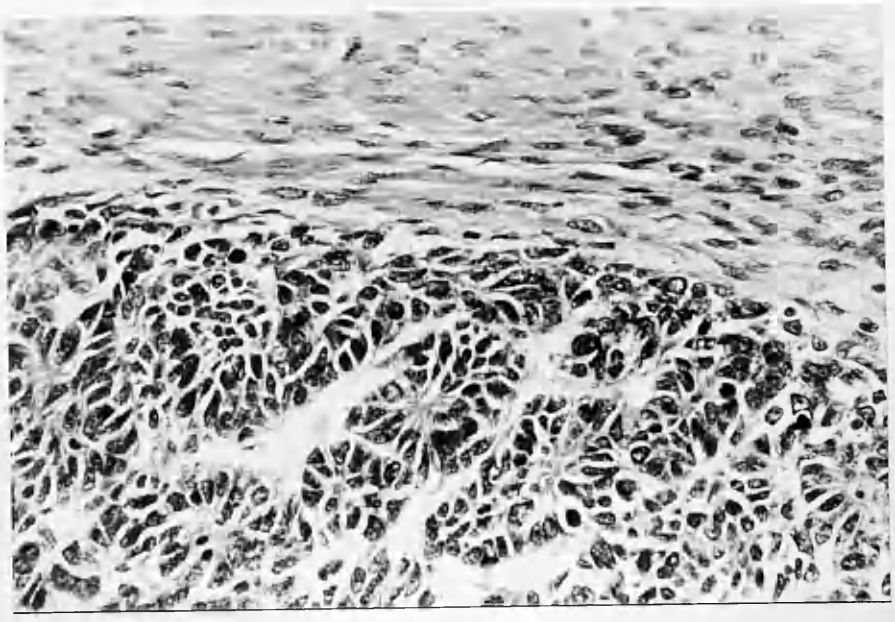


FIGURE 42. The glomeruloid bodies in nephroblastomatosis consist of poorly vascularised tufts lined by a hyperchromatic epithelium. Nephroblastomatosis. H&E.

FIGURE 43. An island of nephroblastoma arising within an area of nephroblastomatosis. Nephroblastomatosis. H&E.

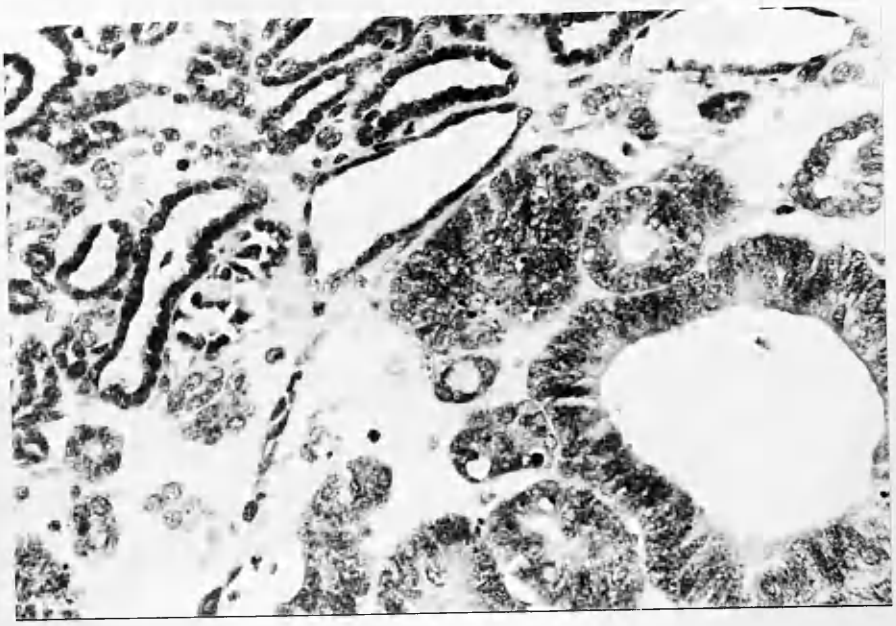
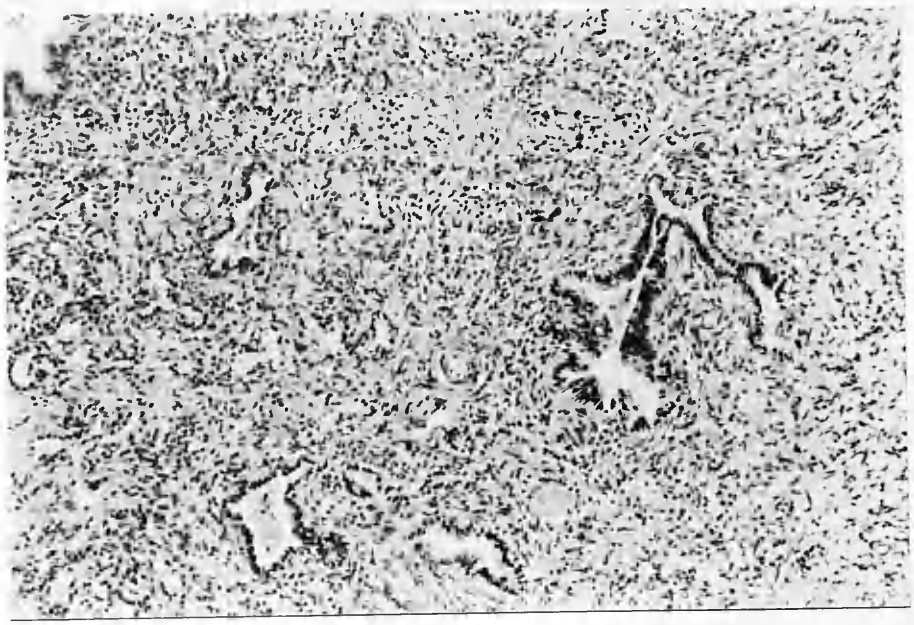


FIGURE 44. In this single case of nephroblastomatosis tissue morphologically resembling fetal lung can be identified. There are immature bronchi, alveoli and a capillary plexus. Nephroblastomatosis. H&E.

FIGURE 45. Some well differentiated tubules in nodular renal blastema show expression of EMA (arrow). Nodular renal blastema. Immunoperoxidase.



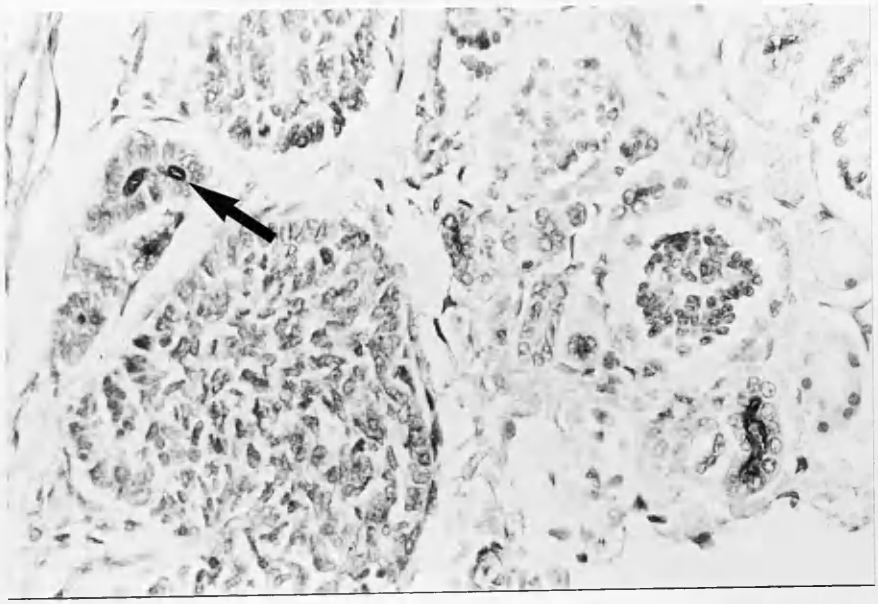
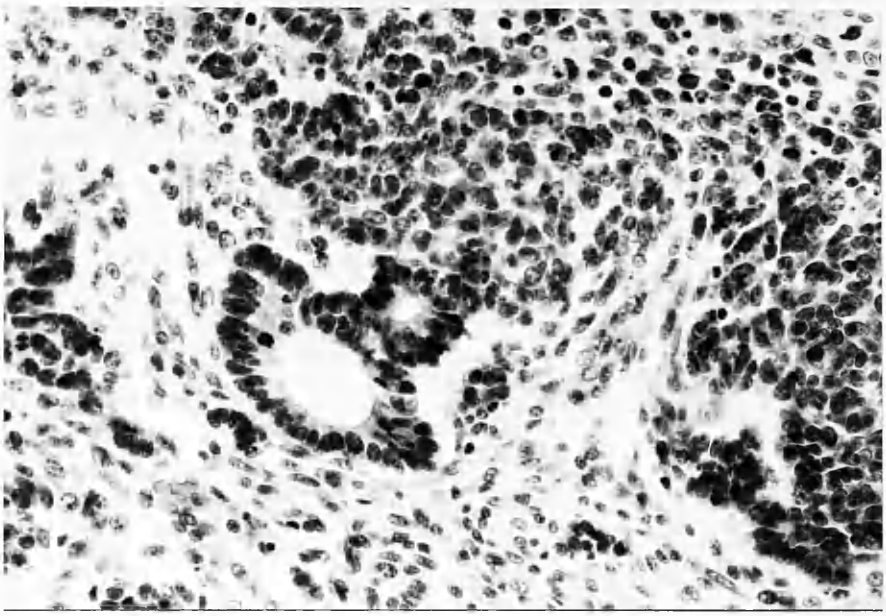


FIGURE 46. The immature tubules of Type A morphology in nephroblastoma consist of tall columnar cells surrounding a central lumen. Nephroblastoma. H&E.

FIGURE 47. Tubules of Type B morphology are well differentiated cuboidal epithelial structures surrounded by a cuff of mesenchyme. Nephroblastoma. H&E.



