J. Anat. (1978), **126**, 2, pp. 303–318
With 19 figures
Printed in Great Britain

Cell death during the postnatal morphogenesis of the normal rabbit kidney and in experimental renal polycystosis

J. A. GARCÍA-PORRERO, J. L. OJEDA AND J. M. HURLÉ

From the Department of Anatomy, Faculty of Medicine of Santander, Santander, Spain

(*Accepted* 4 *July* 1977)

INTRODUCTION

In a previous work (Ojeda, Barbosa & Gómez-Bosque, 1972) we have studied the morphological aspects and the evolution of the renal lesions of the experimental polycystosis model of Perey, Herdman & Good (1967). Our observations showed that this model presents three evolutionary phases. The tubular cysts, which are the predominant lesion of the second phase, suffer a remarkable reduction in number and size during the third phase. This reshaping of the tubular cysts can be explained either on the basis of selective growth inhibition and alteration of cell multiplication rates, or of excessive cell death, or both.

Cell death during normal morphogenesis is a fact. Its morphology, localization and possible utility have been extensively reviewed by Glücksmann (1951), Saunders (1966), Saunders & Fallon (1966) and Ojeda (1974). Cell death has been studied in the regression of the mesonephros: this is a clear example of phylogenetic degeneration; however, there is a lack of information about the role of cell death during the postnatal morphogenesis of the metanephros.

The pathogenesis of renal polycystosis induced by corticoids is still incompletely understood.

Perey *et al.* (1967) have suggested that hypokalaemia is important in the aetiology of cyst formation. Crocker & Vernier (1970) and Crocker (1973), working with fetal and embryonic kidneys, have reported that a low concentration of potassium in the organ culture medium occasionally produces cystic dilatation of the ureteral bud. The role of potassium in the development of polycystic kidneys is not entirely clear, however (Resnick, Brown & Vernier, 1973).

Percy (1975) has observed that the pyrimidine analogues 5-iododeoxyuridine and cytosine arabinoside produces microcystic changes similar to the lesions of the rabbits postnatally treated with corticosteroids. The pyrimidine analogues and the corticosteroids (Ruch, 1969) impair normal cellular division. This suggests the possibility that the polycystic experimental kidney model of Perey *et al.* has, in addition to hypokalaemia, a pathogenesis related to a direct pharmacological action of corticosteroids. Courrier & Cologne (1951) and Jurand (1968) have observed that the malformations induced by corticosteroids are related to abnormal necrotic areas.

Abnormal cell death, which is very prominent in teratogenesis (for a review see Saxen & Rapola, 1969 and Menkes, Sandor & Ilies, 1970), could be the basic cellular lesion in the pathogenesis of the renal polycystosis induced by corticosteroids.

In the present work we have studied, by means of light and electron microscopy,

the normal and abnormal cell death which takes place (i) during the postnatal morphogenesis of rabbit kidney, and (ii) in the experimental renal polycystosis model of Perey *et al.* Histological and ultrastructural lesions not directly related to cell death have not been reported in this work.

Our observations emphasize the role of abnormal cell death in the evolution of cystic lesions.

MATERIALS AND METHODS

In our study we have used litters of newborn rabbits (*Oryctolagus cuniculus*, Spanish giant variety). Some members of each litter were given a single intramuscular injection of methylprednisolone acetate (20 mg/kg); the others were injected with an equal volume of saline solution. More detailed information has been given previously (Ojeda *et al.* 1972).

A total of 51 rabbits between 3 and 48 days old was used. The animals were killed by decapitation under ether anaesthesia, and the kidneys processed for light and electron microscopy.

For light microscopy, kidneys fragments, including both cortex and medulla were fixed in Carnoy solution. Then they were dehydrated, embedded in either paraffin or Paraplast, and serially sectioned transversely at 7 μm. The sections were stained by the Feulgen method and with Harris' haematoxylin–eosin.

For electron microscopy the kidneys were fixed by perfusion through the aorta with 3 % glutaraldehyde in 0·2 M cacodylate buffer at pH 7·3. (Prior to its use, possible contaminants were eliminated from the glutaraldehyde solution by treatment with activated charcoal.) After perfusion fragments of the kidney were immersed in fresh cold fixative for an additional 2 hours, then rinsed in 0·2 M cacodylate buffer and post-fixed in 1 % osmium tetroxide for another 2 hours. Tissue blocks were stained with uranyl acetate, dehydrated in a graded series of acetones and propylene oxide, and embedded in Araldite. Semithin sections were cut with a LKB ultratome III, stained with 0·1 % toluidine blue in 1 % sodium borate solution, and mounted in a drop of Araldite under a coverglass. These sections were used for general orientation, as well as for identification of possible cell death areas. Ultrathin sections of selected areas were then made, mounted on uncoated copper grids, stained with lead citrate (Reynolds, 1963) and examined with a Philips EM 201 electron microscope.

