SHORT TITLE

THE CELL WEB IN THE EPITHELIAL CELLS OF
THE RAT KIDNEY ----- PEREIRA, G.

THE CELL WEB
IN THE EPITHELIAL CELLS OF THE RAT KIDNEY

by
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INTRODUCTION

Several recent light and electron microscopic investigations have focussed new attention upon the fibrous component of the cytoplasm of epithelial cells. A new staining method, the tannic acid-phosphomolybdic acid-amido black (TPA) technique, was briefly presented by Puchtler (1956). It consisted in treating Carnoy-fixed sections with aqueous solutions of tannic acid and phosphomolybdic acid and then in staining them with the acidic dye amido black. Puchtler and Leblond (1958), using the TPA technique on tissues prepared for light microscopy, have shown, in the apical cytoplasm of intestinal epithelial cells, a definite fibrillar structure. This cytoplasmic structure assumed the general appearance of a spider's web spanning the apical cytoplasm immediately below the striated border and inserting on the terminal bars found between the edges of adjacent cells at their free margins. The structure was referred to as a "terminal web", a term introduced by Sauer (1937) to describe a similar structure in ependymal cells stained with iron hematoxylin.

Fawcett (1958), Palay and Karlin (1959), Zamboni (1961), Trier (1962), and others, working on intestinal epithelial cells with the electron microscope, have demonstrated that the terminal web consisted of a feltlike condensation of fine filaments some 50 to 80 Å in diameter. They have also shown that such filaments

converged into the cytoplasmic densification of desmosomes and terminal bars. In fact, the staining of the latter structures with the TPA technique in light microscopy was attributed to the aggregation, at the level of these intercellular attachment sites, of terminal web filaments on the cytoplasmic membrane, which was found to stain very slightly or not with TPA (Puchtler and Leblond, 1958). This notion is also supported by the finding that the desmosomes observed with the electron microscope between cellular remnants in the stratum corneum of the epidermis were not associated with typical tonofilaments (Bloom and Fawcett, 1962) and were not stained by TPA (Kallenbach, 1963).

Leblond, Puchtler and Clermont (1960), investigating a wide variety of TPA stained tissues, have shown that cytoplasmic fibrils could be demonstrated in many cell types. These TPA positive fibrils assumed various configurations; for example, they could take the form of pillars extending from the apex to the base of the cells (goblet cells, surface mucous cells of the stomach, pillar cells of the organ of Corti, etc.); they could be dispersed throughout the cytoplasm (tonofibrils in stratified squamous epithelial cells); they could surround the cells partially (islet of Langerhans cells) or completely (liver cells); they could be located at the base of the cells (basal web in the reticulo-endothelial cells of splenic sinuses); they could extend almost completely throughout the length of cells known to be contractile

(myofibrils of striated muscle, cardiac muscle, smooth muscle and of myoepithelial cells); and so on. The term "cell web" was proposed by these authors to designate all intracytoplasmic fibrillar elements stained with TPA. They also tentatively suggested that the cell web fibrils might contribute in "giving firmness and shape to the cell".

Recently, Kallenbach (1963) has shown in TPA preparations two terminal webs in the intestine absorptive cells, a deeply stained distal web firmly connected to the terminal bars and a lightly stained proximal web attached to the row of proximal desmosomes. McNabb (1964), in his electron microscopic investigation of these cells, has not only confirmed the existence of the two webs, but has also found bundles of filaments coursing the lateral surfaces of the cells from the proximal desmosomes toward their base. He has also demonstrated that the filaments composing such a "lateral web" traveled from desmosome to desmosome down to the level of the nucleus and that, from this level, they continued down the lateral cell walls and across the basal cell membrane, thus constituting a "basal web". The latter was very close to the basal cytoplasmic membrane, and no desmosomes or other attachment sites were seen in relation to it. Both Kallenbach (1963) and McNabb (1964) believed that such an intracellular framework of fibrils (visible by light microscopy) or filaments (seen with the electron microscope) is endowed with strength.

Relatively little attention has been given to the fibrillar component of renal epithelial cells (see Review of Literature).

What is the exact morphological configuration of the cell web in these kidney cells ? How does the cell web vary along the renal tubules ? Does it exist in the renal corpuscle ?

The present study will answer these questions at the light microscopic level by demonstrating and describing in detail the cell web in various renal epithelial cells by means of the TPA technique. Such a detailed study will hopefully give information not only on the structure of the cell web and its attachment sites, but also on its orientation, distribution and possible physiological functions.

REVIEW OF LITERATURE

The historical evolution of our knowledge about the cell web and its properties has been traced in the reviews of Puchtler and Leblond (1958) and Kallenbach (1963), which should be referred to for detailed information concerning the earlier literature on this subject. The following is a brief review of the light and electron microscope literature on the cell web and its attachment sites in general and in the kidney.

On Cell Web Fibrils and Attachment Sites In General

Cell Web Fibrils

More than a hundred years ago, Brettauer and Steinach (1857), in their study of isolated intestinal epithelial cells, suggested that a differentiated layer of cytoplasm at the apex of the cells rendered the latter more resistant to mechanical stresses and deformation. It was not until recently that Sauer (1935, 1937) discovered the net-like fibrillar nature of the terminal web in a surface view of the apex of ependymal cells stained with iron hematoxylin. However, Schmidt (1943) was the first to indicate that the terminal web fibrils were composed of submicroscopic filaments by showing with the polarization microscope a highly birefringent layer in the apical cytoplasm of the intestinal epithelial cells of the tadpole in the plane of the terminal bars. With the advent of the electron microscope, the filamentous substructure of the terminal web of intestinal cells was definitely confirmed by several investigators (Granger and Baker, 1950; Fawcett, 1958; Palay and Karlin, 1959; McNabb, 1964; and others).

Cell web fibrils were also demonstrated in the basal cytoplasm of some epithelial cells. Indeed, at the base of the reticulo-endothelial cells lining the splenic sinuses and in close proximity to the basement membrane, another kind of web, the "basal plates", was described (Mangubi-Kudrjatzewa, 1909). These basal plates constituted a basal web and were confined to the territory

of only one cell; they stained avidly with the tannic acid-phosphomolybdic acid-amido black (TPA) technique (Leblond et al, 1960) and appeared to be homogeneous at the light microscopic level (Leblond et al, unpublished). However, Stoeckenius (1959) showed with the electron microscope that the basal plates had a filamentous substructure and approximated the basal cell membrane which rested upon the basement membrane; this author found no visible relationship of the basal plates with desmosomes or half-desmosomes. Both Mangubi-Kudrjatzewa (1909) and Stoeckenius (1959) suggested that this splenic basal web may be endowed with some contractile properties.

Over the rest of the cytoplasm, the best example of cell web fibrils is the epidermal fibrils, which were first described by Ranvier (1882) in all cells of the Malpighian layer. Heidenhain (1911) thought that the intracellular fibrils in all stratified squamous epithelia had tensile strength in addition to a supporting function, and he introduced the term "tonofibrils" to designate them. While he believed that these tonofibrils passed from cell to cell, Bizzozero (1870) and Schaffer (1927) suggested that they were intracellular and inserted with both ends in desmosomes without crossing the latter. Heidenhain (1911) also found that the orientation and arrangement of these tonofibrils varied greatly in different cell types and were characteristic in any one cell

type; he showed that they were vertically oriented in the basal layer (stratum germinativum) of the epidermis and assumed a horizontal course close to the stratum corneum. Similarly, Schmidt (1926) and Horstmann (1957), working with the polarization microscope, reported the existence of mainly circularly arranged tonofibrils in the outer root sheath of the hair follicle, while they found evidence for circularly and longitudinally oriented fibrils in the inner root sheath; Horstmann (1957), using polarization optics, described tonofibrils running parallel to the long axis of the hair shaft in the cuticle of the mature hair. Since the epidermal tonofibrils stained well with the TPA technique and filled most of the cytoplasm, Leblond et al (1960) referred to them as "dispersed web"; these authors described similar dispersed webs in other stratified squamous epithelia, such as the epithelia of trachea and vagina, and the anterior corneal epithelium. Kallenbach (1963), in a continuation of their work, studied extensively the distribution and orientation of dispersed cell web fibrils in the various layers of the hair follicle as well as in the cells of the enamel organ, and found that they almost invariably terminate in desmosomes.

The epidermal tonofibrils visible with the light microscope appeared under the electron microscope as aggregations of filaments some 50 Å thick (Horstmann, 1957). This finding was confirmed by a number of investigators (Weiss and Ferris, 1954;

Charles and Smiddy, 1957; Bargaen, 1959; Hibbs and Clark, 1959; Petry et al, 1961). In recent years, bundles of fine filaments similar in dimension and arrangement to the tonofilaments in epidermal cells were reported in electron microscopic studies of several simple epithelia. For example, Clark (1963) and Hoshino (1963) described such intracellular filaments in the reticular-epithelial cells of the thymus; these authors noted their particular affinity for membranous sites and their close association with the desmosomes connecting adjacent epithelial cells. According to Kohnen and Weiss (1964), however, the cytoplasmic filaments of thymic epithelial cells making up Hassall's corpuscles often were apparently unrelated to desmosomes. These fibrils were found to stain with TPA (unpublished observations). Similarly, Nachmias (1964) reported the finding of extensive areas of filamentous material in the cytoplasm of amebae (Chaos Chaos); she found that filaments of the order of 70 to 80 Å in diameter ran roughly parallel to the surface of the ameba and were most frequently seen in cytoplasm subjacent to convoluted membrane; she postulated a connection of these filaments with cytoplasmic movements, a notion supported by the finding of Simard-Duquesne and Couillard (1962) that a glycerinated ameba preparation contracted upon addition of adenosine triphosphate and magnesium ion.

A web seems to be present in almost all cells. Indeed, a few

publications of electron microscopic observations of filamentous elements (averaging 70 Å in diameter) in endothelial cells of both non-mammalian and mammalian capillaries, arterioles and arteries appeared in the last decade (Palade, 1953; Fawcett, 1959; Hana, 1960; Rhodin, 1962; Weiss, 1962; L.O. and H.L. Zwillenberg, 1963); Han and his collaborators (1963) described filamentous structures (60-70 Å thick) in the endothelial cells of vessels of the hamster dental pulp, which were attached to desmosomes like the tonofilaments of the epidermis. More recently, Bensch, Gordon and Miller (1964) reported bundled filamentous structures in endothelial cells of guinea pigs' and human pulmonary arteries, arterioles and precapillaries; these authors suggested a contractile function for these filaments, which resembled structurally tonofilaments, because of "their lack of attachment to the cell wall, resemblance to smooth muscle" and parallel grouping in bundles of considerable length".

Terminal Bars

Heidenhain (1892) was the first to describe "terminal bars", a term introduced by Bonnet (1895) to designate rod-like structures occurring in the plane of contact of highly polarized epithelial cells just below the apical border. Subsequently, several investigators reported terminal bars in the iron hematoxylin-stained epithelia of a wide variety of species and regarded them as

accumulations of intercellular cement outlining the free surface of the cells (Zimmermann, 1894; Bonnet, 1895; Cohn, 1895, 1897; Zimmermann, 1898). However, in addition to apical terminal bars, Cohn, (1897) described basal terminal bars in the ameloblasts of kitten following staining with iron hematoxylin. Chlopkow (1928), on the other hand, after resolving the terminal bars in the intestinal epithelium as double lines separated by a lightly stained material, reached the conclusion that the terminal bars consisted of thickened areas of the opposing cytoplasmic membranes of adjoining cells, which a pale staining intercellular cement substance separated.

More recently, Puchtler and Leblond (1958), in their histochemical studies of cell membranes and related structures in the rat thickened areas of the opposing cytoplasmic membranes of adjoining cells, which a pale staining intercellular cement substance separated.

More recently, Puchtler and Leblond (1958), in their histochemical studies of cell membranes and related structures in the rat intestinal epithelium, showed terminal bars, as well as terminal web, to consist only of protein and to stain intensely with the TPA technique. Using this stain, Leblond, Puchtler and Clermont (1960) and Kallenbach (1963) were able to demonstrate terminal bars in various epithelia. This staining method also enabled

Kallenbach (1963) to confirm the existence of apical and basal terminal bars in ameloblasts, as described earlier by Cohn (1897).

Many electron micrographs have shown terminal bars as local specializations of the surfaces of adjacent cells contributing to the greater adhesion and mechanical stability of the tissue; these structures appeared as dense regions on both sides of the cytoplasmic membrane into which fine terminal web filaments converged. For example, Zetterqvist (1956) described the terminal bar in the intestine as two thickened areas of apposed cytoplasmic membranes separated by a space 100 to 200 Å wide and with an accumulation of dense material in the subjacent cytoplasm. However, the structural details of terminal bars were not resolved until Farquhar and Palade (1963) surveyed and investigated these structures in a number of epithelia (including the epithelial cells of the nephron). They described the terminal bar as a bipartite junctional complex: the tight junction (or zonula occludens) located nearest the lumen and the intermediate junction (or zonula adhaerens) which succeeds it. The tight junction extends down for a distance which varies with the epithelium and is characterized by the fusion of the outer leaflets of adjoining cell membranes, with resultant obliteration of the intercellular space. Along this first element of the terminal bar there is relatively little accumulation of dense cytoplasmic

material. The intermediate junction (zonula adhaerens) is located between the tight junction and the first desmosome (or macula adhaerens) and is characterized by an intercellular space (about 200 Å wide), occupied by homogeneous, apparently amorphous material of low density occasionally bisected by a faint band of higher density. Extensive condensation of cytoplasmic filaments, continuous with the terminal web, occurs as a fine feltwork along either side of this junctional element. Occasionally the tightly filamentous mat in the zones of cytoplasmic densification is further condensed into a plate running parallel to the cell membrane from which it is separated by a narrow light zone.

Desmosomes

The term "desmosome" was introduced by Schaffer in 1920. Desmosomes, however, were discovered fifty years earlier by Bizzozero (1870) at discrete loci between adjacent epidermal cells of the Malpighian layer. The author regarded them as attachment plaques to which each cell contributed one half, because a desmosome tends to break in half when pulled apart. He (1870) and Schaffer (1927) visualized the desmosomes in the epidermis as points of intimate contact of two adjoining cells and believed that the epidermal tonofibrils would terminate in them and not pass from cell to cell.

Using the TPA technique, Leblond et al (1960) were able to stain desmosomes in epidermal and intestinal epithelial cells; these structures were seen as strongly TPA-stained granules located in the plane of contact of two neighboring cells, often without relation to the cell apex, and having staining properties similar to those of terminal bars and cell web fibrils. This led these authors to suggest, as it was aforementioned (see Introduction), that the TPA staining of terminal bars and desmosomes was probably due to the accumulation of cell web filaments on these structures.

The morphology of desmosomes has been described in many epithelia by a number of electron microscopists (Odland, 1958; Karrer, 1960; Hama, 1960; Fawcett, 1962; and others). However, it was the investigation of Farquhar and Palade (1963) which settled the problem of desmosome morphology by distinguishing these structures from terminal bars. According to these authors, the desmosome (or macula adhaerens) consists of a wide intercellular space (about 240 Å) bisected by a central disc of dense material and of cytoplasmic filaments converging on the dense plaques disposed parallel to each cell membrane in the subjacent cytoplasmic matrix. Tamarin and Sreebny (1963) determined, by the use of histometric and densitometric methods with electron microscopy, that a desmosome is elliptical in shape and usually has its long axis (which is in the order of 4100 Å) oriented parallel

to the base-apex axis of the cell.

Moreover, some investigators have demonstrated with the electron microscope half-desmosomes closely associated with tonofibrils and attaching the basal cells of the epidermis to the basement membrane (Weiss and Ferris, 1954; and others). While these epidermal half-desmosomes have not yet been seen with the light microscope, Kallenbach (1963), using the TPA technique, has shown similar structures between the apical surface of ameloblasts and the enamel matrix.

On Cell Web Fibrils and Attachment Sites in the Kidney

Cell Web Fibrils

As early as 1891, a layer of granules ("basal bodies") was described in the epithelial cells forming the proximal convoluted tubule of the kidney by Nicolas (1891) and Prenant (1891). Such a layer was seen extending across the apical cytoplasm of the proximal tubular epithelial cells immediately beneath the brush border. Less than fifteen years later, Joseph (1905) indicated that the basal bodies were different from those of cilia. This view was widely accepted by most workers for a quarter of century until Millendorff (1930) noted the netlike fibrillar character of this layer; he denied the presence of isolated granules at the

base of brush border in the epithelial lining of the proximal convoluted tubule; in contradistinction to the earlier workers, he observed "a mainly continuous layer of uneven thickness" stretching across the apex of the cells and seldom presenting a few interruptions.