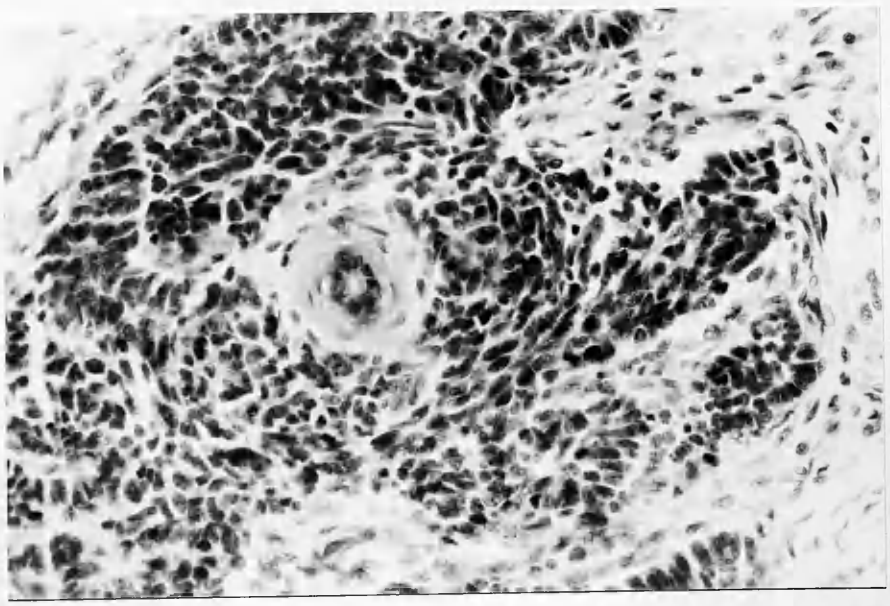


FIGURE 48. Well differentiated tubules in nephroblastoma showing diffuse cytoplasmic staining with CAM 5.2. Nephroblastoma. Immunoperoxidase.

FIGURE 49. A small well differentiated Type B tubule expressing epidermal prekeratin. Note the surrounding cuff of mesenchyme. Nephroblastoma. Immunoperoxidase.

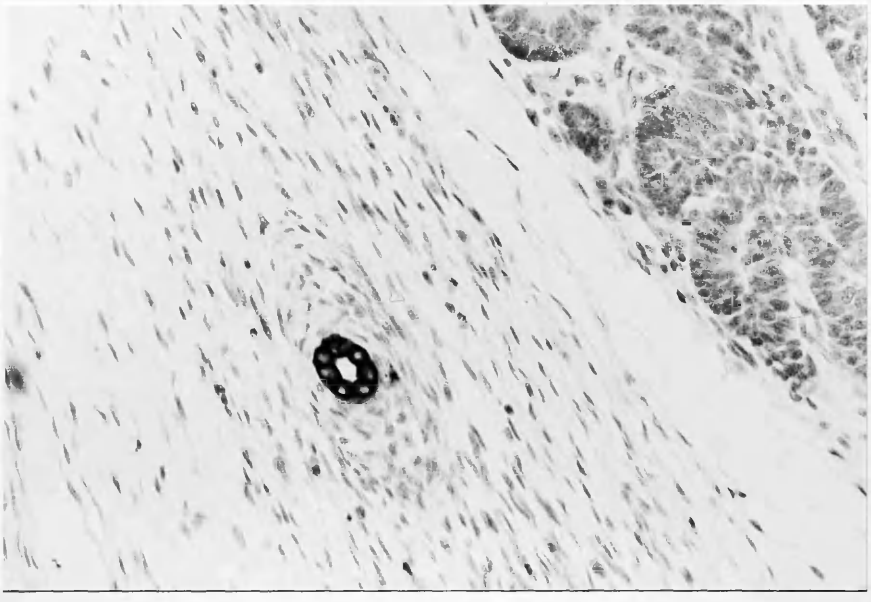


FIGURE 50. In nephroblastoma EMA is seen on the apical surface of the epithelium lining some well differentiated Type A tubules. Nephroblastoma. Immunoperoxidase.

FIGURE 51. Brush border antigen expression is restricted to some well differentiated Type A tubules. Nephroblastoma. Immunoperoxidase.

FIGURE 52. The cytoplasm of the spindle shaped cells of the primitive mesenchyme of nephroblastoma show reactivity for alpha-1-antitrypsin. Nephroblastoma. Immunoperoxidase.

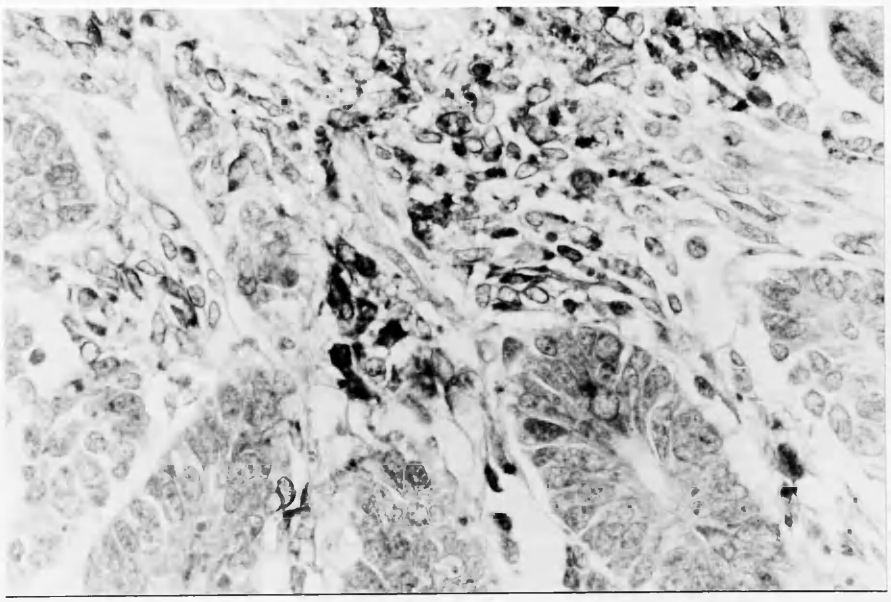
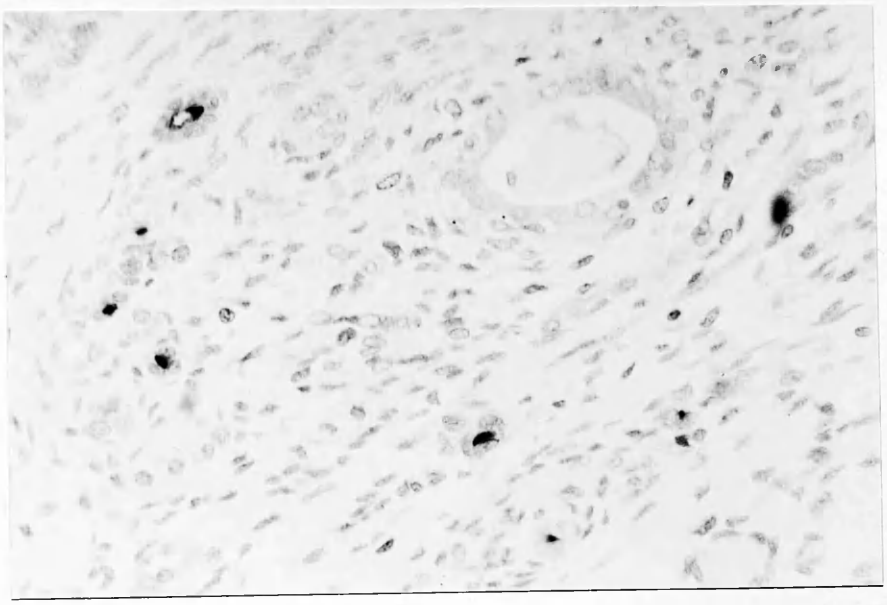


FIGURE 53. AGF 4.36 stains the luminal border of the cuboidal cells lining several terminal branches of a Type B tubule. The larger Type B tubule does not stain. Nephroblastoma. Immunoperoxidase.

FIGURE 54. The columnar cells lining a neoplastic Type A tubule show staining on their luminal surface with AGF 4.36. Nephroblastoma. Immunoperoxidase.



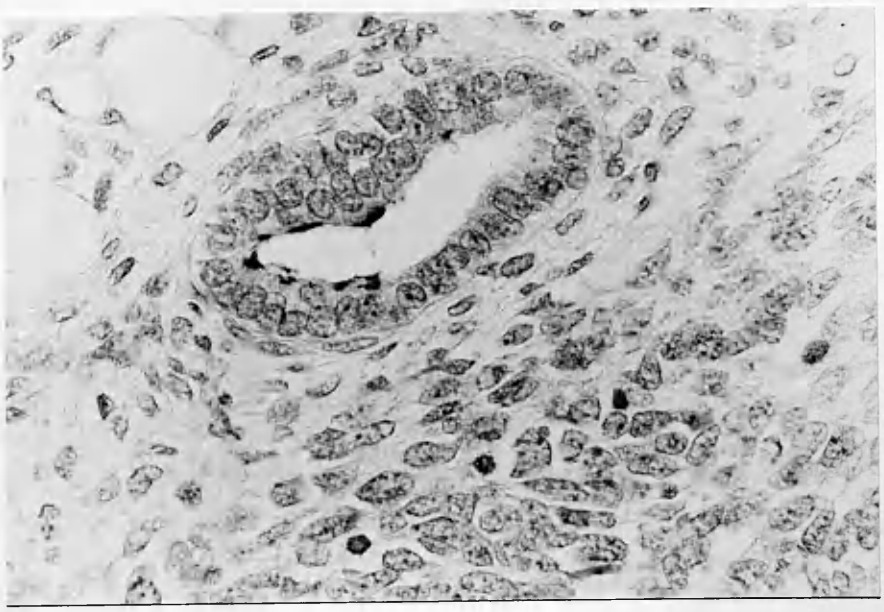
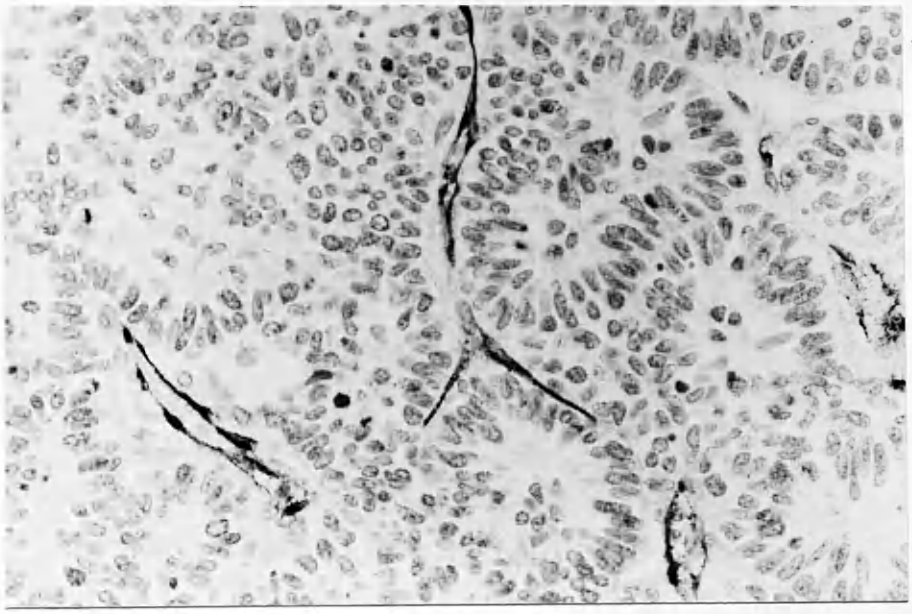


FIGURE 55. The capillary network around the neoplastic tubules in nephroblastoma is demonstrated by the reactivity of the endothelial cells for Factor VIII R antigen. Nephroblastoma. Immunoperoxidase.

