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Histochemistry

The Postnatal Development of the Rat Kidney, with Special Reference to the Chemodifferentiation of the Proximal Tubule *

W.F. Neiss** and K.L. Klehn

Institute of Anatomy (Prof. Dr. T.H. Schiebler, Chairman) University of Würzburg, Koellikerstraße 6, D-8700 Würzburg, Federal Republic of Germany

Summary. New nephron anlages appear in the renal cortex up to the 4th postnatal day (PD). The last anlages to be formed develop into functional nephrons by PD 10, and the cortex appears mature at PD 12 after formation of the cortex corticis. The renal medulla develops by the longitudinal growth of loops of Henle and collecting ducts. The immature medulla cannot be divided into different zones and corresponds structurally to the later inner stripe of the outer zone. The inner zone is formed by PD 8, and the outer stripe of the outer zone by PD 12. The renal medulla is mature at PD 21.

From the start of its development, the renal proximal tubule consists of the pars convoluta and pars recta. In both parts the formation of the brush border is accompanied by the simultaneous appearance of brush border enzymes (alkaline phosphatase, γ-glutamyltranspeptidase, dipeptidylaminopeptidase IV) and lysosomal enzymes (acid phosphatase, acid β-galactosidase, N-acetylglucosaminidase, dipeptidylaminopeptidase II) over the full length of the proximal tubule. During the course of proximal tubule maturation, however, the lysosomal enzyme activities decline in the pars convoluta (with constant brush border enzyme activities), while the brush border enzyme activities increase in the pars recta (with constant lysosomal enzyme activities). The two parts further differ in that they exhibit different lysosomal patterns from the outset, the pars convoluta containing numerous large, highly enzyme-active lysosomes arranged in groups, and the pars recta containing only a few very small lysosomes with low enzyme activity. Thus, even in the newborn rat, the lysosomal pattern of the pars recta already corresponds to that of the mature S_3 segment. The S_1 and S_2 segments of the pars convoluta first differentiate between PD 10 and 21, as the groups of large lysosomes are progressively broken up and the extent of the lysosomal apparatus is diminished, this proceeding in a retrograde direction from the end of the immature pars convoluta.

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** To whom offprint requests should be sent

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Introduction

The development of the rat kidney is very complex. It is not surprising, therefore, that while many problems of renal development have been dealt with (Bargmann 1978, lit.), not all have been clarified. In the present investigation we are concerned with the question of whether relationships between morpho- and chemodifferentiation, especially in the proximal tubule, are demonstrable during postnatal development of the rat kidney. In exploring this question, we shall examine the differentiation of the tubule into segments (S_1, S_2 and S_3 after Maunsbach 1966), giving particular attention to the development of the S_3 segment, inasmuch as recent studies in adult rats (Zabel and Schiebler 1980; Daigeler 1981) have shown that this segment is functionally distinct from S_1 and S_2. In addition, we shall examine the development of more brush border enzymes and lysosomal enzymes than in previous studies (Mühlenfeld 1969; Zeller 1973), employing a differentiated histochemical technique (cf. Lojda et al. 1979). Special note is taken of the ontological relationship of these two enzyme groups in the renal proximal tubule. Finally, we shall consider the development of the other parts of the nephron and examine the maturation of the various kidney regions in respect to each other.

Material and Methods

The studies were performed on the kidneys of 340 Wistar rats of our own random bred closed colony. The animals were kept in standard Makrolon cages at 20° C (±2°) with light-dark changes every 12 h; standard Altromin® feed and tap water were given ad libitum.

All animals were decapitated between 8:00 and 9:00 a.m. under light ether anesthesia according to the following schedule: 2 pairs per day on postnatal day (PD) 1–3, 4 pairs per day on PD 4–9, 6 pairs per day on PD 10–30, 4 pairs on PD 35, 4 pairs on PD 40, and 10 pairs on PD 90.

Histochemistry. All histochemical investigations were performed on the right kidney. The removed organs were frozen on specimen holders in N_2-cooled propane with talc added (Winckler 1970b). Then cryostat sections were prepared at −25° C with a section thickness of 10 µm (cryostat: System Dittes-Duspiva); the specimens were sectioned parallel to the long axis of the renal papilla. Next the cryostat sections were freeze-dried in a Pearse-Edwards Speedivac Dryer under standardized conditions (Winckler 1970a). Several of the dried sections each were mounted on albuminized slides in a 0.5% celloidin solution. After air-drying, the histochemical enzyme demonstrations were performed (procedure of Lojda et al. 1979, unless otherwise indicated).

Brush Border Enzymes. Dipeptidylaminopeptidase IV (DAP IV; substrate: glycyl-prolyl-4-methoxy-2-naphthylamide); γ-glutamyltranspeptidase (γ-GT; substrate: γ-glutamyl-4-methoxy-2-naphthylamide); alkaline phosphatase (after Gomori, aPGO; substrate: 3% 2-glycerophosphate); alkaline phosphatase (aP; substrate: 5-Br-4-Cl-3-bromindoxylphosphate).

Lysosomal Enzymes. Acid phosphatase (sP, after Burstone, modified from Barka and Anderson 1962; substrate: naphthol-AS-TR-phosphate); β-N-acetylglucosaminidase (NaGase; substrate: naphthol-AS-BI-D-acetylglucosaminide); dipeptidylaminopeptidase II (DAP II; substrate: lysyl-alanyl-4-methoxy-2-naphthylamide); acid β-galactosidase (β-Gal; substrate: 5-Br-4-Cl-3-indocyl-β-D-galactoside).

Incubation Times. sP, NaGase, β-Gal, DAP II and DAP IV, 60 min, γ-GT, aPGO and aP, 20 min at 37° C. The preparations were postfixed for 24 h in formol-calcium, rinsed for 10 min with tap water, and covered with glycerine gelatin.