Leblond et al (1960), using the TPA technique on renal tissue prepared for light microscopy, demonstrated a finely granulated layer spanning the apical cytoplasm of proximal tubular epithelial cells immediately beneath the brush border and terminating in terminal bars; they referred to it as a "unilayered terminal web". Such a layer has not yet been clearly illustrated in electron microscopic studies.

Moreover, in the middle of the nineteenth century, Wedl (1850) saw basal fibers on isolated kidney tubules; he described them as cross-rings ("Querringe") similar to those seen on the cuirass of lower animals. Zimmermann (1898) stained the so-called basal fibers ("Basalreifen") with iron hematoxylin. According to the author, these fibers are fine and oriented oblique to the lumen of the tubule; they are situated between the base of the epithelial cells and the basement membrane and run circularly around the tubule. He regarded these fibers as cementing structures which would firmly attach the renal epithelial cells to the basement membrane. He showed such fibers in the lining epithe-

lium of the collecting tubule and the pars intercalata (or Schaltstück) of the distal tubule of the rabbit and also reported the presence of similar basal cementing structures in the corneal epithelium. E. Bizzozero (1901), studying fresh and macerated preparations of human, pig and horse kidneys, confirmed the existence of these obliquely oriented basal fibers in the epithelial cells lining the proximal convoluted tubule and the thick limb of Henle's loop, but not in those forming the thin limb of the loop.

Similarly, Heidenhain (1911) demonstrated, in iron hematoxylin-stained sections, the basal fibers in the proximal convoluted tubule of the mouse kidney. He made the important discovery that each fiber is related to one cytoplasmic lamella, which results from the inflection of the basal cell membrane into the interior of the cell and which he was able to resolve as a rod-like structure at the base of the cells. He also considered the fibers as continuous "cementing rings" which would reinforce the attachments of the cells to the basement membrane by connecting each rod-like lamella to the latter. These views of Heidenhain (1911) were also shared by Frisch (1915).

Three years later, Tasic (1918) made once again a thorough investigation of the basal "Kittfäden" (cementing threads) of Zimmermann (1898) in the dog, cat and human kidney. In contra-

distinction to the findings of earlier workers, this author (1918) discovered that the basal fibers in the proximal tubular epithelial cells are short and discontinuous and that they run in one direction only within the same cell territory (the orientation being always altered somewhat at the cell border). On the other hand, he confirmed the intimate relation of the fibers to the rod-like cytoplasmic lamellae at the base of the cells. Moreover, he demonstrated similar fibers in Bowman's capsule. He also noted that, in the renal medulla, the basal fibers in the epithelial cells lining the collecting tubule become gradually less numerous as one progresses toward the tip of the papilla. Similar results were obtained by Djokic (1919) in the bear and by Belosavitch (1919) in the llama.

Recently, Kallenbach (1963), using the TPA technique, stained the basal fibers in the cells lining the proximal convoluted tubule. However, he suggested that such fibers are intracellular structures, contrasting with the views of previous workers who believed them to be intracellular.

It is to be noted that no mention of a lateral web in renal epithelial cells was made in either light or electron microscopic studies.

Terminal Bars

Zimmermann (1898) was the first to show terminal bars in the proxi-

mal convoluted tubule. These intercellular attachment sites were also demonstrated in the proximal convolution by Leblond et al (1960) and Kallenbach (1963) in TPA preparations. They have been repeatedly described in the nephron with the electron microscope (Takaki et al, 1956; Rhodin, 1958; Miller, 1960; Latta et al, 1961; Maunsbach et al, 1962; and others).

Farquhar and Palade (1963), in their investigation of junctional complexes in a number of epithelia, found that the organization of the terminal bars varies appreciably from one segment of the nephron to another. In the proximal convoluted tubule, the tight junction (zonula occludens) is extremely shallow while the intermediate junction (zonula adhaerens) is deep and usually associated with a broad band of dense cytoplasmic material, so that the adhering zonule would undoubtedly represent the terminal bar seen with the light microscope. In the distal convoluted tubule and the collecting tubule, however, the situation is almost reversed: the first element of the terminal bar is extensively developed and is accompanied on each side by bands of dense cytoplasmic material which continue along the second element (zonula adhaerens) when present. This adhering zonule varies from one intercellular space to another: sometimes it is well defined; sometimes it is poorly developed; finally in many instances it is entirely missing. The authors concluded that the second element of the terminal bar in the distal convolution "is not a continuous

structure around each cell and throughout the epithelium".

Desmosomes

While desmosomes have not yet been clearly demonstrated in the nephron and collecting tubule with the light microscope, they have seldom been described in electron microscopic studies. Farquhar and Palade (1963) have shown that these structures are small and definitely less frequent all along the epithelium of the renal tubules (especially in the distal convolutions) than in other epithelia; a wide interval generally separates a desmosome from the second element of the terminal bar.

In the renal corpuscle, Farquhar et al (1961) reported a special junctional complex in the plane of contact of adjacent podocytes. These authors found the junction line to be extremely tortuous as a result of the extensive interlocking of the foot processes of the epithelial cells. The junctional element appears as a relatively wide gap (200 to 250 Å) bisected by a distinct filamentous line, not by a plate (as in the case of the desmosome). However, the associated fibrillar ectoplasmic differentiation is similar to those of the zonula adhaerens. These findings were confirmed by Rhodin (1962) and by Trump and Benditt (1962).

MATERIALS AND METHODS

Animals

Kidneys and occasionally other tissues from healthy adult male albino rats of the Sherman strain were routinely used throughout this work. These white rats, weighing between 150 and 220g., were obtained from the Rockland Farms (New City, New York) colony.

All animals were maintained with Purina Fox Chow and water ad libitum. The animal quarters of the Department of Anatomy were kept between 80 and 85° F.

Histological Techniques

The TPA Technique

The tannic acid-phosphomolybdic acid-amido black (TPA) technique was the main staining method used in this investigation. When this technique was applied to kidney tissue, the nucleus, the mitochondria and the ergastoplasm of the epithelial cells were often stained in addition to the cell web. An effort was then made to use a technique which will render the cell web as evi-

dent as possible by reducing or eliminating the staining of the other organelles. Empirically, it was found that this could be achieved by substituting the mode of paraffin embedding currently used in the TPA technique by a more elaborate one, called the "double embedding technique". The routine method employed in this work will now be outlined, and its significance discussed in the light of the results obtained with it and with other histological procedures.

The Routine Technique

The technique presently used is in essence similar to the one employed by Puchtler and Leblond (1958) and Kallenbach (1963) with some slight modifications. It can be outlined as follows:

Kidneys were excised from the animal under ether anesthesia, hemisected either longitudinally or transversely, and immediately immersed in an excess of freshly prepared Carnoy's fluid (Romeis, 1948). Fixation in Carnoy's fixative (60 volumes ethanol, 30 volumes chloroform, 10 volumes glacial acetic acid) was for 24 hours at room temperature.

After fixation, each kidney half was infiltrated and embedded according to the following method, referred to as the "double embedding technique":

Dehydrate in ascending strengths of alcohol (70,80,95 and 100%), only if stored in 70% alcohol (a procedure which is not recommended since the tissue is already dehydrated following fixation in Carnoy's fluid).

Clear in a 1:1 mixture of alcohol and methyl benzoate (Fisher Reagent) for 30 minutes and in methyl benzoate (Fisher Reagent) alone for another 30 minutes.

Treat with a 2% solution of nitrocellulose (or celloidin) dissolved in methyl benzoate (Fisher Reagent) for 24 hours in a closed air-tight container to prevent evaporation.

Transfer to a 10% solution of nitrocellulose dissolved in methyl benzoate (Fisher Reagent) for 24 hours (also in a tightly stoppered container).

Pass through two changes of benzene for about 45 minutes each (total $1\frac{1}{2}$ hours).

Transfer to a 1:1 mixture of paraffin and benzene at a temperature a few degrees above the melting point of the paraffin being employed (which is $50-52^{\circ}$ C), for 30 minutes.

Infiltrate with two changes of paraffin (M.P., $50-52^{\circ}$ C) in a vacuum oven heated at $54-56^{\circ}$ C, for about 45 minutes each (total $1\frac{1}{2}$ hours).

Embed in paraffin (M.P., $58-60^{\circ}$ C).

The kidney halves were sectioned at 2mu, stuck to glass slides with albumen-glycerin, and stained with TPA as follows (Kallenbach, 1963):

Hydrate sections through two changes of xylol (5 minutes each), two changes of 95% alcohol (1 minute each), and one change each of 70 and 50% alcohol (1 minute each) to distilled water.

Mordant sections in a freshly prepared 5% aqueous solution of tannic acid for 10 minutes.

Rinse briefly in distilled water (for 5 seconds with agitation).

Mordant in a freshly prepared 1% aqueous solution of phosphomolybdic acid for 10 minutes.

Rinse briefly in distilled water (for 5 seconds).

Dehydrate in one change each of 95% alcohol, absolute alcohol, methanol-glacial acetic acid 9:1 for 5 seconds each (with agitation).

Stain in a saturated solution of amido black 10B (Chroma Gessellschaft, Stuttgart) in methanol-glacial acetic acid 9:1 for 1 minute.

Wash in three changes of methanol-glacial acetic acid 9:1 for 2 minutes each.

Clear in three changes of xylol (3 minutes each).

Mount in balsam.

Stained kidney sections (in which no fading could be observed for two years) showed bluish-green TPA positive sites and brownish-yellow TPA negative sites (Fig. 14). Some of the results obtained with the "routine technique" will now be illustrated.

In any given Carnoy-fixed kidney section treated with the routine

TPA technique, nuclear staining was uniformly intense (Figs. 10-14) and did not turn out to be capricious.

Contrasting with the moderately colored mitochondria, cell web structures were avidly stained and could be readily distinguished from neighboring structures. Figure 14 shows cross sections of the unilayered terminal web and of terminal bars, longitudinal sections of the lateral web, and the so-called rodlets (basal web) in the cells of the proximal convoluted tubule.

Nerve fibers were stained in extraglomerular regions (Fig. 13). Erythrocytes possessed a peripheral ring of TPA positive material immediately beneath the cell surface (Fig. 9). The smooth muscle myofibrils in the walls of the renal blood vessels were intensely stained (Fig. 29).

With the exception of intraluminal fibrin threads, hemoglobin granules or droplets and albumen casts, the routine technique did not stain the extracellular material. Indeed, connective tissue fibers (collagenous and reticular fibers, as well as basement membranes) were always TPA negative; they are shown as brownish-yellow structures in figure 14.

Histological Tests of the "Routine Technique"

In this chapter the effects of different fixatives and different fixation procedures on subsequent staining pattern of renal epithelial cells with TPA will first be discussed. Then, the significance of the mode of washing and embedding used in the "routine technique" will be analyzed.

Test of Carnoy's Fixative

Electron microscopic studies of the kidney have not yet produced a convincing illustration of cell web structures in the renal epithelial cells. It therefore seemed of interest to determine whether these cell web structures observed with the light microscope were artifacts of fixation (in the broad sense of the term).

To test this possibility, kidneys were excised from adult male albino rats under ether anesthesia with a minimum of handling; some were hemisected, whereas others were cut into thin slices with a fresh razor blade. A portion of the renal tissue thus obtained was immediately fixed in an excess of fresh Carnoy's fluid for 24 hours at room temperature; the remaining kidney tissue was left standing on a shelf for times varying from 15 minutes to 12 hours prior to fixation in Carnoy's fixative.

In all the kidney sections studied, the general pattern of TPA

staining did not differ markedly, and the texture of cell web structures remained the same, thus indicating that they are normal cytoplasmic components.

Test of Gluteraldehyde Fixation

Puchtler and Leblond (personal communication) found that TPA staining was impaired after fixation in Bouin's fluid. Puchtler and Sweat (1962) discovered that after fixation in Zenker-formol all cell web structures were TPA negative. Kallenbach (1963) studied the effect of various fixatives on subsequent staining of the cell web with TPA, such as 80% and absolute alcohols, Newcomer with and without dioxane, Cellosolve, Carnoy, formalin, picric acid, mercuric chloride, trichloroacetic acid, Müller and Zenker. Of all the fixatives he tested, only Carnoy's fluid appeared to give superior fixation and staining results with TPA. 80% and absolute alcohols, Newcomer and Cellosolve were almost as good and far superior to the remaining fixatives, which resulted in inconsistent variation, impairment or complete inhibition of TPA staining. Furthermore, in evaluating TPA preparations after fixation in Carnoy's fluid, it was found that mitochondria in the renal epithelial cells, though moderately stained, were generally present in the form of granules instead of rods and that their number and arrangement varied in different segments of the rat nephron.

It therefore seemed advisable to study the results of other fixation procedures with respect to preservation and TPA staining intensity of cellular structures in renal epithelial cells. To explore the optimal condition for cytological fixation, it was necessary to draw upon the pool of electron microscopy fixatives, as most of the fixatives of choice currently used in morphological studies with the light microscope has already been tested. The following experiment was done:

Method

Kidneys as well as a variety of other tissues from two adult male albino rats were fixed by perfusion through the aorta with a cold solution of freshly prepared 6.5% gluteraldehyde in 0.1M phosphate buffer (pH 7.5; Sabatini et al., 1963).

The technique for perfusing the kidneys was as follows: The rats were anesthetized with ether. The heart was exposed and 2 ml. of 0.1% heparin was injected into the right ventricle through a No. 27 needle. The right ventricle was then opened and a cannula connected to a polyethylene tube was tied into the aorta. About 100 ml. of a balanced salt solution at room temperature was then allowed to enter the left ventricle through the tube at a moderate rate, flushing out most of the blood from the animal. The tube was then connected, taking care to eliminate bubbles, to a glass adaptor fed with the gluteraldehyde fixative by a gravity

drip perfusion apparatus at a 4 foot head of pressure. The perfusion lasted for about 30 minutes. After about 75 to 100 ml. of the fixative was used, the kidneys were removed and hemisected. Some kidney halves were placed directly into an excess of buffered gluteraldehyde fixative for 12 hours; others were postfixed in Carnoy's fluid for also 12 hours.

Following fixation all these kidney halves were dehydrated in alcohol, embedded according to the "double embedding technique" and sectioned at 2 mu. To compare the staining by various dyes, all sections were stained by the following methods: (a) TPA, (b) TPA in conjunction with the periodic acid-Schiff (PA-Schiff) technique, (c) Regaud's iron hematoxylin stain (Lillie, 1954, p. 79), and (d) 0.5% aqueous solution of toluidine blue (Chroma Gesellschaft, Stuttgart.).

Moreover, following the perfusion, a small piece of renal tissue was taken from a remaining kidney half and transferred to a drop of fresh fixative where it was diced into blocks. These blocks were placed inside a vial of the gluteraldehyde fixative and fixed for one and one half hours at room temperature. After fixation, the tissue was dehydrated in graded alcohols consisting of 15 minute changes of 30, 50, 70, 90, and 95% alcohol and three changes of absolute alcohol. The tissue pieces were then embedded in Epon according to Luft's procedure (1961) using mixture A

(62 ml. of Epon 812 and 100 ml. of dodecenyl succinic anhydride) and mixture B (100 ml. of Epon 812 and 89 ml. of methyl nadic anhydride) in a ratio of 7:3. Finally, thin sections were cut at $\frac{1}{2}$ μ on a Porter-Blum microtome, mounted on glass slides, stained with a 1% solution of toluidine blue made alkaline by saturating with sodium borate ($\text{Na}_2 \text{B}_2 \text{O}_4 \cdot 8\text{H}_2\text{O}$) for 15 seconds, and mounted in balsam following washing in distilled water and drying on a hot plate (Sandborn, personal communication).

Results

Table 1 summarizes the results obtained with the above staining methods after fixation in gluteraldehyde alone. Staining intensity is expressed by a rating in 4 steps: absent (-), slight (+), moderate (++) , and intense (+++).

Four types of staining patterns were distinguished. In type 1, which was achieved by staining kidney sections with TPA alone, the picture was dominated by the intense staining of the mitochondria in the renal epithelial cells. With the exception of the cell web fibrils in the cells of the parietal and visceral layers of Bowman's capsule and the myofibrils of smooth muscle fibers, cell web structures did not stain. Nuclei were TPA negative, whereas nucleoli were moderately stained. Intraluminal erythrocytes and fibrin threads were colored intensely blue. All other structures were unstained.