FIGURE 56. S-100 protein is seen in the adipose cells in a case of nephroblastoma. Nephroblastoma. Immunoperoxidase.



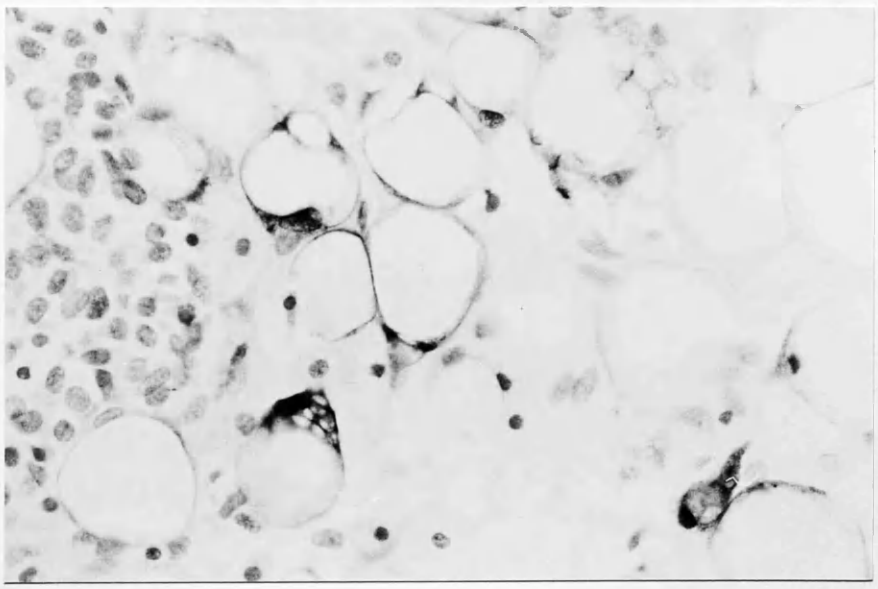


FIGURE 57. A small spindle cell lying adjacent to a blood vessel shows reactivity for S-100 protein. Nephroblastoma. Immunoperoxidase.

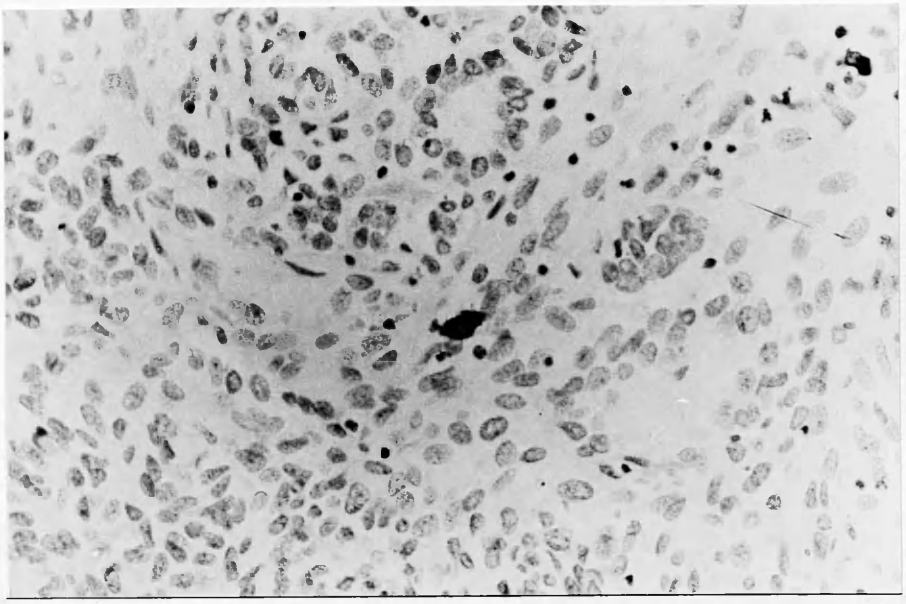
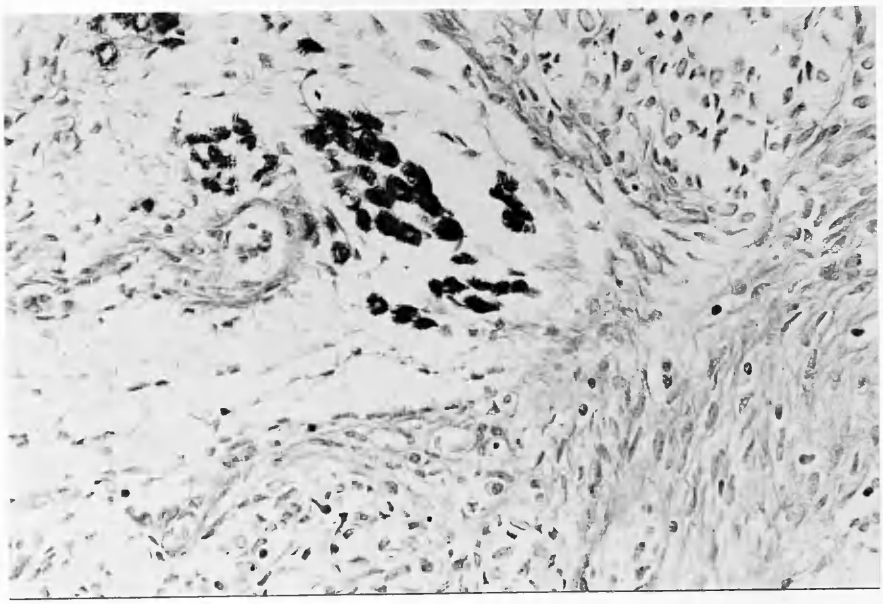


FIGURE 58. Skeletal muscle cells in nephroblastoma stain for myoglobin. Nephroblastoma. Immunoperoxidase.

FIGURE 59. Some cells in the blastemal areas of nephroblastoma also stained for myoglobin. Nephroblastoma. Immunoperoxidase.



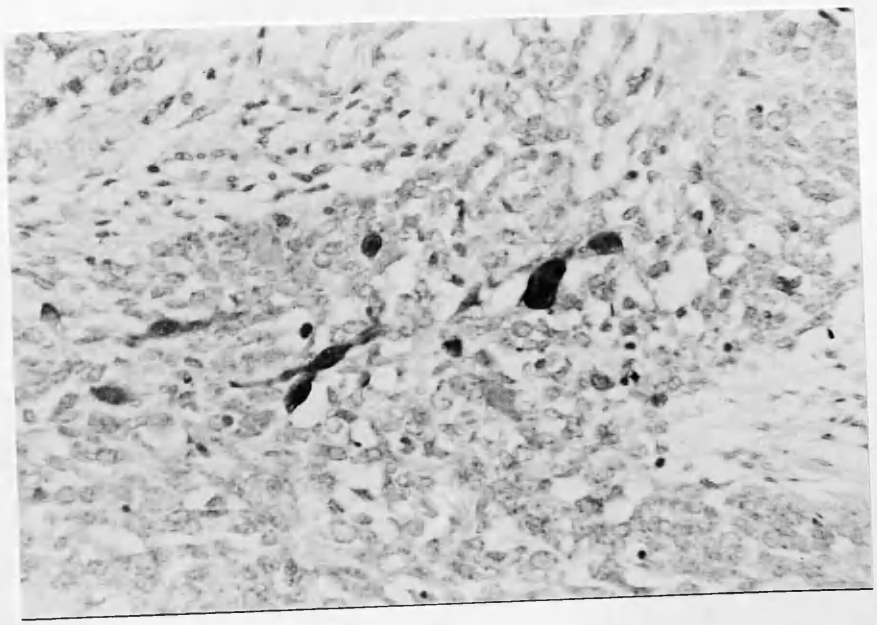
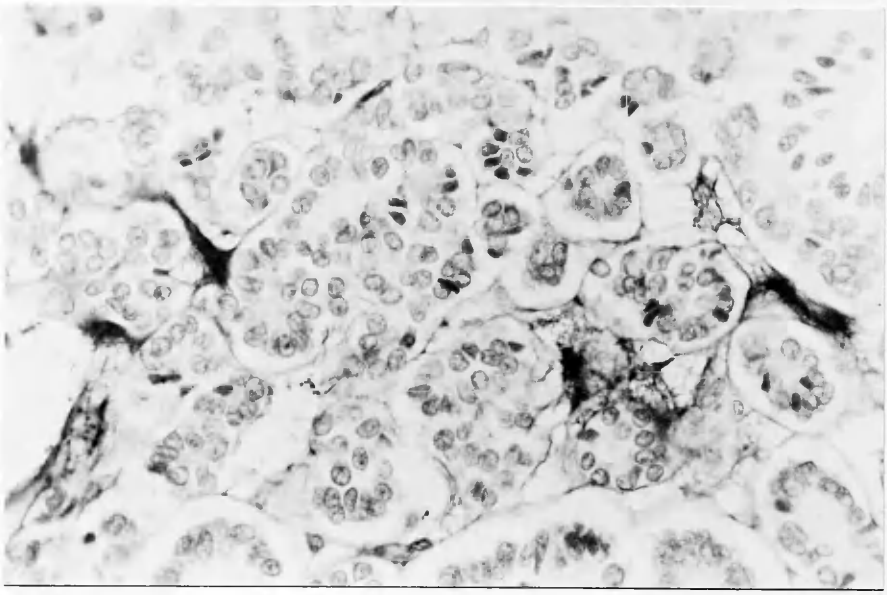


FIGURE 60. Fibronectin is seen associated with the epithelial basement membrane in nephroblastoma. Nephroblastoma. Immunoperoxidase.