Histology. The histological studies were performed on the right kidney. Tissues were fixed in Bouin solution, embedded in paraffin and cut into sections 7 μm thick (parallel to the long axis of the renal papilla). Several sections each were mounted on albuminized slides and stained with azan stain.

Evaluation. An assessment was made 1) of the width of the brush border and intensity of the reaction in the brush borders of the proximal tubules and 2) of the position, relative size and intensity of the histochemical reaction of the lysosomes in the proximal tubule segments.

Nomenclature after Peter (1909) and von Möllendorff (1930). The term "medullary ray bundle" is taken from Heidenhain (1937). The proximal tubule is subdivided, into S_1, S_2 and S_3 segments according to Maunsbach (1966).

Results

Postnatal Day 1–4

Morphology

The kidney of the newborn rat consists of cortex and medulla. The renal cortex is organized into a subcapsular blastema which occupies about one-third of the cortical width, a layer of primitive nephrons in the midcortical third, and a layer of differentiated nephrons which forms the juxtamedullary third of the cortex. The medulla is subdivided into a central zone which is poor in connective tissue and corresponds essentially to the region of the papilla, and an outer zone which is adjacent to the cortex and contains abundant connective tissue. In continuation of this outer zone of the medulla, primitive medullary rays extend through the differentiated juxtamedullary and primitive midcortical layer of the cortex into the blastemic stripe.

Cortex. Up to PD 4 the subcapsular blastemic stripe is seen to contain undifferentiated nephrogenic mesenchyma, renal vesicles and S-shaped bodies, from which Bowman's capsule and all subsequent nephron segments develop, and the terminal ampullae of the collecting ducts with fork-like bifurcations. The primitive midcortical layer of the cortex contains numerous nephrons with hemispherical glomeruli and primitive tubules. In these primitive nephrons Bowman's capsule does not yet show a patent lumen; the podocytes form a continuous layer of cuboideal to columnar epithelium around the capillaries. The primitive tubules have a narrow lumen and a columnar epithelium with basally situated nuclei. It is not possible to distinguish between the proximal and distal convoluted tubules. In the third layer of the renal cortex, directly adjacent to the medulla, are a number of mature renal corpuscles (Fig. 1). The Bowman's capsules of these corpuscles display a broad lumen; the podocytes of the glomeruli already appear largely mature in the paraffin section. Proximal and distal tubules can be distinguished in this cortical region. The immature convoluted parts of the proximal tubules display a high columnar epithelium, prominently stained cell nuclei, and a narrow brush border. The convoluted parts of the distal tubules have a cuboideal epithelium with round-to-oval, centered nuclei and a cytoplasm which is more weakly stained than that of the proximal tubules (Fig. 1).

Medullary rays. In the medullary rays the collecting ducts and loops of Henle are arranged according to age, such that the ontogenetically oldest loops of Henle of the differentiated nephrons from the juxtamedullary cortical layer are located at the periphery of the medullary rays. The epithelium of the descending limbs of these loops resembles that of the pars convoluta of the proximal tubules, except that the cell height is somewhat smaller and the brush border is narrower and more weakly stained. The cell height and width of the brush border decrease continuously in a distal direction and the brush border finally disappears. The ascending limbs of these loops of Henle of the primitive nephrons form the midcortical layer. The epithelium of the collecting ducts is cuboideal and displays cells of varying staining intensity. The epithelium of the primitive Henle loops differs markedly from that of the Henle loops of differentiated nephrons. In these primitive loops, whose tip generally still lies in the medullary ray and has not yet reached the area of the medulla, ascending and descending limb are formed of a uniformly structured cuboideal epithelium. A brush border is not yet evident in the descending limb of these loops. One is struck by the fact that the shortest, youngest and apparently least mature loops of Henle are regularly located in closest proximity to the collecting ducts.

Medulla. In the *region of the papilla* of the renal medulla we find collecting ducts with a high columnar epithelium and numerous bifurcations, as well as the tips of the Henle loops of juxtamedullary nephrons. The descending limbs of these loops in the medulla consist of a low, strongly basophilic squamous epithelium. The turning points of these later long loops and the entire ascending limbs are formed from a cuboideal distal tubule epithelium similar to that seen in the medullary rays (Fig. 2). In this region of the future inner medullary zone, little connective tissue is present between the individual tubules. By contrast, the future outer medullary zone bordering the cortex contains an abundance of connective tissue. The cells of this loose connective tissue are arranged at right angles to the tubules like the rungs of a ladder. The tubules themselves form groups called "medullary ray bundles" (Heidenhain 1937). The same arrangement of Henle loops and collecting ducts is found in the medullary ray bundle as in the medullary ray (see above). A smooth transition occurs between the straight parts (partes rectae) of the proximal tubules of already differentiated nephrons and the immature descending limbs of Henle with their low squamous epithelium. A sharp boundary between inner and outer stripe does not occur within the primitive outer medullary zone, because the already differentiated proximal straight tubules extend for varying distances into the medulla, according to the varying ages of the Henle loops.

Histochemistry

In the newborn rat, high lysosomal and brush-border enzyme activities can be demonstrated in the proximal tubules of differentiated iuxtamedullary nephrons. At the same time, enzyme activities are nearly negligible in the subcapsular blastema and in the primitive nephrons of the midcortical layer. Only a few

small, weakly reacting lysosomes can be found there. They appear to be most common in the primitive glomeruli. Brush border enzymes are not observed at all in the blastema. Thin bands of reaction product are occasionally found in the layer of the primitive nephrons, indicating very weak brush-border enzyme activities on the luminal side of the epithelium of short tubule segments. These segments are regularly located directly at the boundary between the layers of primitive and differentiated nephrons. These weak brush border activities appear to reflect an incipient chemodifferentiation of the proximal tubule epithelium.

