CELLULAR DIFFERENTIATION IN THE KIDNEYS OF NEWBORN MICE STUDIED WITH THE ELECTRON MICROSCOPE*

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Plates 119 to 125

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Renal structure and function are incompletely developed at birth in a number of mammals, including the rat and man. Functional immaturity manifests itself as an inability on the part of the kidney to vary the volume and concentration of the urine (McCance, 1948). In the rat, renal function matures during the first few weeks after birth (Falk, 1955). Detailed studies of the structural changes in kidneys of newborn animals have been confined largely to the rat.

During the first few weeks after birth, the rat kidney grows as a result of the formation of new nephrons in the peripheral zone of the cortex (Kittelson, 1917; Arataki, 1926), and at the same time the cells of the kidney acquire their specific cytological characteristics. Stergios, in an unpublished thesis (1933), has described the development of the brush border in the proximal tubules and the formation of basal striations, the *Stäbchen* of Heidenhain (1874), in proximal and distal tubules. At birth the proximal tubules possess a homogeneous cuticular border which acquires the appearance of a brush border during the first 2 weeks after birth. The basal striations develop during this time by a process of alignment of the mitochondria, which were randomly arranged at birth, into rows perpendicular to the basement membrane. Between 4 and 9 days after birth, the brush border and basal striations achieve a state of organization which renders them birefringent (Olivecrona and Hillarp, 1949). Coincident with the development of the brush border, the cells of the proximal tubules acquire the ability to stain intravitally with trypan blue (Baxter and Yoffey, 1948). The alkaline phosphatase reaction and the periodic acid-Schiff staining properties of the brush border continue to differentiate for several months after birth in the mouse (Longley and Fisher, 1956)

Cytoplasmic droplets that stain with the periodic acid-Schiff technique occur in the proximal tubules of a variety of mammalian fetuses, including the rat and man (Davies, 1954). Their presence is associated with proteinuria, and both droplets and proteinuria disappear soon after birth in most animals. Similar droplets have been produced in the proximal tubules of adult rats by the administration of certain

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foreign and native proteins (Oliver, MacDowell, and Lee, 1954), and Davies has suggested that in the fetal kidney, the presence of droplets indicates the reabsorption of plasma proteins which have passed through immature and abnormally permeable glomeruli.

The cytogenesis of the urinary tubules in human fetuses was described by Policard in 1912. The process is similar to that in the rat, but is more nearly complete at birth. First, the proximal tubules acquire a homogeneous cuticular border. Later, granules or droplets accumulate in the cells of the proximal tubules by what Policard believed to be a transformation of mitochondria. Finally, the cuticular border becomes a brush border, and basal striations develop, first in the distal tubules and then in the proximal tubules.

We have studied cellular differentiation in the kidneys of Swiss albino mice during the first 2 weeks after birth, using the high resolution of the electron microscope.

Materials and Methods[1]

Swiss albino mice three to six months old were used as the source of adult kidneys, and were bred to obtain newborn mice. At about 20 days gestation age six litters were born spontaneously and two litters were delivered by Caesarean section and placed with foster mothers. Littermates were killed at intervals up to 2 weeks after birth. Sections from 32 newborn mice and about 20 adult mice have been examined. The mice were killed by decapitation. Within 2 minutes the kidneys were cut with razor blades into one millimeter cubes, and these pieces were immersed in fixative. Infant kidneys were fixed 1 hour and adult kidneys 2 hours in a mixture of 1 per cent osmium tetroxide and 1 per cent potassium dichromate adjusted to pH 7.2 (Dalton, 1955). The tissues were washed 15 minutes in tap water, dehydrated in ethanol over a period of 1 hour, and imbedded in a mixture of methyl and *n*-butyl methacrylate, polymerized at 45°C. Thin sections were cut using glass knives and either a modified Minot microtome (Dempsey and Lansing, 1953) or a microtome designed by Porter and Blum (1953). They were examined in an RCA EMU 2E electron microscope with a 40 micron objective aperture. Sections 2 microns thick were examined by phase contrast microscopy and after staining with the periodic acid-Schiff technique (Lillie, 1954). The appearance of cells in the electron microscope was compared with the appearance of the same cells in adjacent thick sections studied by light microscopy.

OBSERVATIONS

During the first 2 weeks of postnatal life the kidneys grew to more than twice their size at birth, and changed from soft, pale, translucent organs to firm, red, opaque kidneys similar to those in adult mice except in size. At term the bladders contained very little urine, but from 4 hours after birth onward, most of the mice possessed bladders distended with urine and stomachs filled with milk. Microscopic studies were confined to the renal cortex. Because of poor peripheral fixation in the blocks of tissue, the subcapsular cortex, in which the formation of new nephrons occurs, was not examined. The microscopic appearance of the deeper layers of the cortex changed progressively with age

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until 2 weeks after birth. At that age, the kidneys were indistinguishable histologically from those of adult mice. Very little variation in morphology was observed in comparing mice of the same age, regardless of the manner of their birth.

The kidneys of adult mice and rats have been examined in the electron microscope by Dalton (1951), Sjöstrand and Rhodin (1953), Rhodin (1954), Pease (1955 *a*, *b*, *c*), and others. The fine structure of the renal cortex appears to be similar in the two species, and our observations on the kidneys of adult mice are in accord with the findings already reported (Figs. 1 and 2). In addition, we have observed dark cells scattered along the collecting ducts. These cells were described by Schachowa in 1876. They contain metachromatic granules (Oliver, 1945) and are strongly autofluorescent (Sjöstrand, 1945). Yoshimura and Nemoto (1953) have suggested that they produce an apocrine secretion. In the electron microscope, both the nuclei and cytoplasm of the dark cells appear denser than do those of neighboring light cells. The cytoplasm of the dark cells is filled with small elliptical vesicles and the apical cell membranes bear a number of long microvilli (Fig. 3). Cytoplasmic vesicles and long microvilli may also be found in light cells. In fact, the cells of the cortical collecting ducts form a continuous array, ranging from light cells with few vesicles and microvilli through light cells with many vesicles and microvilli to dark cells with many vesicles and microvilli. Light and dark cells may represent, therefore, different functional states of a single cell type.

The kidneys of newborn mice differ from those of adult mice in being less densely filled with structures visible by light and electron microscopy. In sections of the kidneys of adult mice, the tubules and glomeruli lie in close apposition, the cells of the tubules are filled with dense membranous structures, and the lumina of the proximal tubules are collapsed, under our conditions of fixation (Figs. 1 and 2). In newborn mice the tubules and glomeruli are less closely apposed. the cells of the tubules contain fewer membranous structures, and the lumina of the proximal tubules are patent (Figs. 4 to 6). On the other hand, the kidneys of newborn mice possess certain structures in greater abundance than do those of adult mice. These include the small cytoplasmic granules described by Palade (1955) and thought to contain ribonucleoprotein (Palade and Siekovitz, 1956), irregular dense cytoplasmic inclusions presumed to be lipide, and large round bodies in cells of the proximal tubules (Figs. 5 and 9). The following is a detailed description of the changes in structure by which these differences disappear during the first 2 weeks after birth.

The stroma, at birth, is a loose connective tissue containing stellate cells, a few collagen fibers, and capillaries with narrow lumina and thick endothelium. During the first 2 weeks after birth, the capillaries dilate to fill the interstices between parenchymal elements, the endothelial cells become thinner and the connective tissue is compressed into a narrow space (Figs. 1, 4, 8, and 14).

