Kaliopenic Nephropathy
A Correlated Light and Electron Microscopic Study*

Claude G. Biava, M.D., Ihor Dyrda, M.D., Jacques Genest, M.D., and Sergio A. Bencosme, M.D.

After the report of Perkins Petersen, and Riley\textsuperscript{23} in 1950 it became recognized that the vacuolar degeneration of renal tubular cells repeatedly observed\textsuperscript{3,6,15,16,17,38} in cases of severe acute or chronic intestinal diseases was due to potassium deficiency. Luft \textit{et al.}\textsuperscript{20} found the same type of degeneration in cases of hypopotassemia resulting from causes other than intestinal losses. Relman and Schwartz\textsuperscript{28,33} correlated functional and morphologic findings in potassium deficiency and demonstrated a well defined spectrum of disturbances of renal function associated with hydropic or vacuolar degeneration of kidney tubules. From these studies a new clinical pathologic entity emerged, which Conn and Johnson\textsuperscript{7} recognized as “common denominator” in chronic potassium deficiency states of different origin and for which they proposed the name of kaliopenic nephropathy.

The ultrastructure of the epithelial vacuolar changes characteristic of this type of nephropathy has not been described and constitutes the main object of the present report. The kidney biopsy from a case of kaliopenic nephropathy due to an aldosterone-producing adrenal cortical adenoma (Conn’s disease) was studied by light and electron microscopy. The occurrence of tubular vacuoles in kaliopenia of adrenal origin was first observed by Conn and Louis\textsuperscript{8} in the course of their original studies on primary aldosteronism. It was subsequently

---

Accepted for publication January 2, 1963. From the Department of Pathology, Queen’s University, Kingston, Ontario, and the Department of Clinical Research, Hotel Dieu Hospital, Montreal, Quebec, Canada. Dr. Biava’s present address is Department of Pathology, Mount Sinai Hospital, Chicago, Illinois.

* Supported by the Canadian Medical Research Council.
confirmed in case reports by several investigators, in nephron microdissection studies, and recently in one of our cases of primary aldosteronism.

Of greatest interest in the present study is the finding that the tubular vacuoles seen by light microscopy correspond to expansions of normally existing extracellular spaces and therefore do not conform morphologically with classically held concepts of intracellular vacuolations as a form of hydropic cell degeneration. Also to be described are peculiar fine structural changes of tubular basement membranes which to date remain unique in our experience as well as in the published experience of others.

**MATERIAL AND METHODS**

A 43 year-old housewife was admitted to Hotel Dieu Hospital, Montreal, because of arterial hypertension first noted 15 years ago. During this period she had been complaining of irritability, nervousness, tiredness, and tingling in the extremities. On one occasion she presented an episode of tetany. Polydipsia with polyuria and nocturia were also noted in recent years. Physical examination disclosed a recumbent blood pressure of 220/120 and generalized hyperactive deep tendon reflexes.

The diagnosis of primary hyperaldosteronism was established because of the persistently hypokalemic (serum potassium 2.1 to 2.9 mEq per L.) metabolic alkalosis (pH 7.45, serum bicarbonate 44 mEq per L., pCO₂ 70 mm. of Hg), a serum sodium of 148 mEq per L., and a urine of low specific gravity (1.008 to 1.014) and of alkaline pH (7.5). Creatinine clearance was 75 ml per minute (uncorrected for body weight), and the final sulfonphthalein excretion was 21 per cent in 15 minutes and 56 per cent in 80 minutes. There was no nitrogen retention or abnormality of urinary sediments. The urinary output ranged from 1750 to 2600 ml per 24 hours. A renal arteriogram was normal. Determinations of urinary aldosterone gave values of 29.1 and 51.3 µg per 24 hours (normal range, 2 to 10 µg per 24 hours). Laminography of the dorsolumbar region with and without presacral air insufflation revealed the presence of a tumor of the left adrenal. This was confirmed surgically, and the left adrenal gland was removed. The adrenal cortex contained a round, yellow, nonencapsulated adenoma weighing 10 gm. After the operation the patient was relieved of her symptoms and blood potassium levels returned to normal with disappearance of the metabolic alkalosis, polyuria, and hypohydremia. However, a slight elevation of blood pressure persisted. Dirining the adrenalectomy a biopsy of the left kidney was taken. For light microscopy, tissue blocks fixed in Müller—formol and embedded in paraffin were sectioned at 2 to 5 µ and stained with hema- lum-phloxine-safran, Periodic acid-Schiff, phosphotungstic acid lie matoxylin, and Masson’s trichrome stains. Sections 0.5 to 1.0 µ thick were also cut from osmium-fixed, Epon-embedded blocks and stained with an alkali solution of toluidine blue. For electron microscopy, small blocks of tissue were fixed in buffered 1 per cent osmium tetroxide, dehydrated in ethyl alcohol, and embedded in Epon. 812. Ultrathin sections were cut with a Porter-Blum microtome, stained with lead hydroxide or uranyl acetate, and examined in an RCA EMU-3D electron microscope.