Brush Border Enzymes of the Proximal Tubules. The investigated brush border enzymes display a strictly parallel development. Alkaline phosphatase, γ-glutamyltranspeptidase and dipeptidylaminopeptidase IV always appear simultaneously in all nephrons. The activity distribution of these enzymes along the tubules is also similar for all nephrons within the differentiated juxtamedullary cortical layer. It is also interesting to note that the activities of all brush border enzymes appear to correlate directly with the width and color intensity of the brush borders demonstrable in the paraffin section. However, it is not yet possible at PD 4 to differentiate the S_1, S_2 and S_3 segments of the proximal tubule based on the activity distribution of the brush border enzymes, even in the differentiated nephrons. From the start of the pars convoluta (at Bowman's capsule) to the end of the pars recta (at its smooth junction with the primitive epithelium of the descending limb of Henle), the activity of each brush border enzyme remains approximately constant over the full length of the tubule. In the area of smooth transition between the pars recta and the immature descending limb of Henle, there is a steady decline in the activity of the brush border enzymes (Fig. 3), corresponding to the decrease in the width and color intensity of the brush borders in the paraffin section.

Lysosomal Enzymes of the Proximal Tubule. The activities of the individual lysosomal enzymes (sP, β-Gal, NaGase, DAP II) display a parallel development similar to that of the brush border enzymes. One gains the impression that the lysosomes in both the convoluted and straight parts of the proximal tubule in the rat always contain these enzymes in a specific activity ratio. The activity of β-Gal appears to be the highest in this regard, followed by DAP II, sP and NaGase. The simultaneous appearance of all investigated lysosomal enzymes appears to be closely coupled to the development of the brush border enzymes. In areas where brush border enzyme activities occur (and a brush border is morphologically evident), these lysosomal enzymes are also observed.

Unlike the brush border enzymes, however, the lysosomal enzymes provide a very early means of subdividing the proximal tubule into segments. The pars convoluta of the proximal tubule is filled with huge clusters of large to moderately large, highly enzyme-active lysosomes whose reaction product appears confluent in the light microscope (Fig. 4). This region corresponds to the later segments S_1 and S_2, which, however, cannot be distinguished from each other until PD 10.

The S_3 segment, on the other hand, can already be clearly distinguished. In the neonatal rat as well as later, there is a well-defined boundary between

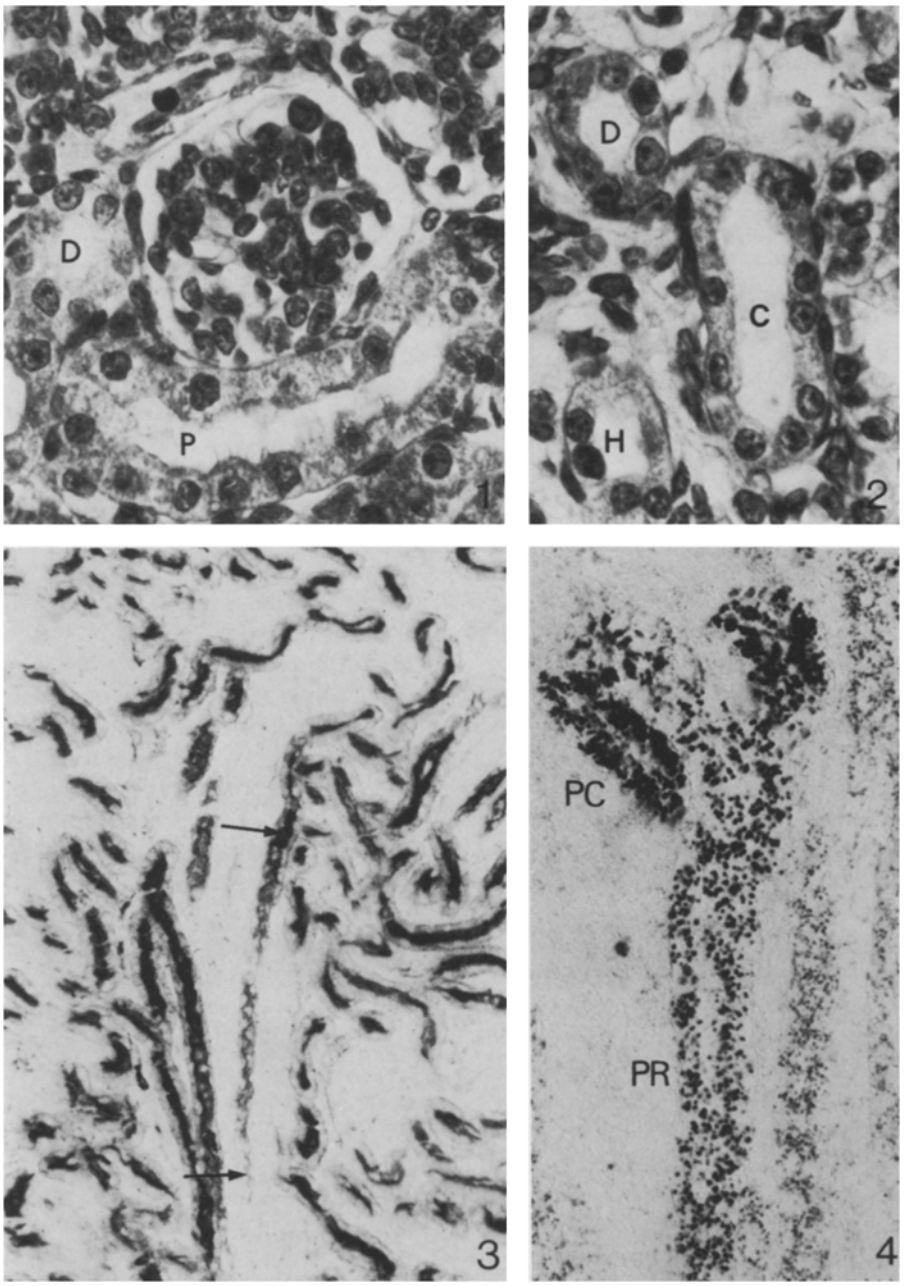

Fig. 1. Postnatal day 1. Glomerulus in the layer of differentiated nephrons. *P* = proximal tubule anlage, *D*, distal tubule anlage. Azan, × 560