RESULTS

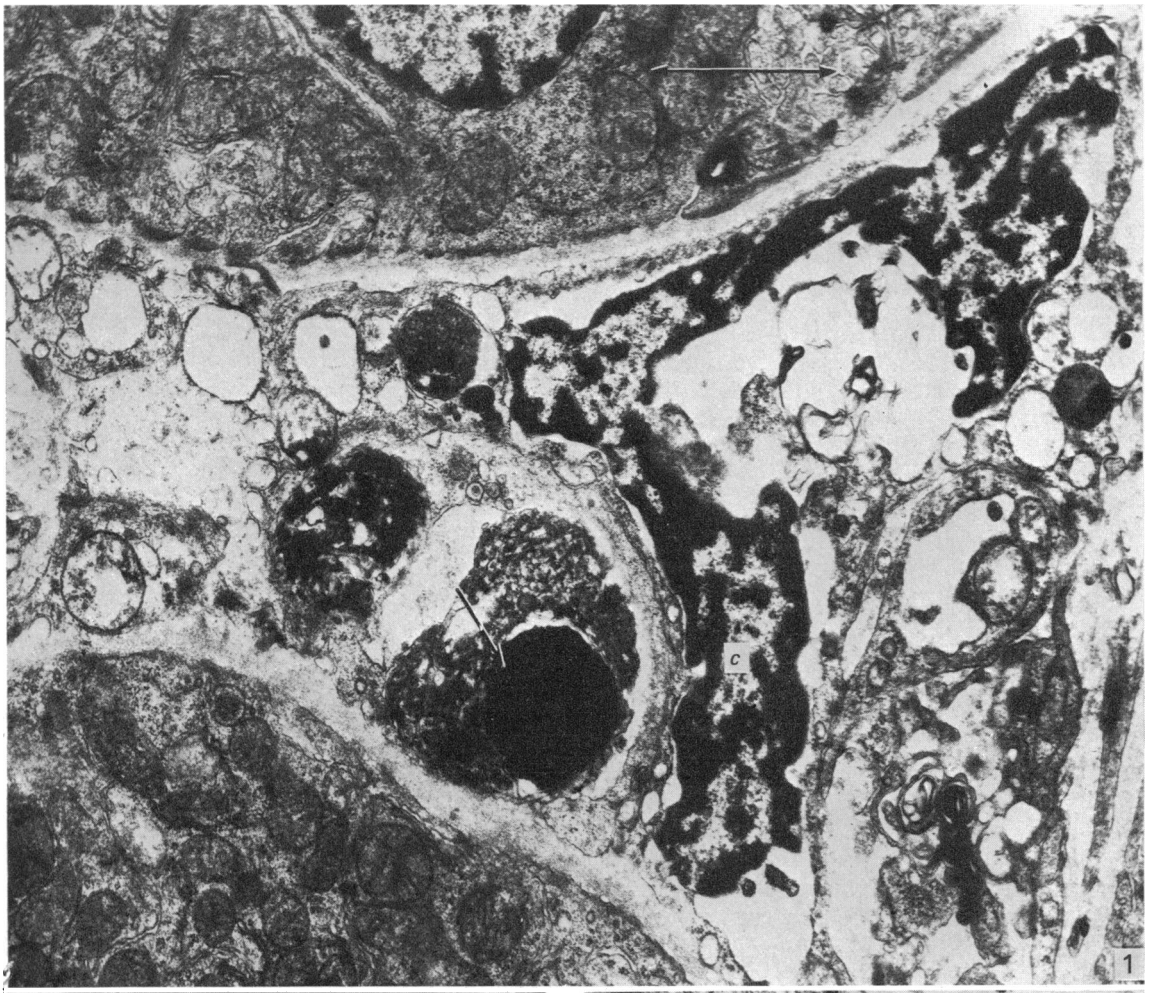
Cell death in the normal kidney

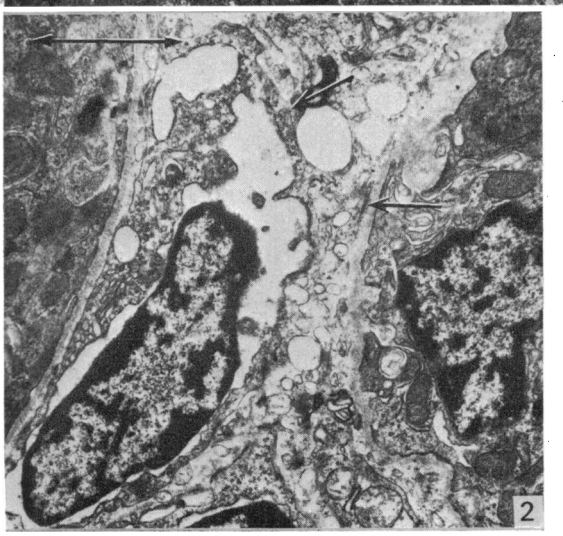
Cell death can be observed occasionally during the postnatal development of the rabbit kidney. It can be classified on the basis of its localization and frequency as follows.

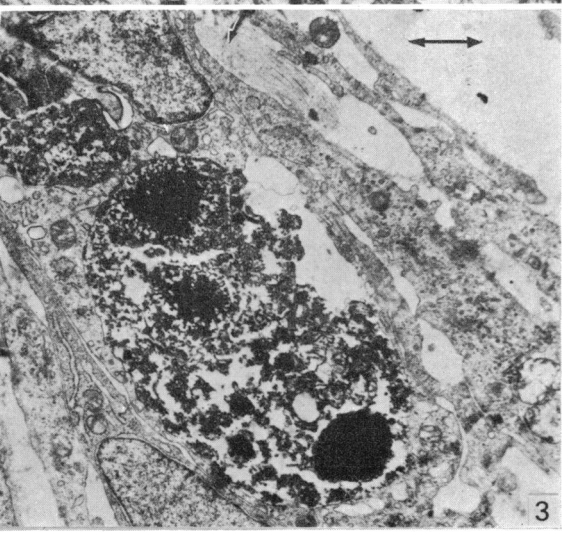
Fig. 1. Two intertubular dead cells in the kidney of a 15 days control rabbit. One of these cells (*c*) shows an irregular nucleus with large clumps of heterochromatin, swollen perinuclear space and cystic mitochondria. The other cell shows a pycnotic nucleus (arrow). Scale, 1 μm.

Fig. 2. Intertubular dead cell of a 12 days control kidney. Note the close association between the degenerated cell and collagen fibrils (arrows). Scale, 2 μm.

Fig. 3. Intertubular phagocyte containing one dead cell with signs of digestion. Note the bundles of microfibrils near the phagocyte (arrow). 4 days control kidney. Scale, 2 μm.


C
1


2


3

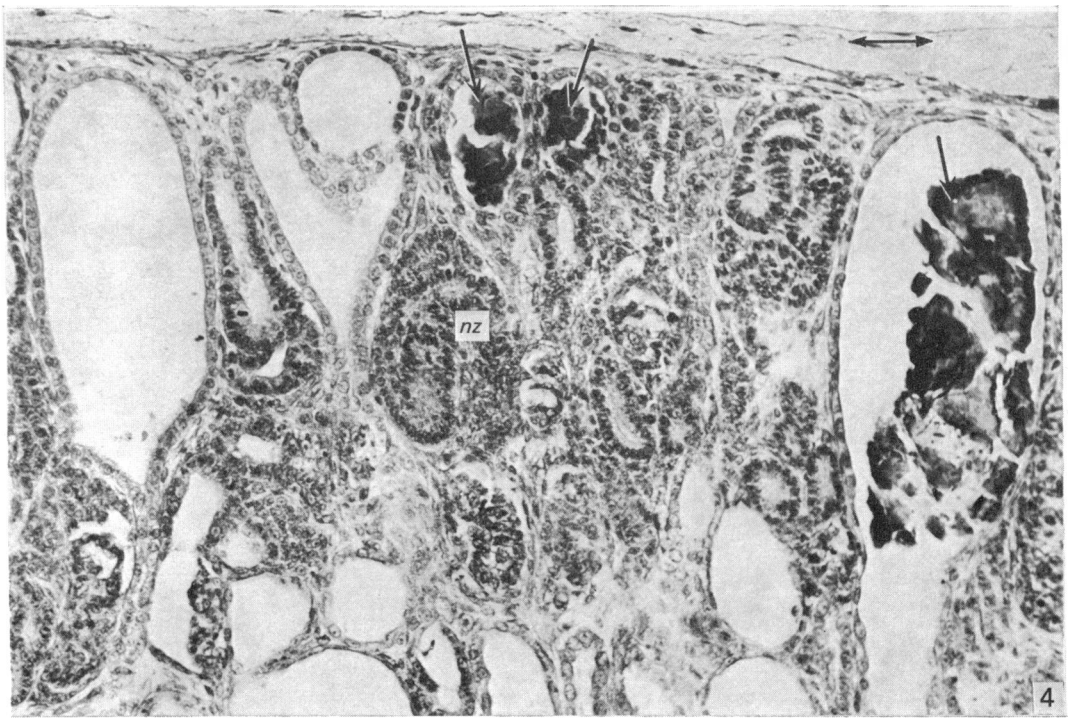


Fig. 4. Frontal section of a kidney 5 days after treatment, showing the presence of an extensive nephrogenic zone (*nz*) and dilatation of the ampullar portion of collecting tubes. Three tubular cysts (arrows) appear filled with cellular debris. Haematoxylin–eosin. Scale, 100 μm.

Intertubular cell death

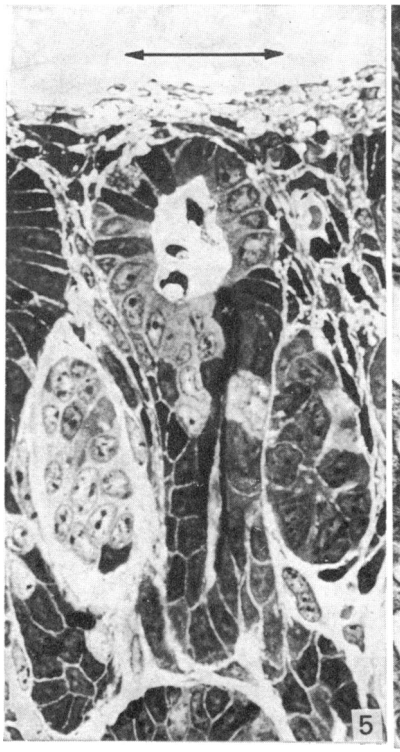
Dead cells can be observed among the tubular portions of the developing nephrons. Specific localization of such cells (e.g. in developing capillaries) could not be determined. They could be classified into three groups:

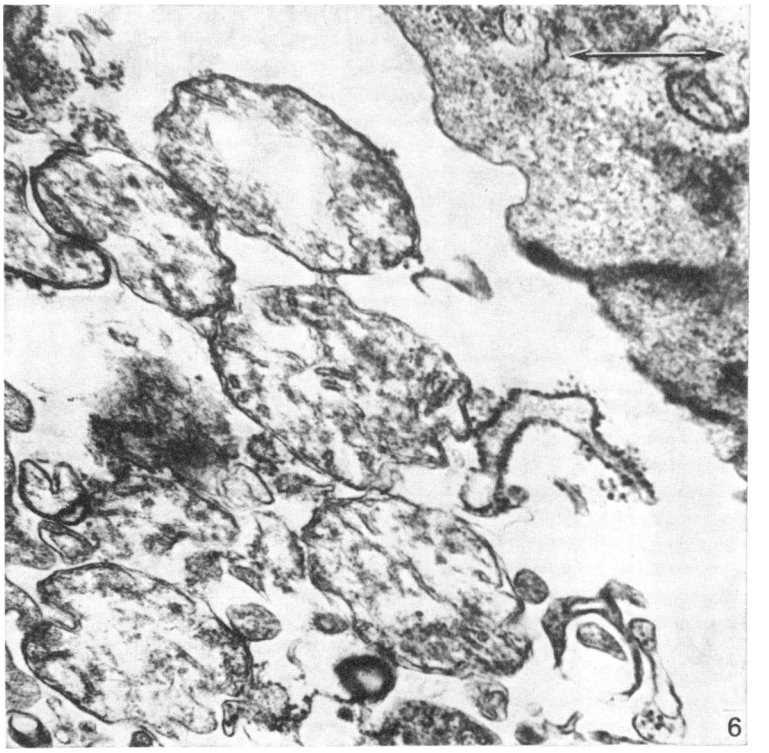
(1) The first kind of dead cell is the most frequent. The nucleus is very irregular, with marked clumping of nuclear material. The most conspicuous feature is the wide separation of the two nuclear membranes, producing vesicles (Fig. 1). These cells present many cellular processes, some of which are extremely long. Mitochondria are few in number and frequently look cystic, with an electron-lucid matrix. They resemble fibroblasts, but they lack the large amount of granular endoplasmic reticulum and the prominent Golgi complex characteristic of such cells. In some cases these dying cells are closely related to collagen fibrils (Fig. 2). No degenerative lesions could be observed in the neighbouring tubules (Fig. 1).