The glomeruli in the deeper layers of the cortex appear to be fully differentiated at birth, but are slightly smaller than those of adult mice (Figs. 4 and 8). During the first 2 weeks after birth, they enlarge gradually by dilation of the capillaries, and at the same time, endothelial and visceral epithelial cells become attenuated.

The juxtaglomerular cells of adult rats have been examined in the electron microscope by Hartroft (1956), and we have observed similar cells in the kidneys of adult mice. We have not searched for them in newborn mice but by chance observed apparently well developed juxtaglomerular cells in one of the mice killed 1 day after birth.

Some cortical tubules in infant mice appear to be relatively undifferentiated. They contain few mitochondria and the cell membranes are simple in contour, so that it is difficult to determine to what portion of the nephron they belong. Other tubules possess, in varying degree, specialized structures which identify them as proximal tubules, distal tubules, or collecting ducts. Tubules identifiable as the thin segment of Henle's loop have not been found in the sections examined by us, although Pease (1955 *c*) has observed them in the rat.

All of the tubules observed in the kidneys of newborn mice possess certain features in common with the tubules of adult mice. Nuclei and nucleoli are uniform in appearance, a basement membrane surrounds each tubule, and a terminal bar is present at the apical end of each junction between adjacent tubule cells (Figs. 4 and 10). The terminal bar consists of a local thickening and increased density of the apposed cell membranes and resembles terminal bars in other epithelia (the adhesion plates of Palade and Porter, 1954; Weiss, 1955 *a*). Terminal bars also resemble the intercellular bridges of skin (Porter, 1954), the intercalated discs of cardiac muscle (Sjöstrand and Andersson, 1954), the adhesion plates between neuroglial cells (Schultz, Berkowitz, and Pease, 1956), and synaptic membrane junctions in the nervous system (De Robertis and Franchi, 1956; Luse, 1956). Each of these sites of cellular juxtaposition is characterized by thickening and increased density of the apposed cell membranes.

The renal tubules of newborn mice differ from those of adult mice in the structure of the mitochondria, the abundance of the cytoplasmic granules of Palade, and the presence of lipide inclusions. At birth, the mitochondria are sparse and small. They contain little homogeneous matrix and completely lack the small, very dense granules frequently found in mitochondria in adult animals (Fig. 5). The granules first appear in the mitochondria within 8 hours after birth. During the first 2 weeks after birth the mitochondria gradually increase in number, grow larger and more rod-like, and accumulate increasing concentrations of homogeneous matrix material. The small cytoplasmic granules of Palade are, for the most part, not associated with ergastoplasmic membranes. They are most abundant in partially differentiated cells (Fig. 9) and least abundant in fully differentiated cells (Fig. 2). Dense, irregular, cytoplasmic inclusions, presumed to be lipide, have been observed in cells of the renal tubules, connective tissue cells, and the parietal cells of Bowman's capsule (Fig. 14). They disappear from the kidney between 3 days and 2 weeks after birth.

The proximal tubules of newborn mice may be identified by the presence of small canals and large vacuoles in the apical cytoplasm similar to those found in the cells of the proximal tubules in adult mice (Fig. 5). The vacuoles and canals may occur

in cells that possess no other indications of differentiation, and probably are the first specialized structures to form in the proximal tubules. As noted in adult mice by Rhodin (1954), the small canals in the apical cytoplasm often appear to be continuous with the apical cell membrane or with the membrane bounding a large vacuole. The walls of the small canals often appear thicker and denser than the cell membrane, as if some amorphous material were adsorbed to their inner surfaces. The vacuoles contain an amorphous material in varying concentrations. In addition, they may contain dense inclusions the size of mitochondria, as well as what appear to be sections of small canals (Figs. 6 and 9). At birth, the apical cell membranes bear variable numbers of scattered, irregular microvilli. The mitochondria are randomly oriented, and the basal cell membranes are relatively simple in contour. Large round cytoplasmic bodies which stain with the periodic acid-Schiff technique are present in many cells of the proximal tubules (Figs. 4 and 8). They resemble the large vacuoles of the apical cytoplasm in that they, too, contain an amorphous material, dense inclusions the size of mitochondria, and what appear to be sections of small canals (Figs. 5, 6, and 10). The large bodies differ from the vacuoles in containing greater concentrations of amorphous material. The dense inclusions which are found within the large bodies and vacuoles may consist of concentric dense lamellae (Figs. 7, 11, and 12), or they may possess the structural characteristics of mitochondria (Fig. 12). The membranes bounding the large round bodies may be single or double. In places they appear to be discontinuous, and the interface between the cytoplasm and the amorphous contents of a body may not be sharp (Fig. 11). Mitochondria occur close alongside the bodies and may appear to be connected with them by membranes (Fig. 11).

During the first 2 weeks after birth, the brush border develops in the proximal tubules as a result of the accumulation of microvilli along the apical cell margins (Figs. 5, 10, and 15). The large round cytoplasmic bodies which stain with the periodic acid-Schiff technique increase in abundance during the first day after birth and decline in number thereafter. Seven days after birth these bodies are smaller in size (Fig. 16), and persist into adult life as dense cytoplasmic inclusions the size of mitochondria, as noted by Rhodin (1954). In adult mice, they are found not only in cells of the proximal tubules, but in cells of the distal tubules and collecting ducts as well. They have also been observed within the lumen in proximal tubules. In appearance, some of these bodies are suggestive of mitochondria altered by the accumulation of a dense or osmiophilic material (Figs. 17 and 18).

Basal striations develop earlier and reach a higher state of organization in the distal tubules than they do in the proximal tubules, but the process of development appears to be similar in the two sites. At birth in most proximal and distal tubule cells, the basal cell membranes are simple in contour, but the lateral cell membranes may be tortuous and interlock with the membranes of adjacent cells (Fig. 10). The mitochondria may be aligned alongside the lateral cell membranes, but otherwise they are randomly oriented. In restricted regions of both proximal and distal tubules, at birth, this arrangement is replaced by one in which groups of mitochondria are aligned perpendicular to the basement membrane and are separated from one another by paired extensions of the cell membranes (Fig. 13). Many of the paired cell membranes may be followed as far as the base of the brush border, where terminal bars

are found, indicating that these are lateral cell membranes between adjacent cells, rather than inflections of the basal cell membranes. Apparently, the cells in these areas are fluted along an axis perpendicular to the basement membrane; and the formation of basal striations, which begins before birth and continues for 2 weeks after birth, appears to be the result of progressive fluting and interlocking of adjacent cells.

The distal tubules of both newborn and adult mice are lined by tall narrow cells with no apparent specialization of the apical cytoplasm. The development of basal striations has been described above.

The cortical collecting ducts in both newborn and adult mice are lined by a low epithelium surrounding a large lumen. The mitochondria are small and arranged at random. At birth the basal cell membranes are smooth except for the areas of junction between adjacent cells, where a variable degree of pleating of the basal cell membranes occurs. We have not observed dark cells in the collecting ducts at birth, but some of the cells contain greater numbers of cytoplasmic vesicles and apical microvilli than others. Dark cells appear between 8 hours and 1 day after birth. During the first 3 days after birth the basal cell membranes of all the cells become tightly pleated, and the collecting ducts resemble those of adult mice.

DISCUSSION

The kidneys of mice and rats grow and differentiate during the first 2 weeks after birth. In the rat, postnatal growth of the kidney is the result of cellular proliferation in the outer neogenic zone of the cortex (see Baxter and Yoffey, 1948). In the present study, poor fixation prevented examination of the peripheral region of the renal cortex in the mouse, so that neither the formation of new nephrons by cellular proliferation nor the differentiation of new glomeruli was observed. The glomeruli in the deeper layers of the cortex appeared to be fully differentiated at birth. The changes in structure of the mouse kidney which were observed in the present study occurred chiefly in the urinary tubules and in the peritubular capillary network. The peritubular capillaries establish close contact with the tubules during the first 2 weeks after birth, by dilation of the capillaries and thinning of the endothelium. The cells of the renal tubules differentiate by acquiring specialized membranous components, and at the same time, certain cytoplasmic constituents are lost.