**RESULTS**

By light microscopy the familiar picture of vacuolated epithelial cells could be readily appreciated in the form of clear vacuoles (Fig. 1) occupying varying portions of cytoplasm in cells of the proximal and distal convoluted tubules and of the terminal ascending segments of
Henle’s loops. Collecting tubules were spared. This type of coarse vacuolation was focal and irregularly distributed, one-third of proximal and distal tubules usually being involved in any given section.

Additional morphologic details could be discerned when thin sections from paraffin or resin-embedded blocks were studied. In these sections a finer type of cellular vacuolation became detectable, which proved to be more widespread than the coarser type mentioned above. Furthermore, its localization within cells could be more accurately defined in that the basal portions of the cytoplasm appeared exclusively or predominantly involved.

The fine vacuolation consisted of the presence in the basal cellular segments of numerous oval or elongated slitlike spaces (Figs. 1 and 2) separated by thin cytoplasmic septa and oriented perpendicularly to the tubular basement membranes. Elongated, radially oriented spaces were seen predominantly in the proximal convoluted tubules, while round vacuoles singly or in clusters were present mostly in the distal tubules. Where the vacuolation was more pronounced it tended to produce a diffuse “clarification” of the basilar cytoplasm of tubular cells.

Under the electron microscope the most striking finding was the widespread separation of the normally juxtaposed parallel double membranes separating the basilar cytoplasmic compartments or lamellae of tubular cells. It is known\(^23, 24, 29, 32\) that such cytoplasmic compartments resting on the tubular basement membranes arise from numerous infoldings or invaginations of the basilar cell membranes and that a complex interlocking between cytoplasmic lamellae of neighboring cells occurs. In any given section this arrangement is responsible for the labyrinthine system of double membranes subdividing the basilar cytoplasm into elongated digit-like compartments containing mitochondria and other cellular organelles. The parallel double membranes result from the close juxtaposition either of adjacent lamellae belonging to the same cells or of interlocked lamellae from neighboring cells. In addition, double membranes are present at the lateral cell boundaries, where they are formed by the juxtaposed single membranes of two adjacent cells. To be emphasized is the fact that the 200-Å gap or potential space (intercompartmental space) between double membranes is an extracellular space which is continuous with the extracellular space present between the base of epithelial cells and the tubular basement membranes.
Fig. 1. Representative proximal convoluted tubule with coarse vacuolation in upper right portion. The diffuse fine vacuolation also discernible in most of the basilar cell segments corresponds electron microscopically to the intermediate degree of separation of basilar membranes shown in Figure 8. Where vacuoles are not visible (lower left portion of tubule) electron microscopy disclosed only minimal separation of basilar membranes as depicted in Figure 7. The glomerulus partly illustrated (lower left corner) shows no abnormalities. Epon embedding, toluidine blue; light micrograph; ×1200.

