ENZYMATIC STAINING REACTIONS IN THE KIDNEYS OF POTASSIUM-DEPLETED RATS *

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The application of various histochemical enzyme techniques to the mammalian kidney has revealed distinctive and reproducible staining patterns within the various portions of the nephron. It is not surprising that most reactions in the cortex are positive, particularly in the proximal convoluted tubules, since the maximum work load of the kidney is performed in these segments. Some enzymatic reactions are also quite distinct in the thin limbs of Henle's loops and the collecting ducts within the medulla.[1]

In potassium deficiency, there occurs an increase in kidney weight, mainly due to hyperplasia of the medulla. Such kidneys have been examined by conventional methods[2-10] and more recently by the microdissection technique.[11] The most outstanding alterations occur in the collecting tubules. Other portions of the nephron reveal less consistent and more variable alterations. These are of the severest degree when weanling rats are given the deficient diet.[12]

Histochemical enzyme staining techniques have been applied to potassium-deficient kidneys by Spargo,[7] Craig and Schwartz,[9] and by Pearse and Macpherson.[13] The results reported have been somewhat contradictory. We have, therefore, investigated a number of histochemical staining reactions in the kidneys of potassium-deficient rats and selected those which demonstrate enzymatic activity in the medullary structures of the normal kidney. An attempt has been made to correlate the remarkable variations in histochemical reactions in the renal medulla with certain functional alterations known to occur in the potassium-deficient kidney.

MATERIAL AND METHODS

Young rats of the Wistar strain, weighing 150 to 200 gm., were used throughout. Thirty rats were fed a potassium-deficient diet and sacrificed after 15 to 25 days. An additional 20 rats were placed initially on a protein-deficient diet supplemented by all the necessary vitamins, as suggested by Spargo.[7] After the initial weights had been reduced 25 per cent, the rats were given a synthetic diet deficient in both potassium

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and sodium. Our experience was in agreement with that of Craig and Schwartz[9] who observed an enhancement of tissue alterations induced in potassium-depleted rats also receiving a low sodium intake.

Following sacrifice, pieces of kidney and heart were fixed in 10 per cent formalin and Rossman's fluid. Sections were stained with hematoxylin and eosin, the periodic acid-Schiff (PAS) technique and occasionally by the Jones silver-methenamine stain.[14]

Histochemical staining was carried out on free-floating, unfixed frozen sections and on sections prepared from thin tissue blocks fixed overnight in cold Baker's formalin. The following methods were used: alkaline phosphatase in formalin-fixed frozen sections according to Gomori (incubation period, 5 to 15 minutes), acid phosphatase in a modification of Gomori's technique using a pH of 6, preferably on formalin-fixed frozen sections (incubation period, 15 to 60 minutes)[15] and adenosine triphosphatase with the technique of Wachstein and Meisel (incubation period, 5 to 15 minutes).[16] For succinic dehydrogenase, unfixed frozen sections were incubated for 5 to 30 minutes in a mixture which contained sodium succinate and Nitro-BT [2,2′-di-p-nitrophenyl-5,5′-diphenyl-3,3′-(3,3′-dimethoxy-4,4′-biphenylene)-ditetrazolium chloride].[17] In earlier experiments, we used the technique of Farber, Sternberg and Dunlap[18] for the demonstration of diphosphopyridine nucleotide (DPN) diaphorase in unfixed frozen sections. Later, the modification of Novikoff and Masek was substituted.[19] Formalin-fixed frozen sections were incubated in a mixture containing Nitro-BT as an indicator and, in addition, reduced diphosphopyridine nucleotide (DPNH) and the buffer (incubation time, 5 to 30 minutes). In some instances the results were checked by the use of fresh frozen sections.

For the demonstration of triphosphopyridine nucleotide (TPN) diaphorase, both the original technique of Farber and co-workers,[18] as well as the modified procedure of Nachlas, Walker and Seligman,[20] were used on fresh frozen sections (incubation time, 15 to 20 minutes). In addition, an incubation mixture which contained only TPNH, Nitro-BT and the buffer was used with both fixed and unfixed frozen sections.

In most instances a normal control rat was sacrificed simultaneously with the experimental rat in order to evaluate possible variations in the staining reactions.