A relatively undifferentiated cell from a renal tubule of a newborn mouse looks simple and uncluttered in the electron microscope. The cell membrane is simple in contour, and the cytoplasm contains relatively few membranous sacs of the kinds associated with the names endoplasmic reticulum (Porter, 1953; Palade and Porter, 1954; Palade, 1956), ergastoplasm (Weiss, 1953), and Golgi complex (Dalton and Felix, 1953). The mitochondria are small and sparse. They contain little homogeneous matrix and completely lack the small, very dense granules found in mitochondria in adult animals. In contrast to this general deficiency of visible structure, undifferentiated cells possess an abundance of the small cytoplasmic granules described by Palade (1955),

and thought to contain ribonucleoprotein (Palade and Siekovitz, 1956). These granules are most numerous in partially differentiated cells and decrease in abundance as the cells of the tubules become fully differentiated. The inverse relationship between cytoplasmic nucleic acid and differentiation has been examined quantitatively in developing erythrocytes by Thorell (1947), who, as a result of this work, proposed that ribonucleic acid takes part in the synthesis of the protein necessary for differentiation.

Some of the products of renal cellular differentiation are elaborations of the cell membrane. The apical microvilli, found in all types of renal tubules, are projections of the apical cell membrane, and the brush border of the proximal tubules is formed of closely crowded microvilli. The vacuoles and small canals found in the cells of the proximal tubules frequently appear to be continuous with the apical cell membrane. Perhaps they represent invaginations of the cell membrane, formed by a process similar to pinocytosis (Lewis, 1931). The basal striations in proximal and distal tubules are bounded by extensions of cell membranes. Heidenhain (1874), Zimmerman (1911), and Graaflin and Foote (1939) have described the cells as being deeply fluted in regions of the urinary tubules which possess basal striations. It appears, from the observations of the present study, that the basal striations develop by progressive fluting and interlocking of adjacent cells. A final example of elaborate cell membranes may be found in the tightly pleated basal cell membranes of the cells in the collecting ducts.

Large round cytoplasmic bodies which stain with the periodic acid-Schiff technique occur in the proximal tubules of the fetal metanephros in a number of mammals, including man (Davies, 1954; see also Policard, 1912). They resemble the cytoplasmic droplets which can be induced in the proximal tubules of adult rats by the administration of certain foreign and native proteins (Oliver, MacDowell, and Lee, 1954). Rhodin (1954) and Miller and Sitte (1955) have used the electron microscope to examine protein absorption droplets in the kidneys of adult mice and rats. In both species, they appear to consist of an amorphous material containing altered mitochondria. The large round bodies, found in the cells of the proximal tubules of infant mice in the present study, stain with the periodic acid-Schiff technique and resemble protein absorption droplets in both the light and electron microscopes. Davies has suggested that the droplets in fetal kidneys represent protein which has passed through abnormally permeable glomeruli. If this contention is correct, the presence of these bodies in newborn mice may indicate a functional immaturity of glomeruli which appear fully differentiated in structure.

The large cytoplasmic bodies in the proximal tubules of infant mice possess a number of features in common with the large vacuoles found in the apical cytoplasm of the same cells. The bodies and vacuoles are similar in size and are both surrounded by dense membranes. They both sometimes contain small

canalicular structures, dense lamellar inclusions, and altered mitochondria. They differ chiefly in their amorphous contents, which are more concentrated in the large bodies than in the vacuoles. These observations are consistent with the hypothesis that the large bodies represent vacuoles which have accumulated a high concentration of amorphous material. From the apparent continuity of the interior of the vacuoles with the lumen of the proximal tubule, by way of small canals, it may be inferred that the amorphous material concentrated in the vacuoles was derived from the contents of the tubular lumen by a process similar to pinocytosis. These suggestions are also consistent with the hypothesis that the bodies represent protein absorption droplets. The disappearance of the large bodies during the first 2 weeks after birth would, according to this interpretation, indicate the disappearance of the amorphous material from the contents of the tubular lumen, that is, from the glomerular filtrate. The observations that the membranes bounding the large bodies may be discontinuous, and that the interface between the cytoplasm and the contents of a body may not be sharp, lead us to suggest that the bodies are disposed of by dissolution in the cytoplasm.

The visceral epithelial cells of the yolk sac in the guinea pig are characterized by irregular microvillous projections of the apical cell membrane, small canals and vacuoles in the apical cytoplasm, and large dense cytoplasmic bodies (Dempsey, 1953). In these features, the visceral epithelium of the yolk sac resembles the proximal tubular epithelium of the newborn mouse kidney. It has been suggested that the yolk sac epithelium is involved in the absorption of a fluid which contains both dissolved and colloidal substances.

Mitochondria appear to be sites for the accumulation of a variety of substances. They segregate the cationic dye, Janus green, *in vivo* and *in vitro* (see Cowdry, 1918). They take up sodium and potassium cations *in vitro* (Bartley and Davies, 1954), and manganous cations *in vivo* (Cotzias and Maynard, 1956). The feeding of silver cations to rats results in the deposition of silver in the mitochondria of various tissues (Dempsey and Wislocki, 1955). Cationic cyanine dyes administered to dogs are concentrated in mitochondria in the kidney (Rennick, Kandel, and Peters, 1956). A dense or osmiophilic material accumulates in the mitochondria of the corneal epithelium in vitamin A–deficient mice (Sheldon and Zetterqvist, 1956). During the first 2 weeks after birth, mitochondria in the mouse kidney accumulate homogeneous matrix and acquire small, very dense granules. These observations prompt us to suggest that the dense bodies resembling mitochondria in Figs. 17 and 18 and perhaps even the concentrically lamellar bodies reported here (Figs. 7, 12, and 16) and described previously by Rhodin (1954), arise through the concentration of dense or osmiophilic substances within mitochondria. Bartley and Davies (1954) and Weiss (1955 *b*) have suggested that the active transport of certain cations may entail their segregation within mitochondria. Accordingly, mito-

chondrial segregation of substances may play a role in their active transport across the renal tubular epithelium, and the concentration of dense materials within mitochondria may be a measure of transport activity.

The wall of the renal tubule, through which materials pass back and forth between the lumen of the tubule and the blood, consists of a number of compartments separated by membranes. These include the cytoplasm of the epithelial cell, the space between the basal cell membrane and the basement membrane of the tubule, the space between the basement membrane of the tubule and the basement membrane of the capillary, the space between the basement membrane of the capillary and the endothelial cell, and perhaps the cytoplasm of the endothelial cell. In addition there are compartments within vacuoles, mitochondria, and nuclei through which materials might pass. Thus there are a number of interfaces or phase boundaries which may function in the selective transport of materials across the tubule wall. Several of the compartments, although they appear to be intracellular, are in a sense extracellular. These include pinocytosis vacuoles and collections of fluid between the paired cell membranes in the basal striations of proximal and distal tubules (Fig. 13).