Fig. 2. Detail of combined coarse and fine vacuolation confined to basal segments of cells. Delicate cytoplasmic septa resting on tubular basement membrane separate fine basilar vacuoles. Epon embedding, toluidine blue; light micrograph; ×2200.
In our case, the epithelial vacuoles seen with the light microscope corresponded to greatly expanded intercompartmental spaces, such an expansion arising through separation of the parallel double membranes (Figs. 3 and 5). The important feature shared by all the vacuoles was that at one or more points they maintained communications with the extracellular space at the base of the cells (Fig. 4 and 6) and were therefore demonstrated to be extracellular in location. The size and shape of the vacuoles varied greatly and were governed by the degree and extent of separation of the double membranes. In general, separation of double membranes occurred first and predominantly along their inner segments, while juxtaposition usually persisted along their outer segments adjacent to the basement membranes, where spreading apart of cytoplasmic lamellae was minimal or absent.

While the focal distribution of the larger vacuoles was similar to that noted by light microscopy, under the electron microscope lesser degrees of separation of double membranes beyond the resolution of the light microscope were nearly always present in cells of proximal and distal convoluted tubules. Figure 7 illustrates minimal separation of basilar membranes of a degree occasionally found in normal tubular cells. An intermediate degree of the same change is presented in Figure 8. When the plane of sectioning was more tangential, the dilated intercompartmental spaces were seen in cross section as circular clear spaces conferring to the cells a characteristic foamy appearance (Figs. 9 and 10). Separation of double membranes with creation of dilated fusiform spaces occurred also at the lateral cell boundaries (Fig. 11) with widening of intercellular spaces. However, the dilation stopped abruptly at the terminal bars or desmosomes near the tubular lumina (Fig. 12). Terminal bars are believed to represent "tight intercellular junctions,"11 probably water-impermeable, so that no abnormal communication resulted between the tubular lumina and the dilated basilar extracellular spaces.

Contrasting with the conspicuousness of the extracellular vacuolation was the paucity of true intracellular changes in the affected tubules. Most remarkable was the general architectural distortion apparently imposed on the cells by the swelling of basilar extracellular spaces (Fig. 13). This produced compression and stretching of basilar cytoplasmic lamellae, which appeared thin and elongated. The cytoplasm itself, however, showed a normal complement of cellular organelles, generally without clear-cut alterations. Intracellular integrity was particularly evident from examination of those portions of basal cytoplasm not subdivided into compartments (Fig. 11) and therefore undisturbed by extracellular vacuolation. Infrequently the cytoplasmic matrix appeared compact and denser than usual, as if dehydrated (Fig. 14).

The tubular basement membranes often exhibited a characteristic thickening and lamination in focal areas with the formation of multiple thin layers separated by spaces filled with numerous vesicular structures (Figs. 14 and 15). These vesicles appeared as round profiles, usually containing finely granular material and varying greatly in size from a few hundred to several thousand Angstrom units. Occasionally, a number of smaller vesicles appeared to be contained in larger ones.

---

**FIG 3.** Electron micrograph of large vacuole in a distal convoluted tubule. Vacuole is formed through separation of inner segments of cytoplasmic compartments and occupies a greatly widened intercompartmental space. X 4500.

**FIG. 4.** Detail of basilar vacuole shown in Figure 3, demonstrating its continuity with extracellular space through intercompartmental spaces of normal width included between parallel double membranes (arrows). X 14,000.

**FIG. 5.** Several basilar vacuoles of a size often difficult to appreciate tinder the light microscope. Each vacuole is outlined by two or more cytoplasmic lamellae. Electron micrograph; X4500.

**FIG. 6.** Detail of Figure 5 to show communications between vacuoles and extracellular space through channels formed by normally juxtaposed (lower vacuole) or moderately separated (upper vacuole) basilar cytoplasmic lamellae. X 14,000.
Fig. 7. Minimal separation of basilar double membranes outlining serpiginous clear spaces. Cytoplasmic organelles and matrix are well preserved. Proximal convoluted tubule. Electron micrograph; ×10,000.

Fig. 8. Intermediate degree of separation of basilar membranes corresponding to pattern of fine vacuolation in Figure 1. Elongated clear spaces are separated by thin and tortuous cytoplasmic lamellae and are extracellular. Electron micrograph; ×8000.

Fig. 9. Foamy appearance of vacuolated tangentially sectioned proximal convoluted tubule. Epon embedding, toluidine blue; light micrograph; ×1000.