Results

Our observations in the kidneys of potassium-deficient rats were similar to those described by various investigators and may be summarized as follows: The collecting tubules in the outer zone of the

medulla showed marked swelling of the cytoplasm and increase in the number of cells with apparent obstruction of the lumens. In the segments of excretory ducts located in the outer medulla there was a marked increase in the dark-staining intercalated cells, as has been pointed out by Oliver and co-workers.[11] The lumens of collecting ducts located in the cortex were often dilated. The collecting ducts close to the papilla showed a fairly normal configuration but were distended by innumerable eosinophilic granules which gave a positive PAS reaction.[7] There was also an increase in PAS staining in other structures of the medulla. Basement membranes stained more strongly, and an increased number of mononuclear cells containing PAS-positive material was seen. The cells in the thin limbs of Henle's loops often had an increased amount of cytoplasm and contained PAS-positive material. The broad ascending limbs of Henle's loops and the distal convoluted tubules appeared essentially normal although they were occasionally compressed by dilated collecting ducts. All structures that stained with the PAS technique also gave a positive staining reaction with the silver-methenamine stain (Fig. 1). Within the cortex, occasional proximal convoluted tubules showed swelling of cells as well as focal atrophy. The atrophy increased with the duration of the potassium deficiency and was more marked in the kidneys of experimental animals after 25 days as compared to those after 15 days.

Histochemical Staining Reactions

Alkaline Phosphatase. There was focal diminution of the staining reaction in proximal convoluted tubules corresponding to the focal atrophy observed in conventional sections. Granules in the collecting tubules did not stain but were clearly recognized by their refractility.

Acid Phosphatase. With the use of a modification of Gomori's method at pH 6.0 in formalin-fixed frozen sections, a consistent staining reaction was noted in the proximal convoluted tubules. Lead deposits indicative of enzyme activity appeared as granules varying from 1 to 3 μ in diameter. The straight or distal portions of the convoluted tubules contained fewer droplets and exhibited some staining of the brush borders. If the incubation period was not extended beyond 30 to 60 minutes, only faint nuclear staining was noted. The ascending broad limbs of Henle's loops exhibited a moderate reaction, as evidenced by the deposit of fine, dust-like granules. A slight although irregular reaction also appeared in the collecting ducts, thin limbs of Henle's loops, and occasional interstitial cells (Fig. 3). In unfixed sections, the lead sulfide deposit appeared in a more diffuse, dust-like fashion in the cortical tubules. Deposits of larger granules

were not seen. Both the ascending limbs of Henle's loops and the collecting ducts revealed a fairly strong reaction after 15 minutes' incubation. After longer incubation, nuclear staining became very marked in unfixed sections.

In the experimental animals there was considerable increase in the overall staining of the medulla; this could be recognized by naked-eye inspection of the sections. The increase was due to the presence of abnormal granules and an actual increase in activity in several medullary structures. This was the case in the collecting ducts, particularly in the segments located close to the papilla. Acid phosphatase activity was less marked in segments located in the outer medulla. There was also a striking increase of staining in the cells which formed the thin limbs of Henle's loops (Fig. 4) and in interstitial stromal cells, particularly near the papilla. In the region of the corticomedullary junction, there was a reduction of the reaction in the ascending limbs of Henle's loops; this was most clearly recognized in unfixed tissue, but was also seen in formalin-fixed frozen sections. Within the cortex, occasional atrophic proximal convoluted tubules showed reduced staining, as in the case of alkaline phosphatase.

Nonspecific Esterase. With naphthol AS as a substrate, there was a marked difference in the staining pattern in fixed and unfixed sections of the normal kidney. In unfixed sections, deposits were diffuse and dust-like in the proximal convoluted tubules. Glomeruli did not react and only slight staining was evident in the ascending limbs of Henle's loops. In the medulla there was rather intense and regular staining in the collecting ducts but only faint and somewhat variable staining in the thin limbs of Henle's loops. In fixed sections, the overall staining was more intense except in the collecting ducts. Proximal convoluted tubules of the cortex contained cells with both fine, dust-like granules and larger granules measuring 1 to 3 μ in diameter. In some cortical structures, obviously the distal convoluted tubules and initial portions of the collecting ducts, esterase activity was limited to isolated scattered cells. There was a slight reaction in cells of Bowman's capsule and in an occasional glomerular epithelial element. Staining in the ascending limbs of Henle's loops was moderately strong (Fig. 5). The thin limbs also exhibited distinct activity, but the collecting ducts stained only weakly (Fig. 7). The epithelium covering the renal papillas was also active. In addition, occasional stromal cells reacted in positive fashion.

