INTRACELLULAR GRANULES OF THE RENAL MEDULLA IN POTASSIUM DEPLETION

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Introduction

In potassium deficiency, the pattern of intracellular granules in the renal medulla is characterized by wide species variation.[1-3] Although these granules form a conspicuous part of the medullary lesion in the rat[4-10] and mouse,[2, 11] they rarely have been described in man.[1, 3, 12] In a case study of two brothers suffering from chronic potassium depletion,[13, 14] histologic lesions were described in the kidneys (Fig. 1). Of particular interest in the elder brother were medullary epithelial and interstitial cells containing discrete and coalescent cytoplasmic granules. More recently, additional staining technics disclosed occasional foci of similar cells in renal tissue from the younger brother. The location and character of the intracellular granules in the two cases suggested a possible relationship to the lesion described in the renal medulla of the potassium-depleted rat.

In the present study, granule-containing cells and cytoplasmic granules in the renal medullary tissue from the two brothers are compared with similar cells and granules observed in rats maintained on a potassium-deficient diet.

Historical Note

In 1941, Liebow, McFarland, and Tennant[11] described histologic changes in the renal medulla of the potassium-depleted mouse and noted "coarse granularity approaching colloid change" in epithelial cells of the papillary ducts. In 1954, Spargo[5] showed that in sections stained by the periodic acid Schiff method granules in the collecting duct epithelium of the potassium-depleted rat were Schiff positive. Craig and Schwartz,[7] in 1957, confirmed Spargo's observation and described in the renal medulla PAS-positive material in interstitial cells of the intertubular spaces and in swollen endothelial cells of capillaries. Additional information concerning the intracellular granules has been obtained from the following procedures: nephron microdissection,[8] histochemical staining for enzymes,[5, 7, 9] elec-

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tron-microscopy,[2, 10, 15] radioautography with Na_{22},[10] and fluorescent immunochemistry.[10] However, despite extensive investigations, the origin and nature of the granules remain in doubt.[2, 3, 9, 10, 15]

In contrast to the numerous experimental studies is the scarcity of information regarding intracellular granules of the renal medulla in human cases of potassium depletion.[1, 3, 12] To the author's knowledge, the presence of such granules in all three medullary cell groups (tubular epithelium, capillary endothelium, and interstitial connective tissue) has not been described in man.

Brief Clinical Summary

The following clinical information concerning the two brothers is presented as evidence of potassium depletion at the time renal tissue was obtained for histologic examination:

1) The elder brother (J.G.) had shown symptoms of chronic potassium depletion for three years prior to his final illness in 1949. During his brief period of hospitalization, the electrocardiogram suggested potassium depletion and blood drawn within 24 hours of death contained a serum potassium of 1.5 mEq. per liter. He died 31 hours after his admission to the hospital as the result of potassium depletion and widespread pneumonia. Renal tissue was obtained at necropsy.*

2) In 1955, the younger brother (R.G.) came under study for potassium-wasting renal disease, symptoms of hypokalemia having been present for 6 years. In 1959, renal tissue for histological examination was obtained at open biopsy. For the preceding seven days he had received potassium supplements and serum potassium had risen from an initial level of 2.5 mEq. per liter to one of 3.3 on the morning of operation. Skeletal muscle obtained at biopsy showed a potassium content of 76.1 mEq. per kilogram fresh tissue (normal range, 91–107[16]).

Abbreviations to Figures

Arl, areolar tissue.
Cap, capillary.
D, silver-positive debris.
e, erythrocyte.
End, endothelium.
Ep, epithelium.
G, PAS-positive globule.
Gr, silver-positive granules.
H, epithelial hyperplasia (?)
I, interstitial cell.
L, tubular lumen.
P, PAS-positive material.
T, tubule.
V, vacuole.

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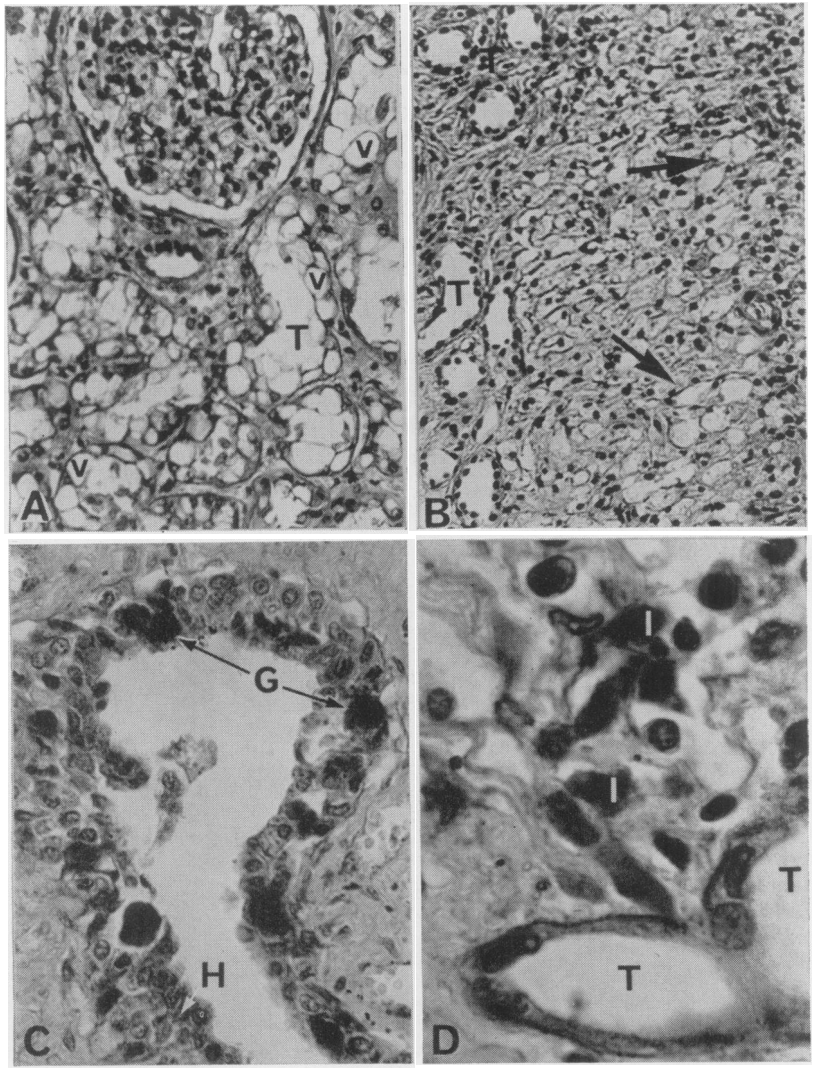

FIG. 1A. *J.G.* Renal cortex. Widespread vacuolization of tubular epithelium. H & E. × 240.