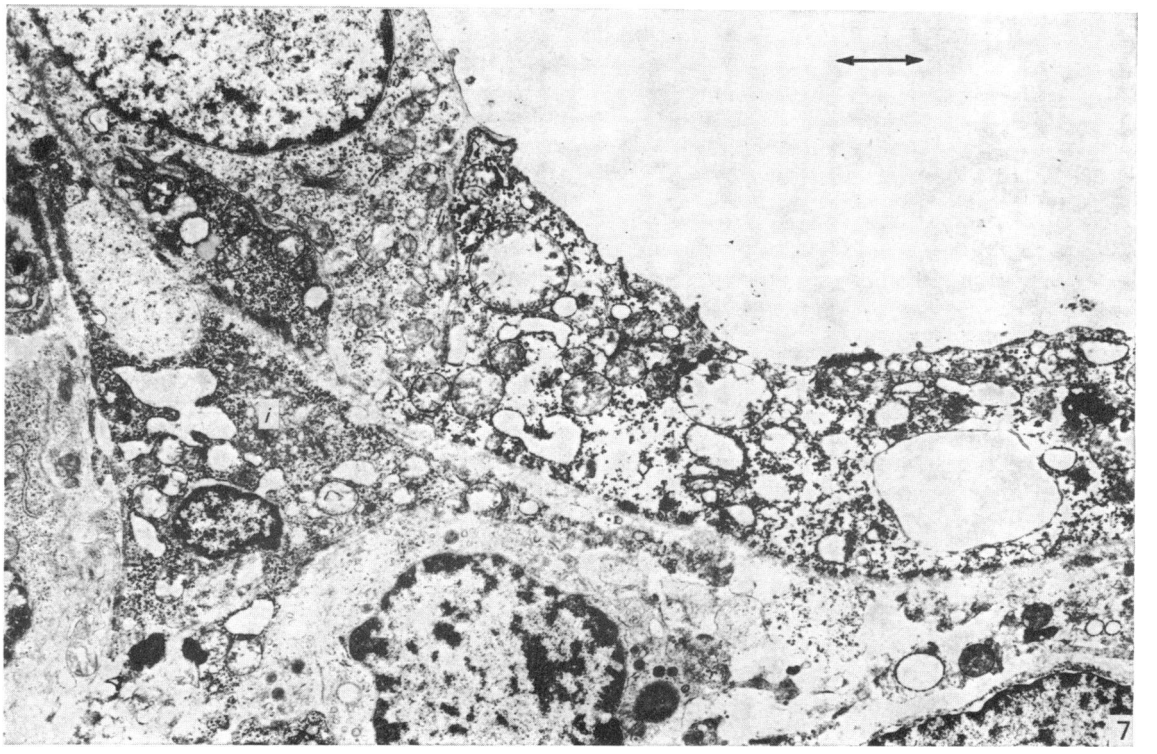
Fig. 5. Dead cells inside dilated terminal ampullae of a collecting tubule. 3 days treated animal. Araldite-embedded section (1 μm) stained with toluidine blue. Scale, 50 μm.

Fig. 6. Free organelles and cellular debris in the cavity of a dilated terminal ampulla. Swollen mitochondria and granular endoplasmic reticulum can be distinguished. 5 days treated animal. Scale, 0·25 μm.

Fig. 7. Cytoplasmic lesions in the wall of a tubular cyst in a kidney 4 days after injection. Note the mitochondrial vacuolization and the dilatation of granular endoplasmic reticulum. *i*, intertubular dead cell. Scale, 2 μm.






i

(2) The second type of intertubular dead cell death shows a pycnotic nucleus with concentrated chromatic material, and the cytoplasmic organelles cannot be distinguished (Fig. 1). These cells are very small and rounded. Sometimes they appear to be surrounded by arms of cytoplasm from an apparently viable intertubular cell.

(3) The third type of dead cell (Fig. 3) has been ingested by a phagocyte (a Type 2 phagocyte following the classification of Ballard & Holt, 1968).

The morphology of these three types of dead cells suggests that in fact they represent successive stages in one continuous necrotic process.

Intertubular cell death is more frequent in the younger stages of development, and it has not been observed after the twentieth postnatal day.

Cell death in the metanephric blastema

The metanephric blastema at different stages of development, and especially at the point of fusion of a renal vesicle with a collecting tube, was carefully observed for signs of cell death. Only two dead cells in animals 3–5 days old were ever seen with the electron microscope, suggesting that cell death in the normal metanephric blastema is a very rare event.

Cell death in the polycystic kidney

The morphological changes in the kidney of a rabbit treated with corticoid follow the stages described previously (Ojeda *et al.* 1972). From the third day after injection until about the fifteenth day the kidney demonstrates immaturity. It shows an extensive nephrogenic zone and a progressive dilatation of the ampullar portions of the collecting tubes (Fig. 4). From the fifteenth to about the fortieth day, tubular cysts become large. After this the kidneys show two types of cysts. The more abundant and characteristic are the glomerular cysts. Tubular cysts, though present, are rare.

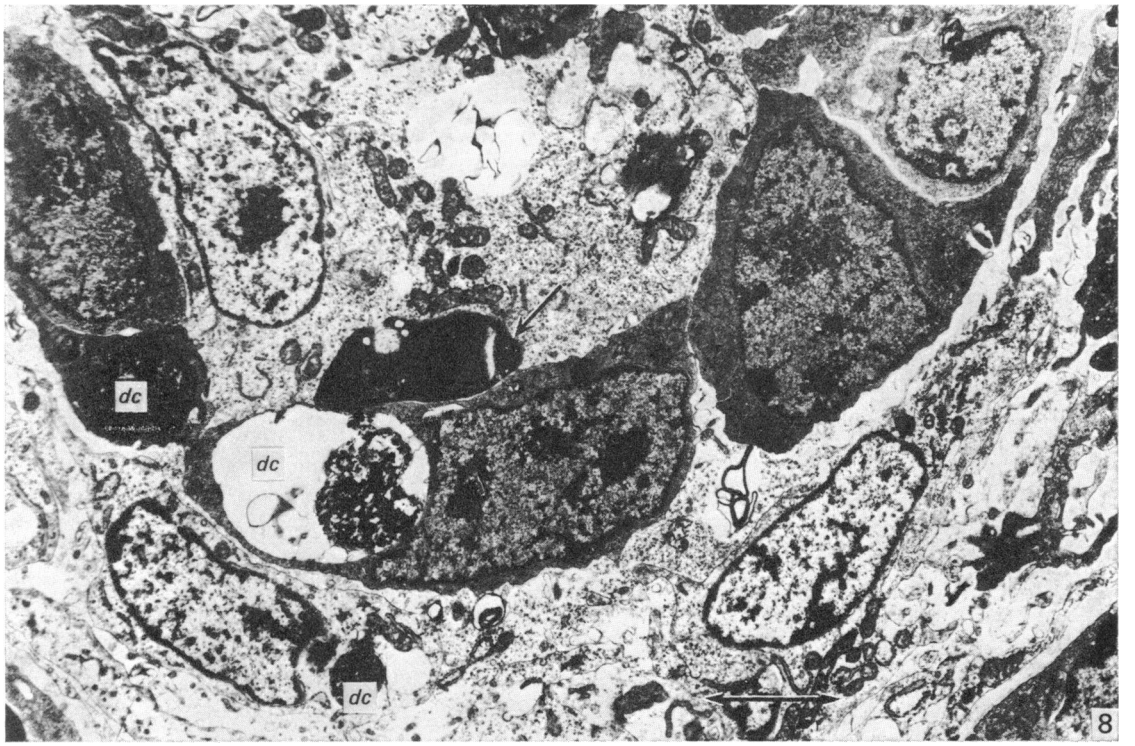
At all stages of cyst evolution cell death can be seen by optic and electron microscopy, the morphology and topography of the dead cells changing with time.