In experimental animals, a reduction of enzyme activity was apparent in some of the proximal convoluted tubules. As in acid phos-

phatase preparations, the ascending limbs of Henle's loops at the corticomedullary junction showed a reduced staining reaction (Fig. 6). The collecting ducts of the medulla exhibited increased staining in segments close to the papilla, and abnormal granules participated in the reaction. There was a most striking increase in esterase activity in the thin limb of Henle's loop; this extended through its entire extent (Fig. 8). Interstitial cells, particularly those located close to the renal pelvis, were likewise the seat of striking esterase activity. An additional feature was the presence of an increased amount of bluish-staining protein in medullary capillaries. It is interesting that all of these phenomena were much more distinct in formalin-fixed than in fresh frozen tissue.

Adenosine Triphosphatase. The normal distribution of adenosine triphosphatase in the rat kidney has been described previously.[21,22] In formalin-fixed frozen sections, activity occurred in glomeruli, capillaries, ascending limbs of Henle's loops and distal convoluted tubules. The proximal convoluted tubules exhibited less activity, and this appeared mainly in brush borders. In the medulla, the inner cell borders of the collecting ducts were stained after 5 to 10 minutes (Fig. 9). Medullary capillaries reacted only after somewhat longer incubation periods.

In the potassium-deficient animals, there was a significant increase in the staining of the outer borders of cells in the collecting ducts, best seen in sections incubated for 5 to 10 minutes (Fig. 10). There was also a moderate reduction in the staining intensity of ascending limbs of Henle's loops and the distal convoluted tubules.

Succinic Dehydrogenase. When Nitro-BT was used instead of neotetrazolium, intense staining was seen within only a few minutes of incubation. Maximum activity was noted in the proximal portions of the proximal convoluted tubules and in the ascending limbs of Henle's loops. If staining was prolonged (15 to 30 minutes), very distinct activity was also seen in collecting ducts, mainly in the outer, and to a lesser degree, in the inner medulla. Within the collecting ducts in the outer medulla, occasional cells showed a very strong reaction. These obviously corresponded to the dark-staining intercalated cells. The thin limbs of Henle's loops reacted only very faintly.

In the potassium-depleted animals, there was a depression of activity in atrophic tubules in the cortex and a distinct although slight overall reduction in the ascending limbs of Henle's loops and in the distal convoluted tubules. The reaction in collecting ducts appeared to be somewhat enhanced, and a larger number of dark-staining intercalated

cells was seen. The thin limbs of Henle's loops stained faintly, but an unequivocal increase in the staining reaction as compared with the control animals could not be detected.

DPN Diaphorase. The normal distribution patterns of DPN diaphorase in unfixed frozen sections has been described previously.[23] In formalin-fixed sections, practically all renal cells, including those of the glomerulus, the thin limbs of Henle's loops, and vessel walls, show activity. In the cortex, the ascending limbs of Henle's loops and the distal convoluted tubules show a somewhat stronger staining than do the proximal convoluted tubules. Formazan deposits indicating enzymatic activity were very sharply localized in the mitochondria. With formalin fixation, however, there was some reduction in staining in the thin limbs of Henle's loops. The intensely staining intercalated cells of the collecting ducts were particularly prominent (Fig. 11).

In the potassium-depleted animals, there was a reduction in staining of some proximal as well as distal convoluted tubules. There was a moderate overall reduction of activity in the ascending limbs of Henle's loops. A larger number of strongly staining cells appeared in the median portions of the collecting ducts; this corresponded to the increase in intercalated cells in potassium deficiency (Fig. 12). There was also a somewhat stronger reaction in collecting ducts in the inner medulla. The atypical granules reacted in positive manner. The thin limbs of Henle's loops exhibited a slight increase in staining, as did some interstitial cells.

TPN Diaphorase. When the original method of Farber and coworkers[18] was used, staining was often spotty. Better and more consistent results were obtained with the technique of Nachlas and his associates.[20] The best results, however, were obtained with formalin-fixed sections when TPNH was used in the incubation mixture. Strongest activity was observed in the proximal convoluted tubules, the ascending limbs of Henle's loops, and in the collecting ducts of the medulla. The distal convoluted tubules stained less intensely with the exception of the macula densa, which could be clearly recognized by its deeper staining.[20] Glomeruli showed only weak activity, but the thin limbs of Henle's loops stained distinctly, particularly in unfixed sections.

In the kidneys of experimental animals, there was increased TPN diaphorase activity in the collecting ducts. There was also a slight increase in the thin limbs of Henle's loops, and a moderate decrease in the ascending limbs of Henle's loops and in those parts of the cortical tubules that had become atrophic. Staining of the macula densa was similar to that of normal controls (Fig. 2).