FIGURE 61. Ki 67 staining can be seen in the nuclei of epithelial cells in nephroblastoma. Nephroblastoma. Immunoperoxidase.



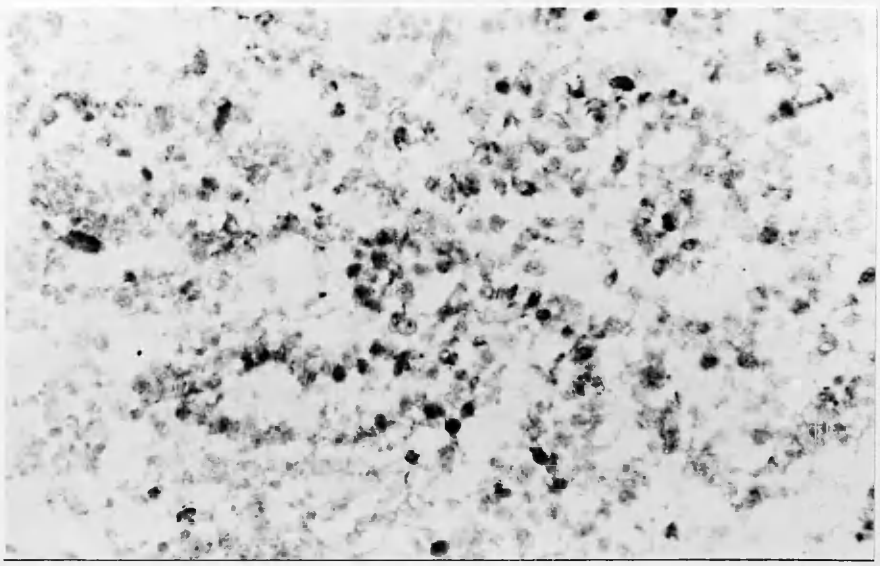
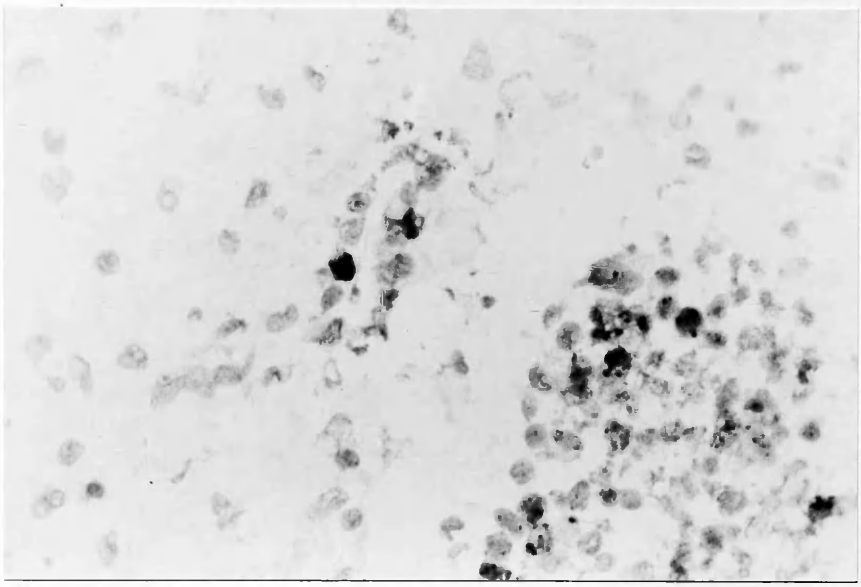


FIGURE 62. A single case of a Type B tubule also shows reactivity for Ki 67. Nephroblastoma. Immunoperoxidase.

FIGURE 63. Tubular epithelial cells in nephroblastoma show staining with HB 21. Nephroblastoma. Immunoperoxidase.



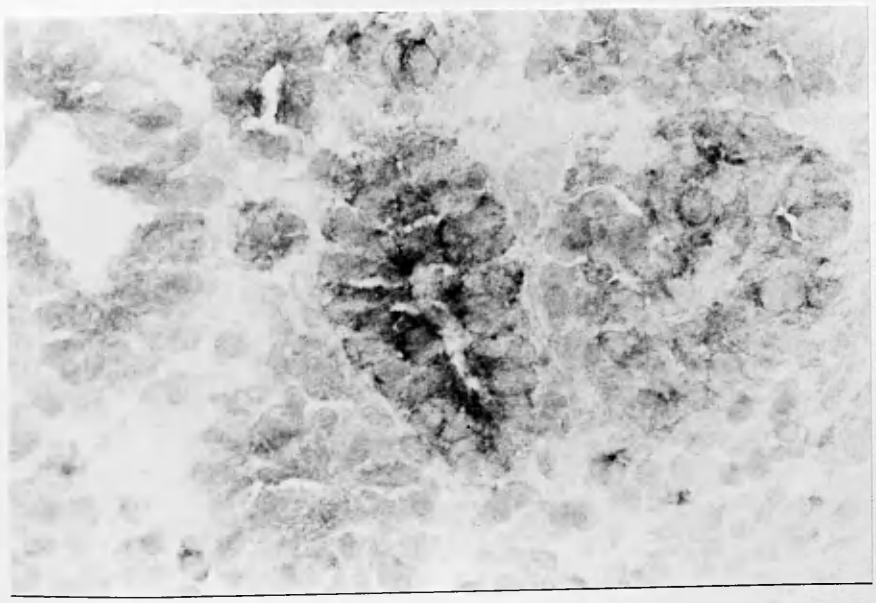


FIGURE 64. Cells containing renin are seen lying adjacent to blood vessels in nephroblastoma. Nephroblastoma. Immunoperoxidase.

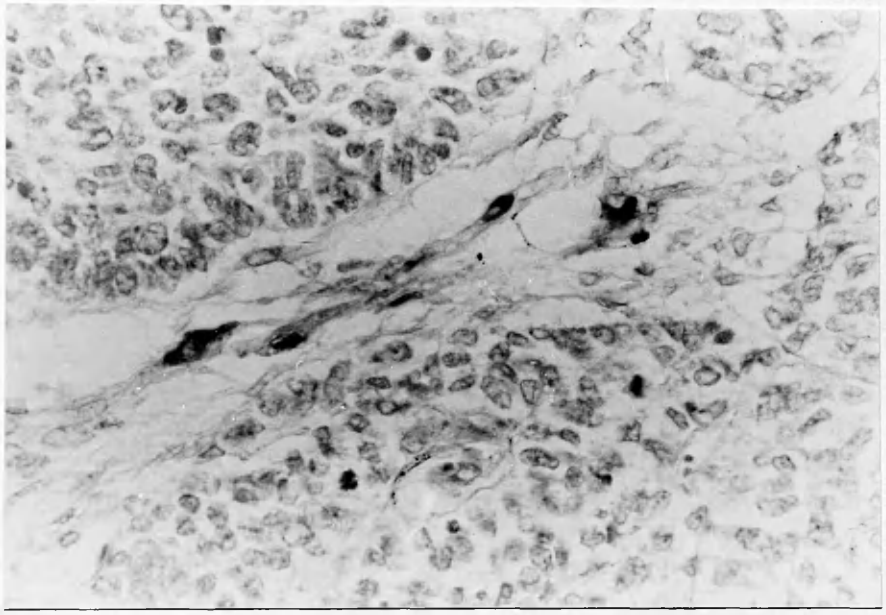
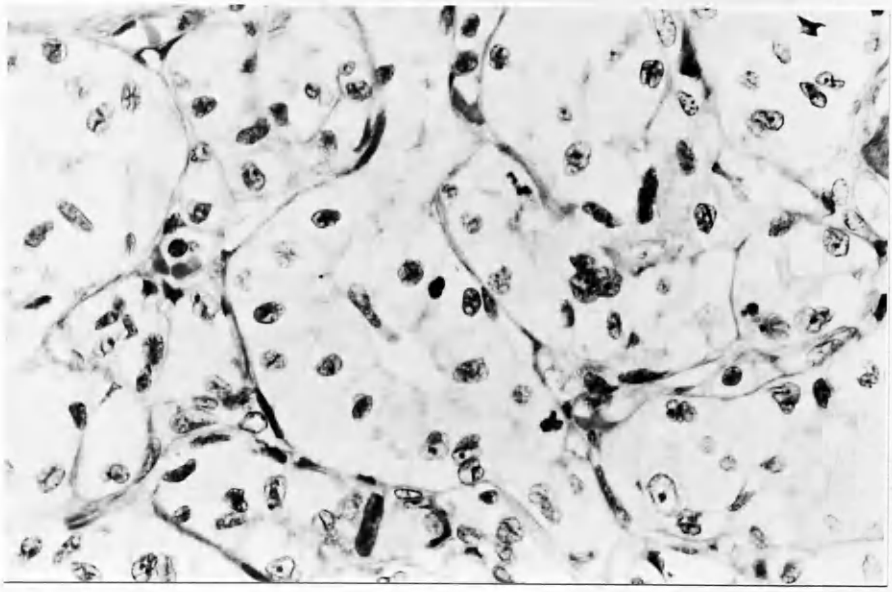


FIGURE 65. Clear cell carcinoma of the kidney in which the cells have clear cytoplasm and a well demarcated cell border. Renal cell carcinoma. H&E.

FIGURE 66. The spindle cell areas of sarcomatoid renal carcinoma show marked pleomorphism. Renal cell carcinoma. H&E.