SUMMARY

The structure of the kidney of the Swiss albino mouse changes progressively during the first 2 weeks after birth. Cells proliferate to form new nephrons, cells differentiate by acquiring specialized membranous components, and certain cytological features which are present at birth diminish in abundance or disappear. The differentiation of the cells of the cortical tubules has been studied using the light and electron microscopes. The tubules are partially and variably differentiated at birth. During the first 2 weeks after birth the brush border develops in the proximal tubules by the accumulation of numerous microvilli on the apical cell margins. Basal striations develop in proximal and distal tubules as an alignment of mitochondria, the result of what appears to be progressive interlocking of adjacent fluted cells. The mitochondria increase in number and size, accumulate homogeneous matrix, and acquire small, very dense granules. The collecting ducts develop tight pleating of the basal cell membranes, and dark cells containing numerous small cytoplasmic vesicles and microvilli appear. At birth there are dense irregular cytoplasmic inclusions presumed to be lipide in renal cells, the cytoplasmic granules of Palade are abundant, and there are large round bodies in the cells of the proximal tubules. The lipide inclusions disappear a few days after birth, and the cytoplasmic granules of Palade diminish in abundance as the cells differentiate. The large round bodies in the proximal tubules consist of an amorphous material and contain concentrically lamellar structures and mitochondria. They resemble the cytoplasmic droplets produced in the proximal tubules of adult rats and mice by the administration of proteins. The large round bodies disappear from

the proximal tubules of infant mice during the first week after birth, but the concentric lamellar structures may be found in adult mice.

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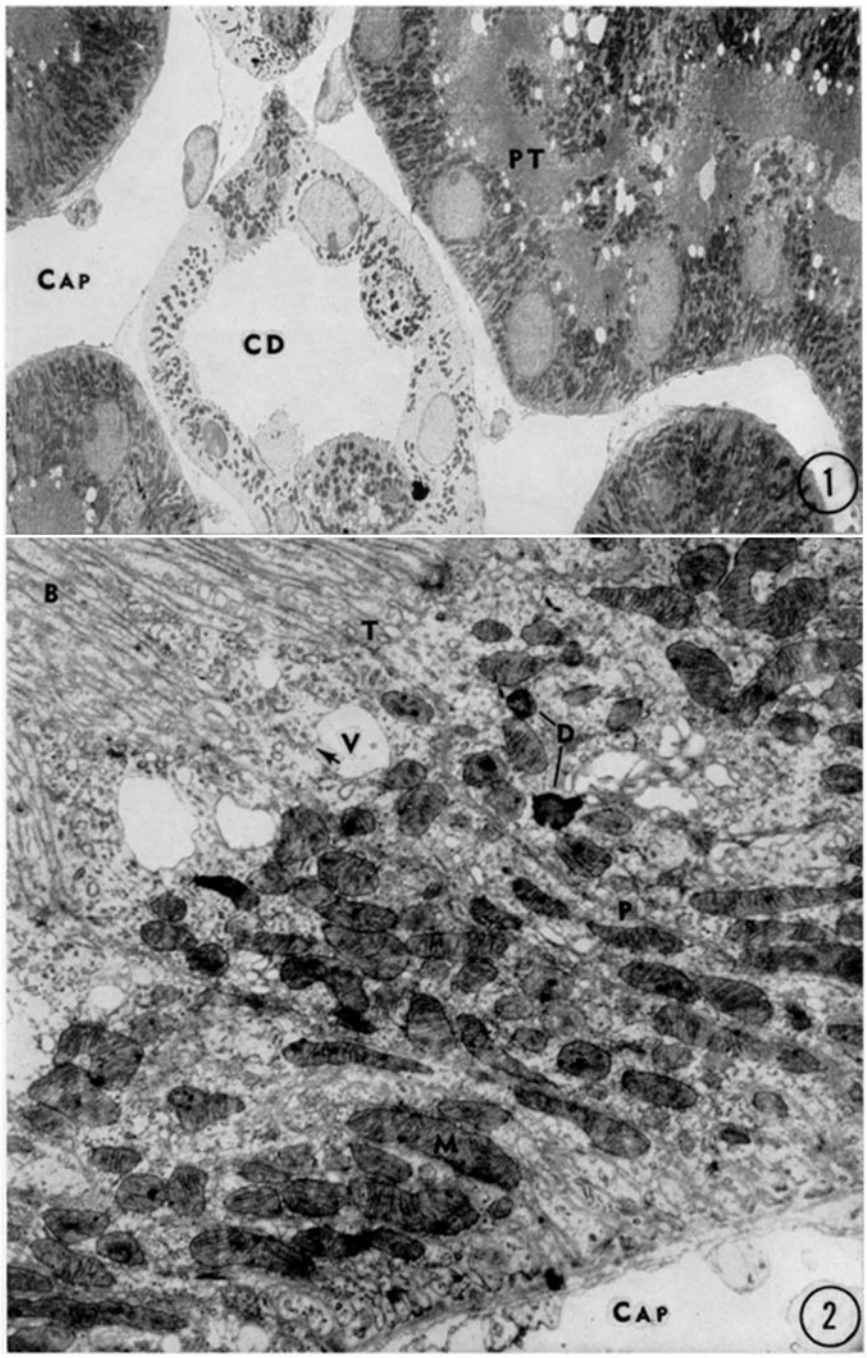
EXPLANATION OF PLATES

All photographs are electron micrographs of thin sections of kidneys from Swiss albino mice fixed in a mixture of osmium tetroxide and potassium dichromate, adjusted to pH 7.2.

PLATE 119

FIG. 1. Electron micrograph of a section from the kidney of an adult mouse. In this low power field proximal tubules (*PT*) and a collecting duct (*CD*) are shown. Under our conditions of fixation, the lumina of the proximal tubules are collapsed and the cells are dense. Large vacuoles are numerous in the apical cytoplasm beneath the brush border, and the bases of the cells are filled with rod-shaped mitochondria aligned perpendicular to the basement membrane. Collecting ducts are lined by a low epithelium surrounding a large lumen. The mitochondria are randomly oriented and dark cells are present. The peritubular capillaries (*Cap*) are large and closely applied to the tubules. × 1,200.