Fig. 2. Postnatal day 1. Outer medullary zone. Medullary ray bundle with collecting duct (*C*), distal tubule (*D*) and immature descending limb of Henle (*H*). Azan, × 560

Fig. 3. Postnatal day 5. γ-Glutamyltranspeptidase. Medullary ray with immature partes rectae. Between the arrows there is a continuous transition from proximal tubule epithelium to primitive epithelium. × 140

Fig. 4. Postnatal day 1. Acid β-galactosidase. Pars convoluta ($S_1 + S_2$, *PC*) and pars recta (S_3, *PR*) of a juxtamedullary proximal tubule. × 350

the S_1+S_2 segment and S_3 at the point where the proximal tubule enters the medullary ray and the pars recta begins (Fig. 4). In the newborn and young rat the S_3 segment corresponds completely to the pars recta of the proximal tubule. Later on, this is no longer the case. From the outset, the lysosomal pattern of the pars recta is similar to that of the mature S_3 segment. Fewer lysosomes are present than in S_1+S_2, moreover, they are quite small and distributed uniformly in the cytoplasm. The total activity of the lysosomal enzymes is markedly lower in the S_3 segment than in S_1+S_2.

In contrast to its abrupt beginning, the transition of the S_3 segment to the low squamous epithelium of the immature descending limb of Henle is a continuous one. The tiny lysosomes decrease steadily in number until they disappear. It is interesting to note that this continuous transition from the S_3 segment to the descending limb of Henle is similar morphologically and histochemically (for both the lysosomal and brush border enzymes).

Differentiation. From PD 1 to 4 the layer of differentiated nephrons in the renal cortex enlarges as development proceeds. Once a proximal convoluted tubule is differentiated, the enzyme activity of its brush border remains more or less constant, whereas the enzyme activity of its lysosomes changes. A slight fall of lysosomal enzyme activity is observed by PD 4 in the segment S_1+S_2 (the differentiated, but not yet mature proximal convoluted tubule) of the oldest juxtamedullary nephrons. The younger convoluted parts, which have just differentiated and are in close proximity to the layer of primitive nephrons, display an appreciably higher lysosomal enzyme activity at PD 4 than the older partes convolutae in nephrons that have differentiated prenatally. During the course of the activity loss, the individual lysosomes in the pars convoluta become somewhat smaller, and the lysosomal clusters appear less dense. In the pars recta (S_3 segment), on the other hand, the pattern of lysosomal enzyme reactions remains unchanged during further development.

Postnatal Day 5–8

Morphology

Cortex. After the formation of new nephron anlages is completed by PD 4 and the subcapsular nephrogenic blastema has disappeared, the cortex still consists of 2 layers on PD 5 (Fig. 5): a narrow subcapsular layer with primitive nephrons and a broader midcortical and juxtamedullary layer containing differentiated nephrons. The last S-shaped bodies are observed in the subcapsular layer on PD 6. Through progressive differentiation of the primitive nephrons, the subcapsular primitive nephron layer becomes progressively narrower up to PD 8 and finally disappears.

Medulla. By PD 8 the medulla is clearly organized into an inner and outer zone, although the boundary between the two zones is not yet as distinct as in the mature kidney. The *inner zone* develops in the medullary substance of

the papilla. Here the thick epithelium of the long ascending loops of Henle gradually narrows and is transformed into the typical thin epithelium of the mature, thin ascending limbs.

The *outer zone* of the medulla is considerably less well developed than the inner zone between PD 5 and 8. The loops of Henle become much more numerous in the outer zone during this period, paralleled by the disappearance of the loose connective tissue "rungs" between the tubules. The differentiated proximal straight tubules also increase in number. As before, however, there is a smooth transition from the pars recta of the proximal tubule with its high cells and brush border to the immature descending limb of Henle with its low squamous epithelium. The oldest partes rectae are located between the medullary ray bundles and already extend into the outer zone for a distance corresponding to the extent of the later outer stripe of the outer medullary zone. The younger partes rectae of the nephrons from higher cortical layers are still appreciably shorter. Since the youngest and shortest proximal straight tubules are located in close proximity to the collecting ducts at the center of the medullary rays, while the older ones are arranged along the flanks of the medullary rays, the individual medullary ray acquires a "stepped" structure, appearing to mature progressively from the apex and sides toward the center and base.

Between the medullary ray bundles, which can still be distinguished in the outer zone of the medulla, the vascular bundles of the renal medulla are formed.

Histochemistry

With the disappearance of the blastemic zone and the displacement of the primitive nephronal layer toward the capsule, the differentiated cortical layer, which contains high brush border as well lysosomal enzyme activities, increases substantially in width.

Brush Border Enzymes. At PD 5 proximal tubules with strongly reacting brush borders are observed over about two-thirds of the cortical width. By PD 8 there is a further increase of reacting cortex; only in the subcapsular zone are there still a few proximal tubule segments with an extremely fine brush border.

In the medulla and medullary ray, the straight parts of the proximal tubules behave differently in the juxtamedullary and midcortical nephrons. The partes rectae of the older *juxtamedullary nephrons,* which lie in the primitive outer medullary zone between the medullary ray bundles, become tortuous, so that a longitudinal section through the medulla in the region of the later outer stripe of the outer zone cuts numerous tubules with strongly reacting brush borders at right angles. This increase of straight proximal tubule segments in the region of the outer stripe between the medullary ray bundles appears to take place mainly by an interstitial longitudinal growth of the partes rectae of the most mature proximal tubules of juxtamedullary nephrons. In contrast, the partes rectae of *midcortical nephrons* elongate by progressive differentiation of the primitive tubules. The immature epithelium of the descending limb of

the loop of Henle continuously forms new enzyme-active brush border in the medullary ray, doing so in a descending direction. As a result, the zone of continuous transition between the straight proximal tubule epithelium and the immature epithelium of the descending limb of Henle, in which the brush border looses its height and disappears, is gradually displaced in a medullary direction.