TABLE 1

RESPONSES OF GLUTERALDEHYDE-FIXED KIDNEY SECTIONS TO VARIOUS DYES

	1 TPA	2 TPA + PA-Schiff	3 Iron hematoxylin	4 Toluidine blue
1. <u>Cell Membranes and Derivatives:</u>				
terminal bars.....	-	-	-	++
terminal web.....	-	-	-	++
brush border.....	-	++	-	+
rodlets.....	-	-	-	++
tonofibrils (in glomerular epithelial cells)....	+++	-	+++	++
basement membranes.....	-	+++	-	+++
 11. <u>Other Structures:</u>				
mitochondria.....	+++	+++	+++	+++
nuclei.....	-	-	-	+++
nucleoli.....	++	++	++	+++
collagenous fibers.....	-	++	-	++
erythrocytes.....	+++	+++	+++	+++ (green)
fibrin threads.....	+++	+++	+++	+++
elastic fibers.....	+	+	+	+
smooth muscle myofibrils.....	+++	+++	+++	+++
glycoprotein-containing granules (in glomeruli and proximal convoluted tubules).....	-	+++	-	+++ (bluish-green)

It is worthy of mention that a similar staining pattern was obtained in all the other tissues examined. However, in addition to mitochondria and nucleoli, the distal terminal web and the terminal bars in intestinal absorptive cells (Fig. 1), the chromatoid body in young spermatids, the zymogen granules in the acinar cells of the pancreas and the salivary glands (Figs. 3 and 4) were deeply stained.

In the kidney, fixation and staining intensity usually tended to be optimal in the vicinity of the intralobular, interlobular and arcuate vessels of the cortical and subcortical zones and the vasa recta of the outer medulla. In longitudinally hemisected kidneys, it was readily observed that the stainability was enhanced in the portions of cortical tissue adjacent to the hilus. In the outer medulla, heavily stained columns of renal tissue were seen radiating from the inner medulla and alternating with lightly stained ones. The general staining pattern was, therefore, dominated by avid staining everywhere, except in subcapsular areas away from the hilus and in alternate columns of outer and inner medullary tissue. It should be mentioned that the arteries and arterioles were always completely depleted of red blood cells by the perfusion, whereas the lumina of capillaries and venous channels were always filled with them. Similarly, the capillaries of juxtamedullary glomeruli contained fewer erythrocytes than those of peripheral glomeruli.

Type 2 staining pattern, which characterized sections treated with the double staining technique, TPA-PA-Schiff, was similar to type 1, excepting that intra-and extra-cellular PA-Schiff-reactive material was well stained. A clear zone or band, completely devoid of organelles, was observed just underneath the brush border of proximal tubular cells and at discrete loci above the tubular basement membranes (between the latter and the well preserved rod-shaped mitochondria). While the granules of certain podocytes were unstained, those in proximal tubular cells showed a positive PA-Schiff reaction.

Type 3, characteristic of iron hematoxylin-stained sections, was surprisingly identical to type 1. The staining pattern was independent of the degree of differentiation.

Type 4 was achieved by staining with toluidine blue. It was characterized, in both paraffin-and Epon-embedded tissue sections, by the moderate staining of the cell web and of the brush border carried by the lining cells of the proximal convolution, and the intense coloration of almost all other kidney structures. Erythrocytes were colored green, while the glycoprotein-containing granules in the proximal tubular cells were stained bluish-green.

Postfixation in Carnoy's fixative resulted in inconsistent variation of the TPA staining. Two types of staining patterns

were distinguished on the same section. The first type was identical to type 1, that is, was characterized by a fairly strong and specific staining of mitochondria and nucleoli in tubular epithelial cells. This staining pattern was encountered exclusively around the large blood vessels of the cortex and outer medulla (namely, the intralobular, interlobular and arcuate arteries and veins, as well as the venous and arterial vasa recta). Fixation (as judged by preservation of cellular structures and staining intensity) was most adequate around cortical and subcortical large blood vessels. It was almost as good around the arterial and venous vasa recta of the outer medulla but far superior to that around the vasa recta in the inner medulla and at the tip of the papilla.

However, in the remaining renal tissue, an essentially "normal", though pale, TPA stain was achieved. (The term "normal TPA stain" is used here to refer to a staining pattern in which mitochondria stain slightly in contrast to the intense coloration of cell web structures). It follows that each kidney section was mottled with two different staining patterns, which gave it a marble-cake appearance.

Finally, it is to be noted that, following fixation in gluteraldehyde (without perfusion) and postfixation in Carnoy's fixative,

a "normal" TPA staining pattern was obtained.

Discussion

This investigation was designed to study the effect of gluteraldehyde fixation on subsequent staining of renal epithelial cells with TPA. The most important finding relates to the treatment of gluteraldehyde-fixed kidney sections with the TPA technique as a fairly specific method for mitochondrial and nucleolar staining. This method is undoubtedly far superior to Altmann's primitive technique for mitochondria and the numerous variants of his technique (Benseley, 1910; Volkonsky, 1928, 1933; Cowdry, 1929; Gabe, 1947; and others). Indeed, this procedure is noteworthy for its simplicity (for it does not require differentiation), the easiness and reliability of its execution, and the constancy of the results obtained with it.

The results also revealed that, in addition to mitochondria and nucleoli, the myofibrils of smooth muscle cells and the cell web fibrils in the cells of the parietal layer of Bowman's capsule and in certain podocytes were intensely stained by this technique, whereas all other cell web structures were TPA negative.

The interpretation of these findings would require an utter comprehension of the chemical mechanism of the TPA reaction, which is at present not fully understood despite the attempts made by

some investigators (Puchtler and Leblond, 1958; Puchtler and Sweat, 1962; Kallenbach, 1963). The TPA method was originally developed as a stain for terminal bars and related structures (Puchtler, 1956; Puchtler and Leblond, 1958), which are presumed to belong to the keratin-myosin-epidermin-fibrin group of proteins. Histochemical studies have shown these cell web structures to consist of only protein (Puchtler and Leblond, 1958). This indicates that the TPA procedure is specific for a certain group of proteins, and Puchtler and Sweat (1962) have pointed out that the TPA reaction appears to be determined by structural chemistry rather than amino acid composition. It, therefore, seems likely that the protein material stained with the present technique, including the cell web structures (myofibrils of smooth muscle cells and cell web fibrils in epithelial cells of renal corpuscle), may have some chemical or stereochemical resemblance.

Moreover, the results of the iron hematoxylin stain were surprisingly identical to those of the TPA stain. No cell web structures were stained in the renal tubular epithelial cells; only mitochondria and nucleoli were TPA positive. Although iron hematoxylin usually tends to stain cell membranes and their derivatives in Carnoy-fixed sections, none of these structures was colored after gluteraldehyde fixation (regardless of the degree of differentiation). Thus, the iron hematoxylin tech-

nique appears to be the only other staining procedure known to give results identical to those of the TPA stain following fixation in gluteraldehyde.

Another important finding relates to the moderate coloration of all cell web structures by toluidine blue under such adequate conditions for cytological fixation. This indicates that these structures are normal constituents of renal epithelial cells.

This notion is also supported by the results obtained in sections treated with the double staining technique, TPA-PA-Schiff.

Indeed, the clear zones or bands (completely free of organelles) which were observed immediately under the brush border of proximal tubular cells and close to the inner surface of tubular basement membranes correspond exactly to the positions of the terminal web and rodlets (basal web), respectively. Rambourg (personal communication) reported a similar clear zone immediately beneath the brush border (in areas where the terminal web was unstained) in kidney sections treated with the double-staining technique, PA-Schiff-toluidine blue.

Lastly, the investigation shows that, after perfusion with and postfixation in gluteraldehyde, there exists a correlation between fixation (as judged by preservation of cellular structures and TPA staining intensity) and the distribution of intra- and extra-renal arterial vessels: the closer the renal tissue to arteries and arterioles, the better the fixation and the stronger

The staining.

The notion that fixation is dictated by the arterial vascular pattern is supported by the results obtained following perfusion with gluteraldehyde and postfixation in Carnoy's fixative. Indeed, with this procedure, a fairly strong and specific mitochondrial and nucleolar staining was only encountered around the arterial vessels of the renal hilus and pelvis, the arcuate, interlobular and intralobular arteries, and some of the arterial vasa recta of the outer and inner medulla. The remaining kidney tissue exhibited an essentially "normal", though pale, TPA stain in which cell web structures and nuclei were stained. When the perfusion was followed by immersion of the renal tissue in gluteraldehyde, a specific mitochondrial stain was achieved in all parts of the kidney section, but was pale in subcapsular locations and in a number of renal tissue columns in the outer and inner medullary zones. Moreover, cursory inspection of the sections uncovered an obvious absence of erythrocytes in all arteries and arterioles, as well as in most juxtamedullary glomerular capillaries, while all other capillaries (including cortical glomerular capillaries) and all venous channels were full of blood cells.

The staining pattern obtained in sections postfixed in gluteraldehyde would appear to be occasioned mainly by the slow rate of diffusibility of gluteraldehyde, coupled perhaps by the particu-

liarities of the renal circulation of albino rats (such as the muscular efferent vessels of juxtamedullary glomeruli, which may, by contracting, prevent the perfusing fluid and the fixative from passing through the venous channels). Indeed, gluteraldehyde is known to be an excellent cytological fixative at the level of the electron microscope (Sabatini et al., 1963), and this work shows that this is true also at the level of the light microscope. However, its use is somewhat limited by its slow rate of penetration. This notion is supported by the experiment in which kidney tissue was fixed by immersion in gluteraldehyde and postfixed in Carnoy's fluid for the same period of time and in which a "normal" TPA stain was achieved, that is, all structures were stained. This implies that gluteraldehyde has even a slower rate of diffusibility than Carnoy's fluid.

Summary

- 1- The effect of gluteraldehyde fixation on subsequent staining of renal tissue with TPA was studied in albino rats. Various dyes were used for comparison with the TPA stain.
- 2- A fairly strong and specific mitochondrial and nucleolar stain was achieved with TPA. With the exception of smooth muscle myofibrils and the cell web fibrils in certain podocytes and in the cells of the parietal layer of Bowman's capsule, no cell web structures were stained.
- 3- Regaud's iron hematoxylin technique is the only other staining

procedure known to stain mitochondria and nucleoli selectively, when it is applied to gluteraldehyde-fixed sections.

4- Toluidine blue, under such optimal conditions for cytological fixation, stained all intra- and extra-cellular material, including cell web structures, thus indicating that the latter are normal constituents of renal epithelial cells.

5- As expected, fixation and staining intensity were shown to be maximum around large renal blood vessels with a progressive decline away from them. This is ascribable primarily to the slow rate of diffusibility of gluteraldehyde and possibly to the particularities of the renal circulation of albino rats.

Test of the TPA Stain on Gluteraldehyde-Fixed Sections

In the preceding test, it was found that gluteraldehyde fixation followed by the TPA method resulted in a fairly strong and specific mitochondrial and nucleolar stain. It therefore seemed advisable to develop a modified technique which would result either in an enhanced stainability of mitochondria and nucleoli or in the staining of other cellular structures. As "normal" TPA preparations are dependent upon a balance between two mordants (tannic acid and phosphomolybdic acid), it was thought that a suitably modified TPA stain could be achieved by upsetting this balance. The following two experiments were performed:

Method

First, gluteraldehyde-fixed kidney sections from the test block were mordanted in tannic acid for times varying from 0 to 60 minutes; after a brief rinse in distilled water, all sections were treated with phosphomolybdic acid for the same times prior to staining with amido black.

Secondly, sections from the test block were mordanted in tannic acid for times varying from 10 minutes to 19 hours; they were then treated identically with phosphomolybdic acid for 10 minutes (as in the "routine technique").

All control sections were stained according to the "routine technique".

Results

The results of the first experiment are summarized in Table 2. The slides were evaluated with regard to the staining intensity of mitochondria (M), nucleoli (N), smooth muscle myofibrils (My), and ergastoplasm (E) by a rating in 4 steps: absent (-), slight (+), moderate (++), and intense (+++). The degree of staining of these structures in the control section was rated as ++.

Amido black colored all tissue components in the unmordanted sections; a similar staining was obtained in a section mordanted

EFFECT OF VARIOUS MORDANTING TIMES ON THE TPA STAIN AFTER
GLUTERALDEHYDE FIXATION

[illegible][illegible]

only in tannic acid. However, Table 2 reveals three more interesting results:

- 1- The ergastoplasm remains completely unstained in sections mordanted in phosphomolybdic acid for 10 minutes or more, regardless of the time for which the sections were treated with tannic acid.
- 2- A moderate staining of mitochondria, nucleoli, smooth muscle myofibrils and a completely TPA negative ergastoplasm resulted when sections were mordanted in tannic acid for a time exceeding that in phosphomolybdic acid, provided that the mordanting time in the latter was 10 minutes or more.
- 3- An intense staining of mitochondria, nucleoli and smooth muscle myofibrils and a completely unstained ergastoplasm was achieved in sections treated with tannic acid for times exceeding 10 minutes and mordanted in phosphomolybdic acid for exactly 10 minutes.

The second experiment confirmed this third result. Indeed, a progressive increase in the intensity of the staining of these structures (excepting the ergatoplasm which remained TPA negative) was obtained in sections mordanted in tannic acid for times exceeding 10 minutes (up to 19 hours) and then treated identically with phosphomolybdic acid for exactly 10 minutes.

Comments

It was concluded that, following gluteraldehyde fixation, the TPA staining intensity can be increased by prolonged treatment of the sections with tannic acid before imbuing them with phosphomolybdic acid (as in the "routine technique"). The chemical mechanism of the TPA reaction in this procedure is at present not completely understood. However, the avid staining of all tissue constituents by amido black (an acid dye) in the unmordanted section indicates that they all contain basic proteins.

Comments on the Double Embedding Technique

Previous histological studies have shown that most fixatives have the disadvantage of inhibiting or interfering with the TPA reaction (Puchtler and Leblond, personal communication; Puchtler and Sweat, 1962; Kallenbach, 1963). Of all the fixatives tested, only Carnoy's fluid insured maximum TPA staining results.

Moreover, it seems that the patterns of precipitated protein are dependent not only upon the fixative used, but also upon the mode of washing and embedding. Indeed, TPA staining tests performed with renal tissue which was infiltrated only with paraffin resulted in a weak and diffuse overall staining of TPA positive structures and in tissue damage. The damage, though variable, most frequently

showed itself as holes or cracks in the specimen, as if an explosion of some sort had taken place during succeeding steps of the processing method. The causes of this pale staining and "explosion damage" are not fully understood, but it is probably due to the fact that paraffin alone does not have sufficient strength to "set" the tissue protein components of such a fragile organ as the kidney, to increase their affinity for TPA and to allow 2 μ sections to be cut adequately.

Kallenbach (1963) obtained good results with the TPA technique by clearing Carnoy-fixed tissues in methyl benzoate containing 1% collodion (a purified form of celloidin) prior to paraffin infiltration and embedding. However, it was found that the affinity of renal tissue protein constituents for TPA could be greatly increased by the "double embedding technique". The latter is a combined low viscosity nitrocellulose (or celloidin) infiltration and paraffin infiltration and embedding.

In this process, the tissue is infiltrated with a celloidin-methyl benzoate solution, using two strengths, namely thin celloidin (2%) and thick celloidin (10%), as exact strengths are not of prime importance. Since no heat is required for this step and high strengths of celloidin are used, shrinkage is negligible and the consistency of the tissue remains unaffected.

Following clearing with two changes of benzene, the tissue is

transferred to a 1:1 mixture of benzene and paraffin (M.P., 50-52° C) for 30 minutes, so as to remove much of the solvent. The tissue is then infiltrated with two changes of fairly soft paraffin (M.P., 50-52° C) in a vacuum oven for a total of only 1½ hours. In this step practically all the solvent is gradually evaporated without the formation of air bubbles and without prolonged treatment in molten paraffin (which tends to cause shrinkage and hardening of tissues). As a result of this, when the tissue is subsequently enclosed in a fairly hard paraffin (M.P., 58-60° C), a consistent, uniform degree of hardness is attained throughout the block.

In conclusion, the so-called "double embedding technique" seems to be the tissue processing method of choice for the TPA staining procedure. Indeed, this process reduces artifacts, for the celloidin, since it is not removed, supports and holds together the fragile structures of the tissue until the time that they are mounted on the slide. Moreover, this mode of embedding appears to affect, as an increased TPA staining intensity was achieved, the pattern of precipitated protein.

Additional Staining Techniques

In addition to the routine staining method, the following techniques were employed to relate the TPA staining results to classical histological findings.

H and E

For routine studies, sections were treated with Harris's hematoxylin for 3 to 4 minutes, rinsed in distilled water, and counterstained by dipping in 1% eosin Y.

Toluidine Blue

Sections were occasionally stained with a 0.5% solution of toluidine blue in water (Pearse, 1954, p.432) to demonstrate cell web structures as well as other basophilic substances.

Iron Hematoxylin

Sections were also subjected to Regaud's iron hematoxylin stain, which is known to show cell membranes and associated structures (Lillie, 1954, p.79). Kidney tissue, following fixation in Carnoy's fluid and embedding according to the "double embedding technique", was deparaffinized and brought to water. Sections were then mordanted in 5% iron alum for 24 hours. After thorough washing in water, they were stained for 24 hours in an alcoholic hemato-

xylin solution; the sections were considered to be adequately stained when, on being taken out of the hematoxylin, they were a homogeneous jet-black and microscopically showed absolutely no cellular detail. This was followed by differentiation in 1% iron alum, controlling the results with a microscope. Finally, the sections were washed in running water for 30 to 60 minutes, cleared and mounted.