FIG. 1B. *R.G.* Renal medulla. Interstitial collections of cells with clear cytoplasm (arrows) adjacent to tubules. H & E. × 150.

FIG. 1C. *J.G.* Renal medulla, terminal collecting duct. Epithelial cells contain PAS-positive intracellular globules (G). Question of epithelial hyperplasia (H). PAS method, hematoxylin. × 380.

FIG. 1D. *J.G.* Renal medulla. Interstitial PAS-positive cells adjacent to tubule. PAS method, hematoxylin. × 1425.

Methods and Materials

Potassium Depletion in Rats: Preparation of Animals

Potassium depletion was produced in weanling Sprague-Dawley rats by means of a potassium-deficient diet (Table 1). Free access was permitted to distilled water which had been passed through a mixed-bed ion exchange resin. A second group of animals received a control diet, identical except for a content of 2.2 per cent potassium chloride. The plan of the experiment was as follows. 1) Two animals from the group receiving the potassium-deficient diet and two from the control group were killed on days 3, 7, 9, 15, and 16. 2) Three rats underwent potassium repletion. Two were killed on day 23, having received the potassium-deficient diet for 15 days and the control diet for eight days. The third was killed on day 31, having received the potassium-deficient diet for 17 days and the control diet for 14 days. 3) Left nephrectomy was performed on eight rats, four of which were then placed on the potassium-deficient diet and the remaining four on the control diet. Half of the nephrectomized rats from each group were killed at the end of 13 days and the remaining half at the end of 16 days.

TABLE 1

*Potassium-Deficient Diet**

Diet	%
Casein (Vitamin-free)	20.0
Alphacel	2.0
Potassium-free Salt Mix	4.0
Sucrose	65.0
Vitamin mix (complete)	2.2
Sodium chloride	0.5
Corn oil (Mazola)	6.3
Salt Mix	**Gm.**
Calcium carbonate	209.0
Magnesium sulfate	98.5
Sodium pyrophosphate ($Na_4P_2O_7 \cdot 10H_2O$)	76.8
Calcium phosphate (tribasic)	205.0
Ferric phosphate $\cdot 2H_2O$	16.0
Cupric sulfate $\cdot 5H_2O$	0.8
Manganese sulfate	0.2
Cobaltous chloride $\cdot 6H_2O$	0.1
Sodium iodide	0.1
Zinc sulfate $\cdot 7H_2O$	0.6

* From Wilson & Kissane,[17] modified by the substitution of an equivalent amount of sodium pyrophosphate for sodium hypophosphate.

Potassium Depletion in Rats: Potassium Content of Muscle

In order to establish the presence of depletion, potassium content of skeletal muscle was determined in rats on control and on potassium-deficient diets for periods of from 13 to 16 days. At the time the animals were killed, approximately one gram of thigh muscle, cleaned of fat and connective tissue by gross dissection, was weighed and placed in a test tube containing concentrated nitric acid. Except for a small supernatant residue of lipid, digestion was complete. The acid solution was brought to 100 ml. with distilled water and a 1:25 dilution was read in a Coleman Junior flame photometer. The standard solution contained a potassium-sodium ratio of 4:1 and the same concentration of nitric acid as the unknown.

Preparation of Renal Tissue for Histologic Study

Animals were killed by decapitation. Kidneys were removed immediately, bisected, weighed, and placed in 10% buffered formalin. Following fixation, the tissue was embedded in paraffin. Blocks of formalin-fixed, paraffin-embedded renal tissue from the two brothers and from a group of patients with miscellaneous diseases were also available. Sections were cut four microns thick and stained 1) with hematoxylin and eosin, 2) by the periodic acid Schiff method, and 3) by Gomori's chromic acid, methenamine-silver technic.[18]* In sections of fixed tissue, the last two procedures stain aldehyde groups formed by oxidation of carbohydrate substances, e.g., glycogen, mucins.[19] Glycoproteins of connective tissue are PAS-positive but remain almost completely unstained by the methenamine-silver method,[19] a fact which may explain the greater clarity of cellular detail noted, on occasion, with this technic.[19, 20]

In order to determine whether or not the intracellular granules were composed of glycogen, a section of renal tissue from each brother, one from certain of the miscellaneous cases, and one from a potassium-depleted rat were incubated in a 1:1000 solution of malt diastase for 45 minutes at 37° C. and then stained by the chromic acid methenamine-silver technic. Sections of tissue were also stained for mast cells, using aldehyde fuchsin,[21]† and for acid mucopolysaccharides, using Alcian blue.

Results

Evidence of Potassium Depletion in Rats Receiving a Potassium-deficient Diet

were compared with values reported in three other series (Table 2). Potas-

* Hereafter, material taking the methenamine-silver stain is referred to as silver-positive.

† Modified as follows: 1) staining time in aldehyde fuchsin was shortened to 45 minutes and 2) staining with hematoxylin preceded staining with aldehyde fuchsin.

TABLE 2

Potassium Content of Rat Muscle (MEq./Kg. Fresh Tissue)

Series	K^+-Depletion Diet			Control Diet		
	Mean	±S.E.	N.*	Mean	±S.E.	N.*
Cotlove et al.[22]	74.7	5.6	8	106	2	15
Offerijns et al.[23]	68	6.1	40	104	7.1	12
Heppel[24]	64.2	2.67	7**	109	—	2**
France	63.3	0.85	8	107	1.27	8

* Number of animals.