Lysosomal Enzymes. With the lysosomal enzymes, as with those of the brush border, there is a considerable increase of reacting tubule segments in the differentiating cortex. In every area where enzyme-active brush borders appear, highly enzyme-active lysosomes are simultaneously found. Whereas the lysosomal enzymes show a strong activity increase in the subcapsular cortex owing to the new formation of differentiated proximal tubule epithelium, there is a moderate to marked decrease of lysosomal enzyme activity in the juxtamedullary and midcortical regions, which is apparent as early as PD 3 and 4. It is evident by PD 5 in the juxtamedullary region and by PD 7 in the midcortical region that the previous confluence of the lysosomal reaction product is no longer present. The individual lysosomes become smaller, and the lysosomal clusters appear less densely packed. Up to PD 8 the segmentation of the proximal tubule corresponds to that of the first postnatal day. An S_1+S_2 segment can be distinguished in the pars convoluta and an S_3 segment in the pars recta of the immature proximal tubule. The most mature S_3 segments are particularly evident between the medullary ray bundles and exhibit relatively few lysosomes of very small size.

Postnatal Day 9–12

Morphology

Cortex. With maturation of the primitive nephrons, the primitive subcapsular layer disappears, and the renal cortex appears to take on a uniform structure. The renal corpuscles of all nephrons are now approximately spherical in shape. Bowman's capsule has expanded, and the capillary loops of the glomeruli are covered with podocytes which correspond light microscopically to those of the mature kidney. The convoluted parts of the proximal tubules of all nephrons possess a brush border from PD 9 on, and they differ markedly from the distal tubules. Only the brush borders of the youngest subcapsular proximal tubules are narrower and less intensely stained than that of the mature proximal tubules of the remaining cortex.

Medulla. From PD 10 on, the boundary between the inner and outer medullary zones is sharply defined. As in the adult rat, the boundary lies at the level of the fornix pelvis renalis.

In the *outer zone* of the medulla, the tissue density increases considerably by PD 12 as a result of growth of the youngest loops of Henle of the subcapsular nephrons. The loose connective tissue of the primitive outer zone disappears entirely. In particular, the medullary ray bundles become markedly thicker and come into close apposition at their edges. As a result, the visible subdivision

of the renal medulla into medullary ray bundles is lost, and the vascular bundles of the outer zone are densely embedded between the collecting ducts and the thick ascending limbs of the loops of Henle. In the region of the *inner stripe of the outer zone,* the thin descending limbs of Henle separate from the thick ascending limbs between PD 9 and 12, coming to lie at the periphery of the vascular bundles.

Maturation of the *outer stripe of the outer zone* progresses well. Straight proximal tubule epithelium develops from the primitive squamous epithelium of the immature Henle loops through a progressive, descending process of differentiation. As a result, the differentiated partes rectae of the proximal tubules of all nephrons elongate so far toward the medulla that most of them successively reach the boundary between the outer and inner stripe of the outer zone by PD 12. Since the partes rectae of the youngest subcapsular nephrons are located in the center of the medullary rays, the portions of the outer stripe lying below the centers of the medullary rays are the last to mature.

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Brush Border Enzymes. At PD 9 the convoluted parts of all proximal tubules display enzyme-active brush borders. These are somewhat sparser in the youngest tubules located directly beneath the renal capsule, but by PD 10 they attain the same level of activity as the brush borders of deeper cortical layers (Fig. 6).

In the medullary rays and outer stripe of the outer zone, the number of enzyme-active brush borders increases further by the progressive, descending differentiation of immature epithelium into straight proximal tubule epithelium. Since the loops of Henle in the medullary ray are arranged in stepped fashion according to their age, and the epithelium matures in a descending direction, it would appear that the brush borders develop from the apex of the medullary ray and from its flanks downward in the direction of the medulla (Fig. 6). Maturation of the brush borders is most advanced in the areas of the outer stripe lying between the medullary ray bundles below the base of the cortical labyrinth. By PD 12 it is evident that the continuous transitions between straight proximal tubule epithelium and the descending limb of Henle have disappeared in these areas of the developing outer stripe. The enzyme-active brush border terminates abruptly at the boundary between the outer and inner stripe of the outer zone. The same sharp change of epithelium is observed in the lysosomal enzymes. It is also noteworthy that from PD 12, the width of the brush border and reaction intensity of all investigated enzymes increases markedly in the partes rectae whose descending differentiation has reached the boundary of the inner stripe. As a result, it becomes possible to clearly distinguish the segments S_1+S_2 and S_3 in the proximal tubules of the juxtamedullary nephrons even on the basis of the brush border enzymes.