Discussion

Many histochemical enzyme staining reactions are regularly reproducible in the mammalian kidney and are, by now, well established.[1] Several observations made in this study, however, deserve comment (Table I). With a modified technique for acid phosphatase, the lead

Table I

Histochemical Renal Alterations in Potassium-Deficient Rats

Enzyme reaction	Changes observed
Alkaline phosphatase	Focal diminution in atrophic proximal convoluted tubules.
Acid phosphatase	Focal diminution in atrophic proximal convoluted tubules. Reduction in ascending limbs of Henle's loops. Marked increase in collecting ducts near papilla, less in outer medulla. Marked increase in thin limbs of Henle's loops and in interstitial stromal cells of medulla.
Nonspecific esterase	Similar to acid phosphatase.
Adenosine triphosphatase	Moderate reduction in ascending limbs of Henle's loops and distal convoluted tubules. Increase in outer cell borders of collecting ducts.
Succinic dehydrogenase	Reduction in atrophic tubules of cortex. Slight reduction in ascending limbs of Henle's loops and in collecting ducts.
DPN diaphorase	Reduction in atrophic proximal convoluted tubules. Moderate reduction in ascending limbs and distal convoluted tubules. Moderate increase in collecting ducts near papilla and greater increase in collecting ducts in the outer medulla. Slight increase in thin limbs of Henle's loops and interstitial cells.
TPN diaphorase	Similar to DPN diaphorase although somewhat less marked.

sulfide deposits indicating enzymatic activity were deposited in the rat kidney as coarse (1 to 3 μ) granules. A similar distribution pattern was found with various azo dye techniques for acid phosphatase[24,25] and for esterase.[25] It should be pointed out, however, that in unfixed frozen sections, such a staining pattern was not apparent. In such preparations, stain deposits occurred as tiny, evenly distributed cytoplasmic granules. It is worth noting that in the kidneys of other species —for instance, the rabbit—acid phosphatase activity is not characterized by the appearance of coarse granules in formalin-fixed sections.[15]

In preparations stained for nonspecific esterase, there were also differences in the distribution patterns in formalin-fixed and unfixed frozen sections. It was particularly noticeable that in formalin-fixed sections, activity in the collecting ducts was suppressed, while in the thin limbs of Henle's loops there was significant staining. In some cortical tubules, obviously the distal convolutions and initial portions of the collecting ducts, only scattered cells gave a positive staining reaction. These were first described by Hess and Pearse[26] using *o*-acetyl-5-bromoindoxyl as substrate. On the basis of studies utilizing

special inhibitors, these investigators considered the reaction to be due to an esterase with cathepsin-like activity. Further investigation will be necessary to substantiate this assumption. The physiologic significance of these cells, which are indistinguishable by conventional techniques, is unknown.

With the techniques for dehydrogenase and DPN diaphorase, and less regularly with the TPN diaphorase technique, dark-staining intercalated cells can be demonstrated readily in the median portions of the collecting ducts[27,28] by their strong staining. The function of these peculiar cells is also unknown at present.

The use of formalin fixation, first advocated by Novikoff and Masek,[19] permits a marked improvement in the techniques designed to demonstrate DPN and TPN diaphorase activity. The simplification of the incubation mixtures, replacing various substrates by DPNH and TPNH and the introduction of Nitro-BT, has contributed to the ease of performance of these techniques. The distribution patterns are essentially similar to those first described by Farber and his co-workers[18] and later confirmed, though somewhat modified, by others. In the case of the kidney, the improved techniques permit definite localization of formazan deposits in mitochondria, as well as differential staining of the macula densa.

Alterations of enzymatic staining reactions in experimental animals were most regularly noted in the medulla. By far the greatest increase of staining was observed with the acid phosphatase and esterase techniques. Obviously the increase occurred in 3 different structural units: the excretory ducts, the thin limbs of Henle's loops, and the interstitial cells. It should be emphasized that alterations in the thin limbs of Henle's loops can be recognized only with difficulty, if at all, in conventionally stained sections. The peculiar granules deposited in collecting ducts reacted with the staining techniques for acid phosphatase, esterase, and DPN and TPN diaphorase. In general, there was considerably less marked increase in activity of oxidative enzymes. However, many more cells in the collecting ducts of the outer medulla gave the strong staining reaction which seemed to be associated with intercalated cells. This is in agreement with the increased number of these cells observed in the kidney in potassium deficiency, described by Oliver and co-workers.[11]