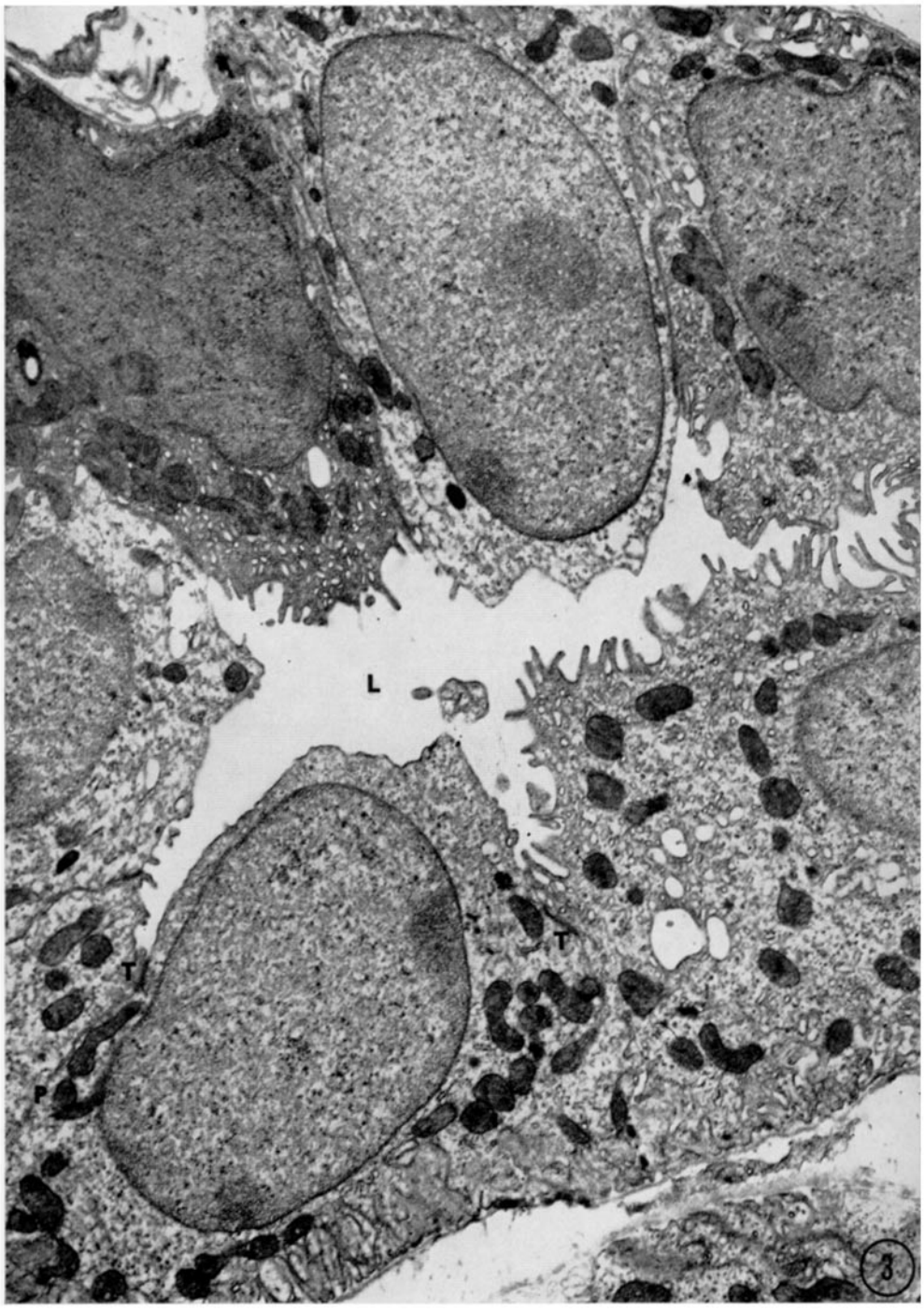
FIG. 2. The wall of a proximal tubule from an adult mouse. The brush border (*B*) is compact and the apical cytoplasm contains small canals and large vacuoles (*V*). Rod-shaped mitochondria (*M*) are numerous and are aligned more or less perpendicular to the basement membrane, with extensions of the cell membranes between them. The contents of the mitochondria include a relatively dense matrix and small, very dense granules. Several dense bodies (*D*), about the size of mitochondria, are present. A pair of lateral cell membranes (*P*), separating adjacent cells, may be followed as far as the brush border, where a terminal bar (*T*) occurs. The terminal bar is seen as a local thickening and increased density of the cell membranes. The small canals in the apical cytoplasm sometimes appear to be continuous with the membranes of the brush border. The membranes bounding the canals often appear to be thicker and denser than the cell membranes, as if some dense material were adsorbed to their inner surfaces. The vacuole marked *V* contains what appears to be a section of a small canal, and at the arrow the membrane bounding the vacuole appears to be continuous with a small canal in the apical cytoplasm. *Cap*: capillary lumen. × 13,000.



(Clark: Renal cellular differentiation)

PLATE 120

FIG. 3. A cortical collecting duct from an adult mouse. The lumen (*L*) is patent and the cells are not much taller than their nuclei. The mitochondria are sparse and randomly oriented. They contain relatively few small, dense granules. The basal cell membranes are pleated but do not enfold mitochondria. Lateral cell membranes (*P*) and terminal bars (*T*) are prominent. A dark cell, present in the upper left, contains numerous small, elliptical cytoplasmic vesicles and long microvilli on its apical margin. The two light cells on the right also contain small cytoplasmic vesicles and long microvilli on their apical margins. By examining numerous collecting ducts, a continuous array of cells can be found, ranging from light cells with few vesicles and microvilli through light cells with many vesicles and microvilli to dark cells with many vesicles and microvilli. × 20,000.



(Clark: Renal cellular differentiation)

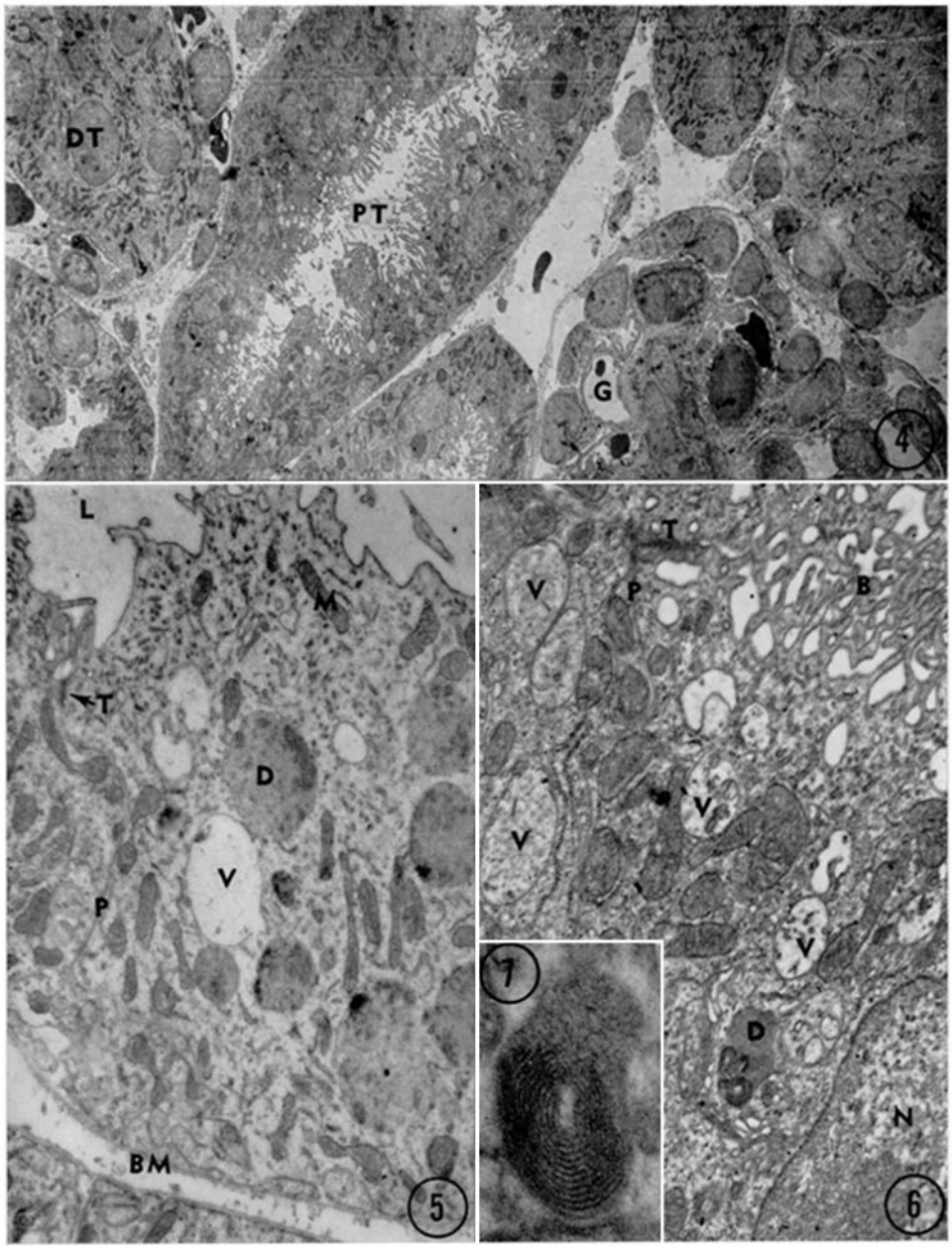
PLATE 121

FIG. 4. A low power field from the kidney of a fetus at term, containing a glomerulus (*G*), proximal tubules (*PT*), a distal tubule (*DT*), and some unclassified tubules. The glomerulus appears fully differentiated, but the endothelial cells and visceral epithelial cells are not as attenuated as those in the glomeruli of adult mice. In the proximal tubules the epithelium is low and the lumina are patent. The brush border is represented by scattered, irregular microvilli and the mitochondria are sparse and randomly oriented. In this field, the intertubular connective tissue is somewhat disrupted. × 1,200.