TPA-PA-Schiff

This double staining technique was used to stain both intra- and extra-cellular material, so that TPA positive structures could stand out against the red-purple background. Following staining with TPA, sections were oxidized for 15 minutes in periodic acid and stained in Schiff reagent for also 15 minutes. They were then washed in three changes of sulfite water for 2 minutes each, dehydrated and mounted in balsam.

PA-Schiff-Hematoxylin

Following treatment with periodic acid and Schiff reagent, some sections were counterstained with Harris's hematoxylin for 3 to 4 minutes.

Gridley's Variant of Maresch's Bielschowsky Silver Solution

To demonstrate reticular fibers, some Carnoy-fixed sections, after

treatment with 0.5% periodic acid for 15 minutes, were sensitized with a 2% solution of silver nitrate for 30 minutes and then stained in Maresch's Bielchowsky silver solution for 15 minutes (Lillie, 1954, p.334). Following washing in distilled water, they were subjected to 30% formalin for 3 minutes, 0.5% gold chloride for 5 minutes and 5% sodium thiosulfate for 3 minutes. The sections were then cleared in xylene and mounted in balsam.

Photomicrography

Photomicrographs were taken with a Leitz Panphot on Kodak Panatomic X sheet film, which was subsequently developed in Kodak Microdol under constant conditions.

To augment the contrast between TPA-stained structures and the background, an orange filter (Corning 3480) was interposed between the light source and the condenser. When it was necessary to further enhance the contrast, a film with orthochromatic lith-type emulsion was employed without the interposition of a filter.

OBSERVATIONS

Topography of the Rat Kidney

In the present work, the rat was chosen not only because it is one of the common laboratory animals, but also because its kidneys are unilobar and relatively simple in structure, and because the renal tubules and blood vessels are arranged in clear-cut zones. For example, the arcuate artery and vein divide the cortex into an outer zone and an inner zone or subcortex.

In TPA preparations, four main zones could be distinguished in the albino rat kidney: (1) a thick outer cortex, characterized by the presence of numerous renal corpuscles; (2) a relatively thin inner cortex, subjacent to the arcuate vessels and completely devoid of renal corpuscles; (3) a strongly TPA-stained outer medulla succeeded by (4) a broad more lightly stained inner medulla.

Moreover, on the basis of the orientation, distribution and number of mitochondria, as well as on the basis of cell web structures, the TPA technique allowed a subdivision of the nephron and the collecting tubule into successive segments.

This subdivision of the renal tubules is summarized in figure 5.

The outer cortex, in addition to the renal corpuscles, comprises principally the convoluted portion of the proximal tubule, part of the ascending portion, the macula densa and terminal convoluted portion of the distal tubule, and the first and part of the second segment of the collecting tubule. The inner cortex (or subcortex) contains the straight portion of the proximal tubule, the ascending portion of the distal tubule and part of the second segment of the collecting tubule. The outer medulla comprises the thin limb of Henle's loop, the terminal part of the second segment of the collecting tubule, and the ascending portion of the distal tubule. Finally, the inner medulla contains mainly the thin limb of Henle's loop and the last two segments of the collecting tubule.

Proximal Convoluted Tubule

The TPA technique allowed a subdivision of the proximal convoluted tubule into two successive stretches, which did not have exactly the same structure.

First or Proximal Stretch

The first stretch of the proximal convolution was mainly located in the outer cortex and was highly convoluted. This part of the nephron was always seen with a partly collapsed lumen filled with

intensely TPA-stained protein precipitate, presumably albumin resulting from orthostatic albuminuria. This segment was lined by large dome-shaped, truncated cells with large and spherical, darkly stained nuclei disposed toward their bases. The apex of these cells was provided with a prominent brush border, which projected into the lumen of the tubule (Figs. 7, 11 and 14). With TPA the brush border was generally unstained (Figs. 7, 10 and 11); when cut tangentially, however, the cytoplasm of its microvilli showed a moderate staining similar to that of the cell body cytoplasm (Fig. 14). The cytoplasm was filled with randomly-scattered, moderately stained granules, most of which corresponded to granular mitochondria (Figs. 7 and 10 to 14); this notion was strongly supported by the large number of mitochondria found to occupy the cytoplasm of these cells on staining gluteraldehyde-fixed sections with TPA (see Fig. 6). After treatment of gluteraldehyde-fixed sections with PA-Schiff, some cytoplasmic granules (or droplets) were found to be PAS positive, thus to contain glycoprotein; they were colored greenish-blue when such sections were stained with toluidine blue (Fig. 8). They ranged in size from small to large and were found in all regions of the cytoplasm, including the basal zone where mitochondria were preserved as rods; sometimes they occurred in distinct rows from the base to the apex of the cells (see Fig. 8).

An avidly TPA-stained granulated layer was seen spanning the apical

cytoplasm of the cells, immediately under the brush border (Figs. 7, 10, 11 and 14). This dome-shaped layer corresponded to the terminal web and was observed to insert into TPA positive terminal bars. These terminal bars, when cut transversely, were cast as delicate but clearly visible dots between the edges of adjacent cells at their free margins (Figs. 7 and 14); when obliquely sectioned, they appeared as short dark bars closely associated with the granulated terminal web (Figs. 7, 10 and 11); in tangential sections through the apex of the cells, they appeared as well stained lines or bars, arranged in hexagons circumscribing the cell apices (Fig. 9). Due to the convexity of the cell apex and to the light staining of the cytoplasm, it was difficult to obtain a clear view of the terminal web in such tangential sections; in some rare locations, however, the terminal web appeared as a tightly knit fibrillar network.

Starting at the terminal bars and running for a short distance along the lateral surfaces of the cells, some strongly TPA-stained material was also seen; this material took the shape of a short curtain suspended to the terminal bars. About half-way between the terminal bar and the basement membrane, this TPA-positive material disappeared and the cell limits became indistinct (Fig. 14). This deeply stained material was, therefore, referred to as a "lateral web".

At the base of the cells and closely applied to the basement

membrane, some intensely TPA positive material could be seen. Such material assumed different shapes depending upon the plane of section of the tubule. In tubular cross sections, this TPA-stained material looked like short and plump spindles which could be followed for some distance within the plane of the section (Figs. 7 and 14). In longitudinally sectioned tubules, the TPA positive material was cut transversely and appeared as single or double dots, as flat bands or as bands with nodular thickenings at their luminal extremity (Figs. 10 and 11). In sections passing tangentially through the base of the cells, the TPA-stained structures were seen as long delicate spindles or as double forked rods (Figs. 12 and 13). Due to their form and texture, these structures were referred to as "rodlets".

The so-called rodlets were found to vary in thickness and in length (Figs. 12 and 13). Measurements of these rodlets indicated that their average length (11 μ) approximated that of the cell diameter toward the basement membrane (10.6 μ). It, therefore, appeared that they remained within the territory of one epithelial cell and never expanded over the length of more than one cell. As the rodlets varied in thickness, it was evident that some of them would be beyond the resolution of the light microscope. However, an estimate of the number of rodlets from longitudinally sectioned tubules revealed that there were, on the average, 7 visible rodlets per nucleus. Not infrequently, in both longitu-

dinal and tangential tubular sections, infoldings of the basal cell membrane of the epithelial cells were seen in the form of TPA negative, faintly drawn striae running circularly around the tubule in close association with the basement membrane. In many instances a number of rodlets were seemingly associated with these cytoplasmic folds and were oriented similarly, that is, circularly around the tubule (Figs. 12 and 13). This association was best demonstrated in sections treated with the double staining technique, TPA-PA-Schiff, in which the rodlets could be clearly demarcated from the PAS reactive inflected basal cell membrane and could be seen to be unmistakably intracellular. Moreover, the rodlets persisted inside the epithelial cell when the base of the latter was detached from the basement membrane.

Second or Distal Stretch

In TPA preparations, the second stretch of the proximal convolution constituted the straight portion and was preferably encountered within the medullary rays and the subcortex (especially in the vicinity of the cortico-medullary junction); it was, to some extent, structurally different from the first stretch. Low cuboidal cells made up the epithelium of this segment. The apex of the cells rarely bulged in a dome-like fashion into the lumen. The latter was completely free of any protein precipitate and was slit-formed when cut longitudinally (Figs. 16 and 17). The cells

carried at their apex a brush border which was more uniform and shorter than in the first part of the proximal convoluted tubule. The cytoplasm was lighter, less granular and seemed to contain fewer mitochondria than that of the cells forming the first portion of the tubule (Figs. 16 to 18). This notion was supported by the observations made in gluteraldehyde-fixed sections stained with TPA or toluidine blue, in which no rows of rod-shaped mitochondria could be seen in the basilar part of the cells (Fig. 15), and in which the glycoprotein granules which occurred in considerable numbers in the first segment were scarce or completely absent.

The "terminal bar-terminal web" combination was essentially similar to that seen in the first stretch of the proximal convolution; however, the terminal web, instead of being dome-shaped in longitudinal sections, was seen in the form of a straight granulated layer extending across the apex of the cells (Fig. 16). In surface view of the epithelial cells, the terminal bars were straight and were arranged in a regular hexagonal pattern. The cell limits were very distinct, owing to the intensely TPA-stained lateral web which was observed to run along the whole length of the lateral surfaces of the cells, that is, from the terminal bars to the basement membrane (Figs. 16 to 18). At discrete loci on the lateral surfaces of the cells, few desmosomes were intimately related to

the lateral web and appeared as strongly TPA-stained granules separated from the terminal bar by a wide interval (Figs. 16 and 18). The so-called rodlets were also encountered at the base of the cells and were almost as numerous as those in the first segment of the tubule (Figs. 16 to 18).

Thin Limb of Medullary Loop of Henle

Definite squamous type of cells bulging into the lumen formed the epithelium of the thin limb of Henle's loop (Figs. 20 and 22), which was seen traversing the outer medulla.

Occasionally, a brush border was seen in the portion of the thin segment nearest the proximal convolution (Fig. 19). The lumen was wide and, as in the distal portion of the proximal convoluted tubule, was free of any protein precipitate (Figs. 20 and 22). Several granules, which were found in gluteraldehyde-fixed sections stained with TPA to correspond to mitochondria (see Fig. 21), gave a beaded appearance to the partly loose, highly attenuated cytoplasm of the squamous cells (Figs. 20 and 22).

The epithelial cells of this segment of the nephron also contained a well-developed cell web. There was a delicate finely granulated terminal web immediately under the cell surface (Figs. 20 and 22). At the base of the attenuated portion of the cells and closely

applied to the basement membrane, TPA positive rodlets formed a well-developed basal web (Figs. 20 and 22); they were less numerous than in the proximal convolution (3-5 per nucleus). These rodlets were also oriented circularly around the tubule, and their cross sections were seemingly associated with some infolding of the basal cell membrane of the epithelial cells. It was difficult to distinguish the TPA positive terminal bars from the rodlets, and no lateral web could be resolved.

Distal Convoluted Tubule

In TPA preparations the distal convoluted tubule could be subdivided into three successive parts; the ascending thick limb of Henle's loop, the macula densa, and the terminal portion.

Thick Limb of Henle's Loop

This part of the distal convolution was easy to find in the outer medulla and subcortex. It was lined by low cuboidal epithelial cells showing an irregular free surface. The nuclei were located in the apical cell zone, causing some bulges into the lumen (Fig. 24). The latter was generally clear and free, except for some occasional pale staining strands, which tended to form a loose meshwork and which, as they were fastened to the terminal bars, probably corresponded to detached apical cell membranes

(see Fig. 24). Owing to the presence of rows of rod-shaped mitochondria, as seen in gluteraldehyde-fixed material (see Fig. 23), the cytoplasm was characterized by very prominent striations in the light basal two-thirds of the cells (Fig. 24).

When the apical cell membranes were not detached and the surface structures could be resolved, there was a simple, finely granulated terminal web underneath the irregular free surface. The terminal web was arched and inserted into terminal bars (Fig. 24). However, where the plane of the section was slightly oblique and cut close to the luminal surface of the cells, a strongly TPA positive coarsely granulated layer was seen under the apical cell membrane and rendered the differentiation between terminal web and terminal bars problematical (see Fig. 24). Examination of tangential sections through the apex of these epithelial cells (such fields are rare and difficult to find) revealed that the terminal bars followed a very sinuous and irregular course (Fig. 25). Such wavy terminal bars would appear several times in slightly oblique sections through the cells and would contribute to give the heavily granulated appearance to the apical TPA positive layer seen in such sections.

As no TPA positive material was seen along the lateral surfaces of the cells, the lateral limits of the latter were indistinct. However, at the bases of the cells and closely applied to the

basement membrane, rodlets were present; they were thinner, more delicate, and seemingly slightly less numerous than in the proximal convolution (Fig. 24). There was also an indication of association of these rodlets with the inflections of the basal cell membranes of the epithelial cells; due partly to the relatively clear cytoplasm of the basilar third of the cells and partly to their large number, these infolded basal membranes were much more prominent than their counterpart in the proximal convolution (see Fig. 24).

Macula Densa and Other Cells of Juxtaglomerular Apparatus

The second portion of the distal convoluted tubule was short and showed some distinguishing features with TPA. It consisted of a group of specialized cells, called the macula densa (a part of the juxtaglomerular apparatus). The cells of the macula densa differed from the other cells of the distal convoluted tubule in several ways. They were intimately related to the cells constituting the other two parts of the juxtaglomerular apparatus, namely, the granular epithelioid cells of the media of the afferent arteriole and the lacis cells of Oberling and Hatt (1960) which form most of the cells of the "Polkissen" or polar cushion of Zimmermann (1933). Such cells made up the wall of the thick limb of Henle's loop on the side next to the afferent arteriole, as the limb approached the vascular pole of the renal corpuscle of

its nephron and attached itself to the arteriole. In TPA preparations, the granular epithelioid cells, when found in the media of the arteriole, were characterized by their round or oval nuclei and the presence in their cytoplasm of avidly TPA-stained fibrils, resembling in all ways the myofibrils of the surrounding smooth muscle cells; their specific granules were not demonstrated by the TPA technique (Fig. 29). However, when located outside the arteriolar wall (in the polar cushion of Zimmermann), they were indistinguishable from the lacis cells, which also contained some TPA-stained fibrils. As shown in figures 26 and 27, the lacis cells usually lay in compact groups away from the arteriolar wall, filling the space between the macula densa, the afferent arteriole and Bowman's capsule, and often extending into the vascular pole of the renal corpuscle; their nuclei were elongated or flattened, and their cytoplasm was rarely visible. It is worthy of mention that, in gluteraldehyde-fixed sections stained with PA-Schiff-hematoxylin, a homogeneous basement membrane-like layer was seen between the base of the macula densa cells and the granular and lacis cells; ~~this~~ layer was often continuous with a PAS reactive fibrillar intercellular matrix, in which the lacis cell nuclei appeared to be embedded and which frequently penetrated between the glomerular capillaries at the vascular pole of the renal corpuscle (Figs. 26 and 27).

Beside having intimate contact with the granular epithelioid and lacis cells, the cells of the macula densa showed some additional peculiarities which distinguished them from the other cells of the distal convoluted tubule. They were tall and narrow; their nuclei were, therefore, more concentrated than in other segments of the tubule and tended to be located basally (Figs. 29 to 31). As it was found, in gluteraldehyde-fixed material, to contain few randomly scattered granular mitochondria, the cytoplasm was not characterized by basal striations (see Fig. 31). The clear, free lumen was sometimes seen to extend close to the base of the cells, thus separating them from one another and from adjacent distal tubular epithelial cells as well (Figs. 29 and 30). Immediately under the irregular apical cell membrane, there was a terminal web, identical to that of the epithelial cells forming the thick limb of Henle's loop. The terminal bars, as in the thick limb, were serrated in tangential sections through the apex of the cells, excepting that, in some transverse sections, they were located nearer the basement membrane than the luminal surface of the cells (Figs. 29 and 30). Owing to the absence of a lateral web, the cell limits were indistinct. No rodlets could be identified at the base of the cells, and the inflected basal cell membranes were much less prominent than in the thick limb of Henle's loop. However, the epithelial cells lining the wall of the tubule on the side opposite the afferent arteriole were identical in all

respects to those forming the first segment of the distal tubule and were provided with rodlets at their bases (see Fig. 29).