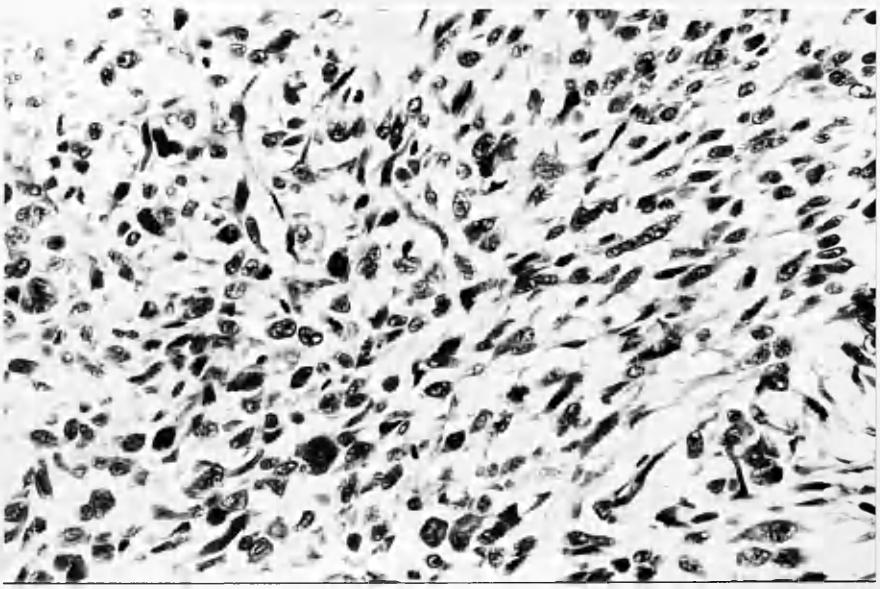
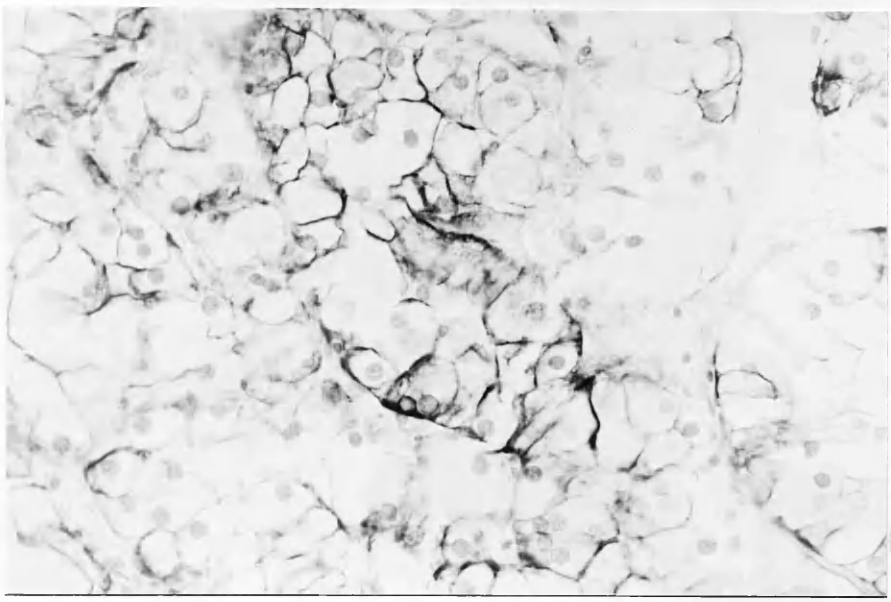


FIGURE 67. The periphery of the cytoplasm of the clear cells in this renal cell carcinoma stain with CAM 5.2. Renal cell carcinoma. Immunoperoxidase.

FIGURE 68. In the granular cell tumours the staining for CAM 5.2 was more diffusely distributed in the cytoplasm. Renal cell carcinoma. Immunoperoxidase.



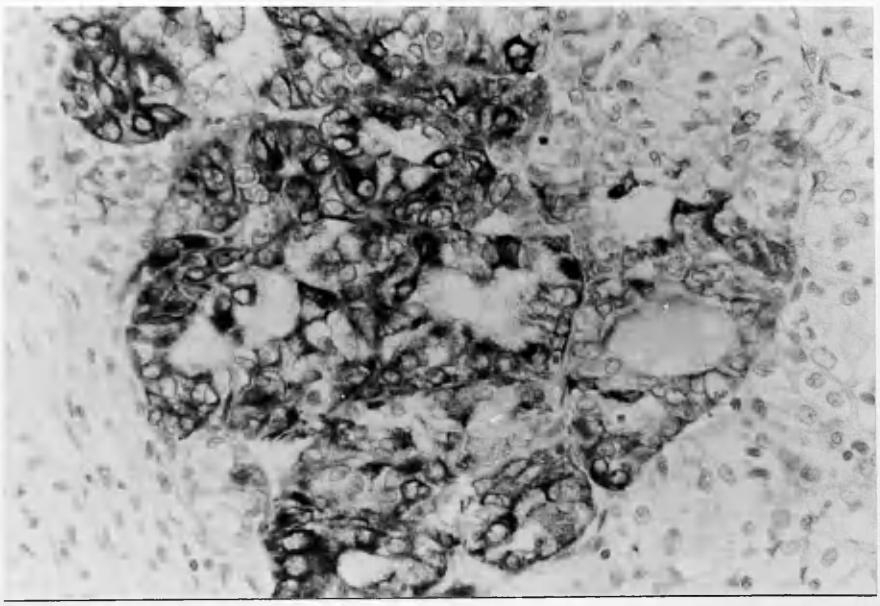
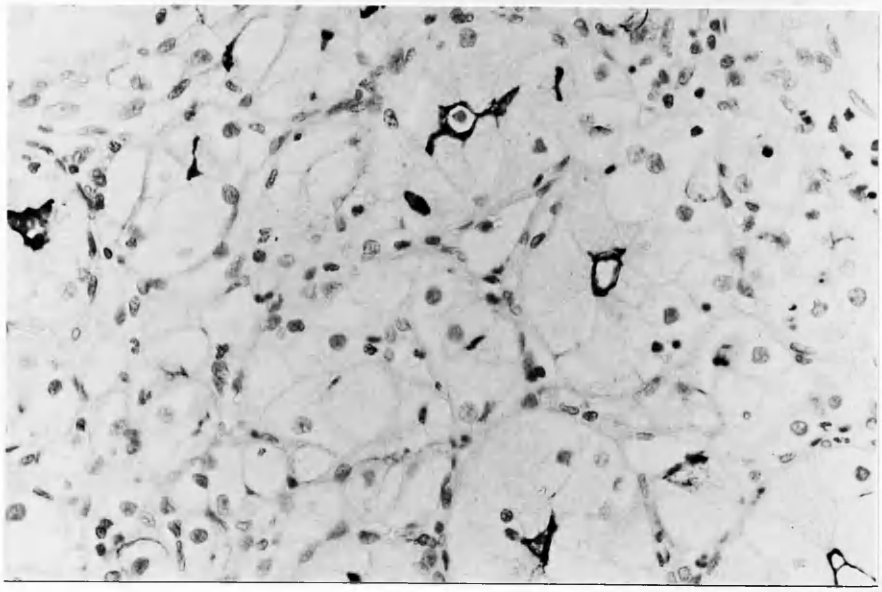


FIGURE 69. EMA is found on the apical surface of the clear cells in this well differentiated renal cell carcinoma. Renal cell carcinoma. Immunoperoxidase.

FIGURE 70. By contrast to Fig. 69 in this less well differentiated carcinoma EMA staining is seen on all cell surfaces. Renal cell carcinoma. Immunoperoxidase.



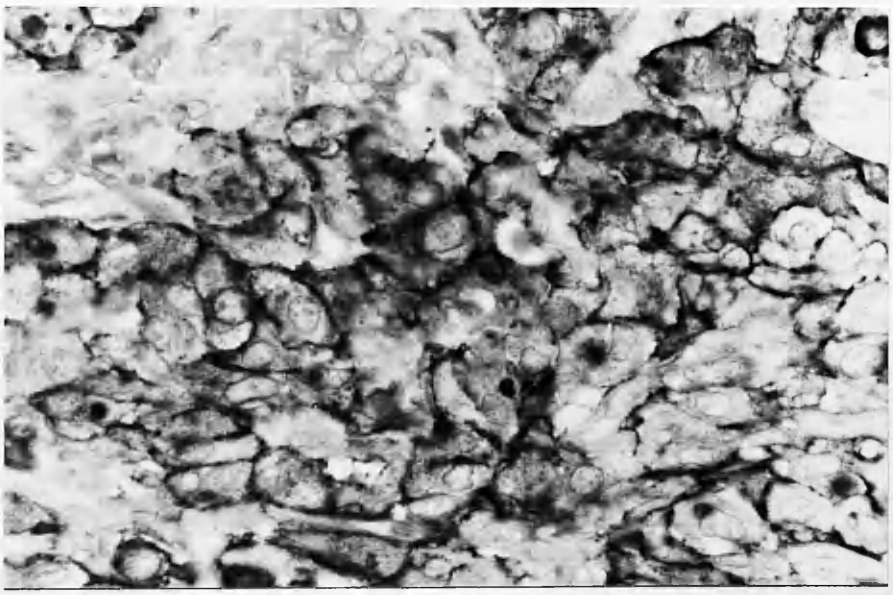
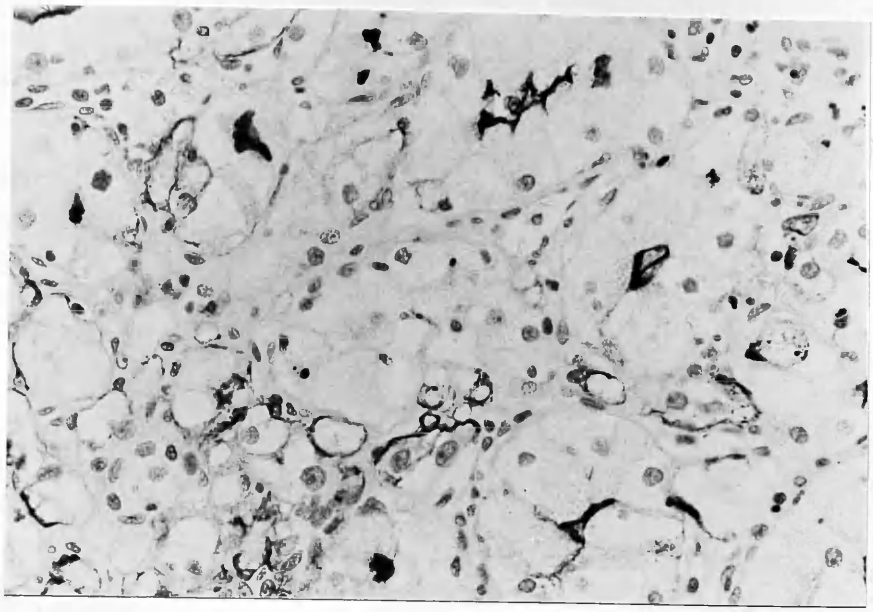


FIGURE 71. Brush border antigen is expressed on the apical cell surfaces of these tumour cells from a clear cell carcinoma. Renal cell carcinoma. Immunoperoxidase.