A significant increase of adenosine triphosphatase activity was encountered in the outer membranes of collecting ducts in the potassium-deficient experimental animals. It should be recalled that adenosine triphosphatase activity in cellular membranes may be of

importance in the cellular transport mechanism. This has been claimed in the case of bile canaliculi,[29] the intercellular membranes of some renal cells,[22] and the secretory capillaries of the pancreas.[30]

Spargo[7] found no increase in acid phosphatase activity in the medulla of potassium-deficient rats, differing in this respect from Craig and Schwartz[9] and Pearse and Macpherson.[13] Spargo and Craig and Schwartz failed to detect the alterations in esterase activity observed by us and by Pearse and Macpherson. The latter investigators described a marked increase in TPN diaphorase and a reduction in DPN diaphorase activity in the medulla of potassium-deficient animals. We, on the other hand, were unable to confirm these observations. While various enzyme reactions were increased in the medulla, there was a uniform reduction of activity in the corticomedullary junction, localized to ascending limbs of Henle's loops. This was noted in preparations stained for adenosine triphosphatase, esterase, acid phosphatase, succinic dehydrogenase and DPN diaphorase. Decrease in activity was least noticeable with the stain for TPN diaphorase. Although conventionally stained sections did not show a significant dilatation of the ascending limbs of Henle's loops in our specimens, it has been pointed out[11] that a certain degree of obstruction of the lumens may occur as the result of proliferation of cells in collecting ducts. It is quite possible that this may lead to a mild internal hydronephrosis which in turn may account for the reduced enzymatic activity in the distal convoluted tubules and ascending limbs of Henle's loops. Such an internal hydronephrosis is apparently much more marked in weanling rats.[12]

A reduction of enzymatic reaction in atrophic tubules noted in occasional proximal convoluted tubules is typical of tubular atrophy resulting from a variety of causative factors.[1] In contrast to Pearse and Macpherson,[13] we were unable to detect an increase in esterase activity due to potassium deficiency in proximal convoluted tubules. On the basis of their histochemical observations, Pearse and Macpherson suggested that potassium deficiency affected protein metabolism, the permeability of tubules to protein, and also the processes of respiration and oxidative phosphorylation, particularly in tubules of the medulla and papilla. Whether changes in histochemical reactions permit such broad conclusions is open to some doubt.

It seems logical, however, to correlate these changes with the functional alterations that characterize potassium deficiency. It has been shown in the rat that potassium deficiency is accompanied by an inability to concentrate urine properly, or to produce a highly acid urine.[10,31] Although there are also alterations in cortical tubules, those

most specific for potassium deficiency are found in the medulla, as has been emphasized.[11]

It has been suggested by Hargitay and Kuhn[32] that the loops of Henle act as a countercurrent multiplier system for the concentration of urine. According to this concept, this mechanism would cause the interstitial tissue of the medulla to be hyperosmotic. This, in turn, would cause diffusion of water out of the collecting ducts with resulting concentration of the urine. Wirz, Hargitay and Kuhn,[33] on the basis of cryoscopic examination of slices made from concentrating rat kidneys, concluded that the osmotic pressure was identical for all tubular structures at a given level. They assumed that there was an increasing osmotic gradient from the cortex which was iso-osmotic with plasma to the tip of the papilla. Further support for the correctness of the "countercurrent multiplier system" hypothesis has been derived from the examination of fluid obtained from various portions of the nephron by Wirz,[34] and by Gottschalk and Mylle.[35] The latter gathered fluid from Henle's loops and adjacent collecting ducts in the papilla of the hamster's kidney. The fluid in both structures had a similar osmolality which was much higher than that of the blood plasma. In contrast, fluid from the cortical segments of the proximal convoluted tubules and the distal portions of the distal convoluted tubules was iso-osmotic and that of the distal convolutions hypo-osmotic to plasma.

In the kidney of the potassium-deficient animal, one is confronted with an obvious paradox. The concentrating power is severely impaired, but a number of enzymatic staining reactions in the two tubular segments of the nephron which are most responsible for urinary concentration are markedly increased. It is true enough that none of the enzymes demonstrated by histochemical methods are known to be implicated directly in the concentrating mechanism. It is obvious, however, that these enzymes participate in cellular metabolic processes, and an increase in their activity indicates increased cellular metabolism. In potassium deficiency, the hyperplasia of epithelium in the collecting ducts, particularly of intercalated cells, and the associated increase in enzymatic staining reactions in both the collecting ducts and the limbs of Henle's loops, could be interpreted as an expression of increased cellular activity aimed at overcoming the depressing effect of the deficiency on the normal function of these cells. Such an interpretation could also explain the increase in enzymatic activity in interstitial cells which, according to the theory of Hargitay and Kuhn,[32] participate in the concentrating mechanism. Indeed, Ullrich, Drenckhahn and Jarausch[36] have measured the osmotic pressure in the interstitial cells close to the tip of the papilla where the increase in histochemically

demonstrable phosphatase and esterase activity is most distinct, and found it practically identical with that of the urine.