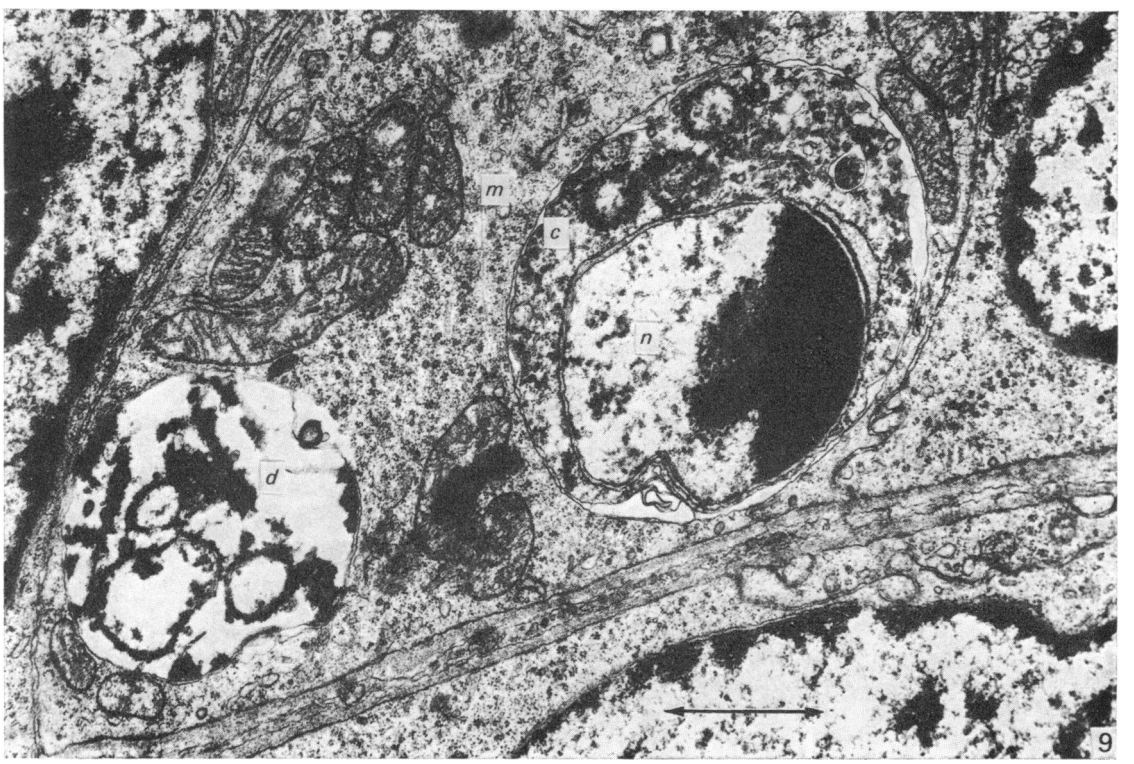
The stage of immaturity

From the third day after injection the ampullate blind ends of the collecting tubes show progressive dilatation. Dead cells (Fig. 5) and cellular debris (Fig. 6) can be seen by light microscopy in the cavities of about 60 % of the tubular cysts forming between the fourth and fifth days (Fig. 4). By the end of the period of immaturity (twelfth day) the number of cysts showing dead cells and cellular debris has decreased to about 40 %. The cells forming the walls of the ampullae show lamellar bodies and a remarkable vacuolization of the cytoplasm. The mitochondria and the granular endoplasmic reticulum are swollen (Fig. 7). Some cells show signs of irreversible deterioration. The nucleus shows a condensation and margination of chromatin, and the perinuclear cisterna is widened. These cells can be considered as dying.

Fig. 8. Phagocytes and one free degenerated cell (arrow) in the nephrogenic tissue of a 5 days treated animal. The dead cells (*dc*) ingested by the phagocytes are in course of digestion. Scale, 4 μm.

Fig. 9. Epithelial cell containing one dead cell in which nucleus (*n*) and cytoplasm (*c*) can be distinguished. Note the close association of the phagocyte to the neighbouring cells, and the lack of degenerative signs in its cytoplasm. *m*, microtubules; *d*, digestive vacuole with cell fragments. 9 days treated animal. Scale, 2 μm.


dc
dc
dc
8


m
c
n
d
9

The above mentioned changes take place mainly during the first days after injection, and they have not been observed in equivalent structures in control animals. From the sixth day cell death is essentially restricted to the metanephrogenic mesenchyme.

It must be noted that the aggregation of nephrogenic tissue seems to be greater in the treated animals than in the control rabbits. This is clear in the semithin sections. In this nephrogenic tissue the dead cells are grouped in small clusters of 3–5 cells. The electron micrographs show two kinds of dead cell. The first is small and dense and free (Fig. 8). All the elements of these cells show increased electron density, the chromatin is clumped into large polar plaques, the cytoplasm is very poor in organelles, and they appear very closely packed. A remarkable feature is a great reduction in surface area.

The second kind of dead cell has been ingested by an epithelial cell of the metanephric blastema. These epithelial phagocytes are very numerous, and they can be divided into two successive stages, following the classification of Ballard & Holt (1968). In the early stage (Stage 1) there are 1–2 dead cells in each phagocyte (Fig. 9), and the dead cells show few signs of digestion. They exhibit a degenerating nucleus, in which the chromatic and non-chromatic material is fragmented, and a cytoplasm with dilated endoplasmic reticulum and debris. The nature of the contents of the other digestive vacuoles in the phagocytic cells is not known. In the later stage (Stage 2) the number of ingested dead cells increases to 2–4 in each phagocyte, and the dead cells show signs of digestion. The nucleus and cytoplasm cannot be clearly distinguished. Some degenerating mitochondria, dense bodies and membranous material can be seen in the digestive vacuoles.

Electron micrography reveals certain general characteristics of the phagocytes. They possess numerous free ribosomes and microtubules (Fig. 9); their endoplasmic reticulum is very scarce; an extensive Golgi complex with associated coated vesicles is found in the cytoplasm, and the ultrastructure is generally very well conserved. Degenerating phagocytes like those described in various other tissues in necrobiosis were never seen (Dawd & Hinchliffe, 1971; Hurlé, Lafarga & Ojeda, 1977).

Frequently the phagocytes have a dark cytoplasm which contrasts with the light cytoplasm of adjacent cells (Fig. 8). They appear closely associated with the metanephric epithelial cells. The digestive vacuoles of these phagocytes are usually located at the basal pole. Frequently phagocytes can be seen located in the parietal layer of Bowman's capsule of the developing renal corpuscle (Fig. 10). No degenerative lesions or phagocytes can be seen in the visceral layer.

We have observed intertubular dead cells similar in morphological characteristics

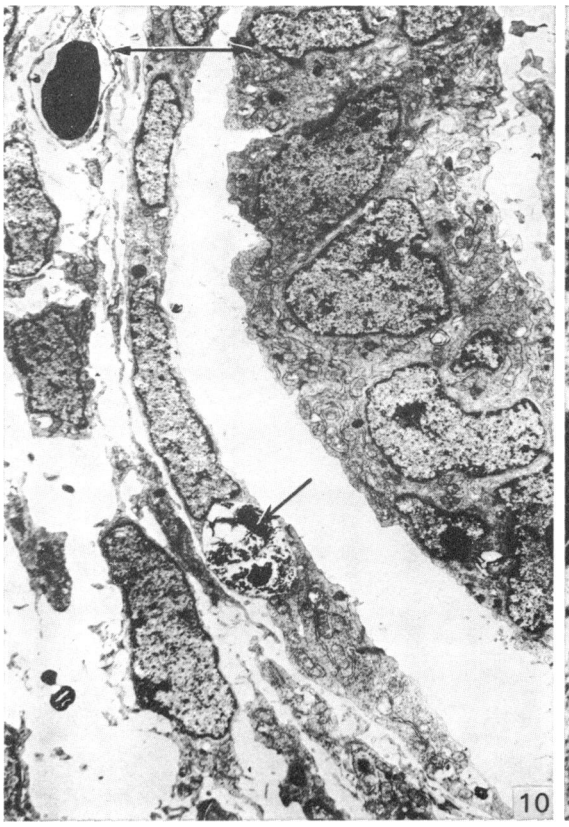
---

Fig. 10. Immature renal corpuscle with a digestive vacuole (arrow) in one cell of the parietal layer of Bowman's capsule. The visceral layer does not show degenerative lesions. 5 days treated animal. Scale, 5 μm.