Lysosomal Enzymes. Starting on PD 11 or 12, the pars convoluta of the proximal tubule shows maturation with regard to the lysosomal enzymes. In the part of a proximal convoluted tubule distant from the glomerulus, the lysosomes

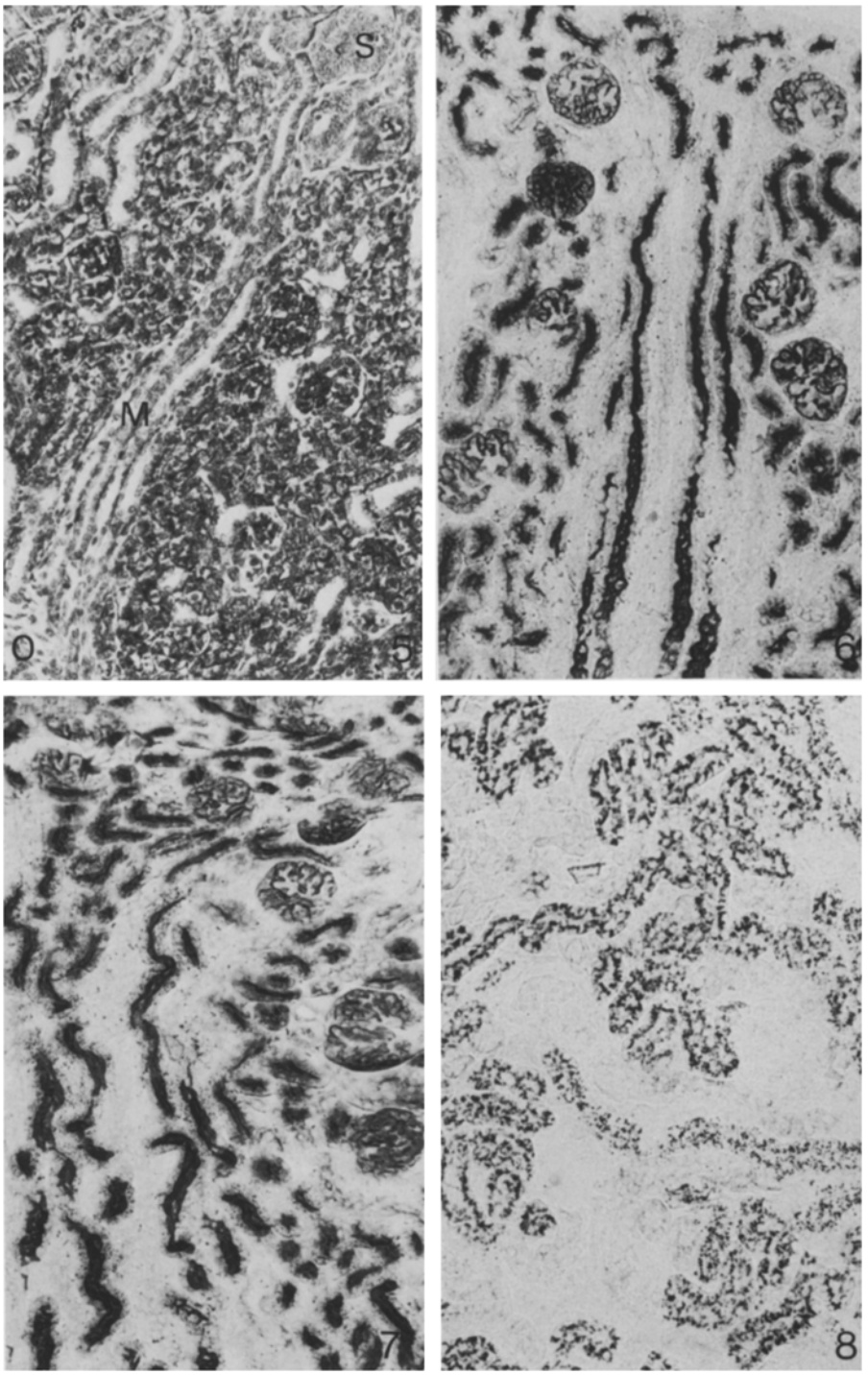

Fig. 5. Postnatal day 5. The renal cortex consists of a subcapsular layer (*S*) with primitive nephrons and a broad layer with differentiated nephrons. *M*, medullary ray, *O*, outer medullary zone. Azan, ×140

Fig. 6. Postnatal day 11. Dipeptidylaminopeptidase IV. Intensive reactions in the brush borders of the tubules of the entire renal cortex and numerous partes rectae of a medullary ray. ×140

Fig. 7. Postnatal day 15. Dipeptidylaminopeptidase IV. Medullary ray and outer stripe contain many partes rectae with strongly reacting brush borders. ×140

Fig. 8. Postnatal day 21. Acid β-galactosidase. Cortex with S_1 and S_2 segments. ×140

take up a palisade arrangement like that seen in the S_2 segment of adult rats (Maunsbach 1966). The individual lysosomes of these palisades appear to be of a rather uniform size. In the part of a proximal convoluted tubule close to the glomerulus (S_1 segment), on the other hand, we still observe the cluster-like arrangements of lysosomes that are typical of the immature S_1+S_2 segment. The S_2 segment appears to mature before the S_1 segment.

Postnatal Day 13–15

Morphology

Cortex. The volume of the renal cortex is increased by interstitial longitudinal growth of the tubules.The entire cortical layer increases in thickness, and tubule loops are displaced over the apices of the medullary rays, which by PD 12 extend up below the renal capsule. The result of this is the secondary development of the cortex corticis by PD 15 through growth displacement. This structure contains only proximal and distal convoluted tubules; apparently the glomeruli are not involved in such displacements.

Medulla. Only a few changes are evident in the renal medulla compared to prior observations. The transition from the straight proximal tubule epithelium to the thin epithelium of the descending limb of Henle is no longer continuous but abrupt, causing the boundary between the inner and outer stripe to become more clearly defined. The partes rectae of all proximal tubules in the medullary rays and in the outer stripe now display a high columnar epithelium and a wide, deeply stained brush border. In the proximal straight tubules of the older nephrons, the brush border is broader and more deeply stained than in the proximal convoluted tubules of the same nephrons.

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Brush Border Enzymes. The *cortex* appears to be mature at PD 13 with regard to the brush border enzymes. The brush borders of all partes convolutae of the cortical proximal tubules demonstrate approximately equal enzyme activity levels. Regional differences in the activity of the brush border enzymes are no longer apparent from one cortical layer to the next.