FIG. 5. The wall of a proximal tubule from a fetus at term. The lumen (*L*) is patent and the brush border is represented by only an occasional microvillus. The apical cell membrane appears to be denser and thicker than the cell membranes in other locations, and the apical cytoplasm contains small canals and vacuoles (*V*), similar to those in adult mice. Large round bodies of irregular density (*D*), bounded by membranes which may be discontinuous, are present in the cytoplasm, and the boundary between the bodies and the cytoplasm is indistinct in places. The mitochondria (*M*) are sparse and randomly oriented. They are poor in matrix and lack the small, very dense granules found in renal mitochondria in adult mice. The basal cell membranes are relatively simple in contour. A pair of lateral cell membranes (*P*), belonging to adjacent cells, end at the lumen in a terminal bar (*T*) and are tortuous in their basal portions. Mitochondria appear to be aligned along these lateral cell membranes. The basement membrane (*BM*) resembles those of adult mice. × 9,000.

FIG. 6. The wall of another proximal tubule at term. The brush border (*B*) exhibits more microvilli than that in Fig. 5. The vacuoles (*V*) in the apical cytoplasm contain membranous structures, including what appear to be small canals like those in the apical cytoplasm. The three vacuoles to the left contain an amorphous material. A large round body (*D*) containing denser ring-shaped structures is present near the nucleus (*N*). *T*, terminal bar; *P*, lateral cell membrane. × 20,000.

FIG. 7. Part of a cell from a proximal tubule at term. The dense body, similar to that shown at *D* in Fig. 6, is about the size of a mitochondrion and contains concentric dense lamellae. × 75,000.

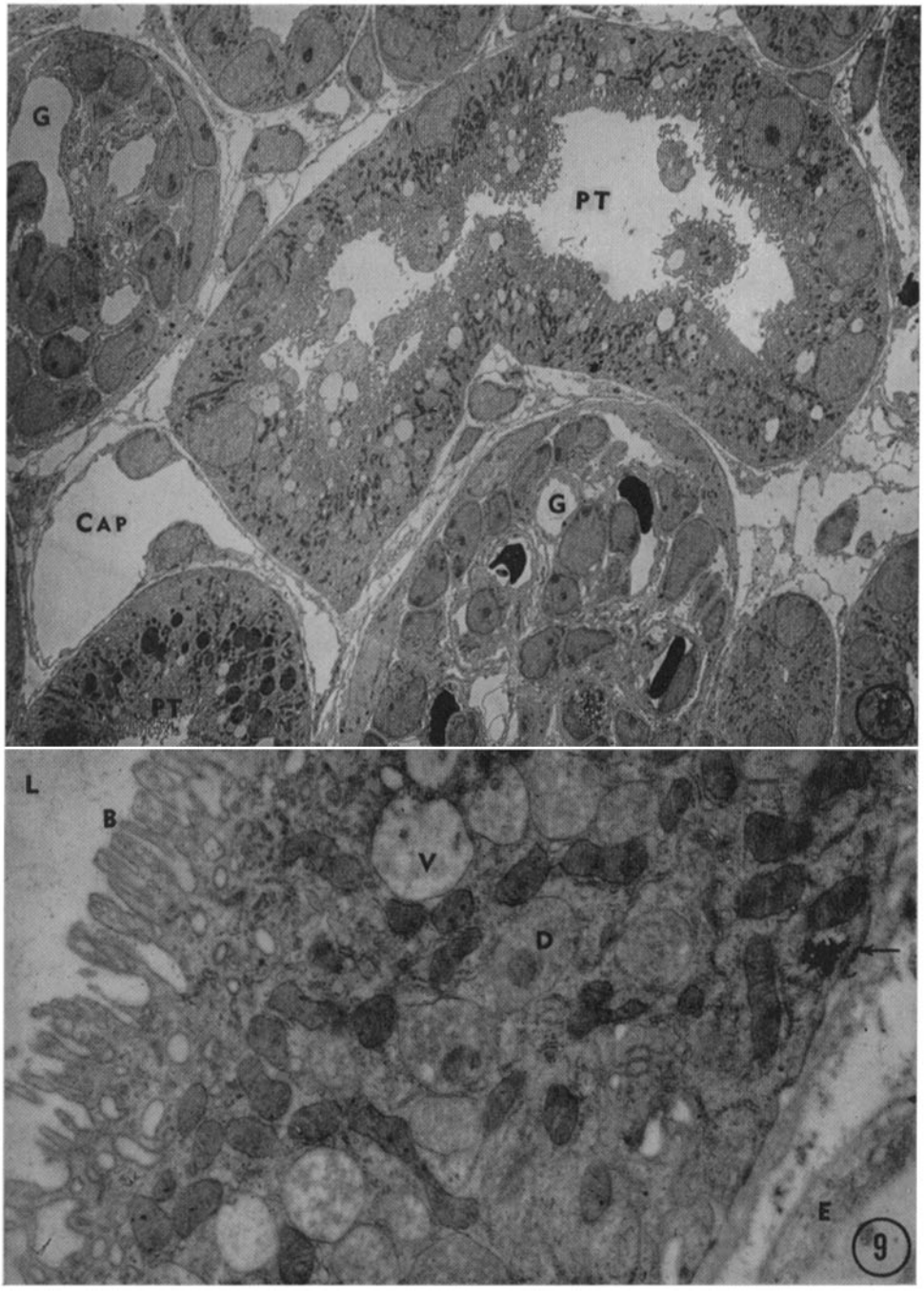


(Clark: Renal cellular differentiation)

PLATE 122

FIG. 8. A low power field from the kidney of a mouse killed 1 day after birth, containing glomeruli (*G*), proximal tubules (*PT*), and unclassified tubules. The glomeruli are similar to those in full term fetal mice. The chief difference between the proximal tubules 1 day after birth and those at term is the greater abundance of vacuoles and large round bodies in the cells 1 day after birth. The mitochondria are randomly oriented. *Cap:* capillary lumen. × 1,200.

FIG. 9. The wall of a proximal tubule 1 day after birth. The lumen (*L*) appears to contain a small quantity of amorphous material. The brush border (*B*) consists of a moderate concentration of irregular microvilli, and there are numerous vacuoles (*V*) of varying sizes in the apical cytoplasm. Large round bodies (*D*), containing varying concentrations of an amorphous material, are present throughout the cytoplasm. The mitochondria are randomly oriented but appear to be somewhat denser than those in full term fetal mice and contain small, very dense granules. The small cytoplasmic granules, described by Palade (1955) and thought to be ribonucleoprotein, are numerous but are not prominently associated with ergastoplasmic membranes. The basal cell membranes are relatively simple in contour, and at the arrow is a dense, irregular cytoplasmic inclusion presumed to be lipide. *E:* endothelial cell. × 15,000.