Fig. 10. Corresponding appearance of the tangentially sectioned tubule shown in Figure 9 under the electron microscope. Communications of the inner vacuoles with the basal extracellular space are not evident, as they do not lie in the plane of sectioning. ×8000.
DISCUSSION

Electron microscopy of renal tubules has defined the existence within tubular basement membranes of distinct intra- and extracellular spaces morphologically separated and of different physiologic significance. Of particular concern is the differentiated structural pattern of the basal segments of tubular cells, with its interlocking of intercellular cytoplasmic compartments and extracellular intercompartmental spaces. The importance of this morphologic organization is borne out by our finding that the apparent cellular vacuolization characteristic of kaliopenic nephropathy is due to expansion of the basal intercompartmental spaces, presumably attended by an accumulation of extracellular fluid in these spaces. It is incorrect to call this change vacuolar cell degeneration, in view of the extracellular location of vacuoles and the good preservation of intracellular structures. In line with the nomenclature used in a previous study, we refer to the dilation of intercompartmental spaces as sub-basilar vacuolation. Although defined by the electron microscopic appearance, this term can be used, we believe, in light microscopy when sufficiently thin sections are used and vacuoles or elongated clear spaces giving negative staining reactions for glycogen and fat are seen exclusively or predominantly at the base of epithelial cells (Figs. 1, 2 and 9).

The absence of consistent intracellular abnormalities, except for architectural distortion, in the face of extreme sub-basilar vacuolation, is in keeping with current concepts concerning the reversibility of
Fig. 13. Architectural distortion associated with severer dilation of intercompartmental spaces in a proximal convoluted tubular cell. Electron micrograph; ×5500.

Fig. 14. Unusually dense cytoplasmic matrix in main body and basilar cytoplasmic lamellae of proximal convoluted tubular cell. Note three intercalated basilar lamellae (arrows) of normal density. These may represent interlocked lamellae belonging to a normal neighboring cell. Basement membrane (BM) is swollen and laminated and contains vesicular deposits. Electron micrograph; ×6500.

Fig. 15. Deposits of round vesicular structures between layers of markedly swollen tubular basement membrane. Electron micrograph; ×11,500.
functional and histologic renal lesions in potassium deficiency states after removal of the causative factors and repair of the potassium losses. Our ultrastructural data would appear to corroborate the experience of several investigators, as reviewed by Conn and Johnson, to the effect that reversion of such lesions can usually be expected.

The functional significance of sub-basilar vacuolation does not yield easily to analysis, since there is want of detailed information regarding the submicroscopic localization of renal functions in the normal basilar apparatus of tubular cells. A basilar apparatus consisting of cytoplasmic compartments separating a labyrinth of extracellular spaces has been described in a great variety of cells known to be involved in the transport of large amounts of water and solutes and in the establishment of chemical and osmotic gradients across their membranes.\textsuperscript{3,10,23,39} Experimentally, enlargement of intercompartmental spaces has been reported in rats\textsuperscript{30} under conditions of very active transport of substances, such as Diodrast and phenolsulfonphaleini, from peritubular capillaries to tubular lumina, it has been observed in rabbits after glucose loading associated with sodium deprivation.\textsuperscript{31} In the rat, sub-basilar vacuolation was a prominent finding associated with polyuria in uranium poisoning,\textsuperscript{33} after neurohypophysectomy, and when a 5 per cent glucose solution was substituted for drinking water.)\textsuperscript{8} In the latter instances the vacuolation was thought to reflect decreased fluid passage from cells to peritubular capillaries, probably related to failure of a postulated cellular pumping mechanism.\textsuperscript{32} Recently a prompt and rapidly reversible dilation of intercompartmental spaces of proximal tubular cells was reported in rats injected with saline.\textsuperscript{5} Under these conditions, however, the change was believed to be a manifestation of increased water reabsorption exceeding the rate of passage through the tubular basement membrane.