** Number of observations. (For purpose of analysis, 16 rats on a low potassium diet were pooled into 7 groups of 2 or 3 animals each and 4 rats on a control diet were pooled into 2 groups of 2 rats each.)

sium repletion, also, was confirmed by muscle analysis. Potassium content of muscle from the two rats receiving the potassium-deficient diet for 15 days and the control diet for eight days was 103 and 104 mEq. per kilogram fresh tissue. For the animal receiving the potassium-deficient diet for 17 days and the control diet for 14 days, the figure was 101 mEq. per kilogram. Unilateral nephrectomy did not affect significantly the concentration of muscle potassium. This is shown by the mean values, expressed as milliequivalents per kilogram of fresh tissue, for each of a group of four rats: a) those having intact kidneys and receiving the potassium-deficient diet, 64.3 (range, 62.9–66.3); b) those having unilateral nephrectomy and receiving the potassium-deficient diet, 62.6 (range, 59.8–66.7); c) those having intact kidneys and receiving the control diet, 110 (range, 107–112); and d) those having unilateral nephrectomy and receiving the control diet, 105 (range, 103–111).

Rats receiving the potassium-deficient diet failed to gain weight. The following figures are taken from the 16 animals used for muscle analysis. Mean body weight for the eight potassium-depleted rats was 60 Gm. on day 1 and 61 Gm. on day 13. Corresponding weights for those on the control diet were 61 Gm. and 103 Gm. Figures for the unilaterally nephrectomized rats closely approximated those of the non-nephrectomized animals.

A constant finding in the potassium-depleted rat is renal hypertrophy.[5, 11] Eight animals with both kidneys intact were killed between days 13 and 16, inclusive. The mean kidney weight of the four on the potassium-deficient diet was 1.36 Gm. (2.34 Gm. per 100 Gm. body weight), while the corresponding figure for the four on the control diet was 0.91 Gm. (0.91 Gm. per 100 Gm. body weight).

Pattern of Intracellular Granules and Granule-containing Cells in the Renal Medulla

Renal tissue from the two brothers and from potassium-depleted rats was examined histologically. PAS-positive, silver-positive, diastase-resistant granules, unstained by aldehyde fuchsin and by Alcian blue, were present in three groups of medullary cells, i.e., 1) tubular epithelium, 2) interstitial connective tissue, and 3) capillary endothelium. The intracellular material occurred in the form of fine and coarse discrete granules, irregular masses composed of coalescing granules, and globules. The smallest granules, sharply defined with methenamine-silver, were often poorly stained by the PAS technic. The globules, on the other hand, were seen best when stained by this latter method. They were characterized by sharp margins, a smooth homogeneous appearance and variable size. Occasionally they were larger than the cell nucleus.

In addition to the potassium-depleted rats, the potassium-repleted rats, and the rats receiving the control diet, 15 patients dying of miscellaneous diseases* and three cases of traumatic death provided renal tissue for comparison with tissue from the two brothers. Histologic data from the rats, from the two brothers, and from the additional clinical cases were as follows.

Potassium-depleted rats. Animals killed after receiving the potassium-deficient diet for three days showed coarse granules in the epithelial cells of terminal collecting ducts. Tubules were in close approximation to each other and intertubular capillaries were inconspicuous. An occasional capillary endothelial cell contained a few silver-positive granules and, rarely, several elongated granule-containing cells were seen lying together in an intertubular space. In the group of rats killed at the end of two weeks, the lesion was more advanced. Collecting duct epithelium in many places was hypertrophied, packed with coarse granules, and, in some instances, showed disintegration of the cell membrane. The lesion was most conspicuous in the region of the papillary tip where a coalescence of silver-positive granules was present in some cells and occasional globules were seen (Fig. 2B). Intertubular capillaries were prominent and the swollen endothelial cells contained silver-positive granules (Fig. 2C). Marked proliferation of interstitial connective tissue was not present, but numerous granule-containing fusiform and stellate cells were present in the intertubular spaces (Figs. 2B, 2D, 2E). As a rule, the granules in the interstitial cells were stained more strongly by the PAS method than were the granules in the adjacent tubular epithelium. Rats which had undergone unilateral nephrectomy before receiving the potassium-deficient diet showed slightly

* Diagnoses listed in appendix.