Terminal or Convoluted Portion

The terminal or convoluted portion of the distal tubule was encountered in the outer cortex shortly after the macula densa. While the cells forming this segment shared several major structural features with those of the thick limb of Henle's loop, they were different from them in only minor ways. The distal convoluted tubule cells were cuboidal in the terminal portion. As in the thick limb, the basilar two-thirds of the cells were characterized by very prominent striations (Fig. 32); the nuclei were located in the apical cell zone close to the clear lumen, and the free surface was irregular in shape. The "terminal bar-terminal web" combination was similar to that of the cells lining the thick limb or macula densa; however, in oblique sections through the apex of the cells, the terminal bars, though still irregular, were found to follow a less sinuous course, to be less serrated than those in other parts of the distal tubule (Figs. 33 and 34). No lateral web was seen on the lateral surfaces of the cells (the lateral limits of the cells were therefore indistinct), but at the base of the cells and closely applied to the basement membrane some rodlets were present; they were as numerous as those in the cells of the thick limb.

Collecting Tubule

On the basis of the shape, number and arrangement of the mitochondria of its lining cells, as well as on the basis of the cell web structures demonstrated in the epithelial cells along its length, the collecting tubule was divided into four segments.

First Segment

The first or initial segment of the collecting tubule was short and confined to the outer cortex. It corresponded to the connecting part, called "Verbindungsstück" by Peters (1927), Möllendorff (1930) and Sjöstrand (1944). As the terminal portion of the distal tubule, it was convoluted and its lining cells were of cuboidal height (Fig. 37). With TPA three types of cells could be identified in its epithelium: ordinary lining cells, transitional cells with a light appearance, and intercalated cells with a dark appearance.

The ordinary lining cells contained, in their basilar two-thirds, a moderate number of long filamentous mitochondria, which, in Carnoy-fixed TPA preparations, retained their perpendicular orientation and formed a basal palisade similar to that in the basal cytoplasm of distal tubular cells (Fig. 37). This notion was supported by the finding that, in gluteraldehyde-fixed material,

such a palisade was found in the basal cytoplasm of the majority of the lining cells, while the apical cytoplasm appeared light due to the presence of only few granular mitochondria (see Figs. 35 and 36). The surface of these cells was provided with tiny microvillous projections, which were resolved by the light microscope probably because the fixative used had caused occasional adhesions of several villi. Underneath the microvilli, a simple, finely granulated terminal web stretched across the apex of the cells, extended into the microvilli and inserted into avidly stained terminal bars (Fig. 37). The latter, when seen in tangential sections through the apex of the cells, were not straight; they were still of the wavy type, though less serrated than those in the lining cells of the terminal portion of the distal tubule (Fig. 38). As in the distal tubule, no lateral web was recorded between neighboring ordinary lining cells. The bases of the cell were completely devoid of rodlets.

The transitional cells with a light appearance were much less numerous than the ordinary lining cells and contained few filamentous and granular mitochondria which were randomly scattered and which had lost their perpendicular orientation (see Figs. 35 and 36). They seemed to arise by progressive transformation of the cells forming the terminal portion of the distal tubule. In all other respects (including cell web structures), they were similar to the ordinary lining cells.

The intercalated cells with a dark appearance constituted a distinctive type of cells. They were interspersed singly or in small groups among the other epithelial cells and did not occur in significant numbers in the lining epithelium (Fig. 37). As the ordinary lining cells and the cells of the terminal portion of the distal tubule, they were of cuboidal height. In fact, they were similar to the distal tubular epithelial cells, excepting that the mitochondria were granular and more abundant (Fig. 37). Examination of gluteraldehyde-fixed sections stained with TPA revealed that the dark appearance of the cytoplasm was primarily due to its rich content of relatively avidly stained granular mitochondria (Figs. 35 and 36); however, contrasting with their appearance in Carnoy-fixed material, these dark cells were taller and narrower than the other cells. As in the ordinary lining cells, the surface of the intercalated cells carried microvilli, underneath which a "terminal bar-terminal web" complex was found (Fig. 37). While no rodlets were recorded, the cell borders were often distinct owing to the presence of a fine lateral web (Fig. 37).

Second Segment

The second segment of the collecting tubule was lengthy and extended from the outer cortex to the distal portion of the outer medulla. It was referred to as the "arched collecting tubule" by

Greep (1954). Its epithelial lining comprised two classes of cells: light and dark, or intercalated, cells. The light cells occurred in significant numbers and had a relatively high cuboidal shape. They were characterized by a large pale nucleus which occupied about half of their clear cytoplasm and which often occasioned a bulging of the cell apex into the lumen of the tubule (Figs. 40 and 41). In both Carnoy- and gluteraldehyde-fixed sections stained with TPA, the cytoplasm was found to contain very few granular mitochondria (Figs. 39 to 41). The cell surface was irregular and uneven and did not appear to carry microvilli (Figs. 40 and 41). These cells showed a poorly developed terminal web (if any) and a relatively well-stained lateral web which was often seen running along their lateral surfaces from the terminal bars to the basement membrane (Figs. 40 and 41). The light cells contained no rodlets at their bases.

The dark cells were generally lower than the light cells. The nucleus was often smaller and darker than that of the light cells and was located basally. As the lining epithelial cells of the initial segment of the tubule, the surface of the dark cells was provided with pronounced microvillous projections and showed a heavily stained terminal web underneath the microvilli (Figs. 40 and 41). The TPA positive terminal bars seen at the junction of the apices of the two types of cells were found to be straight

in tangential sections passing through the apex of the cells (Fig. 42). However, while they gave a regular hexagonal outline to the apex of the dark cells, they were barely visible at the apex of the light cells (Fig. 42). Not infrequently, the distinctively avidly stained cytoplasm of the dark cells was observed to be interposed between a light cell and the basement membrane. Consequently, the lateral cell membrane of a dark cell (which was rendered distinct by the intense TPA staining of the lateral web) was often seen to bifurcate without making any contact with the basement membrane and to pass underneath the adjacent light cell, thus separating the base of the light cell from the strip of dark cell cytoplasm (Figs. 40 and 41). As in the light cells, no rodlets were found at the base of the dark cells.

Third Segment

The third segment of the collecting tubule was also lengthy and traversed the whole proximal portion of the inner medulla. Its epithelial lining was formed by a single type of cuboidal cells, which contained few scattered mitochondria, as seen in glutaraldehyde-fixed material (Fig. 43). These cells showed a delicate terminal web associated with well developed terminal bars (Figs. 44 and 45). These terminal bars were also straight and outlined the cell apex in a regular hexagonal pattern. At the base of the cells occasional rodlets were visible (Figs. 44 and 45). Occasionally a lateral web was resolved.

Fourth Segment

The fourth segment of the collecting tubule, also known as the papillary or excretory duct, extended throughout the distal portion of the inner medulla (or tip of the papilla). Its lining cells were large and columnar. Contrasting with the epithelial cells covering the other portions of the collecting tubule, their free surface was smooth. In addition to a well developed "terminal bar-terminal web" complex, they had a very prominent lateral web, that is, some TPA stained fibrils connected to the terminal bars and running along the lateral walls of the cells toward the basement membrane (Fig. 46). No rodlets were resolved at their base, and in top views of the cells the terminal bars were straight and had an hexagonal arrangement in circumscribing the apex of the cells (Fig. 47).

Surface of Papilla

The surface of the papilla was lined by a simple cuboidal or columnar epithelium, which was continuous with the lining epithelium of the fourth portion of the collecting tubule and which consisted of two types of cells. As the cells forming the terminal part of the collecting tubule, the type of papillary epithelial cells which predominated were characterized by a deeply TPA-stained "terminal bar-terminal web" combination as well as a prominent lateral web (Fig. 48). The second type of papillary cells were

scattered singly among the first class of cells; they were intensely TPA positive throughout and slender in shape (Fig. 48). As they showed signs of nuclear and cytoplasmic degeneration, they were regarded as degenerating cells.

Renal Corpuscle

Capsule of Bowman

In the parietal epithelial cells, the identification of cell web structures in several locations was not impeded by TPA positive protein precipitate in the capsular space. A cell web was seen in the form of strongly stained fibrils running in both the apical and basal cytoplasm of the parietal squamous cells, parallel to their long axis (Figs. 50 to 53). These longitudinally oriented fibrils were often observed to be discontinuous and seemed to be confined to the territory of only one epithelial cell (Fig. 49). They always showed a particular affinity for membranous sites, excepting the nuclear membrane.

In some longitudinal sections through the parietal epithelium, the apical and basal fibrils were found to fuse between the oval nuclei where the cell body was highly attenuated, but they did not appear to insert into desmosomes or terminal bars (Figs. 50, 51, 53 and 56). In other planes of cut in which the base of the epithelial cells was sectioned tangentially, the basal fibrils

could be followed for some length and were closely applied to the TPA negative capsular basement membrane (Fig. 49). In such sections they were frequently seen in the form of intensely stained single dots or short bars, arranged in a more or less parallel fashion, linked together by delicate TPA positive strands, and embedded in the inner surface of the capsular basement membrane (Fig. 50). Indeed, in these tangential sections, the inner surface of the basement membrane was observed to be markedly irregular, and to exhibit fingerlike masses pressing upward for short distances into the bases of the cells and invaginating their basal cell membranes in the process (see Fig. 50). The resulting cytoplasmic folds were usually seen as faintly drawn striae interdigitating with the fingerlike masses of the basement membrane and containing within them the dots or short bars of the basal fibrils.

Moreover, in some planes of cut, the fibrils were seen in the form of a continuous circumferential fibrillar layer which completely surrounded the cell and which was located immediately under the cell membrane (Fig. 57). In obliquely sectioned parietal epithelial cells, this circumferential layer was observed to be discontinuous and to consist of short bars (Fig. 55). In cross sections through the cells, these short bars were substituted by coarse granules (Fig. 54). When the base of the cells was cut

transversely, however, only the basal fibrils appeared in cross section (Fig. 58).

The elongated visceral epithelial cells, known as podocytes, covered the outer surface of the glomerular basement membrane, their inner surfaces conforming to its curvature. They were seen to fill most of the capsular space between the glomerular capillaries. Some podocytes showed a very intense TPA staining of their cytoplasm, which impeded the resolution of any fibrillar structure. This cytoplasmic coloration was homogeneous in some cases and accumulated into masses in others; not infrequently, it extended into the cytoplasmic processes which characterize these cells. Such cells tended to be located on the outer surfaces of the peripheral capillaries and seemed to be in intimate contact with three or even four capillaries (see Figs. 59 and 60).

Other podocytes were characterized by the presence in their cytoplasmic bodies of TPA positive material which assumed the form of fibrils which often extended into the long cytoplasmic arms of these cells (Figs. 56 and 62). Such fibrils were sometimes wavy in appearance and frequently showed nodular thickenings (Fig. 63). These TPA-stained fibrils were not seen to insert into any special intercellular attachment sites. However, in some planes of cut, rows of deeply TPA-stained very fine granules were sometimes resolved in close proximity to the glomerular basement membrane

(see Fig. 64). Such fine granules, on the basis of their staining intensity, size and position, were considered to be desmosome-like structures connecting the foot processes of neighboring podocytes which are known to interdigitate.

It is worthy of mention that the fibril-containing podocytes seemed to be phagocytic as their cytoplasm usually contained granules which occurred in considerable numbers and varied in size from a small to large (Fig. 61). Since these granules showed a positive PA-Schiff reaction, they presumably contain glycoprotein. It is interesting to note that such granules were TPA positive and displayed a series of colors ranging from deep blue to purple when subjected to the double staining technique, TPA-PA-Schiff. It is also noteworthy that they were stained only in Carnoy-fixed sections, thus contrasting with the granules in proximal tubular cells which were visible only in gluteraldehyde-fixed material. Finally, these glycoprotein-containing granules were often replaced by large coarse bodies, which were present singly or in pairs and which usually occupied a juxtannuclear position (see Fig. 62). With the double staining technique, TPA-PA-Schiff, such bodies often displayed a TPA positive reticulum, in the meshes of which a PAS reactive mucoid material accumulated.

Glomerulus

In the glomerulus the inner surface of the TPA negative glomerular

basement membrane was lined by few visible endothelial cells. The identification of the latter was rendered somewhat difficult by the intense TPA staining of some protein precipitate in the lumina of the glomerular capillaries, as well as by the ring of TPA positive material under the surface of each intraluminal erythrocyte. These endothelial cells showed no cell web structures.

Only the nuclei of the relatively few lacis cells which penetrated the glomerulus between the capillaries were visible. Indeed, most of their cytoplasm was embedded in a TPA negative fibrillar intercellular matrix, which reacted positively on staining with PA-Schiff and which was continuous with the glomerular basement membrane (see Figs. 26 to 28).

DISCUSSION

In the following chapters, the subdivision of the nephron and the collecting tubule into successive segments will be discussed. This will be followed by an analysis of and a discussion on the terminal web and terminal bars, the lateral web and desmosomes, and the basal web in renal tubular epithelial cells. Lastly, the cell web fibrils in the lining cells of Bowman's capsule will be analyzed.

Subdivision of Nephron Segments and Collecting Tubule

Proximal Convoluted Tubule

Zimmermann (1911), in his study of cat and dog kidney, subdivided the proximal convoluted tubule into two parts: a convoluted part and a more distal straight part (or pars recta). More than a quarter of a century later, Sjöstrand (1944) made an extensive light- and fluorescent-microscopic investigation of the mouse kidney and noted structural differences in the lining epithelium of the proximal convolution which he subdivided into four successive segments. Using the electron microscope, Rhodin (1958a,

1958b) paid considerable attention to the classification suggested by Sjöstrand (1944). However, the findings of Rhodin (1958a, 1958b) tallied well with those of Zimmermann (1911), for he could distinguish, on the basis of the morphological characteristics of the cells lining the proximal convoluted tubule, only two main portions: a proximal part, representing the convoluted part in Zimmermann's (1911) classification or the first two parts in Sjöstrand's (1944) classification, and a terminal part, corresponding to the "pars recta" noted by Zimmermann (1911) or the third and fourth parts described by Sjöstrand (1944).

Rhodin (1958a, 1958b), in his electron microscopic investigation of the mouse, rat, cat and human kidney, confirmed the earlier finding of Zimmermann (1911) that the cell borders in the terminal part of the proximal convolution are much more prominent and straight than in the proximal part and that they do not interdigitate. Indeed, prior to Zimmermann's (1911) discovery, Schachowa (1876), who first described the inflections of the basal cell membrane in the proximal convolution, had clearly demonstrated with Golgi type impregnations that the proximal tubular epithelial cells are interlocked; this author believed that the interlocking of neighboring cells occurred along the whole length of the proximal convoluted tubule.

Other distinguishing features of the terminal part of the proximal

convolution were also pointed out by Rhodin (1958b). He noted that the infoldings of the cell membrane in the basilar portion of the cells lining this segment of the tubule are fewer and less pronounced than in the proximal part. He also reported that the microvilli carried by these cells are coarser, less abundant and seemingly shorter than their counterpart in the proximal portion of the tubule. Finally, according to this author, the cytoplasm of these cells is characterized by a great reduction in the number of mitochondria which, in contrast to the initial segment of the tubule, are mostly oval or spherical and are randomly distributed.

From the evidence presented in this work, the subdivision of the proximal convoluted tubule into two successive stretches, on the basis of the orientation and number of mitochondria as well as on the basis of cell web structures, seems reasonable, especially that it is in full accordance with the classification suggested by Zimmermann (1911) with the light microscope and later by Rhodin (1958b) with the electron microscope. Indeed, in TPA preparations, the cells of the second stretch of the proximal convolution differ in several ways from the other proximal tubular cells. First, the brush border is shorter than its counterpart in the first stretch. Secondly, the mitochondria are fewer and granular in appearance, and therefore are not arranged in rows as in the first

stretch. Finally, the cell limits are more distinct than those of the cells forming the first stretch of the tubule (a feature which will be discussed below).

Distal Convolute Tubule

As early as 1895, Landauer pointed out that the cell limits of the distal convolute tubule are straight toward the luminal part of the cell and interdigitate with neighboring cells in the basal part. The basal cell membrane in the distal tubule was found to be extensively folded (Pease and Baker, 1950; Dalton, 1951; and others). Rhodin (1958a, 1958b) reported that the infolded basal membranes of the distal tubular cells are much more prominent and appear to be more numerous and more complicated than their counterpart in the proximal convolution; this author also noted that they are less deep and that the interlocking of cells is less conspicuous toward the terminal portion of the distal tubule. While Pease (1955) and Rhodin (1958b) described varying numbers of microvilli in the distal tubular cells, Kurtz (1964) found that the structures are uncommon in this segment of the nephron and attributed their presence in the electron microscopic preparations of Pease (1955) and Rhodin (1958b) to poor methods of fixation.

Mollendorff (1930) suggested that the distal convolute tubule includes the thick ascending limb of Henle's loop, the macula

densa, and the intercalated part (pars intercalata or "Schaltstück"). Since Rhodin (1958b) found with the electron microscope that the general cellular structure of the thick limb of Henle's loop is similar, if not identical, to the cells of the distal tubule, he followed the classification suggested by Müllendorff (1930).