FIGURE 72. A similar distribution of AGF 4.48 is seen in a well differentiated carcinoma. Renal cell carcinoma. Immunoperoxidase.



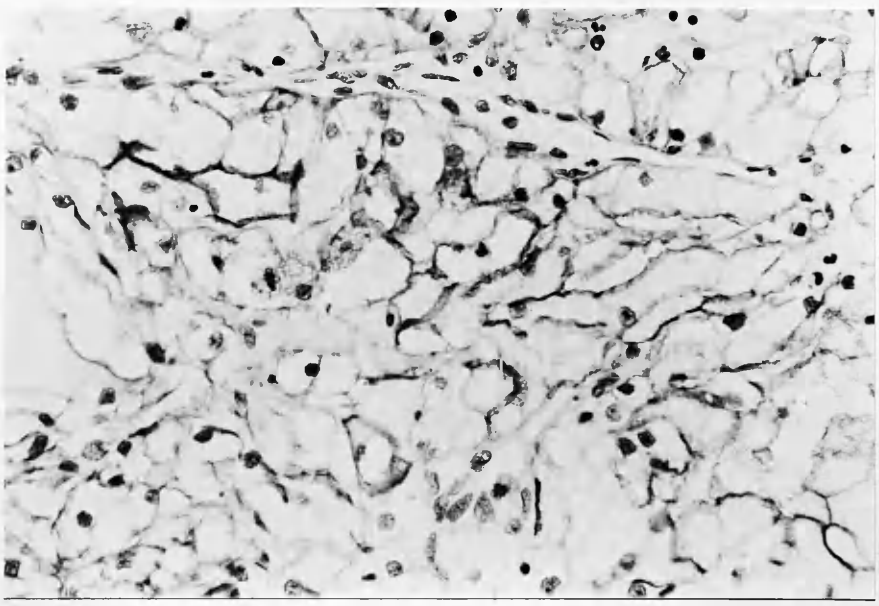


FIGURE 73. Staining for alpha-1-antitrypsin is seen in some of the cells in this clear carcinoma. Adjacent cells are negative. Renal cell carcinoma. Immunoperoxidase.

FIGURE 74. The spindle cells of this sarcomatoid carcinoma also expressed alpha-1-antitrypsin. Renal cell carcinoma. Immunoperoxidase.

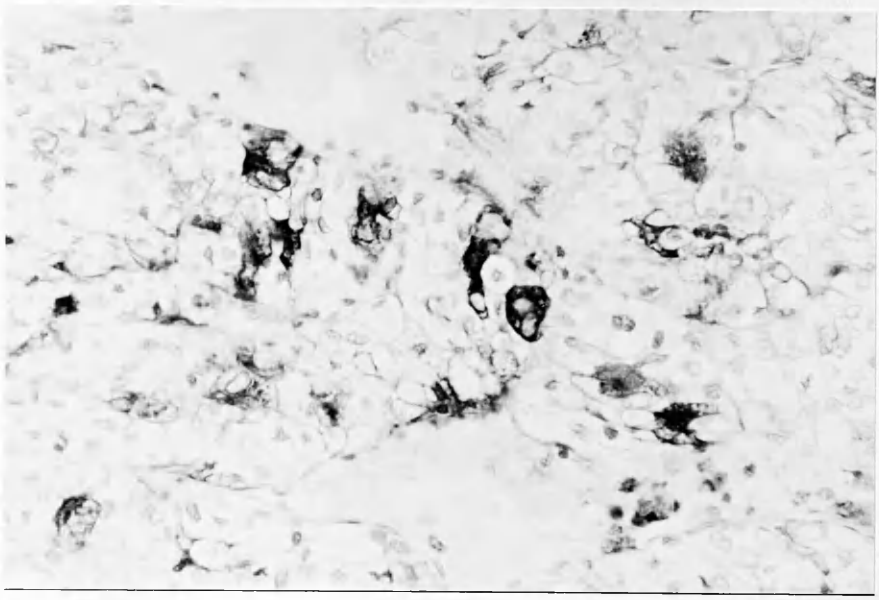
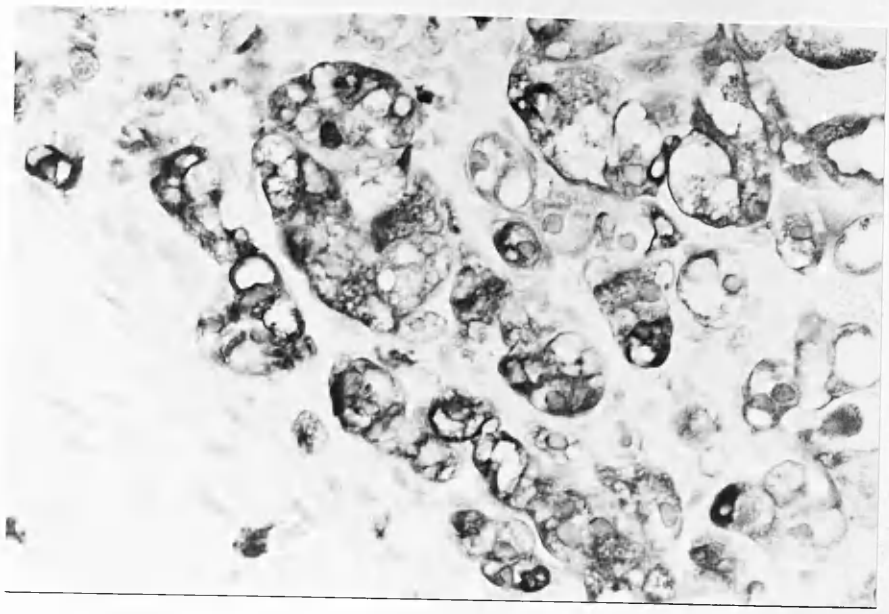


FIGURE 75. Cytoplasmic staining for ferritin is present in this renal cell carcinoma. Renal cell carcinoma. Immunoperoxidase.

FIGURE 76. Some spindle cells in the stroma of a tumour stain for ferritin. Renal cell carcinoma. Immunoperoxidase.



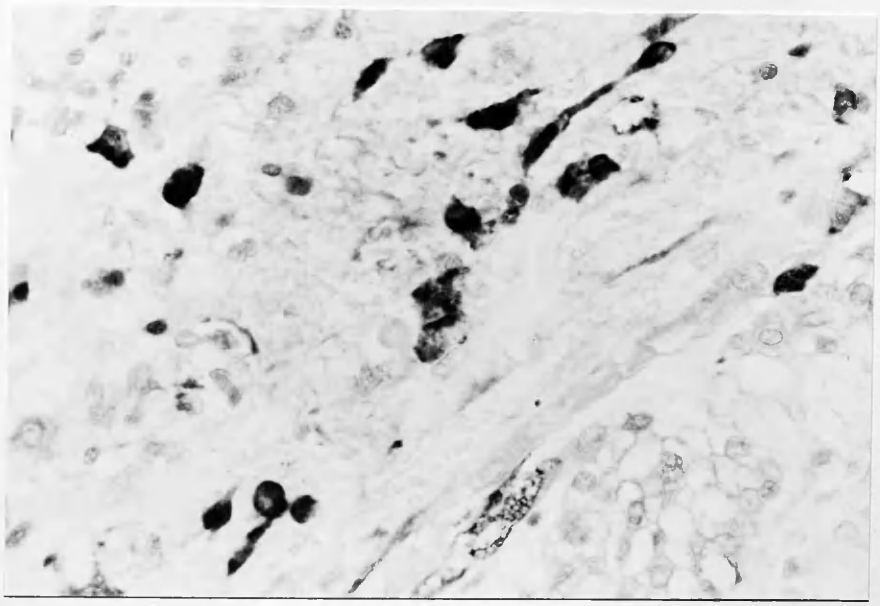
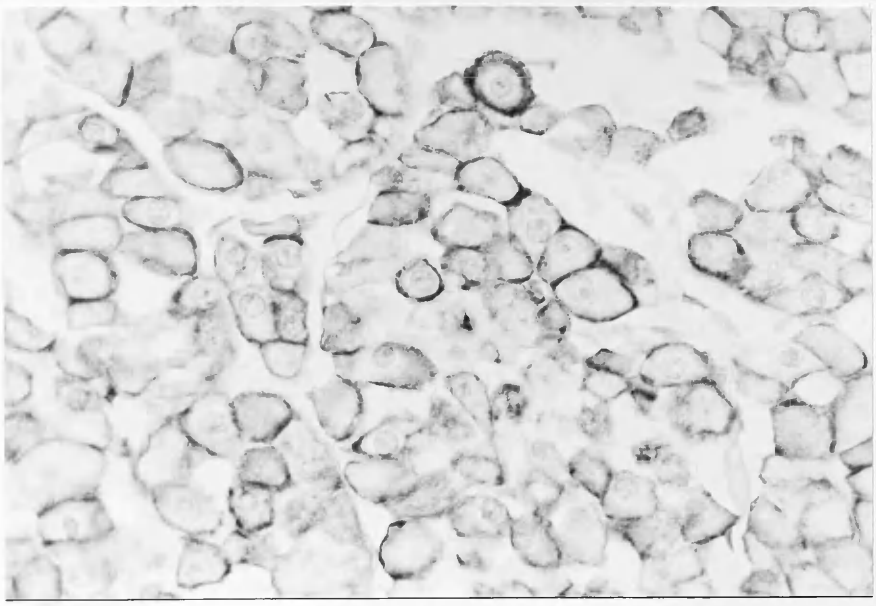


FIGURE 77. Cytoplasmic staining for vimentin is present in the tumour cells in this case. Renal cell carcinoma. Immunoperoxidase.

FIGURE 78. The thin sinusoidal type of blood vessels in renal cell carcinomas show endothelial staining for Factor VIII R antigen. Renal cell carcinoma. Immunoperoxidase.