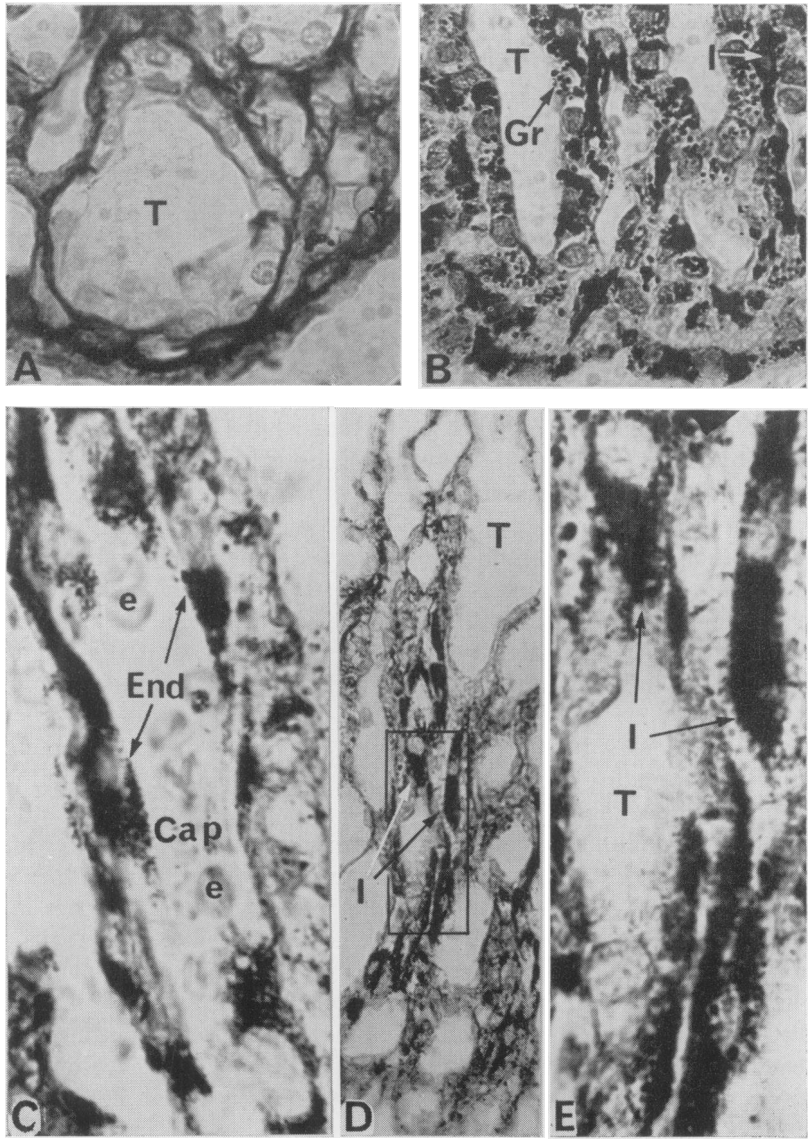

FIG. 2A. Rat maintained on control diet. Renal papillary tip showing terminal collecting tubules with normal epithelium. Methenamine-silver, nuclear fast red. × 480.

FIG. 2B. Potassium-depleted rat. Renal papillary tip showing terminal collecting tubules. Epithelial cells contain silver-positive granules (Gr). Intertubular spaces are widened and contain silver-positive, interstitial cells. Methenamine-silver, nuclear fast red. × 480.

FIG. 2C. Potassium-depleted rat. Capillary endothelial cells containing silver-positive granules. Methenamine-silver, nuclear fast red. × 1425.

FIG. 2D. Potassium-depleted rat. Renal papilla showing silver-positive, interstitial cells in intertubular spaces. Methenamine-silver, nuclear fast red. × 400.

FIG. 2E. Higher magnification of rectangular area shown in Figure 2D. × 1425.

more intense, but essentially similar, histologic changes when compared to the corresponding animals with intact kidneys.

Intracellular granules similar to those described in the potassium-depleted rats were not found in any of the rats receiving the control diet. Peritubular capillaries were inconspicuous and no proliferation of interstitial cells was seen. Three rats underwent potassium repletion after receiving the potassium-deficient diet for approximately two weeks, and in these animals the intracellular granules were absent. Capillaries appeared normal and interstitial cells were rarely seen, but a considerable amount of amorphous silver-positive debris was present among the epithelial cells of the papillary ducts.

J.G. (AVN 98-49). Renal tissue from the potassium-depleted elder brother showed many medullary cells containing granules. Where the lesion involved the tubular epithelium (Figs. 1C, 3B, 3C), the highest concentration of granules and granule-containing cells was in the region of the terminal collecting ducts and adjacent papilla. In the same area, granules were present to a lesser degree in the epithelium of certain smaller tubules thought to be loops of Henle. The lesion in the human was considerably less intense than that seen in the animal and the concentration of granules was less uniform. Epithelial cells with many coarse granules occurred immediately adjacent to cells with none, and tubules with many granule-containing cells were often in close proximity to tubules with normal-appearing epithelium.

Proliferation of interstitial connective tissue in the intertubular spaces (Fig. 1D) extended from cortico-medullary junction to renal papilla. The reaction was most conspicuous at the midportion of the medulla where there appeared to be a relative reduction in the number of tubular elements. Interstitial cells varied greatly in size and shape, some being oval, others stellate or fusiform.

The abnormal intracellular material in both the epithelial and the interstitial cells was PAS-positive, silver-positive, and diastase-resistant. It occurred not only in the form of fine and coarse granules but also as large globules. As noted in the rat, the granules in the interstitial cells were more strongly stained by the PAS method than were the granules in the tubular epithelium.

Both fully formed and budding capillaries were abundant in the interstitial connective tissue and contained swollen endothelial cells packed with silver-positive, diastase-resistant granules (Figs. 4A, 4B). It frequently was difficult or impossible to be certain whether a given silver-positive cell was an interstitial fibrocyte or an endothelial cell in a budding capillary. Sections of heart, lung, lymph node, liver, and spleen, incubated with diastase and stained with methenamine-silver, were examined for the