In the present work, Müllendorff's (1930) classification is adopted, but not for the same reason as Rhodin (1958b). It is agreed that the cells forming the thick ascending limb as well as the macula densa and "pars intercalata" share many major structural features. For example, in gluteraldehyde-fixed TPA preparations, they all contain, with the exception of the cells of the macula densa, rod-like mitochondria in their basal cytoplasm; as reported by Kurtz (1964), their apex was not found, in Carnoy-fixed material, to be provided with microvillous projections. However, from the evidence presented in this work, these cells show some important structural differences, especially as far as the cell web is concerned (a topic which will be discussed in detail in the next chapters), thus justifying the subdivision of the tubule into three successive segments.

Collecting Tubule

Sjöstrand (1944), in his historical review of the histology of the collecting tubule, noted in the mouse kidney a relatively

abrupt transition from the distal convolution to the "connecting piece" of the collecting tubule. Using the electron microscope, Rhodin (1958a, 1958b) described the cortical portion of the collecting tubule which he subdivided into two segments: an initial connecting part followed by the arched portion of the tubule. This author confirmed the earlier finding of Sjöstrand (1944) that the transition from the distal tubule to the connecting part is abrupt. He also extensively described the "light" and "dark" or "intercalated" cells noted in earlier studies (see Müllendorff, 1930; Sjöstrand, 1944) in the arched collecting tubule. The light cells are more abundant and taller than the dark cells, which occur with greatest frequency in some of the cortical segments of the collecting tubule.

More recently, Young and Wissig (1964) found that the abrupt transition from the distal tubule to the connecting part of the collecting tubule observed by Sjöstrand (1944) in the mouse apparently does not occur in the rat. On the basis of the types of cells lining the collecting tubule, Young and Wissig (1964) divided the latter into four successive segments. The short first segment corresponds to the "connecting piece" and consists of three classes of cells: ordinary lining cells with randomly scattered mitochondria which predominate in number, transitional cells reminiscent of distal tubular cells, and occasional dark

or intercalated cells. The lengthy second segment begins in the cortex and terminates in the outer portion of the inner medulla; it is lined by ordinary lining cells similar to those of the first segment as well as scattered intercalated cells, which are generally taller. The third segment traverses most of the inner medulla and is formed by a single type of cuboidal epithelial cell. The fourth segment is confined to the tip of the papilla and is also lined by a single type of low or medium columnar cell.

The classification presented in this work tallies well with that suggested by Young and Wissig (1964), except for three features of the first segments of the tubule: first, the dark cells are generally lower than the light cells in Carnoy-fixed sections, but taller in gluteraldehyde-fixed sections; secondly, the dark cells, as noted also by Rhodin (1958, sometimes penetrate between the basement membrane and the bases of the light cell, thus justifying the name "Schaltzelle" (covering cell) given to them by Audrejewitsch (1919) and Müllendorff (1930); thirdly, what Young and Wissig (1964) regarded as "transitional cells" was found to be the predominant cell type and was, therefore, referred to as "ordinary lining cells". The positive significance of the cell web structures in the lining epithelium of the collecting tubule will be discussed below.

Terminal Web and Terminal Bars

The epithelial cells lining the first stretch of the proximal convoluted tubule possess a dome-shape simple terminal web which extends immediately beneath the brush border and terminates in the straight terminal bars outlining the apex of the cells in a regular hexagonal pattern. From the evidence presented in this work, such a terminal web forms a tightly knit fibrillar network under the brush border. Thus, the apical region of the cells forming the first stretch of the proximal convolution would be a dome sustained by a framework of criss-crossing cell web fibrils. Such an arrangement would explain the granular texture which is often recognized in cross sections of the terminal web.

From the evidence of cross and longitudinal sections of the second stretch of the proximal convolution, it is ascertained that the apex of the cells lining this segment of the nephron seldom bulges in a dome-like fashion into the slit-formed, free lumen and carries a brush border which is more uniform and shorter than its counterpart in the first stretch of the tubule. It follows that, in contradistinction to the first portion of the proximal tubule, a relatively straight simple terminal web stretches across the apex of the cells and inserts on the straight terminal bars circumscribing their apex.

Leblond et al (1960) and later Kallenbach (1963) reported that the brush border of proximal tubular cells as well as the striated border of intestinal absorptive cells are TPA negative. Recently, McNabb (1964) clearly demonstrated with the electron microscope that the microvilli making up the striated border of intestinal epithelial cells contain a central core of straight filaments which extend into the apical cytoplasm as a rootlet and connect with the terminal web. This author also showed that the filamentous component of the microvilli of the striated border has the same dimension as that of the terminal web to which it is connected. An illustration of such a fibrous core in the microvilli constituting the brush border of proximal tubular cells was not found in the large volume of electron microscopic work on the kidney. As a set-off, in a few electron micrographs (Ham and Leeson, 1961; Fig. 466; Farquhar and Palade, 1963, Figs. 19, 20; Kurtz, 1964, Fig. 13), a hazy material was seen at the level of the terminal web and extended into the cytoplasm of the microvilli. Moreover, contrary to the description of Rhodin (1958b) who stated that there were no formed structures in the microvilli, Kurtz (1964) showed that the cytoplasm of the microvilli does contain "small particles common to the general basic ground substance of the rest of the cell".

In the present work, however, the microvilli forming the brush

border were found to be generally TPA negative, excepting that in some planes of cut their cytoplasm showed a moderate TPA staining similar to that of the cell body cytoplasm. This moderate coloration of the cytoplasm of these microvilli cannot be attributed to the presence of cell web fibrils, since the operational definition of the term "cell web", as seen by light microscopy, is "an intracellular fibrillar component of the cytoplasm which stains strongly with TPA". It is still an open question as to whether filamentous cores similar to those found by McNabb (1964) in the microvilli of the striated border of intestinal absorptive cells exist in the microvilli making up the brush border of proximal tubular cells. From the evidence presented in this work, the presence of filamentous cores cannot be ascertained, and it seems likely that such cores, even if they existed, would not be demonstrated by the TPA technique and, therefore, would have to be regarded, just like those of the striated border microvilli, as not being a part of the cell web.

In the other segments of the nephron, as well as in the collecting tubule and the cells coating the surface of the papilla, the terminal web is simple and terminates in terminal bars. However, with the exception of the dark or intercalated cells of the first two segments of the collecting tubule, the terminal web is much finer than that spanning the apical cytoplasm of the cells forming

the proximal convolution. Indeed, a strongly TPA-stained dense terminal web stretches across the apex of the dark cells and inserts on the terminal bars; an important feature of this web is that it extends into the microvilli which are resolved in TPA preparations probably because the fixative used has caused occasional adhesions of several villi. The only renal tubular epithelial cells that seem to lack a terminal web are the light cells in the second segment of the collecting tubule.

The cells of the proximal convoluted tubule are known to be primarily concerned with absorption (Bloom and Fawcett, 1962). While an explanation of the function of the dark and light cells lining the second segment of the collecting tubule has not yet been found, Andrejewitsch (1919) and later Yoschimura and Nemoto (1953) attributed the difference between dark and light cells to different functional states of the same cell type in an apocrine-type secretion process. Clark (1957) shared also the view that these cells only represent different functional states, a concept which seems to be supported by the finding that potassium depletion in rats causes an increase in the number of dark cells (Oliver et al, 1957). Thus, there seems to be ample evidence that the dark cell is a modified version of the light cell and that the latter is involved in a secretion of apocrine type while the former is mainly concerned with absorption. This would probably explain why the light cell is seemingly devoid of a terminal web. Moreover, the terminal web

in the dark cells is as dense as that in the proximal tubular cell, contrasting with the fine terminal web described at the apex of other renal tubular epithelial cells, which are known to have a secretory function. This observational analogy between the proximal tubular cells and the dark cells of the collecting tubule (which are both absorptive cells) seems to indicate that the terminal web is well developed in cells involved in absorption, a concept which is supported by the dense distal terminal web described by Kallenbach (1963) in intestinal absorptive cells.

No electron microscopic investigation of renal epithelial cells was made. However, the data given in the literature on the ultrastructure of the kidney were carefully verified. There was no convincing illustration of the terminal web, though certain structures corresponding to the terminal web could be identified in some electron micrographs (Farquhar and Palade, 1963, Figs. 19, 20; Kurtz, 1964, Fig. 13; and others). In such electron micrographs, as it is illustrated in figure 67, the terminal web appears as a hazy band, which is not divided into an amorphous layer and a filamentous layer and which stretches across the apex of the cells, separating, in the case of proximal tubular cells, the brush border from the cell body and terminating in the terminal bars. As it was aforementioned, this hazy material was seen to extend into the cytoplasm of the microvilli making up the brush border or carried

by other renal tubular cells (Ham and Leeson, 1961, Fig. 466; Farquhar and Palade, 1963, Fig. 21).

Terminal bars were shown in the proximal convoluted tubule by Zimmermann (1898). Contrary to the terminal web, they have been described repeatedly in all renal tubules with the electron microscope (Pease, 1955; Takaki et al, 1956; Rhodin, 1958a, 1958b; Miller, 1960; Latta et al, 1961; Farquhar and Palade, 1963; Kurtz, 1964; and others).

From the evidence presented in this work, the terminal bars are straight bar-type structures which outline in a regular hexagonal pattern all the renal tubular cells and those covering the surface of the papilla, except the cells lining the distal convoluted tubule and the first segment of the collecting tubule where they are wavy or serrated. Such wavy-type terminal bars have never been described with either the light or the electron microscope. However, Farquhar and Palade (1963), in their investigation of junctional complexes in a number of epithelia, noted a striking difference between the terminal bars in the proximal convolution and those in the distal convolution and the collecting tubules. Indeed, in the proximal convoluted tubule, the tight junction (or zonula occludens) is extremely shallow while the intermediate junction (or zonula adhaerens) is deep and usually associated with a broad band of dense cytoplasmic material. In the distal and collecting tubules, however, the situation is almost

reversed, for it is the zonula occludens which is extensively developed. The zonula adhaerens varies from one intercellular space to another: sometimes it is poorly developed; sometimes it is well defined; finally in many instances it is entirely missing. This led these authors to conclude that the second element of the terminal bar in the distal convolution "is not a continuous structure around each cell and throughout the epithelium". However, the evidence presented in this work tends to question this statement. It would seem more reasonable to assume that the zonulae adhaerentes in the distal tubule are not in the same plane as the zonulae occludentes. This would not only explain why the intermediate junctions in this segment of the nephron are often missing, but also account for the serrated appearance of the terminal bars in TPA preparations.

Still better, Bulger (1964) discovered that, contrary to the opinion of earlier workers, that slender cytoplasmic compartments, which were usually thought to be confined to the apical cell region, extended the full thickness of the epithelium from lumen to base, particularly in the lining cells of the ascending thick limb of Henle's loop and to a lesser extent in the other distal tubular cells. This author noted that these small compartments were connected to neighboring cells by junctional complexes and that they seemed to be "thin radial processes of columnar cells similar to those known to exist on the cells of the loop of Henle".

This finding would thus also justify the serrated appearance of the terminal bars in the distal convolution. The fact that the terminal bars become gradually less serrated, as one proceeds from the ascending thick limb of Henle's loop toward the first segment of the collecting tubule, suggests that, distal to the macula densa, there is a progressive straightening of the lateral cell membranes, a notion which is supported by the scarcity or absence of interdigitations between the light and dark cells lining the second segment of the collecting tubule (Rhodin, 1958b) and confirmed in the present study.

Finally, the fact that, in contrast to the straight terminal bars outlining the apex of the dark cells of the collecting tubule, those circumscribing the apex of the light cells, which do not seem to possess a terminal web, are not stained by TPA constitutes further evidence that the staining of terminal bars with TPA is presumably due to the aggregation of cell web fibrils on these intercellular attachment sites. This is in full accordance with the concept put forward by Leblond et al (1960) and later by Kallenbach (1963).

In conclusion, with the exception of the light cells of the collecting tubule, all renal tubular epithelial cells possess terminal web fibrils which are inserted on terminal bars. The latter are straight in all the cells coating the surface of the papilla as well as in

all renal tubular segments, except the distal convolution and the first part of the collecting tubule where they are serrated. The terminal web is denser in absorptive cells (proximal tubular cells and dark cells of collecting tubule) than in secretory cells (other renal tubular epithelial cells), and seems to extend into the microvilli carried by the dark cells of the first two segments of the collecting tubule. Such cell web fibrils probably function in the mechanical support of the apical cytoplasm of the renal tubular epithelial cells where stress is likely to be exerted.

Lateral Web and Desmosomes

With the exception of the cells forming the thin and thick limbs of Henle's loop, the terminal portion of the distal tubule and the initial segment of the collecting tubule, the limits of all renal tubular cells as well as those coating the surface of the papilla are distinct in TPA preparations. This is due to the intensely TPA-stained lateral web which runs along the lateral surfaces of the cells from the terminal bars toward the basement membrane. This concept is supported by the fact that the combined thickness of two lateral cell membranes and the intermediate space is about 250 Å, while the effective resolution of the light microscope is 0.2 or 2,000 Å (Eloom and Fawcett, 1962). Thus, theoretically, the thickness of lateral cell membranes would keep them

beyond the range of the light microscope.

The lateral web is continuous with the terminal web at the level of the terminal bars and, in sections, has a granular texture. This would suggest that the lateral web has a filamentous substructure and forms a curtain of tightly knit cell web fibrils along the lateral surfaces of the cells.

While the lateral web extends to only about half-way between the terminal bar and the basement membrane in the cells of the first stretch of the proximal convolution, it runs along the whole length of the lateral surfaces of the cells forming the second stretch of the tubule. Many light and electron microscopists have repeatedly demonstrated that the cells lining the first portion of the proximal convoluted tubule are interlocked, but that the cells are less fluted close to the lumen as compared with the basal parts (Belosavitch, 1919; Pease and Baker, 1950; Rhodin, 1958b; and others). On the contrary, in the terminal portion of the tubule, interlockings of neighboring cells are rare (Rhodin, 1958b). It can be concluded from these findings that lateral web fibrils are present only along the straight portion of the lateral sides of proximal tubular cells and are not visible or absent where interdigitations occur. This also holds for the cells of the macula densa and the last three parts of the collecting tubule as well as for the cells lining the surface of the papilla, where interlockings rarely take place (Rhodin, 1958b; Kurtz, 1964;

and others). Thus lateral web fibrils seem to show a particular affinity for straight lateral cell membranes.

Another evidence that lateral web fibrils run only along straight lateral cell membranes is found in the second segment of the collecting tubule. Rhodin (1958b) reported that, while interlockings may be seen between the dark and light cells of this segment of the tubule, they are exceedingly rare between adjacent dark cells. In the present work, no lateral web fibrils was found between neighboring dark cells, whereas they were distinct between light and dark cells; moreover, these fibrils sometimes extended between the base of a light cell and the attenuated portion of a dark cell cytoplasm where the latter is interposed between the light cell and the basement membrane.

Beside the lateral web described by McNabb (1964) in his electron microscopic study of intestinal absorptive cells, a convincing illustration of the lateral web was not found in the literature. However, in some electron micrographs, as it is represented in figure 67, a hazy ectoplasmic material, which was also encountered at the level of the terminal web, was seen coursing the lateral surfaces of some renal tubular epithelial cells (see Ham and Leeson, 1961, Fig. 466; Farquhar and Palade, 1963, Figs. 19 to 23).

Desmosomes have never been described in the renal tubular epithelial

cells with the light microscope. Recently, however, Farquhar and Palade (1963), using the electron microscope, have shown these structures to be small and definitely less frequent all along the epithelium of the renal tubules (especially in the distal convolution) than in other epithelia. These authors also reported that a wide interval generally separates a desmosome from the zonula adhaerens of the terminal bar.

In the present investigation, few distinct desmosomes are seen at discrete loci in the plane of contact of neighboring proximal tubular cells and adjacent dark and light collecting tubular cells. They appear as strongly TPA-stained granule-type structures located at some distance from the terminal bar. This finding is in agreement with that of Farquhar and Palade (1963). As it was aforementioned, the TPA staining of desmosomes, like that of terminal bars, is probably due to the accumulation of cell web fibrils on these attachment sites, especially that the lateral web is attached to them.

In conclusion, a fish net-like lateral web extends from the terminal bar toward the basement membrane along the straight portion of the lateral surfaces of renal tubular cells. Such a web seems to be absent wherever interlockings of neighboring cells occur and runs from desmosome to desmosome located at discrete loci in the plane of contact of two adjoining cells.