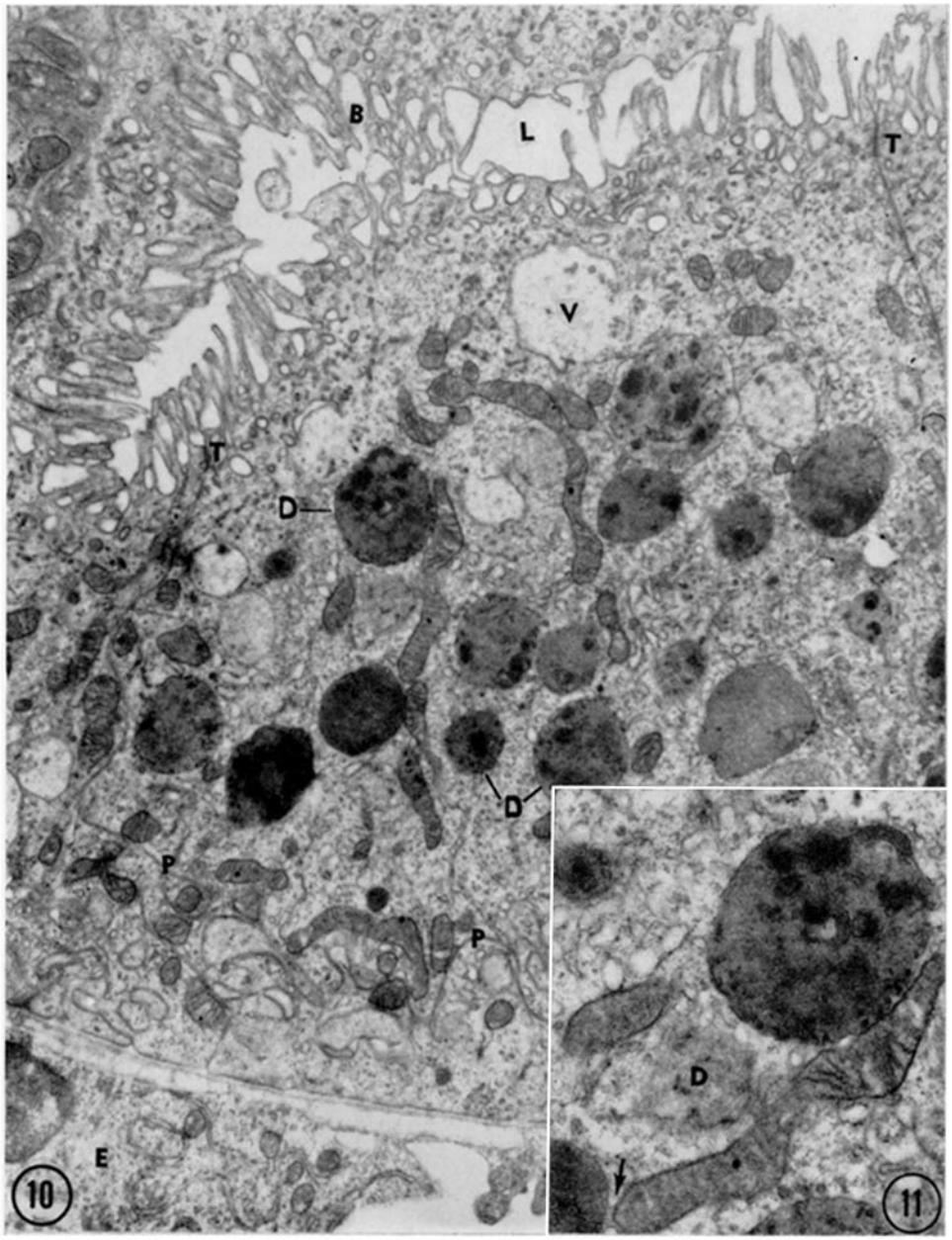


(Clark: Renal cellular differentiation)

PLATE 123

FIG. 10. A proximal tubule 1 day after birth. Numerous large round bodies of irregular density (*D*) are present in the cytoplasm. Several of them are closely associated with mitochondria. The basal cell membranes are relatively simple in contour, but the lateral cell membranes between adjacent cells (*P*) are tortuous in their basal portions and mitochondria appear to be aligned along them. *B:* brush border, *L:* lumen of tubule, *T:* terminal bar, *V:* vacuole. *E*, endothelial cell. × 10,000.

FIG. 11. An enlargement of one of the dense bodies shown in Fig. 10. The large round body in the upper right contains two series of concentric dense lamellae and its bounding membrane appears to be discontinuous. The round body marked *D* consists of an amorphous material indistinctly separated from the surrounding cytoplasm. Mitochondria are closely associated with these bodies, and one appears to be connected with the dense body in the lower left by membranes (arrow). × 20,000.

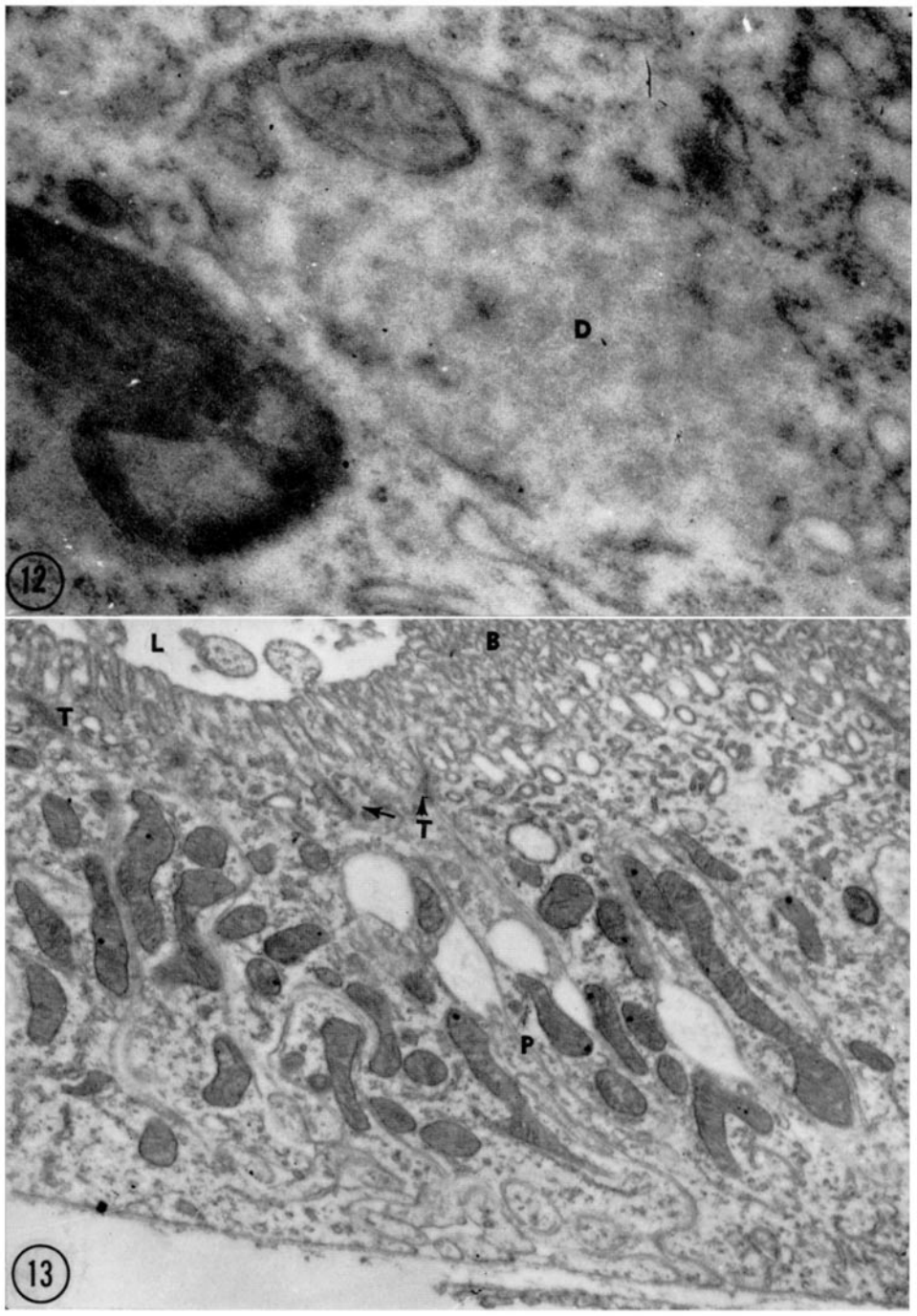


(Clark: Renal cellular differentiation)