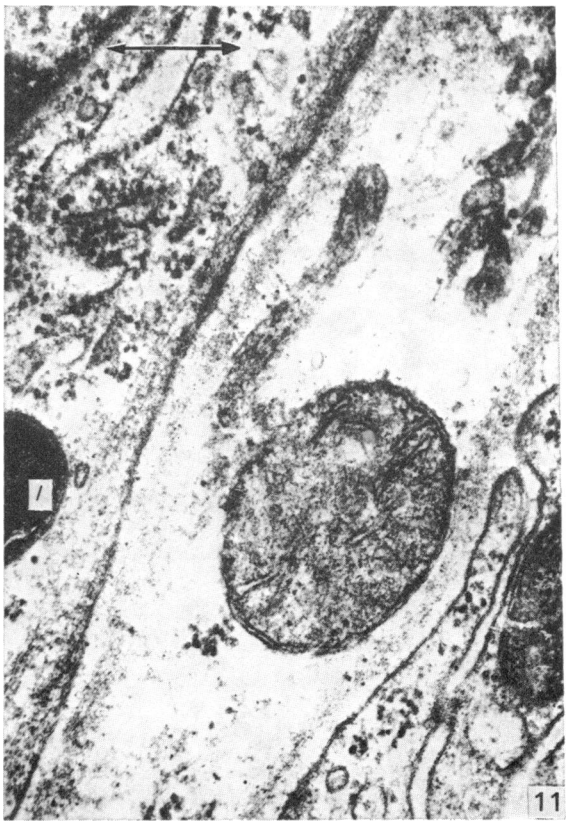
Fig. 11. Free mitochondria between the basement membranes of two tubules. *l*, lysosome. 15 days treated animal. Scale, 2 μm.

Fig. 12. Cytoplasmic vacuolation in the cells of the wall of one tubular cyst. Note that damaged cells alternate with normal cells (arrows). *p*, phagocyte. Araldite-embedded section (1 μm), stained with toluidine blue. 20 days treated animal. Scale, 25 μm.

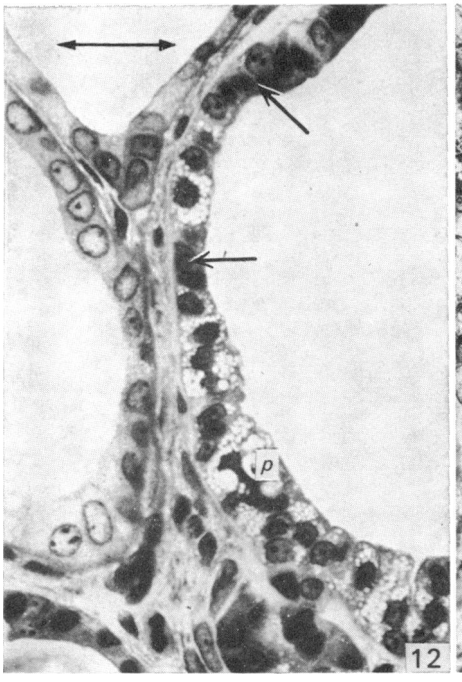
Fig. 13. Epithelial cell from the wall of one tubular cyst. Note the widened perinuclear cisterna (*pc*) and the extremely distended endoplasmic reticulum. *L*, lumen of the cyst; *b*, basement membrane; *ly*, lysosome. 20 days treated animal. Scale, 1 μm.



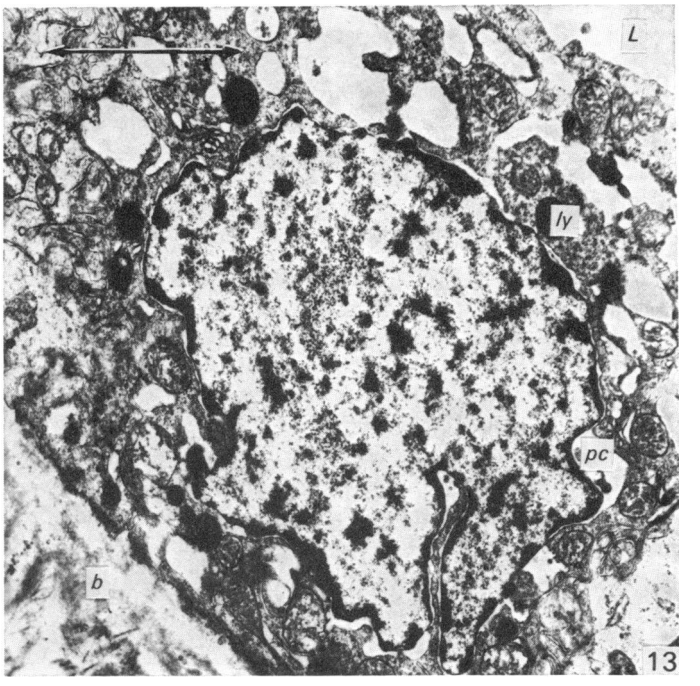
10



11



12



13

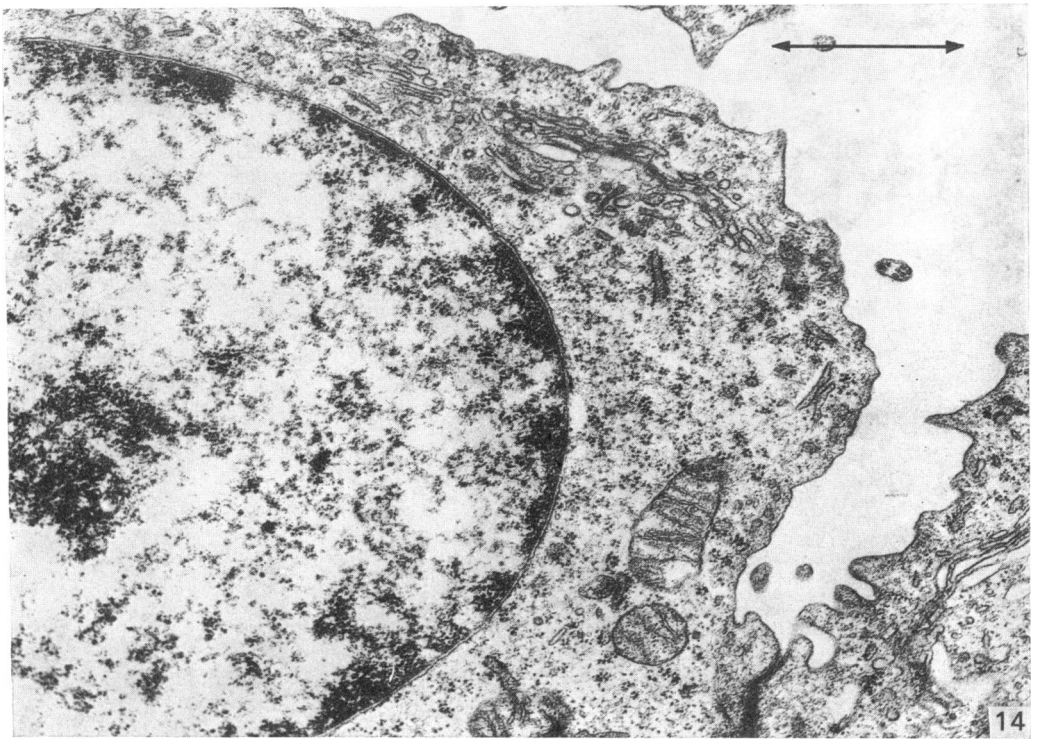


Fig. 14. Cell of a non-cystic collecting tube in a kidney 20 days after injection. Notice the well preserved ultrastructure and compare with the cell shown in Fig. 13. Scale, 0·5 μm.

and frequency to those described in the control animals. In some cases we have found free cytoplasmic organelles among the basement membranes of developing tubular cysts (Fig. 11).