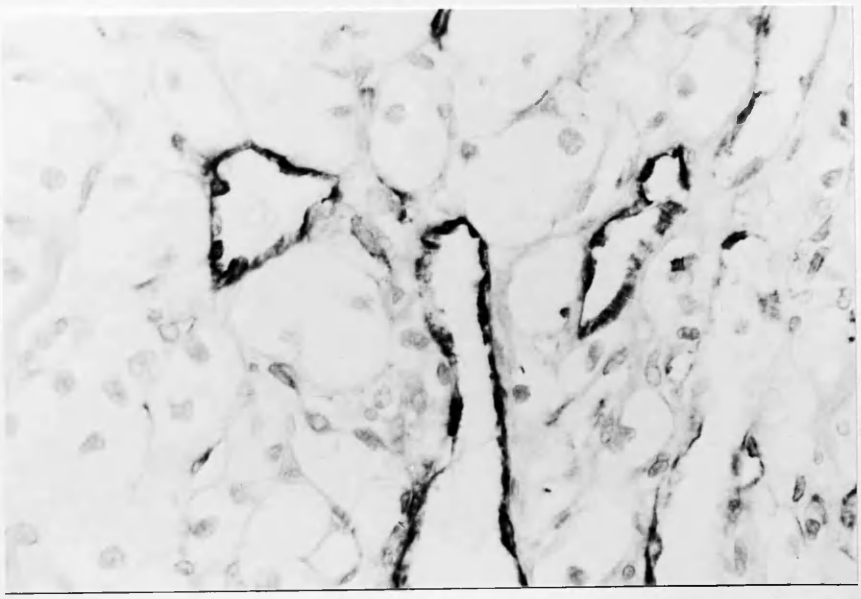


FIGURE 79. Fibronectin is detected at the basal margin of these epithelial tumour cells. Renal cell carcinoma. Immunoperoxidase.

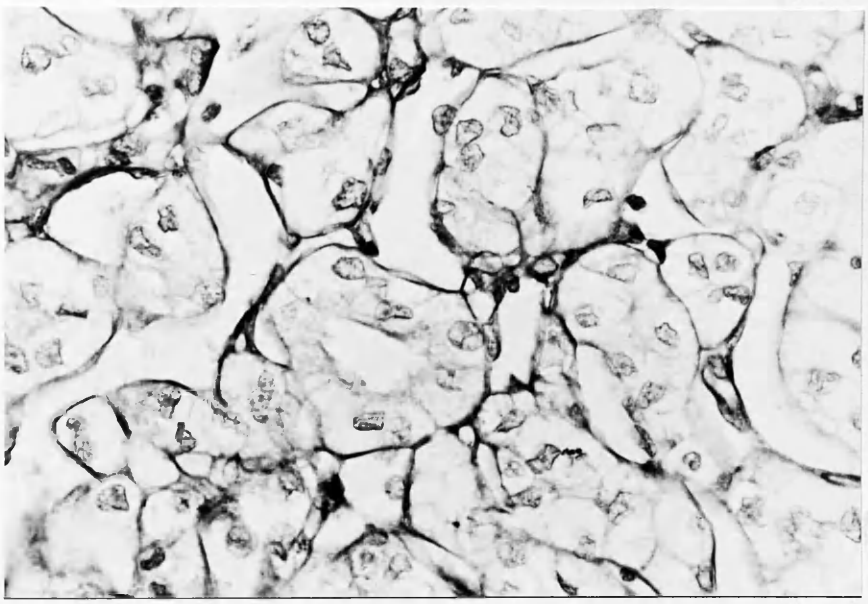


FIGURE 80. Only a very occasional tumour cell in renal cell carcinomas expressed the nuclear antigen detected by Ki 67. Renal cell carcinoma. Immunoperoxidase.

FIGURE 81. There is strong reactivity with HB 21 on the tumour cells in this carcinoma. Renal cell carcinoma. Immunoperoxidase.

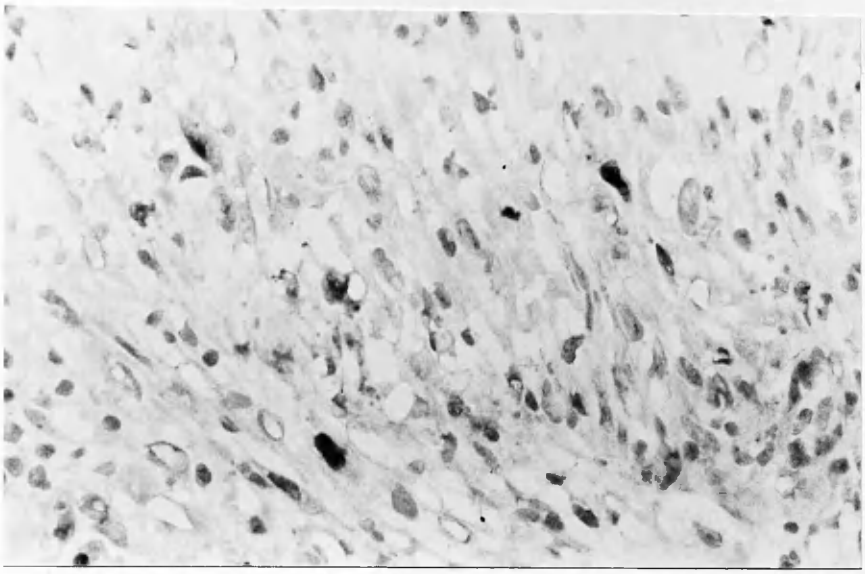


FIGURE 82. Renin containing spindle are located adjacent to the blood vessels in this renal cell carcinoma (arrow). Renal cell carcinoma. Immunoperoxidase.

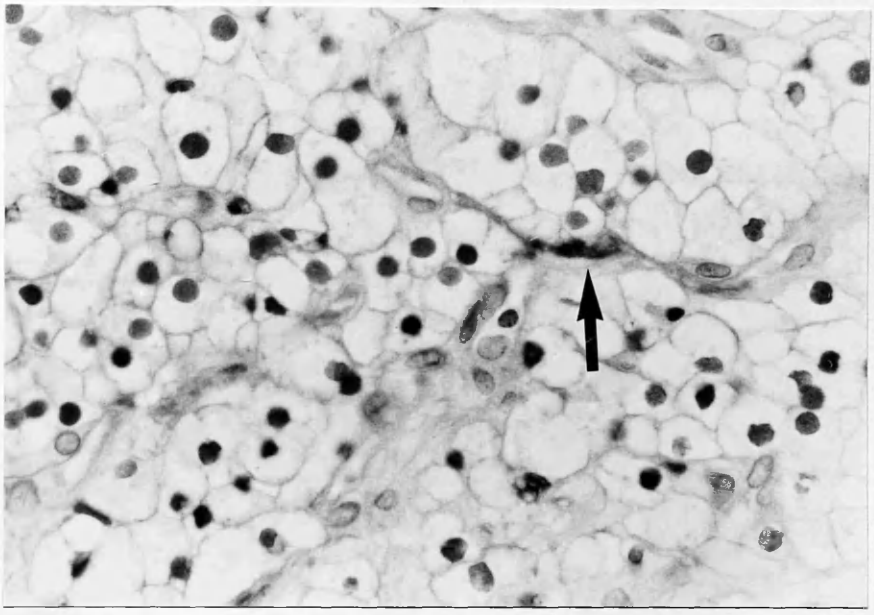
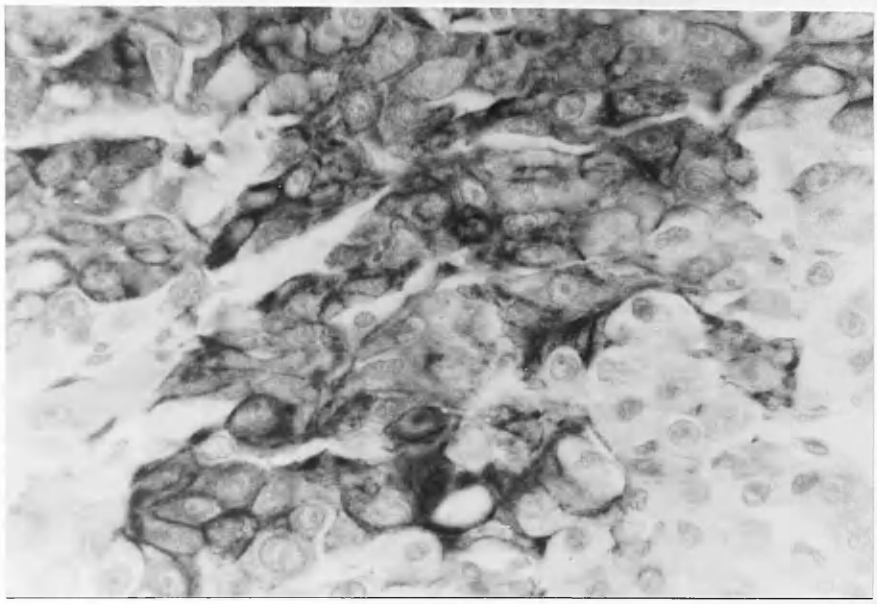


FIGURE 83. Diffuse cytoplasmic staining for CAM 5.2 is seen in this oncocytoma. Renal oncocytoma. Immunoperoxidase.

FIGURE 84. The oncocytomas showed some cytoplasmic staining for EMA. Renal oncocytoma. Immunoperoxidase.