Altogether it seems reasonable to assume that the changes found in the medulla in potassium deficiency of the rat are not a primary consequence of potassium depletion, but rather a nonspecific compensatory phenomenon aimed at overcoming the biochemical defect which occurs in renal cells deprived of this essential electrolyte.

Summary

Young albino rats of the Wistar strain were fed a complete synthetic diet deficient in potassium. Various enzymatic histochemical techniques were applied to the kidneys of such animals after they had received the experimental diet for 15 to 25 days. There occurred a striking increase in acid phosphatase and nonspecific esterase activity in the renal medulla, in the thin limbs of Henle's loops, in scattered interstitial cells, and, to a somewhat lesser degree, in collecting ducts. A distinct increase of adenosine triphosphatase activity was observed in the outer cell borders of collecting ducts. Stains for oxidative enzymes revealed less marked increase of activity in medullary structures.

There was an overall reduction in acid phosphatase, nonspecific esterase, adenosine triphosphatase, succinic dehydrogenase, DPN and TPN diaphorase activities in the ascending limbs of Henle's loops and distal convoluted tubules. The striking TPN diaphorase activity of the macula densa remained unchanged. Occasional atrophic tubules in the cortex revealed loss of staining by all techniques used.

The significance of the observations is discussed in relation to the severe functional alterations that occur in potassium deficiency, particularly the impairment of the kidney to concentrate urine properly. In the light of the "countercurrent multiplier system" theory of renal concentration, it is thought that the striking increase of enzyme activity in medullary cells implicated in this mechanism may indicate an attempt by these cells to overcome the depressing effect of potassium deficiency.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

Counterstains were not used in any of the sections illustrated.

FIG. 1. Inner portion of the medulla in a rat after 14 days on the potassium-deficient diet. Paraffin section stained by Jones's periodic acid silver-methenamine technique. A positive reaction is given by the granules in the collecting ducts as well as by the cytoplasmic substance in the thin limbs of Henle's loops and interstitial cells. Basement membranes also stain strongly. × 700.

FIG. 2. Renal cortex of a rat after 25 days on the potassium-deficient diet. Formalin-fixed frozen section stained for TPN diaphorase. There is a marked reduction of enzymatic activity in atrophic proximal convoluted tubules. The cells of the macula densa on the upper left of the glomerulus show intense staining. × 450.

FIG. 3. Inner portion of the medulla of a normal control rat. Formalin-fixed frozen section stained for acid phosphatase. Fine granular deposits of lead sulfide indicating enzymatic activity are seen mainly in collecting ducts and to a lesser degree in the thin limbs of Henle's loops and interstitial cells. × 360.

FIG. 4. Inner portion of the medulla of a rat after 15 days on the potassium-deficient diet. Formalin-fixed frozen section stained for acid phosphatase. There is intense activity, most marked in the thin limbs of Henle's loops. Compare with Figure 3. × 360.

FIG. 5. Corticomedullary region in a normal rat kidney. Formalin-fixed frozen section stained for nonspecific esterase. The ascending limbs of Henle's loops show moderate staining in contrast to the much stronger staining of the proximal convoluted tubules shown at the upper edge of the photograph. × 90.

FIG. 6. Corticomedullary region of the kidney shown in Figure 4. Formalin-fixed frozen section stained for nonspecific esterase. Note the distinct diminution of enzymatic staining in the ascending limbs of Henle's loops as compared to the normal control section in Figure 5. × 90.