In the period of immaturity of the polycystic kidney neither infiltration with hematogenous cells nor signs of inflammation were observed.

The stage of tubular cysts

At this stage the metanephrogenic zone has disappeared. Dying and dead cells can be seen in the walls of tubular cysts and among the basement membranes of adjacent tubules.

Within the wall of the tubular cysts, among healthy epithelial cells, groups of

---

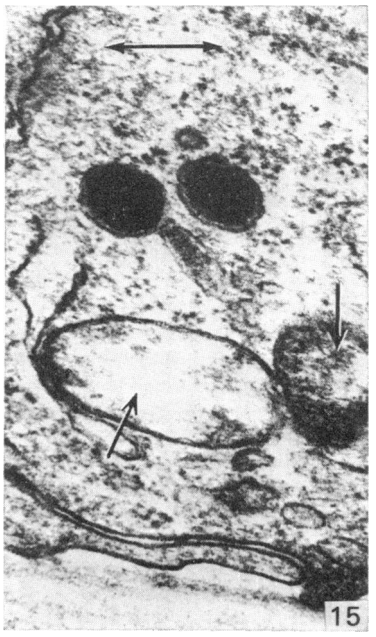
Fig. 15. Lysosomes in the wall of a tubular cyst in a kidney 20 days after injection. Two swollen mitochondria (arrows) are to be seen. Scale, 0·25 μm.

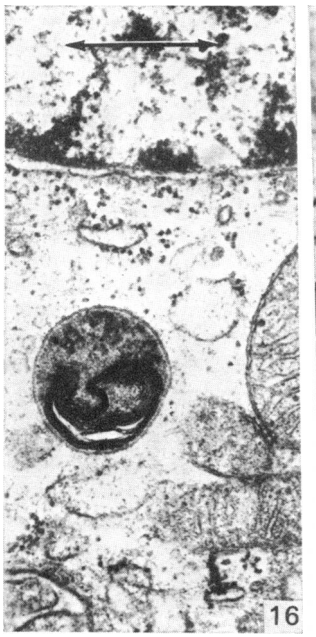
Fig. 16. Myelin-like figure in a lysosome of the wall of a tubular cyst. 22 days treated animal. Scale, 0·5 μm.

Fig. 17. Two digestive vacuoles (arrows) in the wall of a tubular cyst in a kidney 22 days after injection. Araldite-embedded section (1 μm) stained with toluidine blue. Scale, 25 μm.

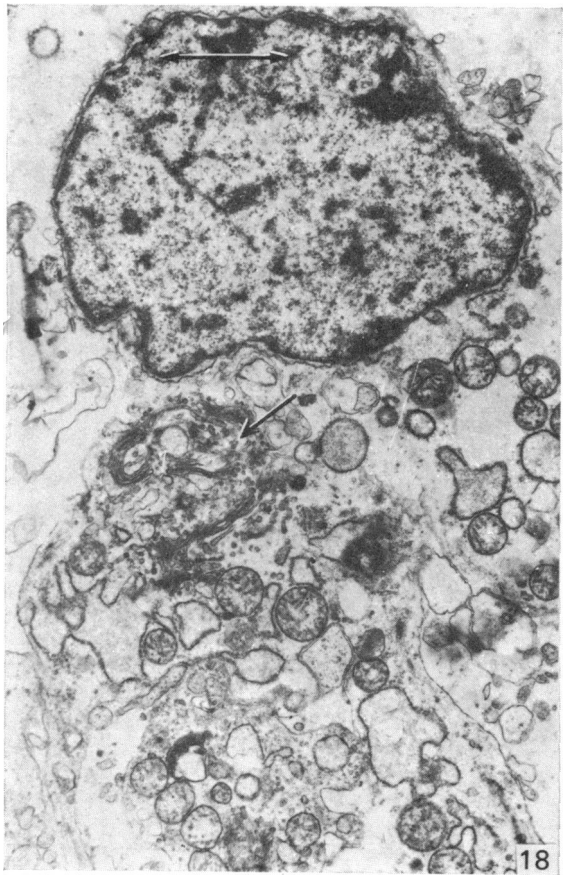
Fig. 18. Free dead cell in the lumen of a tubular cyst in a kidney 26 days after injection. Note the well preserved nucleus and the Golgi apparatus (arrow). Scale 2 μm.

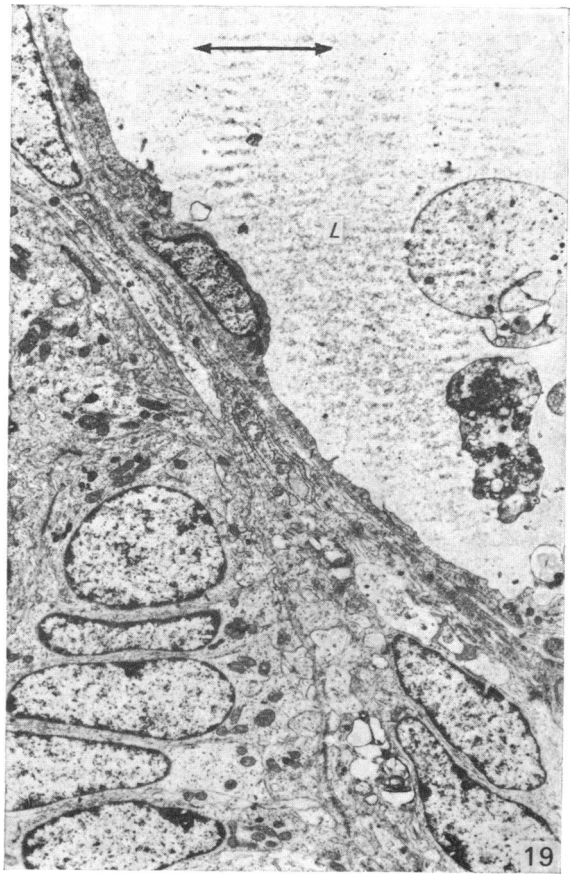
Fig. 19. Cyst produced by dilatation of Bowman's capsule. Amorphous and cellular debris are observed in the lumen (*L*). 37 days treated animal. Scale, 5 μm.


15


16


17


18



19

10–15 cells showing cytoplasmic alterations suggestive of degeneration can be found; these dying cells are located mainly in regions where two or more cysts come close together. Between the twentieth and the twenty sixth day after injection about 30 % of the tubular cysts have degenerated cells in their walls. The most conspicuous feature of the dying cells at the light microscopic level is the vesiculation of the cytoplasm (Fig. 12). The electron microscope shows that some of the large tubular cysts are lined by degenerating epithelium. The mitochondria of these epithelial cells are swollen and show a reduced number of cristae, being finally transformed into empty vacuoles with a decreased number of dense grains in their matrix. Some cells show an extremely distended smooth-surfaced endoplasmic reticulum (Fig. 13). Free ribosomes can also be observed. These lesions have never been observed in the cells of the non-cystic collecting tube, whose ultrastructure is well preserved (Fig. 14), nor in the cells of the collecting tubes of the control animals.

In the cytoplasm of degenerating cells we have found numerous dense bodies (Fig. 13) limited by a single membrane. Located between the electron-dense matrix and the membrane there is an electron-lucid rim (Fig. 15). Some of these dense bodies show myelin-like figures, and these can be observed in cyst cells which do not show any other type of lesion (Fig. 16). The nucleus shows moderate changes. Some cells show an indented nucleus with a swollen perinuclear space and vesiculation of the nuclear membrane. Occasionally nuclei with dense chromatin can be observed. The changes mentioned above are frequently present in a single cell. Dead and dying cells seem not to lose their attachments to adjacent cells.

Phagocytes are present in the walls of the tubular cysts (Fig. 17). These cells are of epithelial origin and have ultrastructural characteristics similar to the ones of the period of immaturity described above.

Between the twenty second and the thirty seventh day after injection about 12 % of the tubular cysts are partially occupied by amorphous material, cellular debris and dead cells. Electron microscopy demonstrates a remarkable vacuolation of the cytoplasm of these cells. The mitochondria as well as the endoplasmic reticulum are swollen and the cell membrane is broken; the structure of the nucleus remains unchanged, however (Fig. 18). Intertubular dead cells are present throughout the life span of tubular cysts.