PLATE 124

FIG. 12. Part of a proximal tubule cell 8 hours after birth. The field contains a large round body and part of a second one. The body on the left contains concentric dense lamellae. The one filling the center of the field and marked *D*, contains a mitochondrion. The membranes bounding this body are discontinuous, and the boundary between the amorphous interior of the body and the surrounding cytoplasm is indistinct. × 75,000.

FIG. 13. The wall of a proximal tubule 1 day after birth. In this region the wall of the tubule contains numerous rod-shaped mitochondria aligned more or less perpendicular to the basement membrane and separated from one another by extensions of the cell membranes, a condition resembling the basal striations found in the proximal and distal tubules of adult mice. Several of the paired extensions of cell membranes (*P*) may be followed as far as the base of the brush border (*B*), where they end in terminal bars (*T*). Therefore these paired membranes appear to be lateral cell membranes between adjacent cells rather than inflections of the basal cell membranes. The large vacuoles in this field lie between paired extensions of the cell membranes and are therefore extracellular. *L:* tubule lumen. × 12,000.



(Clark: Renal cellular differentiation)

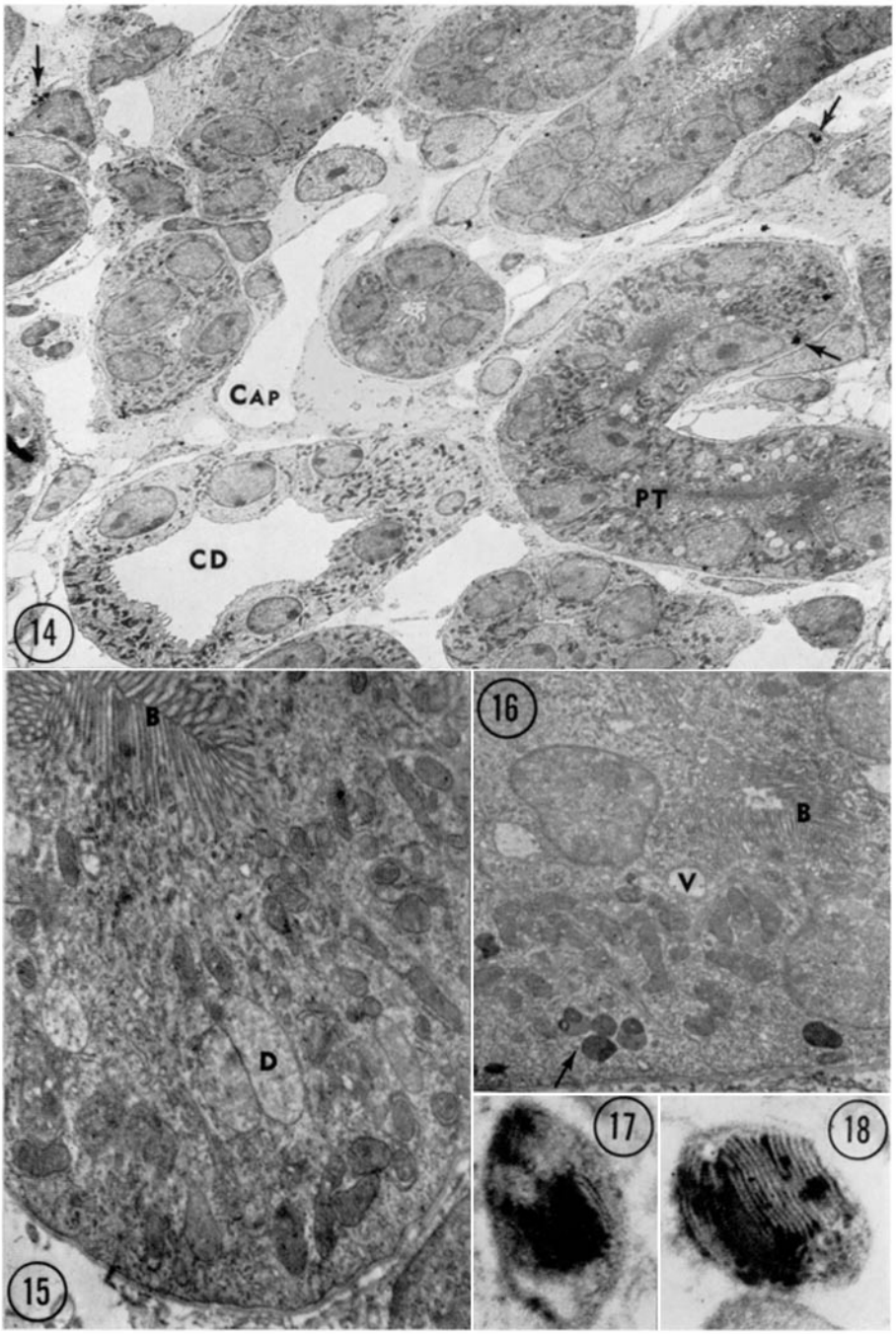
PLATE 125

FIG. 14. A low power field from the kidney of a mouse killed 3 days after birth. The lumen of the proximal tubule marked *PT* is collapsed but the mitochondria still appear to be randomly oriented. The collecting duct (*CD*) contains a cell slightly darker than its neighbors, with long microvilli along its luminal border. Cytoplasmic inclusions presumed to be lipide are present in cells of the tubules and connective tissue (arrows). The peritubular capillaries (*Cap*) do not fill the intertubular spaces as completely as they do in adult mice. × 1,200.

FIG. 15. The wall of a proximal tubule 3 days after birth. The lumen is collapsed and the brush border (*B*) consists of tightly packed microvilli. A few large round bodies containing low concentrations of amorphous material are present (*D*). The basal cell membranes are simple in contour and the mitochondria are randomly oriented. × 10,000.

FIG. 16. A proximal tubule 7 days after birth. A small, patent lumen is present and the brush border (*B*) consists of tightly packed microvilli. Round bodies of irregular density are present in the bases of the cells (arrow) but are smaller than the bodies found in younger mice. The denser, ring-shaped portions of these bodies consist of concentric lamellae which can be resolved by higher magnification. The basal cell membranes are simple in contour and the mitochondria are randomly oriented. *V:* vacuole. × 6,000.

FIGS. 17 and 18. Dense cytoplasmic bodies from cells in the cortical collecting ducts of adult mice. These bodies are the size of mitochondria, and in appearance, they are suggestive of mitochondria altered by the accumulation of a dense or osmiophilic material. × 54,000.



(Clark: Renal cellular differentiation)