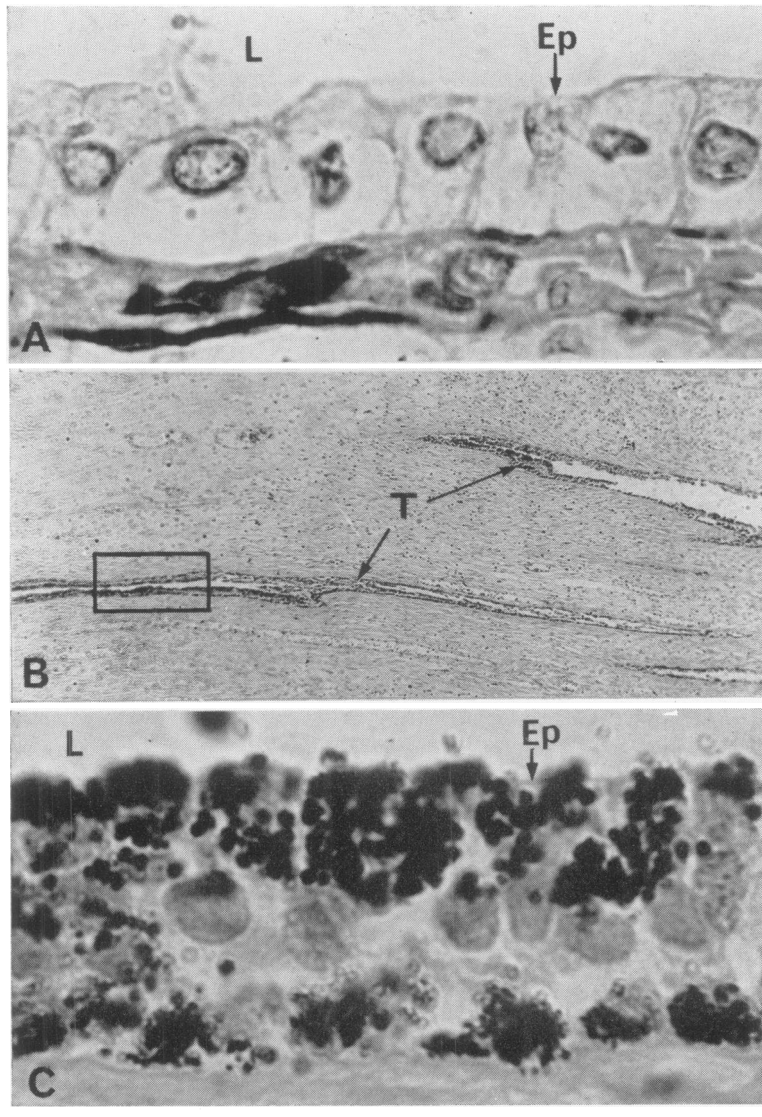

FIG. 3A. *A.M.* Traumatic death, "normal" control. Terminal collecting tubules showing normal epithelium. Methenamine-silver, nuclear fast red. × 1425.

FIG. 3B. *J.G.* Renal medulla. Terminal collecting tubules lined with silver-positive, epithelial cells. Methenamine-silver, nuclear fast red. × 200.

FIG. 3C. Higher magnification of epithelium from within rectangular area shown in Figure 3B. × 1720.

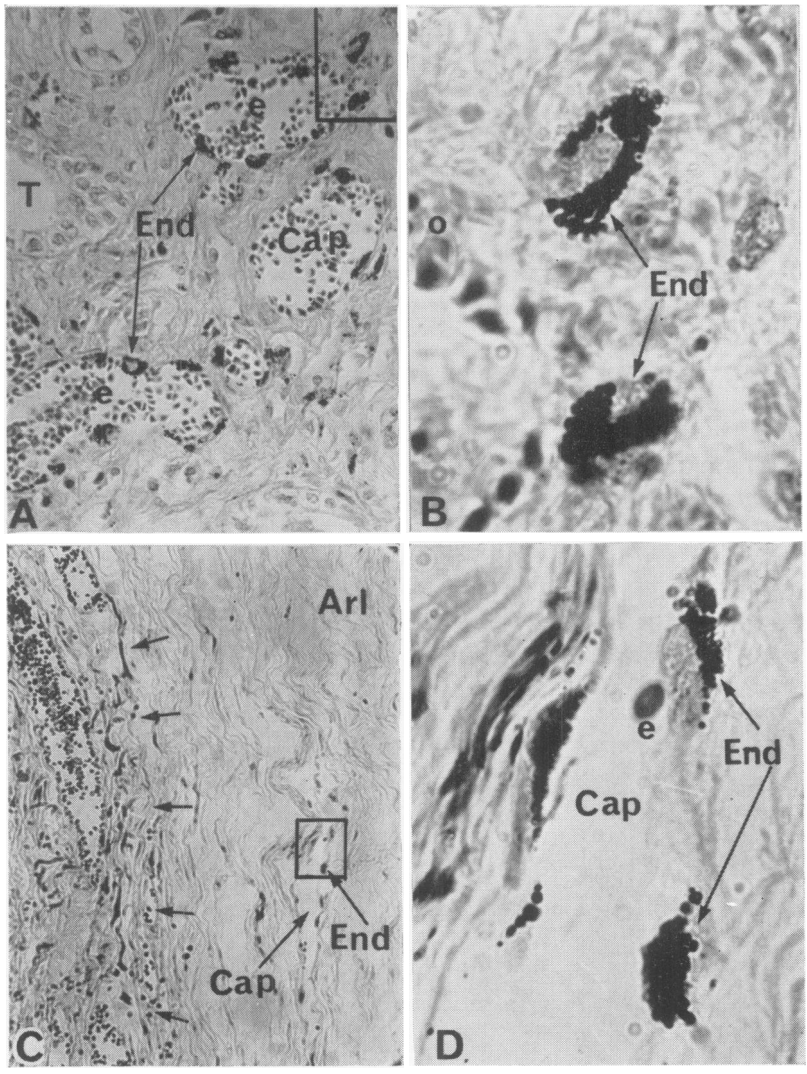

FIG. 4A. *J.G.* Renal medulla, area of interstitial fibrosis. Capillaries with endothelial cells containing silver-positive granules. Methenamine-silver, nuclear fast red. × 280.

FIG. 4B. Higher magnification of rectangular area shown in Figure 4A. × 1425.

FIG. 4C. *J.G.* Renal medulla with kidney capsule (arrows) adjacent to renal pelvis. Exrta-capsular areolar tissue (Arl) showing capillary containing silver-positive, endothelial cells. Methenamine-silver, nuclear fast red. × 100.

FIG. 4D. Higher magnification of rectangular area shown in Figure 4C. Capillary with endothelial cells containing silver-positive granules. × 1425.

presence of granule-containing capillary endothelial cells. No such cells were found, nor were any seen in the renal cortex. However, in the extracapsular areolar tissue, immediately adjacent to the renal pelvis, silver-positive granules were present in the endothelial cells of a few capillaries (Figs. 4C, 4D).

R.G. (S-597-59). Renal tissue from the partially repleted younger brother showed considerably fewer granule-containing medullary cells. Except for the rare occurrence of a few intracellular silver-positive granules, the epithelial cells of collecting tubules appeared normal. However, the terminal collecting ducts were not present in the specimen. In several places, large cells containing globules of PAS-positive material were present in the nests of interstitial cells characterized by clear cytoplasm (Fig. 5B). Also localized to these areas of clear cells, and absent from the adjacent columns of tubules, were accumulations of silver-positive amorphous debris (Fig. 5A), resembling that found in the vicinity of the terminal collecting duct epithelium in the potassium-repleted rat. Scattered through the medulla were occasional clumps of capillaries with swollen endothelial cells filled with large and small silver-positive, diastase-resistant granules (Fig. 5C).