In this period of tubular cysts, but more especially in the later stages, a second type of cyst formed by dilatation of the capsular space of Bowman can be observed. This type of cyst reaches its full development in the final stage in the evolution of the polycystic kidney described by us (Ojeda *et al.* 1972). The walls of glomerular cysts are formed by an epithelium of flat cells typical of the parietal layer of the renal corpuscle. Inside the capsular cavity an amorphous material, and in some cases cellular debris, can be seen (Fig. 19).

DISCUSSION

Good fixation of kidney, and especially developing kidney (Larsson, 1975), is usually considered difficult, and so it might be tempting to some to interpret the cellular lesions reported here as the consequences of autolysis associated with poor fixation. Three arguments are against this hypothesis. In the first place, degenerative lesions were seen in the kidney of control animals only in the interstitial cells and only during the first twenty days of postnatal development – never later. In the second place, we did not find degenerative lesions in the experimental animals, nor

in prenatal glomeruli which are generally considered difficult to fix (Johnston, Latta & Osvaldo, 1973), nor in other parts of the kidney such as the convoluted tubules, nor in the cells next to the degenerating ones. Finally, the images of phagocytosis cannot be explained as artefacts produced by a poor fixation. The observed lesions *must* be interpreted as evidence of necrobiosis in the kidneys of living animals.

Some of the intertubular dead cells observed in the kidneys of the control animals can be equated to the 'cells with dark electron-dense nuclei' described by Leeson (1961) in the young hamster. Bulger & Nagle (1973) have described, in adult rabbits' kidneys, interstitial cells showing condensed chromatic material like the Type 2 cells described here. However, the cells described by us differ in such respects as scarcity or absence of Golgi cisternae and lack of lipid droplets.

Macrophages have been observed in kidney interstitial tissue by several authors (Kirkman, 1943; Morrison & Schneeberger-Keeley, 1969; Romen & Thoenes, 1970); however, phagocytes with ingested dead cells like those described in the present study have not been reported up to now in the normal rabbit kidney. Leeson (1961), arguing from the decrease in the number of 'cells with electron-dense nuclei' with increasing age, suggested that such cells either disappear completely or differentiate into other cell types. Buss & Gusek (1968) have observed that, during the postnatal development of the interstitial cells of rat kidney, initially undifferentiated cells are later transformed either into fibroblasts or into cells presenting necrobiotic alterations similar to those reported here. From our study it is possible to postulate that dead cells either disintegrate in the intertubular space or are digested by phagocytosing cells.

The possible role of intertubular cell death in the postnatal development of kidney is not clear. It could indicate the disposal of a surplus of undifferentiated metanephric cells that have not participated in the development of the kidney. Leeson (1961) suggested that basement membranes of the tubules derive partially from this intertubular cell death; this is in agreement with our observations, for there was sometimes a clear association between the dead cells and the extracellular material, especially with the collagen fibres. Several authors (Hughes, 1943; Ojeda & Hurlé, 1975) have associated cell death with the appearance of extracellular material in the chick embryo, but experimental proof is lacking.

Cell degeneration has been reported by Ilies (1971) during several stages of the development of the metanephros. In our study cell death was observed in the subcortical metanephric zone of normal animals; its significance is unknown.

The cell death observed in the kidney of treated animals is abnormal in intensity and topography; nevertheless its role in the pathogenesis of this 'model' of polycystic kidney is not clear. It is possible that cell death decreases too drastically the mass of cells in the metanephric zone; there are many examples (Grobstein, 1966, 1967) to indicate that a certain minimum mass of cells is often necessary if differentiation is to take place properly. Gossens & Unsworth (1972) have suggested that, in mouse kidney tubulogenesis, the capacity for autonomous differentiation from the condensed stage to the elongated one and then to the coiled tubule stage is critically dependent upon the mass of available non-induced mesenchymal tissue. The decrease of metanephric cell mass could explain the persistence of a nephrogenic zone and the prolonged renal immaturity, and perhaps the formation of the glomerular cysts. Furthermore, it has been pointed out by us (Ojeda *et al.* 1972) that the persistence of the nephrogenic zone could explain the dilatation and excessive branching of the collecting tubules.

A possible alternative explanation is that cell death in the metanephrogenic zone is the consequence of poor induction by the cystic collecting tube.

Cell death in the tubular cysts appears very early. It is possible that the cell detritus liberated into the cavity of the terminal ampullae retains water because of the oncotic pressure of its proteins, and this increases intracystic pressure. However, the cysts are always in communication with the tubular system (Ojeda *et al.* 1972) and, because of this, distension can hardly be a decisive factor in the pathogenesis of this model of polycystic kidney.

The cell death observed in the walls of the fully developed tubular cysts could, however, explain the change in the shape of the cysts that we have described previously (Ojeda *et al.* 1972).

The morphological changes in the cysts brought about by cell death could be very important, but it is very difficult to deduce the pathogenesis of the polycystic kidney from the morphology of the lesions.

We can only speculate about the mechanism of cell death in this model of polycystic kidney induced by corticoids. Our electron microscope studies have shown that, during the period of immaturity, the basic lesions are condensation of chromatin and increase in the number of free ribosomes. This type of necrosis is very frequently observed in teratogenic studies using substances which disturb replication, transcription and translation (Schweichel & Merker, 1973).

Corticosteroids can produce cell death in the kidney by their mitostatic effect (Ruch, 1969), and this is in agreement with Kallen's theory (1965) that cell death is a consequence of aberrations in mitosis.

Lamellar bodies such as those observed in the cells of the terminal ampullae of the treated animals have been attributed to impurities in commercial glutaraldehyde (Schultz & Case, 1970), but with this in mind we used a highly purified glutaraldehyde. We have demonstrated a high content of glycogen in the walls of the tubular cysts (García-Porrero & Ojeda, 1975), and on this basis the lamellar bodies could be attributed to the transformation of glycogen into lipids (Curgy, 1968).

The pathological cell death in the kidneys of rabbits treated with methylprednisolone acetate is a paradoxical phenomenon, since corticosteroids have a cell-protecting effect attributed to their capacity to stabilize lysosomal membranes (Weissmann, 1975). However, in some cases it would appear that corticosteroids are able to provoke cell injury and even cell death.

At the stage of tubular cysts the dying cells show swollen mitochondria and a notable increase in the number of primary lysosomes, supporting De Duve's (1964) 'suicide bag' theory of cell death.

Morrison & Panner (1964) observed that experimental hypokalaemia leads to the formation of large numbers of lysosomes in the cells of rat renal papillae. Wiener *et al.* (1968), using quantitative electron microscopy, found that cortisone induces a two to three fold increase in the number of lysosomes per unit area of cytoplasm, attributable to a decreased turnover of these organelles as a result of an increase in their membrane stability. This finding is in agreement with the results obtained by Jurand (1968).

However, the precise 'cause and effect' relation between lysosomes and cell death is not clear. The release of lysosomal enzymes is probably a manifestation rather than a cause of cell death (De Duve, 1964). It is likely that a combination of factors causes cell death; e.g. changes in blood supply can be a basic mechanism.

Taken all in all the results of this study suggest that cell death *is* an important

factor in the evolution of the lesions in the renal polycystosis induced by corticosteroids, and probably in the genesis of this condition as well.

SUMMARY

We have studied, by means of optic and electron microscopy, the normal and abnormal cell death that takes place during the postnatal morphogenesis of rabbit kidney, and in the experimental renal polycystosis produced by methylprednisolone acetate.

In the normal kidney intertubular cell death can be observed during the first 20 days of the postnatal development. However, cell death in the normal metanephric blastema is a very rare event.

In the polycystic kidney numerous dead cells can be seen between the third and forty eighth days after injection. The topography and morphology of the dead cells depend on the stage in the evolution of the disease. In the 'stage of renal immaturity', dying and dead cells are present in the nephrogenic tissue, in the dilating collecting tubules and in the intertubular spaces. In this stage the cellular pathology is essentially nuclear. In the stage of tubular cysts, the dead cells are mostly located in the walls of cysts, with some dead cells, but mostly cellular debris in their lumina. At this stage the cellular pathology is basically cytoplasmic. The dead cells are eventually digested by what appear to be phagocytes of tubular epithelial origin.

It is suggested that cell death is an important factor in the evolution of the lesions of renal polycystosis induced by corticosteroids, and probably in the initiation of the pathological process as well.