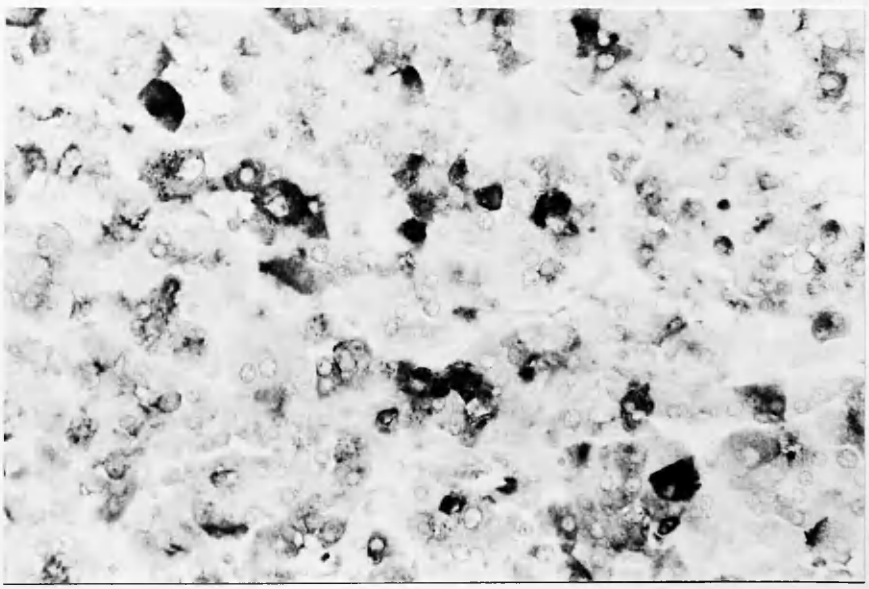
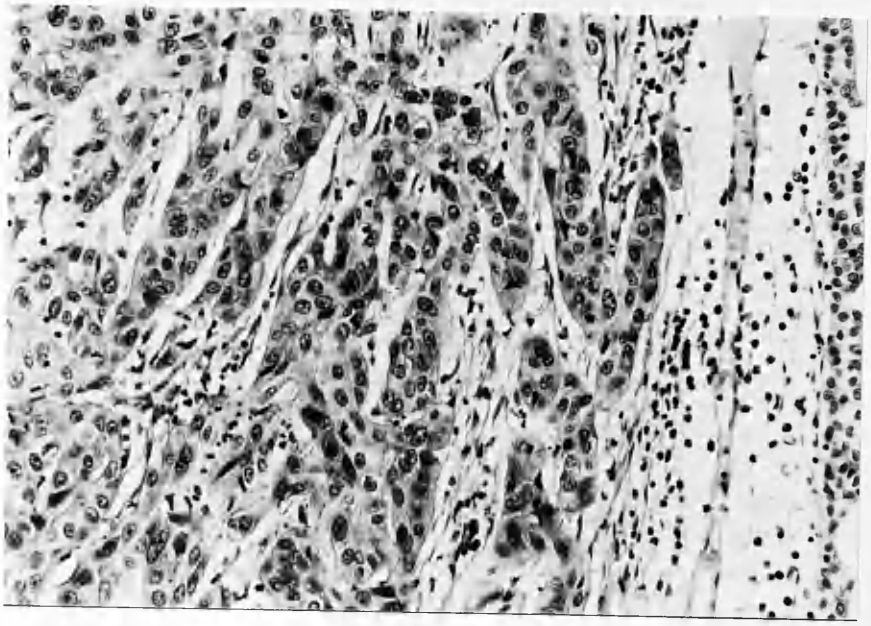


FIGURE 85. In the medulla the collecting duct carcinoma can be seen to consist of closely packed papillary structures composed of cuboidal epithelial cells. Collecting duct carcinoma. H&E.

FIGURE 86. Anastomosing tubules of the tumour, lined by pleomorphic tumour cells, infiltrate the renal parenchyma. There is a marked peritubular desmoplastic reaction. Collecting duct carcinoma. H&E.



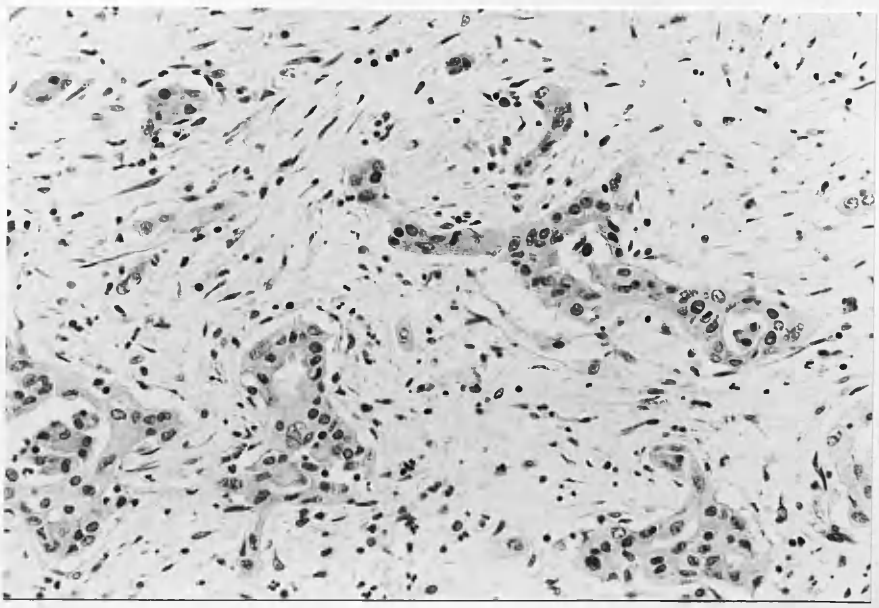
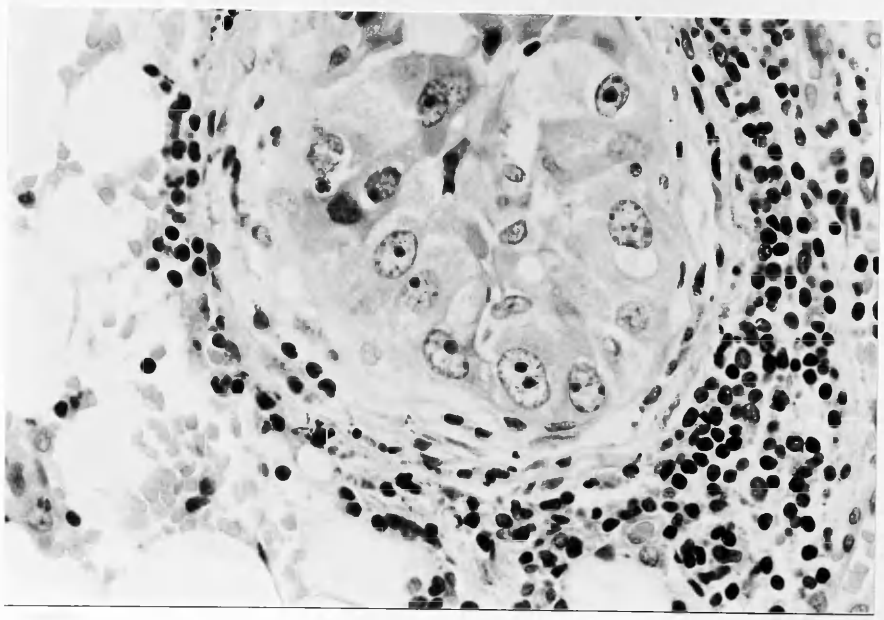
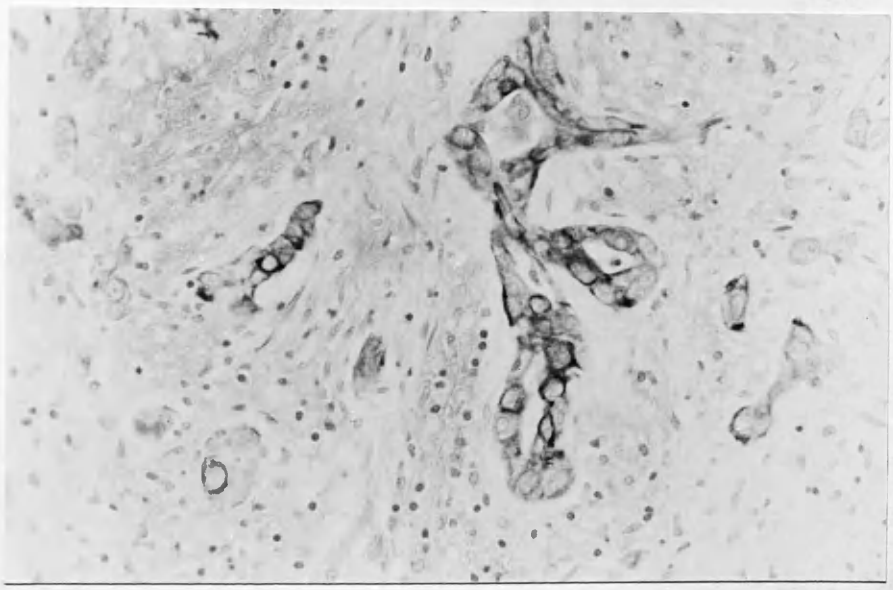


FIGURE 87. The tumour cells in the infiltrating portion of the tumour are highly atypical, cuboidal with vesicular nuclei and prominent nucleoli. Collecting duct carcinoma. H&E.

FIGURE 88. The anastomosing tubules of the infiltrating portion of the tumour showing cytoplasmic staining for epidermal prekeratin. Collecting duct carcinoma. Immunoperoxidase.





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APPENDIX 1.

Dilutions of Primary Antibodies.

ANTIBODY	DILUTION
CAM 5.2	1/30
PKK1	1/200
EPIDERMAL PREKERATIN	1/360
VIMENTIN	1/50
EMA	1/500
HMFG2	1/20
BRUSH BORDER	1/200
ALPHA-1-ANTITRYPSIN	1/200
ALPHA-1-ANTICHYMOTRYPSIN	1/180
DESMIN	1/100
MYOGLOBIN	1/200
FIBRONECTIN	1/1000
S-100 PROTEIN	1/250
FERRITIN	1/600
RENIN	1/1200
HCG	1/5000
AFP	1/1000
CEA	1/150
AGF 4.48	1/1
AGF 4.36	1/1
C14	1/4
HB21	1/400

KI 67 1/5

NDOG1 1/8

APPENDIX 2.

DILUTIONS OF SECONDARY ANTIBODIES.

PROCEDURE	ANTIBODY	DILUTION
PAP	SWINE ANTI-RABBIT	1/400
	RABBIT PAP COMPLEX	1/400
PAP	RABBIT ANTI-GOAT	1/80
	GOAT PAP COMPLEX	1/500
INDIRECT PEROXIDASE		
	RABBIT ANTI-MOUSE	1/40
AVIDIN BIOTIN COMPLEX		
	ANTI-MOUSE ABC	1/75

APPENDIX 3.

PUBLICATIONS ARISING FROM THIS THESIS.

Lindop G B M, Fleming S (1984) The immunocytochemical localisation of renin in renal cell carcinoma. Journal of Clinical Pathology 37: 27-31.

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