BASAL WEB

As it was aforementioned (see Review of Literature), Wedl (1850) was the first to describe the so-called rodlets or basal fibers ("Basalreifen") in isolated kidney tubules. Zimmermann (1898), and later Bizzozero (1901) and Heidenhain (1911) stained these rodlets with iron hematoxylin in a wide variety of species and considered them to be extracellular basal cementing rings ("Kittfaden"), which would reinforce the attachments of the cells to the basement membrane by connecting each rod-like cytoplasmic lamella to the latter. Tasic (1918), on the contrary, showed them to be short and discontinuous cementing lines at the base of the cells lining the proximal convolution. More recently, Kallenbach (1963), using the TPA technique, demonstrated these structures at the base of proximal tubular cells and believed that they were intracellular.

From the evidence presented in this work, the so-called rodlets are single or forked structures located at the base of the cells lining the proximal convolution, the thin and thick limbs of Henle's loop, the terminal portion of the distal convoluted tubule and the third segment of the collecting tubule. They run circularly around these renal tubular segments in close proximity to the basement membrane (see Fig. 65).

The fact that the length of the rodlets approximates the diameter of an individual epithelial cell toward the basement membrane, coupled with the facts that they stain avidly with TPA and persist inside the epithelial cell when the base of the latter is detached from the basement membrane, seems to indicate that they are intracytoplasmic structures. Indeed, beside fibrin threads, TPA positive structures are intracellular. The rodlets cannot be regarded as "half-desmosomes", that is, special attachment sites which sometimes occur between cells and extracellular structures, since they do not remain firmly pinned to the basement membrane when all the rest of the basal cell surface sags away from the latter. The intense TPA staining of the rodlets suggests that they have a filamentous substructure. Thus, contrary to the views of Zimmermann (1898) and Tasic (1918), the hypothesis that the rodlets are intracytoplasmic fibrillar structures making up a basal web is favored, a concept which is in full accordance with the operational definition of the term "cell web" as a fibrillar component of the cytoplasm which stains with TPA.

It has been found with Golgi type impregnations (Möllendorff, 1930) and with the electron microscope (Ruska et al, 1957; and others) that the basal cytoplasm of the renal tubular cells in which rodlets are found is divided into a multitude of cytoplasmic compartments or lamellae formed by the numerous infoldings of the

cell membrane near the basement membrane. These compartments are thus limited by the basal cell membrane. Most of them emanate from the same cell, but some are derived from the neighboring cells, so that adjacent cells interdigitate. Although no mention of rodlets has been made in electron microscopic studies, these structures are visible in some electron micrographs (Rhodin, 1958b, Figs. 10, 22; Latta et al, 1961, Fig. 4; Ham and Leeson, 1961, Fig. 466; Farquhar and Palade, 1963, Fig. 21) as hazy ectoplasmic masses confined to the base of the cytoplasmic compartments near the basement membrane, as it is illustrated in figures 66 and 67. Such compartments correspond, in fact, to the TPA negative striae which are seen in intimate association with the rodlets, and these hazy masses which they contain represent not slight thickenings or local specializations of the cell membrane, but rather bundles of cell web filaments. It, therefore, seems very likely that the rodlets are located within the cytoplasmic compartments, the majority of which, from the evidence presented in this work, run circularly around the renal tubules.

Ruska et al (1957) have clearly shown that the basal cell membrane of proximal tubular cells occasionally makes an indentation before it is reflected again into the interior of the cell, thus subdividing partially some cytoplasmic compartments at the base of these cells (see Fig. 67). This division of single compartments

may account for the fact that the rodlets sometimes appear as paired structures when the tubule is cut longitudinally and as forked spindles in tangential sections through the base of the tubular epithelial cells.

According to Rhodin (1958b), the basal part of distal tubular cells shows more cytoplasmic compartments than that of proximal tubular cells; in this segment of the nephron, such compartments are often very narrow and become less deep in the lining cells of the connecting part of the collecting tubule. This author also reported that the inflections of the basal cell membrane are short and give rise to narrow cytoplasmic compartments in the cells forming the first two segments of the collecting tubule. Latta et al (1962) pointed out that basal infolded membranes are much fewer in the cells of the macula densa than in the other lining cells of the distal tubule. Kurtz (1964) also noted that the cells of the macula densa seem to have very few cytoplasmic compartments, which are only focally seen in the collecting tubule cells. Moreover, deep infolded membranes running the whole way from the basement membrane to the lumen were described only in the attenuated portion of the squamous cells lining the thin limb of Henle's loop by Pease (1955), Rhodin (1958b), and others. The present investigation revealed that the rodlets vary in thickness; they are prominent and abundant at the base of the

lining cells of the proximal convolution; they are found only in the attenuated portion of the cells forming the thin limb of Henle's loop; they are much finer and less readily visible in all the distal tubular cells (excepting the cells forming the macula densa); finally, they are absent along the whole length of the collecting tubule (excepting the third segment) and at the base of the cells coating the surface of the papilla. All these light microscopic findings, when correlated to the electron microscopic morphology of the tubular epithelial cells, tend to indicate that rodlets occupy the bases of only long cytoplasmic compartments close to the basement membrane and are absent wherever these compartments are short. In addition, an intimate relationship seems to exist between the size of the compartments and that of the rodlets, a concept which is supported by the finding that the rodlets are much finer and less prominent in the distal tubule than in the proximal convolution. It is, therefore, very likely that the size of a rodlet is dictated by the dimensions of the cytoplasmic compartment in which it occurs.

In conclusion, the majority of renal tubular cells possess a basal web which assumes the shape of single or forked rodlets of variable thickness, which are confined to the territory of only one epithelial cell and which run circularly around the tubules, in close proximity to the basement membrane. The rodlets thus

indicate that the folds of the basal cell membrane are oriented along a given axis, that is, mainly circularly around the renal tubules. Each rodlet is conceived as a bundle of cell web filaments, which extends in the base of a cytoplasmic compartment, close to the basement membrane, from one end of a tubular epithelial cell to the other, and which does not terminate, as far as can be seen now, in desmosomes or other special attachment sites. There appears to be no connection between the lateral web and the rodlets. On the basis of the size and distribution of these structures, it is felt that they occupy the bases of only long cytoplasmic compartments. Such a basal web is equivalent to the basal plates in the reticulo-endothelial cells of splenic sinuses, which also stain with TPA (Leblond et al, 1960) and appear to give rigidity to the base of the cell.

As the rodlets are presumably contained within the bases of only long cytoplasmic compartments, it seems reasonable to regard them as basal web filaments insuring rigidity to these compartments and making the latter more resistant to stress. Since Ruska et al (1957) reported that individual compartments may be folded, it is not unconceivable that the long cytoplasmic compartments of tubular epithelial cells, owing to the presence of the basal web, may be rigid, while the short ones have more flexibility.

Cell Web Fibrils in the Renal Corpuscle

Capsule of Bowman

Very little attention has been paid to the parietal epithelial cells of Bowman's capsule in electron microscopic investigations of the kidney. It seems that electron microscopists, once they are engaged in the study of the renal corpuscle, become entangled by the tentacular processes of the octopus-like podocytes or allow themselves to be nursed in the cytoplasmic arms of the mesangial or intercapillary cells. Indeed, it is only recently that Kurtz (1964) surveyed briefly the parietal epithelial cells in the rat, rabbit and human kidney and found that the attenuated portions of neighboring cells are interlocked without special attachment plates. Although this author did not report the presence of filamentous structures in the cytoplasm of these cells, a hazy ectoplasmic differentiation, similar to that seen in some electron micrographs of tubular epithelial cells (see Fig. 67), was visible in one of his electron micrographs, both immediately beneath the apical surfaces of the cells and at their bases in close proximity to the basement membrane (Kurtz, 1964, Fig. 2).

Such a hazy ectoplasm most probably corresponds to the TPA-stained cell web fibrils which run in both the apical and the basal cyto-

plasm of the parietal epithelial cells, parallel to the long axis of the latter. These cell web fibrils always show a particular affinity for membranous sites, excepting the nuclear membrane. They tend to converge toward each other and to fuse between the nuclei of adjacent parietal epithelial cells where the cell body is highly attenuated, and do not appear to be associated with special attachment sites, a notion which is supported by the finding of Kurtz (1964) that adjoining parietal epithelial cells interdigitate without junctional complexes.

Contrary to the report of Tasic (1918), no rodlets were recorded in the lining cells of the parietal layer of Bowman's capsule. Perhaps, Tasic (1918) was misled by the particular arrangement of the cell web fibrils at the base of the parietal epithelial cells. Indeed, in some tangential sections through the bases of these cells, the cell web fibrils are seen as single dots or short bars, linked together by delicate TPA positive strands and embedded in the inner surface of the capsular basement membrane, which exhibits fingerlike masses pressing upward for short distances into the bases of the cells and invaginating their basal cell membranes in the process (see Fig. 50). This arrangement of the cell web fibrils is reminiscent of that of the tonofibrils which were discovered by Clark (1963) with the electron microscope in the subcapsular epithelial cells of the mouse thymus (see Review of

Literature) and which stain with TPA (unpublished observations). The fact that no rodlets are present in the parietal epithelial cells provides further evidence that these structures are present only in renal epithelial cells whose basal cytoplasm shows deep cytoplasmic compartments limited by the inflected basal cell membrane.

In gluteraldehyde-fixed material, the cell web fibrils in the parietal epithelial cells and the smooth muscle myofibrils are the only cell web structures which stain with TPA (see Materials and Methods), thus indicating that the cell web fibrils are different from the rodlets (which are TPA negative in such preparations) and bear a close chemical or stereochemical resemblance to smooth muscle myofibrils. Moreover, Brodie (1914) found that Bowman's capsule can expand very much. It, therefore, seems likely that, in addition to a supporting function, the cell web fibrils in the cytoplasm of the parietal epithelial cells may be endowed with some contractile properties. The contractile function of these fibrils is strongly suggested by their lack of attachment to the cell membrane, their similarity to smooth muscle myofibrils, as well as their parallel arrangement.

In the visceral layer of Bowman's capsule, only the phagocytic podocytes seem to contain cell web fibrils, for the latter could not be identified in the dark podocytes. The deep coloration of

the dark podocytes is not attributable to the presence of cell web structures or mitochondria, since they did not show an intense TPA staining in gluteraldehyde-fixed material. It seems more likely that they correspond to the RNA-rich podocytes described in histochemical studies by Pease (1955) in the rabbit and rat kidney, and later by Foster and Riad (1963) in the rabbit kidney. These authors suggested that these cells may be actively concerned in protein synthesis.

The cell web fibrils in the phagocytic podocytes are sometimes wavy in appearance and frequently show nodular thickenings. They do not seem to terminate in special attachment sites, although tiny TPA-stained desmosome-like structures appear to occur at the level of the foot processes of these podocytes. Such attachment sites were described with the electron microscope by Farquhar et al (1961), and later by Rhodin (1962) and Trump and Benditt (1962). As mentioned before (see Review of Literature), Nachmias (1964) found extensive areas of filamentous material in the cytoplasm of amebae (animals which are known to nourish themselves by phagocytosing foreign material)and postulated a connection of these filaments with cytoplasmic movements, a concept shared by Simard-Duquesne and Couillard (1962) who discovered that a glycerinated ameba preparation contracted upon the addition of ATP and magnesium ion. Moreover, Pease (1955) believed that

the foot processes of podocytes "do more than simply offer support" and suggested that these cells may have elastic or contractile powers.

It seems conceivable that the fibril-containing podocytes, which, like amebae, are phagocytic, may have contractile properties. This notion is strongly supported by the findings that their fibrils are sometimes wavy, and that, in gluteraldehyde-fixed sections, they stain with TPA, like smooth muscle myofibrils, while the cell web of the renal tubular cells is unstained.

In conclusion, the parietal epithelial cells and certain podocytes contain in their cytoplasm deeply TPA-stained cell web fibrils. Such fibrils do not seem to terminate in special intercellular attachment sites; in gluteraldehyde-fixed material, they stain with TPA like smooth muscle myofibrils and show a particular affinity for membranous sites. It is suggested that these cell web fibrils, in addition to a supportive function, may be endowed with contractile powers.

Glomerulus

In the glomerulus, the endothelial cells of the glomerular capillaries are seemingly devoid of a cell web. The lacis cells of Oberling and Hatt (1960), which contain a small amount of TPA-

stained fibrils when they lie in the polar cushion of Zimmermann (1933) in close proximity to the macula densa of the distal convoluted tubule, seem to lose these fibrils when they penetrate the centrolubar region of the glomerulus and come to lie between the glomerular capillaries, embedded in a PA-Schiff positive fibrillar intercellular matrix which is continuous with the glomerular basement membrane. Perhaps these lacis cells in the centrolobular region of the glomerulus correspond to the "mesangial" or "intercapillary cells" so often described in electron microscopic studies (Yamada, 1955; Mueller and Mason, 1955; Latta, 1961; Farquhar and Palade, 1962; and others).

SUMMARY AND CONCLUSIONS

The tannic acid-phosphomolybdic acid-amido black (TPA) technique was used to study the "cell web" (a supporting fibrillar component of the cytoplasm which is usually associated with or inserted on terminal bars and desmosomes) in the epithelial cells of the adult rat kidney. At the apex of all tubular epithelial cells (excepting the light cells lining the second segment of the collecting tubule) and immediately beneath the surface membrane projections (brush border, microvilli), the cell web assumed the shape of a tightly knit fibrillar network which spanned the apical cytoplasm of the tubular cells and inserted on the terminal bars; this structure was referred to as "terminal web". With the exception of the serrated terminal bars circumscribing the apex of the distal tubular cells and the cells forming the first cortical segment of the collecting tubule, all terminal bars were straight and outlined the apex of the tubular epithelial cells in a regular hexagonal pattern.

On the lateral surfaces of all tubular cells, excepting those lining the distal convolution, some TPA-stained fibrils were seen running from the terminal bars toward the base of the cells. Such fibrils formed a fish net-like layer which coursed down the

lateral cell wall, traveling from desmosome to desmosome located at discrete loci in the plane of contact of two neighboring cells. These fibrils were resolved only along lateral cell surfaces which are known to be straight and constituted a "lateral web".

Lastly, at the base of all tubular cells, with the exception of those forming the macula densa of the distal convoluted tubule and the cortical and papillary collecting ducts, some coarse fibrils took the shape of single or forked rods of varying thickness and length. These rod-like structures, for which the name "rodlets" was proposed, were confined to the territory of only one epithelial cell and were oriented mainly circularly around the renal tubules in close proximity to the basement membrane, thus making up a "basal web". The latter was seemingly not related to the lateral web or any special attachment sites.

In Bowman's capsule, however, the cell web was present in a dispersed form. The parietal epithelial cells contained cell web fibrils extending in both the apical and the basal cytoplasm and showing a particular affinity for membranous sites (excepting the nuclear membrane) without special attachment sites. In certain visceral epithelial cells or podocytes, cell web fibrils were found; they were sometimes wavy in appearance and frequently showed nodular thickenings.

In conclusion, in most renal tubular epithelial cells, cell web fibrils were found below the apical, lateral and basal cell surfaces, making up a framework for the support of the cytoplasm.

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Fig. 1 Small intestinal epithelium of adult rat.

Terminal web (TW), terminal bars (TB) and mitochondria are stained exclusively.

Perfusion fixation with 6.5% gluteraldehyde and TPA (x1200).

Fig. 2 Liver of adult rat.

Mitochondria and nucleoli are stained selectively in the parenchymal cells.

Perfusion fixation with 6.5% gluteraldehyde and TPA (x1200).

Fig. 3 Pancreas of adult rat.

Zymogen granules (Z G), mitochondria and nucleoli are the only structures stained in the acinar cells.

Perfusion fixation with 6.5% gluteraldehyde and TPA (x1200).

Fig. 4 Striated duct of sublingual gland of adult rat.

Mitochondria are stained exclusively in the cells lining the striated duct. Zymogen granules (Z G) and a few mitochondria are selectively stained in the neighboring serous demilune cells.

Perfusion fixation with 6.5% gluteraldehyde and TPA (x1200).

Fig. 5 Schematic drawing to show the subdivision of the various nephron segments and of the collecting tubule. This classification of the renal tubules of the adult rat is based not only upon the orientation, number and distribution of the mitochondria in the lining epithelial cells, but also upon the cell web structures present in these cells.

Proximal convoluted tubule. First stretch.

- Fig. 6 Cross section of a proximal convoluted tubule. Note the fairly strong and specific staining of mitochondria and nucleoli in the epithelial cells. Note also a prominent TPA negative brush border at the apex of the cells.

Perfusion fixation with gluteraldehyde and TPA (x1200).

- Fig. 7 In this transversely sectioned tubule, the epithelial cells have terminal bars (TB), a finely granulated terminal web (TW). At the base of the cells, longitudinally sectioned rodlets (R) stretch within the plane of the section.

Carnoy fixation and TPA (x1200).

- Fig. 8 Longitudinal section through a proximal tubule. Note the rows of glycoprotein-containing granules (G) in the epithelial cells.