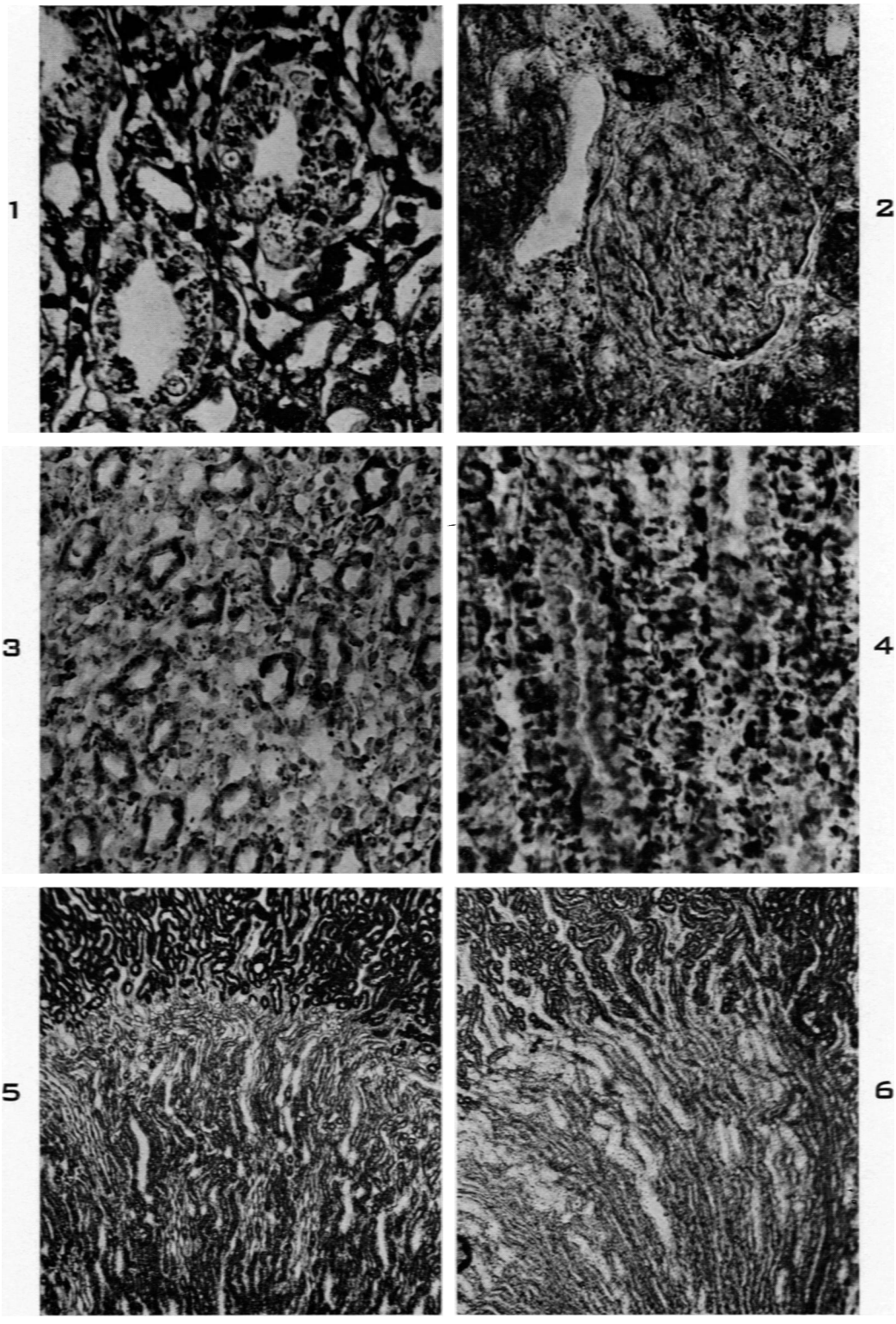
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FIG. 7. Inner renal medulla of a normal rat. Formalin-fixed frozen section stained for nonspecific esterase activity. The thin limbs of Henle's loops and occasional interstitial cells show more staining than the collecting ducts. × 360.

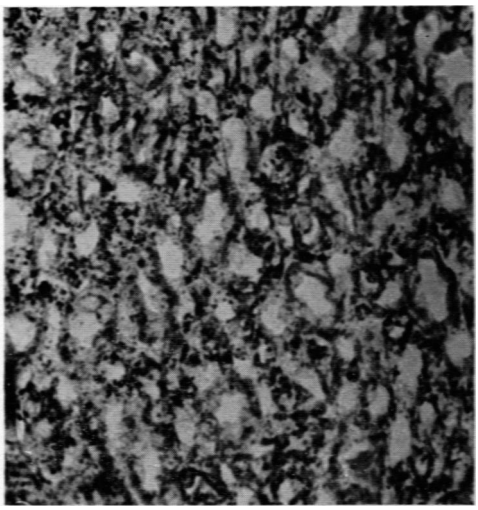
FIG. 8. An adjacent area in the kidney shown in Figures 4 and 6. Formalin-fixed frozen section stained for nonspecific esterase. There is striking staining of the thin limbs of Henle's loops and interstitial cells, and a somewhat less intense reaction in the collecting ducts. Compare with Figure 7. × 360.