In the *medullary rays* and *outer stripe of the outer zone,* the descending differentiation of immature epithelium into straight proximal tubule epithelium reaches its conclusion. At PD 15 the epithelium of the descending limbs of all loops of Henle in the medullary ray and outer stripe displays enzyme-active brush borders (Fig. 7). In all nephrons there is now a sharp epithelial transition between the pars recta of the proximal tubule and the thin descending limb of Henle, and the outer stripe of the outer zone shows a distinct boundary with the inner stripe. The continuous transitions between the partes rectae and the descending limbs of Henle, which are the strict rule up to PD 4, are

no longer observed. The width and enzyme activity of the brush borders of the partes rectae of midcortical and subcapsular nephrons also increase markedly on PD 14 or 15, with the result that the S_3 segment is clearly defined in all nephrons during the demonstration of brush border enzymes.

Lysosomal Enzymes. At PD 13 the partes convolutae of the proximal tubules of all nephrons appear to react uniformly in terms of the lysosomal enzymes. The earlier activity differences between the convoluted parts of midcortical and subcapsular nephrons are no longer observed. S_2 segments with typical palisades are found throughout the cortex at PD 15. The S_1 segment is not yet as mature as S_2. While the immature cluster-type lysosomal pattern has for the most part disappeared (the lysosomes are somewhat larger than in S_2 and less uniformly distributed), the pattern is not yet identical to that in the adult rat. The S_3 segments appear mature throughout the kidney with respect to the lysosomal enzymes investigated. The lysosomes are less numerous in S_1 and S_2, are markedly smaller and show a diffuse distribution throughout the cell.

Postnatal Day 16–21

Morphology

With the appearance of a sharp boundary between the inner and outer stripe of the outer medullary zone and the development of the cortex corticis, the morphogenesis of the kidney is largely complete at PD 15. Thereafter the outer stripe undergoes some further development, expanding by interstitial longitudinal growth of the proximal straight tubules. This is accompanied by a considerable increase in the cell height of the epithelium and in the width and color intensity of the brush border in all proximal straight tubules. By PD 21 the brush border of the pars recta is in all nephrons significantly broader and more intensely stained than the brush border of the pars convoluta. As a result of this, the medullary rays and the outer stripe become clearly visible by PD 21, even to the unaided eye, thus forming the subcortical zone.

The renal structure does not change further after PD 21, although there is a uniform enlargement of all parts of the organ at least up to the 30th day of life.

Histochemistry

Brush Border Enzymes. The histochemical organization of the renal cortex and the outer stripe of the outer zone is for the most part complete by PD 15. From then until PD 21 there is a considerable increase in the length and enzyme activities of the S_3 segments. The outer stripe of the outer zone is broadened considerably by interstitial longitudinal growth of the partes rectae of all proximal tubules. This is accompanied by a powerful increase in the activity of

the brush border enzymes in all S_3 segments, especially in the case of γ-GT and DAP IV, and less so in the case of aP. The brush borders have also broadened. At PD 21 the brush border enzymes in the kidney have attained the distribution pattern characteristic of adult rats.

Lysosomal Enzymes. In the pars convoluta of the proximal tubule, the lysosomes of the S_1 segment increase markedly in size by PD 21 but are still smaller than in the adult animal (Fig. 8). In the S_3 segment the increase of brush border enzyme activities is not accompanied by a rise of lysosomal enzyme activities. This contrasts sharply with the first step in the neogenesis of the straight proximal tubule epithelium (see above), where the occurrence of the lysosomal enzymes and the brush border enzymes go hand in hand.

The organization of the proximal tubule into the S_1, S_2 and S_3 segments is essentially complete by PD 21. The S_3 segment is already fully mature. The S_1 and S_2 segments already display the typical mature lysosomal patterns from a relative standpoint, although the overall lysosomal apparatus of the cortex is not yet as complex as in the adult.

Discussion

The development of the rat kidney begins prenatally and extends through the third week of postnatal life. The cortex and medulla must be considered separately owing to their different modes of development. The development of the *renal cortex* is characterized by the appositional formation of new nephron anlages on the one hand, and by the interstitial growth and differentiation of existing nephron anlages on the other. In agreement with Larsson (1975) and Kazimierczak (1978), we find that a nephronogenic blastema is present only up to PD 4. We do not believe that the formation of new nephron anlages is possible beyond that stage. This conflicts with the reports of other authors (Arataki 1926; Baxter and Yoffey 1948; Boss et al. 1963; Speller and Moffat 1977), who have described the appearance of new nephron anlages beyond PD 4. According to our findings, however, the period after PD 4 is marked by the differentiation and maturation of the last nephron anlages that have already formed. This process continues until about PD 10. On this day we find that all investigated brush border enzymes demonstrate high activities in all the convoluted parts of the proximal tubules in the cortex, as Mühlenfeld (1969) has described for aP.

The *medulla* is ontogenetically younger than the cortex, maturing at a later time. It is noteworthy that the development of the inner zone of the renal medulla is complete before that of the outer zone (cf. Mühlenfeld 1969; Ricciarelli 1970; Speller and Moffat 1977). Initially the *inner zone* corresponds histologically to a primitive inner stripe, in which the loops of Henle of the juxtamedullary nephrons lie in close-packed arrangements beside the collecting ducts. The definitive inner zone develops as thin epithelium takes the place of thick epithelium in the distal tubule, doing so in an ascending direction from the papillary apex (cf. Ricciarelli 1970; Neiss 1981).

The primitive *outer medullary zone* differs from the developing inner zone

by its abundance of loose connective tissue arranged in a "ladder rung" pattern. Within this connective tissue the collecting ducts and loops of Henle form loose groups, called medullary ray bundles, which continue the medullary rays of the cortex into the medulla. The medullary ray bundles of the immature rat kidney bear a remarkable resemblance to the medullary ray bundles in the fetal human kidney (Heidenhain 1937). These bundles represent the organizing element of the immature outer zone. During the course of medullary maturation, they become so closely apposed that they are no longer distinguishable in the rat at PD 15.