Support from the Marcelino Botin Foundation, Santander, Spain, is gratefully acknowledged.

The authors wish to thank Dr F. J. García-Sancho for his invaluable help in the preparation of this manuscript.

REFERENCES

BALLARD, K. J. & HOLT, S. J. (1968). Cytological and cytochemical studies on cell death and digestion in the foetal rat foot: the role of macrophages and the hydrolytic enzymes. *Journal of Cell Science* **3**, 245–262.

BULGER, R. E. & NAGLE, R. B. (1973). Ultrastructure of the interstitium in the rabbit kidney. *The American Journal of Anatomy* **136**, 183–204.

BUSS, H. & GUSEK, W. (1968). Untersuchungen über die interstitiellen Zellen der Nierenrinde. Ein Beitrag zur Frage der Matrix mesenchymaler Nierengeschwülste der Ratte. *Virchows Archiv B*1, 251–268.

COURRIER, R. & COLOGNE, A. (1951). Cortisone et gestation chez la lapine. *Compte rendu hebdomadaire des séances de l'Academie des sciences, Series D* **232**, 1164–1166.

CROCKER, J. F. S. & VERNIER, R. L. (1970). Fetal kidney in organ culture: abnormalities of development induced by decreased amounts of potassium. *Science* **169**, 485–487.

CROCKER, J. F. S. (1973). Human embryonic kidneys in organ culture: abnormalities of development induced by decreased potassium. *Science* **181**, 1178–1179.

CURGY, J. J. (1968). Influence du mode de fixation sur la possibilité d'observer des structures myéliniques dans les hépatocytes d'embryons de poulet. *Journal de Microscopie* **7**, 63–80.

DAWD, D. S. & HINCHLIFFE, J. R. (1971). Cell death in the 'opaque patch' in the central mesenchyme of the developing chick limb: a cytological, cytochemical and electron microscopic analysis. *Journal of Embryology and Experimental Morphology* **26**, 401–424.

DE DUVE, C. (1964). Lysosomes and cell injury. In *Injury, Inflammation and Immunity* (ed. L. Thomas, J. Uhr and L. Grant), pp. 283–311. Baltimore: Williams & Wilkins.

GARCÍA-PORRERO, J. A. & OJEDA, J. L. (1975). Comportamiento de la fosfatasa alcalina, glucógeno y ácido ribonucleico en la poliquistosis renal experimental por corticoides. *Anales del Desarrollo* **47**, 77–82.

GLÜCKSMANN, A. (1951). Cell death in normal vertebrate ontogeny. *Biological Reviews* **26**, 59–86.

GOSSENS, C. L. & UNSWORTH, B. R. (1972). Evidence for a two-step mechanism operating during *in vitro* mouse kidney tubulogenesis. *Journal of Embryology and Experimental Morphology* **28**, 615–631.

GROBSTEIN, C. (1966). What we do not know about differentiation. *American Zoology* **6**, 89–95.

GROBSTEIN, C. (1967). Mechanisms of organogenetic tissue interaction. *National Cancer Institute* **26**, 279–299.

HUGHES, A. F. W. (1943). The histogenesis of the arteries of the chick embryo. *Journal of Anatomy* **77**, 266–287.

HURLÉ, J. M., LAFARGA, M. & OJEDA, J. L. (1977). Cytological and cytochemical studies of the bulbus of the chick embryo heart: phagocytosis by developing myocardial cells. *Journal of Embryology and Experimental Morphology* **41**, 161–173.

ILIES, A. (1971). La topographie et la dynamique des zones nécrotiques normales chez l'embryon humain de 11-30 mm. CR. V. Les zones nécrotiques des ebauches de l'appareil digestif, pulmonaire, cardiaque et de l'appareil uro-génital. Etude histologique et histochimique. *Revue roumaine d'embryologie et de cytologie* **8**, 55–66.

JOHNSTON, W. H., LATTA, H. & OSVALDO, L. (1973). Variations in glomerular ultrastructure in rat kidneys fixed by perfusion. *Journal of Ultrastructure Research* **45**, 149–167.

JURAND, A. (1968). The effect of hydrocortisone acetate on the development of mouse embryos. *Journal of Embryology and Experimental Morphology* **20**, 355–366.

KALLEN, B. (1965). Degeneration and regeneration in the vertebrate central nervous system during embryogenesis. In *Progress in Brain Research* **14**, pp. 77–96. Amsterdam: Elsevier Publishing Company.

KIRKMAN, H. (1943). The number and distribution of macrophages and fibroblasts in kidneys of albino rats with emphasis on twenty-five day males. *American Journal of Anatomy* **73**, 451–482.

LARSSON, L. (1975). Effects of different fixatives on the ultrastructure of developing proximal tubule in the rat kidney. *Journal of Ultrastructure Research* **51**, 140–151.

LEESON, T. S. (1961). An electron microscope study of the postnatal development of the hamster kidney. With particular reference to the intertubular tissue. *Laboratory Investigation* **10**, 466–480.

MENKES, B., SANDOR, S. & ILIES, A. (1970). Cell death in teratogenesis. In *Advances in Teratology* (ed. D. H. M. Woollam), pp. 169–215. London: Logos Press.

MORRISON, A. B. & PANNER, B. J. (1964). Lysosome induction in experimental potassium deficiency. *The American Journal of Pathology* **45**, 295–303.

MORRISON, A. B. & SCHNEEBERGER-KEELEY, E. E. (1969). The phagocytic role of renal medullary interstitial cells and the effect of potassium deficiency on this function. *Nephron* **6**, 584–597.

OJEDA, J. L., BARBOSA, E. & GÓMEZ-BOSQUE, P. (1972). Morphological analysis of renal polycystosis induced by corticoids. *Journal of Anatomy* **111**, 399–413.

OJEDA, J. L. (1974). *La muerte celular en la morfogénesis normal y patológica.* Secretariado de Publicaciones. Universidad de Santander.

OJEDA, J. L. & HURLÉ, J. M. (1975). Cell death during the formation of tubular heart of the chick embryo. *Journal of Embryology and Experimental Morphology* **33**, 523–534.

PERCY, D. H. (1975). Teratogenic effects of the pyrimidine analogues 5-iododeoxyuridine and cytosine arabinoside in late fetal mice and rats. *Teratology* **11**, 103–118.

PEREY, D. Y. E., HERDMAN, R. C. & GOOD, R. A. (1967). Polycystic renal disease: a new experimental model. *Science* **158**, 494–496.

RESNICK, J. S., BROWN, D. M. & VERNIER, R. L. (1973). Oxygen toxicity in fetal organ culture. I. The developing kidney. *Laboratory Investigation* **28**, 437–445.

REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology* **17**, 208–212.

ROMEN, W. & THOENES, W. (1970). Histiocytäre and fibrocytäre Eigenschaften der interstitiellen Zellen der Nierenrinde. *Virchows Archiv B* **5**, 365–375.

RUCH, J. V. (1969). Action d'un glucocorticoide sur l'embryon et les tissus embryonnaires. *Archives de Biologie* **1**, 108–112.

SAUNDERS, J. W. JR. (1966). Death in embryonic systems. *Science* **154**, 604–612.

SAUNDERS, J. W. JR. & FALLON, J. F. (1966). Cell death in morphogenesis. In *Major Problems in Developmental Biology* (ed. M. Locke), pp. 289–314. New York: Academic Press.

SAXEN, L. & RAPOLA, J. (1969). *Congenital Defects*, pp. 104–111. New York: Holt, Rinehart & Winston.

SCHULTZ, R. L. & CASE, N. M. (1970). A modified aldehyde perfusion technique for preventing certain artifacts in electron microscopy of the central nervous system. *Journal of Microscopy* **92**, 69–84.

SCHWEICHEL, J. U. & MERKER, H. J. (1973). The morphology of various types of cell death in prenatal tissues. *Teratology* **7**, 252–266.

WEISSMANN, G. (1975). The effects of steroids and drugs on lysosomes. In *Lysosomes in Biology and Pathology* vol 1, pp. 276–295. Amsterdam: North-Holland Publishing Company.

WIENER, J., LOUD, A. V., KIMBERG, D. V. & SPIRO, D. (1968). A quantitative description of cortisone-induced alterations in the ultrastructure of rat liver parenchymal cells. *Journal of Cell Biology* **37**, 47–58.