From these experimental data it is evident that the basilar apparatus of renal tubular cells is capable of undergoing definite changes under a variety of physiologic and pathologic stimuli, in connection with our ease, the experimental findings (10 not particularly help our understanding of the pathogenesis of sub-basilar vacuolation in potassium deficiency, but the multiplicity of experimental conditions under which the vacuolation has been observed does discourage attempts to construe it as a lesion specific to hypokalemia. Rather, the same consideration would depict sub-basilar vacuolation as a morphologic common denominator produced by various stimuli such as cell poisons, electrolyte imbalances, and hormonal dysfunctions, all capable of interfering with some basic cellular mechanism, probably located at the cell membrane.

Current concepts of renal function\textsuperscript{2} and recent advances in the elucidation of membrane transport mechanisms\textsuperscript{34} would suggest that the basic cellular mechanism involved may be the “Na+ pump.” In the kidney, the energy-requiring process of active sodium extrusion from the cell is presumed to be the primary event with which several other important renal functions are closely integrated, namely, reabsorption of water and chloride, potassium and hydron secretion, acidification, and concentration of urine. Active sodium reabsorption may influence, and in turn it may be influenced by, any one component of this system of integrated functions, for instance potassium availability, and it is finely modulated by a number of factors, among which aldosterone plays a major role. According to Pitts,\textsuperscript{26} Giebish,\textsuperscript{13} and Whittentbiny,\textsuperscript{37} considerable physiologic evidence would place at the outer (basilar) wall of tubular cells the position of time Na+ pump responsible for the active transport of Na+ from tubular lumina to peritubular capillaries, while the inner (apical) cell wall would allow the passive passage by diffusion of the NaCl and water reabsorbed under the driving force of the basilar Na+ pump. It becomes possible, therefore, to envision the basilar epithelial cell membranes revealed by the electron microscope as like the site of the complex system of enzymes and “carrier” substances postulated\textsuperscript{14, 34} to constitute the Na+ pump. In this position the Na+ pump could influence both the physicochemical state of the membranes amid the amount and. movement of time reabsorbate leaving the cell and flowing within intercompartmental spaces to reach interstitium
and peritubular capillaries alter passage through basement membranes. Interference with the activity of the Na+ pump may conceivably produce separation of basilar cell compartments and accumulation of fluid within intercompartmental spaces by way of alterations in the physicochemical state of basilar cell membranes, by inadequacies of the forces regulating the amount and movement of reabsorbate, or both. Abnormalities in the renal handling of sodium and expansion of the extracellular fluid volume observed in potassium deficiency, particularly in primary aldosteronism, would support this interpretation. In this sense, among the men’s renal and systemic functional changes defining the physiopathologic spectrum of primary aldosteronism and potassium deficiency, our case would indicate that physiologic expansion of extracellular spaces has its most prominent and clearest morphologic expression in the form of sub-basilar vacuolation of kidney tubules.

It is likely that the focal swelling and lamination of the tubular basement membranes are also a consequence of accumulation of fluid in extracellular spaces. The nature and significance of the vesicular deposits between layers of the basement membranes are not apparent at present. The possibility that they bear some relationship to abnormal steroid secretion or potassium deficiency will have to be studied by the observation of additional cases and also by experiments.

**SUMMARY**

The fine structure of the vacuolar changes characteric of kaliopenic nephropathy was studied in a case of primary aldosteronism with potassium deficiency. Under the electron microscope the epithelial vacuoles corresponded to dilated extracellular spaces arising from separation of normally juxtaposed cytoplasmic lamellae or compartments at the base of tubular cells. Dilation of these spaces resulted in varying degrees of cellular architectural distortion, but the fundamental cytoplasmic structures appeared generally unaltered. The changes described were referred to as sub-basilar vacuolation. Their degree and location produced morphologic patterns of coarse and fine tubular vacuolation which could be correlated with light microscopic appearances in thin sections, although minimal degrees of sub-basilar vacuolation and extracellular location of vacuoles could be detected only at the resolution afforded by the electron microscope.

The findings have been tentatively interpreted as representing a morphologic counterpart of the hypernatremia and expansion of extracellular fluid volume present in aldosteronism and potassium deficiency. They may be related to altered function of the sodium pump, which presumably is located in the basilar cell membranes and is sensitive to such factors as potassium availability and aldosterone levels.

**REFERENCES**


2. Berliner, R. W. Mechanisms involved in the formation of diluted and concentrated urine, In