Perfusion fixation with gluteraldehyde and toluidine blue (x1200).

Proximal convoluted tubule. First stretch.

Fig. 9 Surface view of the apex of proximal tubular cells. The terminal bars (TB) are straight and outline the cell apex in a regular hexagonal pattern.

Carnoy fixation and TPA (x1200).

Fig.10 Part of a longitudinally sectioned tubule. At the base of the epithelial cells, cross sections of rodlets (arrows) are seen as single dots, double dots or short bars.

Carnoy fixation and TPA (x1200).

Proximal convoluted tubule. First stretch.

Fig.11 The longitudinally sectioned tubule shows the cross sections of several rodlets, some of which are indicated by arrows. The rodlets in this field appear as short bars (left arrow), double dots (right arrow) or single dots. Note a longitudinally cut rodlet on the left of the arrows.

Carnoy fixation and TPA (x1200).

Figs. 12,13 Tangential sections through the base of the lining epithelium of the proximal convoluted tubule. Rodlets, indicated by arrows, appear as single or forked structures running circularly around the tubule. These rodlets are discontinuous and vary in size and in length.

Note nerve fiber (N) in fig. 13.

Carnoy fixation and TPA (x1200).

Fig.14 Color micrograph of a cross section through the first stretch of a proximal convoluted tubule. This field shows bluish-green TPA positive sites and brownish-yellow TPA negative sites. The tubular epithelial cells have terminal bars (TB) and a terminal web (TW). Along the lateral surfaces of the cells, TPA-stained fibrils extend from the terminal bars toward the base of the cells, forming a lateral web (LW). Several longitudinal sections of rodlets (R) are closely applied to the brownish-yellow basement membrane.

When the generally TPA negative brush border is obliquely sectioned, it shows a moderate staining similar to that of the cell body cytoplasm. This staining, therefore, is not attributable to cell web fibrils.

Carnoy fixation and TPA (x1650).

Proximal convoluted tubule. Second stretch.

- Fig.15 This field shows cross sections of the second stretch of the proximal tubule (P) and of the thick limb of Henle's loop (D). The staining of mitochondria is strong and specific. The cytoplasm of the lining cells of P contains fewer mitochondria than the cells forming the first stretch of the proximal convolution. Note also the granular appearance of the mitochondria in P.

Perfusion fixation with gluteraldehyde and TPA (x1200).

- Fig.16 The longitudinally sectioned tubule shows a deeply stained terminal web (arrow) inserting on terminal bars and continuous with a finely granulated lateral web (LW). Cross sections of rodlets (R) are present at the base of the epithelial cells.

Note the slit-formed tubular lumen and the short brush border.

Carnoy fixation and TPA (x1200).

- Fig.17 The two transversely cut tubules show lateral webs (arrows) running from the terminal bars to the basement membrane.

Carnoy fixation and TPA (x1200).

- Fig. 18 Longitudinal section through a proximal tubule. Associated with the lateral webs and at a distance from the terminal bars are desmosomes (d).

Carnoy fixation and TPA (x1200).

Thin limb of medullary loop of Henle.

- Fig.19 Cross section through the portion of thin limb nearest the proximal convolution. Note a proximal tubular cell with its characteristic brush border among the thin limb lining cells.

Carnoy fixation and TPA (x1200).

- Fig.20 Longitudinal section through the thin limb. A simple terminal web, indicated by arrow, stretches in a dome-like fashion across the apex of the cell and terminates in terminal bars (TB). At the base of the cells, note cross sections of rodlets (R).

Carnoy fixation and TPA (x1200).

- Fig.21 Three cross sections of thin limbs (TL) and two cross sections of thick limbs (D). Note the particular arrangement of the relatively few mitochondria in the thin limbs (TL), giving a beaded appearance to the lining epithelial cells.

Perfusion fixation with gluteraldehyde and TPA (x1200).

- Fig.22 Cross section of a thin limb. This field shows longitudinally sectioned rodlets (R) only in the attenuated portion of the cytoplasm of the lining cells. The arrow indicates the terminal web. No terminal bars are visible in this field.

Carnoy fixation and TPA (x1200).

Thick limb of Henle's loop.

Fig.23 Longitudinal section through the thick limb (D). Note the numerous rod-shaped mitochondria in the basilar two-thirds of the lining cells.

Perfusion with gluteraldehyde and TPA (x1200).

Fig.24 Longitudinal section through the thick limb. The epithelial cells have a terminal web (TW) which inserts on terminal bars. When the apex of the cells is cut tangentially, as indicated by arrows, the deeply stained cross sections of terminal bars are very concentrated, indicating that these structures are serrated. At the base of the cells, several cross sections of fine rodlets (R) are seen in close proximity to the basement membrane.

Carnoy fixation and TPA (x1200).

Fig.25 Surface view of the apex of the cells lining the thick limb. The terminal bars are wavy, as it is indicated by the arrow.

Carnoy fixation and TPA (x1200).

Macula densa and other cells of the juxtaglomerular apparatus.

- Fig.26 Section passing through the macula densa of the distal convoluted tubule (MD) and the other cells of the juxtaglomerular apparatus. The lacis cells forming the polar cushion of Zimmermann (P) are closely associated with the macula densa cells (MD) and are embedded in a PAS positive fibrillar intercellular matrix. These lacis cells, as indicated by the arrows, penetrate the glomerulus between the glomerular capillaries.

Perfusion fixation with gluteraldehyde and PA-Schiff-Hemat (x800).

- Fig.27 Section passing through the macula densa (MD) and the polar cushion of Zimmermann (P). The PAS positive basement membrane of the macula densa (MD) is continuous with the fibrillar intercellular matrix in which the lacis cells are embedded (P).

Perfusion fixation with gluteraldehyde and PA-Schiff-Hemat. (x800).

- Fig.28 Same field as fig. 27 at a higher magnification. In this field, three types of cells are distinguished in the renal corpuscle: glomerular capillary endothelial cells (E), Lacis cells (L) or "mesangial cells", and podocytes (PY).

Perfusion fixation with gluteraldehyde and PA-Schiff-Hemat. (x1200).

Macula densa of distal convoluted tubule.

- Fig.29 Cross section of the macula densa. A TPA negative basement membrane persists between the afferent arteriole (A), the macula densa (MD) and the glomerulus (G). While longitudinal sections of rodlets (R) are present on the side of the tubule opposite the arteriole (A), no rodlets are visible on the side next to the arteriole (A).

Carnoy fixation and TPA (x1200).

- Fig.30 Cross section of the macula densa. The tubular lumen sometimes penetrates between the macula densa (MD) cells, as indicated by the right arrow, so that the terminal bar comes to lie closer to the basement membrane than to the cell apex. The left arrow points at a cross section of a terminal bar at the free margins of two macula densa (MD) cells, on which the terminal web inserts.

Carnoy fixation and TPA (x1200).

- Fig.31 Cross section of the macula densa. This field shows the macula densa (MD) and part of the renal corpuscle (G). The epithelial cells next to the glomerulus contain very few mitochondria, while the other tubular cells have the characteristic basal mitochondrial palisade of the distal convolution.

Perfusion fixation with gluteraldehyde and TPA (x1200).

Terminal portion of distal convoluted tubule.

Fig.32 Cross section of the terminal portion of the distal tubule. This field shows that the basal palisade of mitochondria is still present in this part of the distal tubule (D).

Perfusion fixation with gluteraldehyde and TPA (x1200).

Figs. Surface views of the apex of the cells lining the
33,34 terminal portion of the distal tubule. The arrows indicate that the terminal bars are serrated or of the wavy type in this part of the distal tubule.

Carnoy fixation and TPA (x1200).

First segment of collecting tubule.

- Fig.35 Longitudinal section through the first segment of the collecting tubule. The ordinary lining cells still have the basal palisade of mitochondria as in the distal tubule. Arrows indicate "dark" or "intercalated" cells.

Perfusion fixation with gluteraldehyde and TPA (x1200).

- Fig.36 Longitudinal section through the first segment of the collecting tubule. This field shows a transitional cell in which the mitochondria have lost their perpendicular orientation, as indicated by arrow.

Perfusion fixation with gluteraldehyde and TPA (x1200).

- Fig.37 Longitudinal section through the first segment of the collecting tubule. The ordinary lining cells as well as the dark cells have a prominent terminal web (TW) and terminal bars. No rodlets are present.

Carnoy fixation and TPA (x1200).

First segment of collecting tubule.

Fig.38 Surface view of the apex of the cells forming this segment of the collecting tubule. The arrow indicates that the terminal bars are still serrated, but to a lesser extend than their counterpart in the distal convolution. A dark cell is seen.

Carnoy fixation and TPA (x1200).

Second segment of the collecting tubule.

Fig.39 Longitudinal section through the second segment of the collecting tubule. Dark cells are intercalated between light cells. The dark appearance of the intercalated cells is mainly due to the presence of numerous granular mitochondria.

Perfusion fixation with gluteraldehyde and TPA (x1100).

Figs. 40,41 Longitudinal sections through the second segment of the collecting tubule. The dark cells have terminal bars (TB) and a dense terminal web (TW). A terminal web cannot be resolved below the irregular surface of the light cells. As indicated by arrows, the attenuated portion of the dark cell cytoplasm is often interposed between the base of a light cell and the basement membrane; owing to the presence of a lateral web, the cell limits are distinct between neighboring dark and light cells, not between adjoining light cells or adjacent dark cells.

Carnoy fixation and TPA (x1100).

Second segment of collecting tubule.

Fig.42 Surface view of the light and dark cells forming this segment of the collecting tubule. Only the terminal bars, as indicated by the arrow, which outline the apex of dark cells are visible. This is attributable to the absence of a terminal web under the cell surface of light cells, thus confirming the concept that the TPA staining of terminal bars is due to the aggregation of cell web fibrils on these attachment sites.

Carnoy fixation and TPA (x1100).

Third segment of collecting tubule.

Fig.43 Cross sections of the third segment of the collecting tubule. The cytoplasm of the lining cells of this segment is occupied by very few granular mitochondria.

Perfusion fixation with gluteraldehyde and TPA (x1100).

Figs. 44,45 Cross sections of the third segment of the collecting tubule. The epithelial cells of this segment have terminal bars (TB) and a terminal web (TW). Few longitudinal sections of rodlets (R) are seen at the base of the cells.

Carnoy fixation and TPA (x1100).

Fourth segment of collecting tubule.

- Fig.46 Longitudinal section through the terminal portion of the collecting tubule. The epithelial lining cells have terminal bars (TB), a terminal web (TW) and a lateral web (LW). The latter extends from the terminal bar to the basement membrane. No rodlets are present. In close proximity to the collecting duct is a venous vasa recta filled with erythrocytes.

Carnoy fixation and TPA (x1200).

- Fig.47 Surface view of the apex of the cells lining the terminal segment of the collecting tubule. The terminal bars (TB) are straight and circumscribe the apex of the cells in a regular hexagonal pattern.

Carnoy fixation and TPA (x1200).

Surface of papilla.

- Fig.48 The epithelial cells coating the surface of the papilla are columnar or cuboidal and have a terminal web (TW), terminal bars, and a lateral web (LW). As indicated by the arrows, some of these lining cells show signs of nuclear and cytoplasmic degeneration.

Carnoy fixation and TPA (x1200).

Parietal epithelial cells of Bowman's capsule.

- Fig.49 The longitudinally sectioned parietal epithelial cells show discontinuous cell web fibrils (F) in their basal cytoplasm. At the apex of the squamous epithelial cell on the right, a deeply TPA-stained fibril runs immediately beneath the surface membrane.

Carnoy fixation and TPA (x1200).

- Fig.50 In this field, the longitudinally sectioned parietal epithelial cells contain deeply stained fibrils in both their apical and basal cytoplasm, which, as indicated by the arrows, tend to fuse between the nuclei where the cell body is highly attenuated. In the upper right corner, the section passes tangentially through the base of the parietal epithelial cells; in this region the TPA negative capsular basement membrane can be seen to exhibit fingerlike masses pressing upward and infolding the basal cell membrane in the process. This is responsible for this particular appearance of the basal fibrils (F).

Carnoy fixation and TPA (x1200).

Parietal epithelial cells of Bowman's capsule.

Figs. 51 to 53 In these longitudinally sectioned parietal epithelial cells, apical (AF) and basal fibrils (BF) run immediately under the surface and basal cell membranes and tend to fuse where the cell body is attenuated (as indicated by arrows). No special attachment sites are associated with these cell web fibrils.

Carnoy fixation and TPA (x1200).

Figs. 54,55 The parietal epithelial cells are sectioned transversely. The fibrils, as indicated by the arrows, are seen in cross section immediately under the cell membrane without special attachment sites.

Carnoy fixation and TPA (x1200).

Parietal epithelial cells of Bowman's capsule.

Fig.56 In the longitudinally sectioned parietal epithelial cell, the apical and basal cell web fibrils, as indicated by the arrow, converge toward each other and fuse. In a neighboring visceral epithelial cell (or podocyte), a coarse fibril (F) can be seen in the cytoplasm.

Carnoy fixation and TPA (x1200)

Fig.57 This field shows tangentially sectioned parietal epithelial cells. The obliquely cut cell web fibrils (F) form a circumferential layer immediately under the cell membrane without special attachment sites.

Carnoy fixation and TPA (x1200).

Fig.58 In this field, the base of a parietal epithelial cell is cut transversely. The cell web fibrils (F) thus appear in cross section.

Carnoy fixation and TPA (x1200).

Visceral epithelial cells of Bowman's capsule.

- Fig.59 This low power photomicrograph shows two dark podocytes in the lower left corner. The intense TPA staining of the cell body of these podocytes extends into their long cytoplasmic processes.

Carnoy fixation and TPA (x550).

- Fig.60 This field shows the same dark podocytes at a higher magnification. These cells do not seem to contain cell web fibrils and appear to be in close contact with three, or even four, glomerular capillaries. However, strongly TPA-stained fibrils (F) showing nodular thickenings are present in the cytoplasmic process of a lighter podocyte.

Carnoy fixation and TPA (x1200).

- Fig.61 This field shows phagocytosed PAS positive granules in the cytoplasm of a podocyte (arrow).

Carnoy fixation and TPA-PA-Schiff (x1200).

Visceral epithelial cells of Bowman's capsule.

- Fig.62 This photomicrograph shows a tangentially sectioned podocyte as well as a longitudinally cut parietal epithelial cell in the lower right corner. The cytoplasm of the podocyte contains numerous relatively straight TPA-stained fibrils (F); it also contains a large coarse body (as indicated by the arrow) consisting of a TPA positive ring and a TPA negative core within.

Carnoy fixation and TPA (x1200).

- Fig.63 This field shows a podocyte with a deeply TPA-stained wavy fibril (F) in its cytoplasm.

Carnoy fixation and TPA (x1200).

- Fig.64 In this photomicrograph, as it is indicated by the arrow, a row of tiny TPA-stained granules is seen in the cytoplasm of a podocyte. Since these granules are closely applied to the basement membrane of the glomerular capillary (clear area), they are regarded as desmosome-like structures connecting the foot processes of adjacent podocytes.

Carnoy fixation and TPA (x1200).

Fig.65 This schematic diagram of part a proximal convoluted tubule of an adult rat kidney is devised to give a 3- dimensional concept of the rodlets. These structures are represented by single or forked rods or spindles of varying thickness and length running circularly around the tubule in close proximity to the basement membrane.

Fig.66 Electron micrograph ($\times 47,500$) of part of the base of a proximal tubular epithelial cell reproduced from A.W. Ham and T.S. Leeson (1961, fig. 466). The basal cell membrane adjacent to the homogeneous basement membrane is inflected into the interior of the cell as a series of parallel double membranes, thus dividing the basal cytoplasm of the tubular cell into a number of cytoplasmic compartments or lamellae. At the base of each of these compartments, there is a hazy ectoplasmic differentiation, which may represent the rodlets (basal webs) seen by light microscopy.

Fig.67 Schematic diagram of a renal proximal tubular epithelial cell as seen in electron micrographs to correlate light and electron microscopic findings. The strongly TPA-stained terminal web observed in the lining epithelial cells of the proximal convolution is seen in a few electron micrographs as a hazy ectoplasmic layer spanning the apical cytoplasm immediately below the microvilli of the brush border and inserting on the terminal bars. A similar ectoplasmic differentiation is sometimes recognized along the lateral surfaces of the cells in intimate association with the few desmosomes located at discrete loci in the plane of contact of two neighboring cells; this would thus correspond to the lateral web seen by light microscopy. Finally, at the base of the cytoplasmic compartments in close proximity to the basement membrane, as seen in figure 66, similar hazy ectoplasmic masses are distinguished; such masses, as it is illustrated in this diagram, may be divided as a result of the indentation sometimes made by the basal cell membrane before it is reflected again into the interior of the cell; these masses would thus represent the single or forked rodlets seen in TPA preparations.