FIG. 9. Inner medulla of a normal rat kidney. Formalin-fixed frozen section stained for adenosine triphosphatase activity after 5 minutes of incubation. Only the inner cell borders of collecting ducts are stained. Capillaries show almost no activity after this short incubation period. × 450.

FIG. 10. Inner medulla of the same kidney shown in Figures 4, 6 and 8. Formalin-fixed frozen section stained for adenosine triphosphatase activity after 5 minutes' incubation. There is markedly increased staining in the outer cell borders of collecting ducts. Compare with Figure 9. × 450.

FIG. 11. Outer portion of the renal medulla in a normal rat. Formalin-fixed frozen section stained for DPNH diaphorase activity. Staining in collecting ducts is most striking in some intercalated cells. The thin limbs of Henle's loops show weak activity. × 360.

FIG. 12. Outer portion of the medulla of the same kidney shown in Figures 4, 6, 8 and 10. Formalin-fixed frozen section stained for DPNH activity. There is a general increase in staining of cells composing the collecting ducts. There is also some increase in the staining intensity of the thin limbs of Henle's loops. Compare with Figure 11. × 300.



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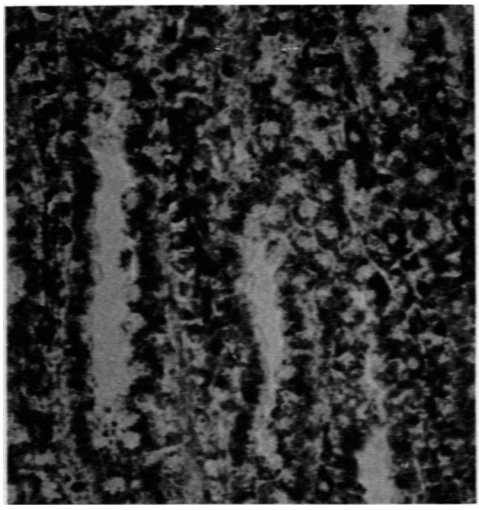

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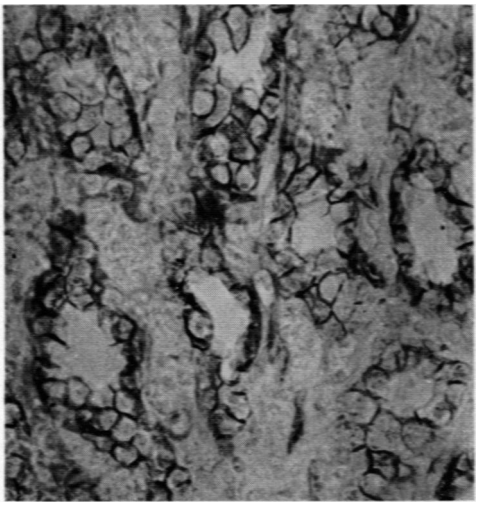

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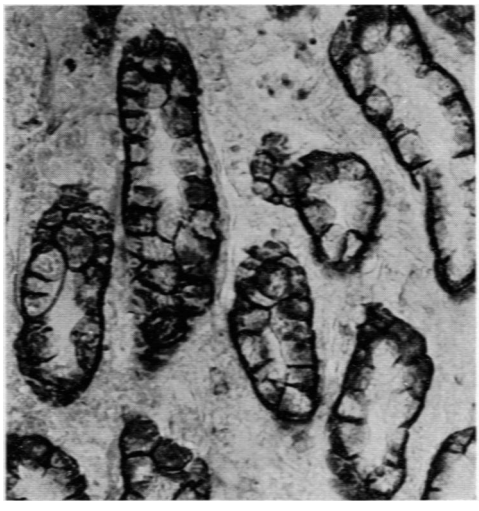

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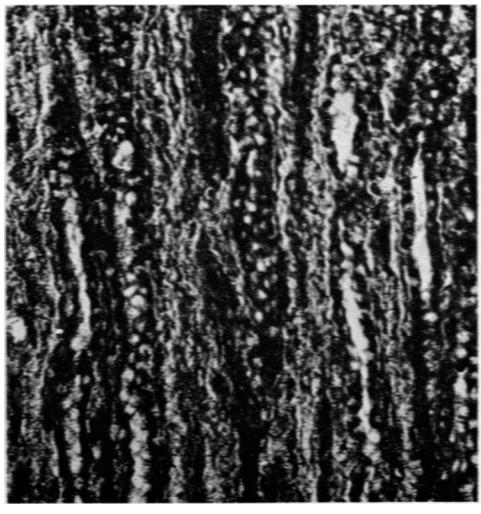

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