Additional clinical cases. Renal tissue from three human cases in which death followed trauma within 24 hours and in which potassium depletion was considered highly improbable was stained by the PAS and methenamine-silver methods. Occasional discrete silver-positive, PAS-positive granules were seen in epithelial cells of the terminal collecting ducts and adjacent papilla. However, in no instance were intracellular granules numerous nor the number of affected cells high. Granule-containing interstitial cells were not seen. None of the capillaries contained endothelial cells with silver-positive granules. In renal tissue from 15 cases dying of miscellaneous diseases, the frequency with which intracellular PAS-positive, silver positive granules involved the epithelium of terminal collecting ducts and adjacent papilla was greater than that seen in the three cases of traumatic death. With two exceptions, however, the lesion did not approach the intensity of that seen in the elder brother. Five of the miscellaneous cases are described briefly.

G.W. (AVN 12-55). A 22 year old white male with cardiac enlargement of unknown cause died as the result of chronic congestive heart failure. He received mercurial diuretics and, during the last three days of illness, adrenal cortical steroids. Proteinuria was present. Serum potassium two days before death was 6.4 mEq. per liter and the electrocardiographic changes did not suggest hypokalemia. Histologic examination of the kidney showed many PAS-positive, silver-positive, diastase-resistant granules in the epithelium covering the papillary tip (Fig. 6A) with but little involvement of the terminal collecting ducts.

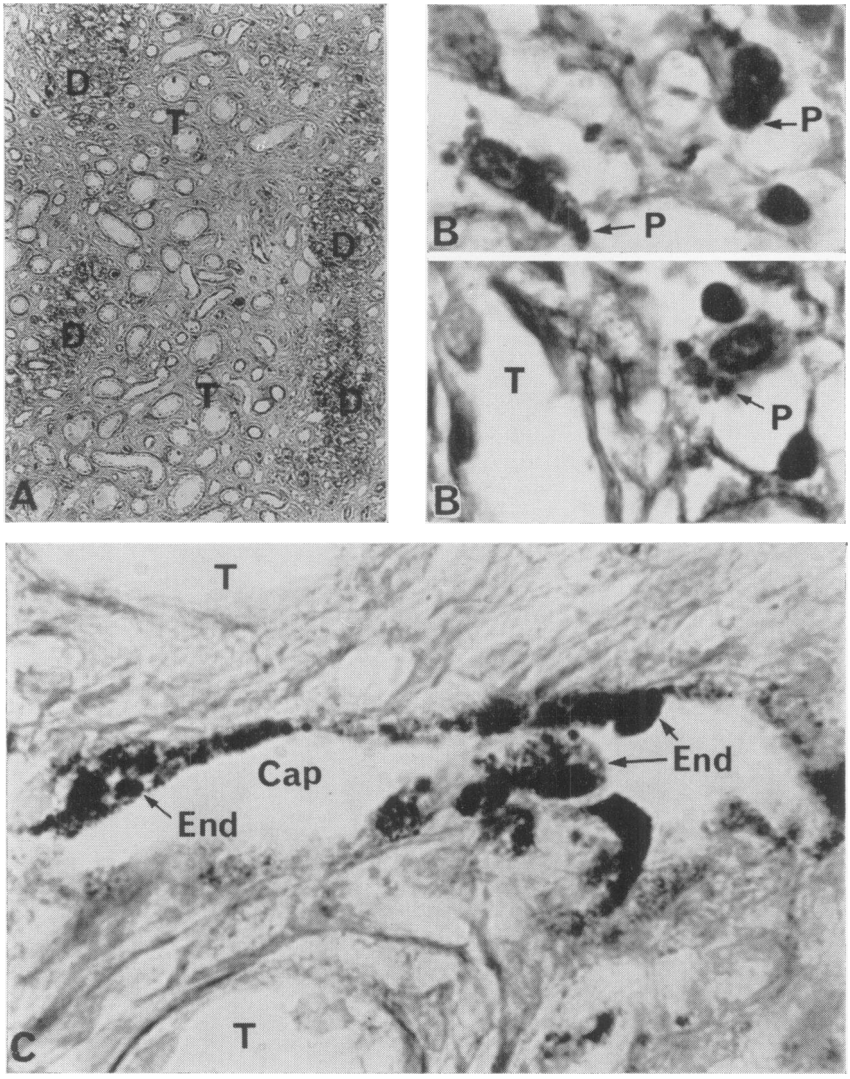

FIG. 5A. *R.G.* Renal medulla, for comparison with Figure 1B. Silver-positive debris (D) is restricted to areas of cells with clear cytoplasm in the intertubular spaces. Methenamine-silver, nuclear fast red. × 50.

FIG. 5B. *R.G.* Renal medulla showing cells with clear cytoplasm. Higher magnification of area shown in Figures 1B and 5A. Occasional interstitial cells containing PAS-positive material (P). PAS method, hematoxylin. × 1425.

FIG. 5C. *R.G.* Renal medulla, area of interstitial fibrosis. Capillary with endothelial cells containing silver-positive granules. Methenamine-silver, nuclear fast red. × 1425.