Between PD 1 and 15 the *outer stripe* forms within the outer medullary zone by the neogenesis of straight proximal tubule epithelium. Before maturation of the outer stripe, all nephrons display a differentiated proximal tubule with a convoluted part and a short straight part and a differentiated distal tubule which are connected to each other by a primitive tubule segment with low squamous epithelium. This immature segment of the nephron initially forms the major portion of the descending limb of Henle's loop. Its presence explains why the cortex and inner medullary zone appear mature, while the outer zone located between them appears immature. The differentiation of the outer stripe is accomplished by the descending transformation of the primitive squamous epithelium of the descending limb of Henle in the medullary rays and later outer stripe into straight proximal tubule epithelium.

The *histogenesis and chemodifferentiation of the proximal tubule* follows a highly differentiated course. From the very outset, as soon as proximal tubule epithelium is present, a distinction can be made between the pars convoluta and pars recta by histochemical methods on the basis of variations in the lysosomal pattern. This is not possible by the light microscopic examination of stained paraffin sections. The pars convoluta is distinguished essentially by the presence of numerous large, highly enzyme-active lysosomes. In the pars recta, by contrast, the lysosomes are small, few in number, and have a low enzyme activity. Despite this difference, brush border enzymes and lysosomal enzymes can be demonstrated in both segments simultaneously with the appearance of brush border. However, this similar developmental pattern is limited to the phase in which the proximal tubule epithelium arises from primitive epithelium. Thereafter the pars convoluta and pars recta develop in different ways: the pars convoluta grows interstitially in length, while the pars recta develops through the descending differentiation of primitive epithelium. The activities of the lysosomal enzymes decrease in the pars convoluta (with no change in brush border enzyme activities), while the activities of the brush border enzymes increase in the pars recta (with no change in lysosomal enzyme activities). The functional significance of these observations remains unclear.

One could theorize that the simultaneous appearance of the brush border, brush border enzymes and lysosomal enzymes in the initial phase of epithelial differentiation marks the start of tubule epithelial function. This hypothesis is supported by the observations of Baxter and Yoffey (1948, trypan blue), Larsson and Maunsbach (1975, horseradish peroxidase) and Schaeverbeke and Cheignon (1980, horseradish peroxidase), who found that intravitally injected tracer is phagocytized in the pre- and postnatal rat kidney by all cells of the *pars convoluta* that possess a brush border.

The demonstrable enzyme activities of the proximal tubule epithelium are transformed during further development. Clusters of highly enzyme-active lysosomes appear over the full length of the newly formed pars convoluta (Davies 1954; Yoshimura and Nakamura 1965; Pugh 1967; Zeller 1973; our finding), so that by PD 10 there is no difference between the later S_1 and S_2 segments (Zeller 1973; our finding). Following a rapid fall of the lysosomal enzyme activities, the lysosomal clusters are progressively reduced and broken up by PD 21 in a process that proceeds in a retrograde direction from the distal end of the pars convoluta. The segments S_2 and S_1 differentiate in succession. Functionally, this peculiar development might be the result of a decreasing tubular protein load. Davies (1954) and Schaeverbeke and Cheignon (1980) have shown that the urine of fetal and neonatal rats contains considerably larger protein molecules in a very much higher concentration than the urine of older animals. According to the electron microscopic findings of Schaeverbeke and Cheignon (1980), this proteinuria is chiefly due to the increased protein permeability of the immature glomerular basement membrane.

The varying development of lysosomal and brush border enzymes (in both the pars convoluta and pars recta) is consistent with the fact that even in the mature kidney, the two enzyme groups have different tasks to perform. Thus, the brush border is associated with the splitting and direct reabsorption of "small linear peptides" (Carone et al. 1979, lit.), while the lysosomal apparatus is concerned with the degradation of larger, phagocytized proteins (Bargmann 1978, lit.).

Little is known about the development of the *pars recta* of the proximal tubule. Interestingly, we found that even the immature pars recta displays the typical lysosomal pattern of the S_3 segment, while only the mature pars recta possesses the very broad, enzyme-active brush border characteristic of the mature S_3 segment (Maunsbach 1966). Apparently immature S_3 segments occur even in the newborn rat (Fig. 4). Zeller (1973) did not observe S_3 segments before PD 10, but our findings indicate that the oldest S_3 segments are just reaching maturity at this time.

If we shift our attention from the individual differentiation of the proximal tubules to the collective development of all proximal tubules, we recognize the familiar pattern of inward-to-outward maturation of the renal cortex. In the newborn rat, differentiated proximal tubules with an enzyme-active brush border and lysosomal apparatus are found only in the cortical third nearest the medulla. By PD 5 they are found in the two-thirds nearest the medulla, and by PD 10 throughout the cortex.

The partes rectae of the proximal tubules also mature successively in the medullary rays and medulla. Owing to the age-dependent arrangement of the loops of Henle, the highly enzyme-active brush borders of the mature partes rectae appear to be arranged in stepped fashion in the medullary rays and medulla (Mühlenfeld 1969; our finding). Starting from the flanks of the medullary rays and descending from their apices, the medullary rays and outer stripe of the outer zone mature by PD 15. Ontogenically and probably as well functionally, the outer stripe of the outer zone is the youngest of all kidney regions.

In summary, it may be said that from the outset the S_3 segment possesses different cytochemical properties than the S_1 and S_2 segments, and also differs markedly from them in its further development. On the other hand, the S_1 and S_2 segments develop along essentially similar lines. This observation supplements experimental findings on the hormonal regulation of the renal proximal tubule (Schiebler et al. 1970; Zeller 1973; Schiebler and Danner 1978; Zabel and Schiebler 1980; Daigeler 1981), which indicate that too in the adult animal, the S_3 segment is quite distinct, both morphologically and functionally, from the S_1 and S_2 segments.

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