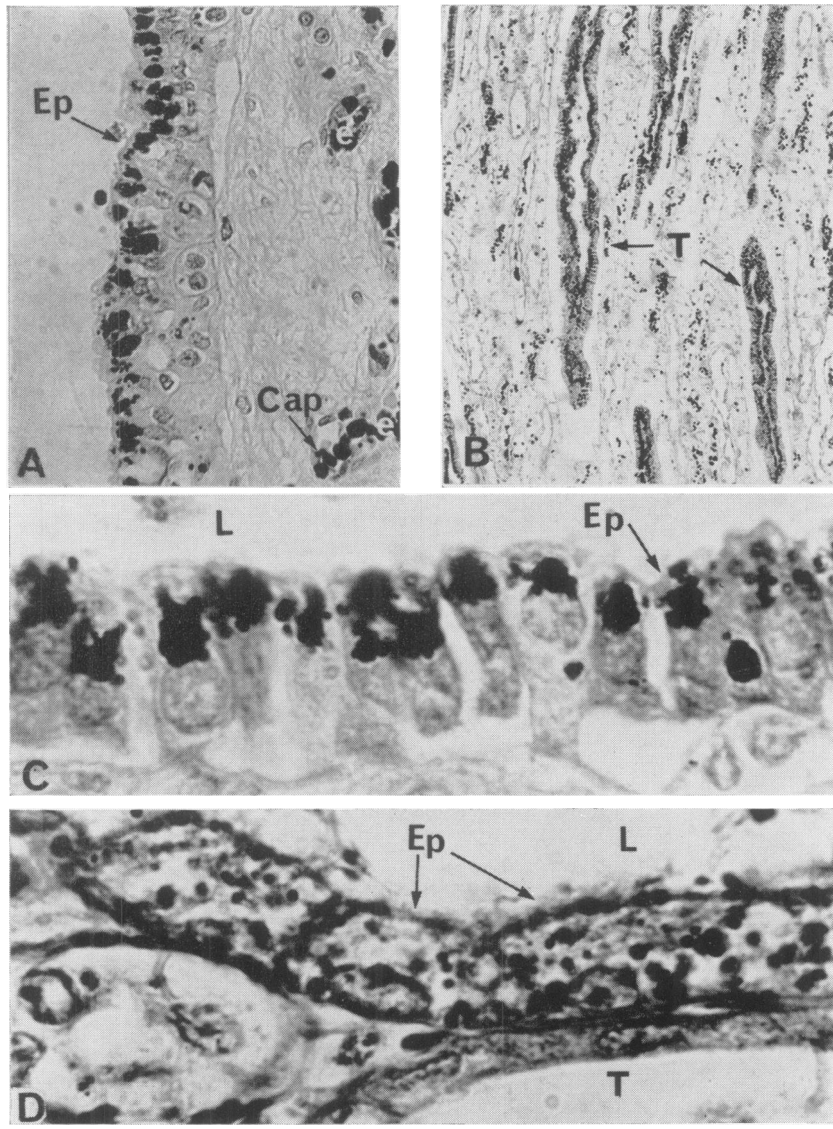

FIG. 6A. *G.W.* Death from congestive heart failure. Renal medulla, papillary tip. Epithelial cells with silver-positive granules. Capillaries contain silver-positive erythrocytes. Methenamine-silver, nuclear fast red. × 400.

FIG. 6B. *T.G.* Death from disseminated lupus erythematosus. Renal medulla, papillary zone. Collecting tubules lined with silver-positive epithelial cells. Methenamine-silver, nuclear fast red. × 180.

FIG. 6C. *T.G.* Higher magnification of terminal collecting duct epithelium. For comparison with Figure 6B. Methenamine-silver, nuclear fast red. × 1425.

FIG. 6D. *E.W.* Death from congestive heart failure. Renal medulla, papillary zone. Collecting tubule with epithelial cells containing silver-positive granules. Methenamine-silver, nuclear fast red. × 1425.

T.G. (*AVN 110-60*). A case of disseminated lupus erythematosus dying one week after admission to the hospital. Determination of serum potassium was not made but the electrocardiogram did not suggest a diagnosis of potassium depletion. Proteinuria was present. Adrenal cortical steroids and potassium chloride were administered during the final 24 hours; no diuretics were given. The patient died in congestive heart failure. Histologically, many of the terminal collecting ducts contained a high concentration of epithelial cells with silver-positive granules (Figs. 6B, 6C). Epithelial cells of the proximal convoluted tubules did not show the vacuolization frequently associated with potassium depletion. Neither granule-containing interstitial cells nor endothelial capillaries with silver-positive granules were present in the renal medulla.

E.W. (*AVN 14-52*). A 40 year old white male with rheumatic valvular heart disease. Death was the result of longstanding congestive heart failure. Proteinuria was present. Serum potassium averaged 4.1 mEq. per liter during the final month of illness and was 3.9 mEq. per liter seven days before death. He received mercurial diuretics but no adrenal cortical steroids. On histologic examination a few of the terminal collecting tubules showed epithelial cells containing silver-positive granules (Fig. 6D). No silver-positive cells were present in the interstitial spaces. Intracellular granules were not present in the capillary endothelium.

J.E. (*AVN 67-61*). A case of chronic pyelonephritis. In the areas of medullary fibrosis, no abnormal intracellular granules were seen either in interstitial fibrocytes or in capillary endothelium. A few silver-positive granules were present in the epithelial cells of terminal collecting ducts and the adjacent papilla.

J.P. (*AVN 88-52*). A case of plasma cell myeloma with extensive involvement of the kidney. Proteinuria was present. Irregular masses of PAS-positive, silver-positive granules were present in the epithelial cells of several terminal collecting ducts. Many medullary tubules were dilated and contained PAS-positive material in the tubular lumen. Although proliferation of interstitial cells was present, the fibrocytes were PAS-negative. No granules were seen in the capillary endothelium.

Discussion

Although histologic findings in the potassium-depleted rats of the present study were in essence those previously described by other investigators,[5-10] two points may be mentioned. First, with regard to the intracellular globules, Milne and Muehrcke[6] described the occurrence of similar large globules among the intracellular granules in the more seriously affected tubular cells of the papillary tip. Second, the absence of more extensive proliferation of interstitial cells is attributed, in the present study, to the fact that all of the potassium-depleted rats were killed within a period of 16 days.

Intracellular granules produced by potassium depletion in the renal medulla of the rat have been compared with similar granules seen in two brothers suffering from chronic potassium depletion. The findings indicate that the lesion found in the elder brother closely resembles that seen in the rats. The medullary intracellular granules in the human and in the animal tissue have the following in common: 1) they are found in epithelium of the terminal collecting ducts and papilla, in interstitial cells of the intertubular spaces, and in capillary endothelium; 2) they are PAS-positive, silver-positive, before and after diastase digestion, and in capillary endothelium are stained more intensely with methenamine-silver than by the PAS technic; and 3) where granules involve epithelium and endothelium, the cells are frequently hypertrophic. Tissue sections from the younger brother, who was partially repleted with potassium, showed 1) silver-positive, diastase-resistant granules in capillary endothelium and, to a lesser degree, in tubular epithelium, 2) occasional large PAS-positive interstitial cells in areas of intertubular fibrosis, and 3) silver-positive amorphous debris localized to interstitial nests of cells with clear cytoplasm. These findings suggest that the younger brother, when in a state of severe potassium depletion, may have had the fully developed lesion observed in the elder brother.

The results of the present study do not explain the origin of the intracellular granules. Opinions differ as to whether the granules are,[15, 25] or are not,[2, 10] derived from mitochondria. Lack of agreement exists, also, concerning the importance of mucoprotein complexes absorbed from the tubular lumen.[2, 9, 10, 26] On the basis of evidence currently available it would seem unwise to assume that the abnormal granules in all three medullary cell groups (tubular epithelium, interstitial connective tissue, and capillary endothelium) are of the same composition or arise in response to the same stimulus.

It seems probable, however, that the intracellular granules may originate under one or more of the following three sets of conditions. *First*, as a unique response to potassium depletion affecting, in the rat, all cells in the zone of the papillary tip.[10] *Second*, as the result of absorption or phagocytosis of mucoprotein. Evidence favoring this view is obtained from studies suggesting that potassium deficiency in rats increases proteinuria and may lower the threshold for protein absorption in epithelial cells of terminal collecting ducts.[2, 9, 26] In the renal medullary extravascular tissues of the potassium-depleted rat, Pearse and MacPherson[9] described small lakes of plasma-like mucoprotein and suggested that metabolism of this substance might be responsible for the PAS-positive material in the numerous interstitial cells. *Third*, as an elaboration of mucoprotein material for a specific purpose, e.g., the production of extra-cellular ground

substance. Elaboration of mucopolysaccharide by capillary endothelium has been described by Curran.[27] In addition, Popper, Paronetto, Schaffner, and Perez,[28] from a study of hepatic fibrosis, described capillary endothelial cells rich in PAS-positive granules in the region of actively proliferating ductules. In sections of renal medullary tissue from the potassium-depleted elder brother, granule-containing cells of the capillary endothelium and of the interstitial connective tissue were usually seen in fairly close association. In the mid-zone of the medulla, they were more numerous and conspicuous than granule-containing cells in the adjacent tubular epithelium. In the potassium-depleted rat, proliferation of these mesenchymal cells was observed in the narrow cleft-like intertubular spaces of the renal papilla.

The presence of PAS-positive, silver-positive intracellular granules observed in the terminal collecting duct epithelium of occasional cases in which potassium depletion appeared unlikely is unexplained. Since the characteristic finding of silver-positive granules in capillary endothelium was not seen in these cases, the possibility exists that some, or all, of the intracellular granules were related to the presence of proteinuria and the absorption of mucoprotein from the urine.

Summary

A study of intracellular granules of the renal medulla in two potassium-depleted brothers is described and the lesion compared with that produced in rats maintained on a potassium-deficient diet. On the basis of the findings it is postulated that the abnormal intracellular granules observed in the two brothers are the result of potassium depletion and correspond to similar changes seen in the experimental animals. Theories regarding the origin of the intracellular granules are briefly discussed.

Appendix

Diagnoses on 15 Cases Dying of Miscellaneous Diseases

1. Staphylococcus endocarditis with septic infarcts to brain and cerebral hemorrhage.
2. Multiple sclerosis, bronchopneumonia.
3. Rheumatic valvular heart disease with chronic congestive heart failure.
4. Cardiac enlargement of unknown cause with chronic congestive heart failure.
5. Systemic lupus erythematosis, hemorrhagic nephritis.
6. Subacute interstitial pulmonary fibrosis.
7. Chronic pyelonephritis.

8. Endocardial and myocardial necrosis of unknown cause with chronic congestive heart failure.
9. Arterial hypertension with intracranial hemorrhage.
10. Cushing's syndrome, renal necrosis following shock.
11. Eosinophilic granuloma, myelogenous leukemia.
12. Myocardial fibrosis of unknown cause with chronic congestive heart failure.
13. Diabetes mellitus with glomerulosclerosis.
14. Diffuse systemic sclerosis with skin and renal involvement.
15. Plasma cell myeloma with renal involvement.

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DISCUSSION

Dr. Frank Gardner (Boston): Does the capillary stain that you showed of the granules exist anywhere besides in the kidneys?

Dr. France: Thus far, as regards both rat and human tissue, I have not seen the lesion in organs other than the kidney, nor have I seen it in the renal cortex.

Dr. Thomas Chalmers (Boston): Dr. Hershel Jick at our hospital has demonstrated that patients with cirrhosis have a concentrating defect which is unique because when receiving a normal salt diet, their maximal ability to re-absorb water under a mannitol load is normal in spite of their inability to elaborate a normally concentrated urine. Cirrhosis patients have been suspected for a long time to be deficient in potassium. We were unable to correct the concentrating defect by administering potassium to these patients, but I should like to ask if these granules have been seen by you in patients with cirrhosis of the liver.

DR. FRANCE (Closing): Occasionally, in patients with longstanding proteinuria one may find medullary tubules in which there are small and large PAS-positive, silver-positive granules. It is my impression that these granules may not in all instances originate from a single cause. Perhaps some are the result of protein absorbed from the tubules. However, I think there are also granules which are not related to protein absorption and that the granules in the capillary endothelium fall into this second group. These granules, therefore, may be more truly representative of potassium depletion.