INFLUENCE OF EXPERIMENTAL KIDNEY DAMAGE ON HISTOCHEMICALLY DEMONSTRABLE LIPASE ACTIVITY IN THE RAT. COMPARISON WITH ALKALINE PHOSPHATASE ACTIVITY

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PLATES 1 and 2

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The introduction of methods capable of demonstrating both alkaline (1, 2) and acid phosphatase (3) in tissue sections has led to interesting results concerning the distribution of these enzymes. The kidney was found to contain a large amount of alkaline phosphatase, predominantly in the cortical portion (2, 4–6). Under various experimental conditions changes in the amount and distribution of alkaline (7–12) as well as of acid phosphatase (11) have been described.

In the present investigation, lipase activity was studied in the normal kidney of different animals and under varying experimental conditions in the rat, using Gomori's recently published technique (13). Sections were also stained for alkaline phosphatase activity in order to compare the influence of experimental damage on two different, microtechnically demonstrable enzymes.

Material and Methods

Kidney sections from normal dogs and rats of the Wistar strain, rabbits, mice, hamsters and guinea pigs were used. Kidney damage in rats was caused by subcutaneous injections of mercury bichloride and uranium nitrate, dietary choline deficiency, and ligation of the ureter.

Lipase activity was demonstrated by Gomori's method with some modifications. This technique is based on the deposition of insoluble calcium palmitate or stearate if the sections are incubated with a water-soluble ester of palmitic or stearic acid in the presence of calcium chloride. The calcium palmitate formed at the site of lipase activity is visualized by transforming it into its lead salt and demonstrating the lead by changing it into dark brown sulfide. The tissue was fixed in ice cold acetone for 24 hours, with one or two changes of the acetone, placed for 24 hours in absolute alcohol, and left for 1 to 2 hours in xylol and for another 2 hours in paraffin at 56°C. By using this procedure, serial sections can be stained for lipase as well as alkaline and acid phosphatase activity. Instead of a maleate buffer, the acetate barbital-sodium buffer of Michaelis (14) was used in the incubation mixture. 5 parts of a solution containing 9.714 gm. sodium acetate, $3H_2O$ and 14.714 gm. barbital-sodium (sodium diethylbarbiturate) in 500 cc. CO_2-free water, 5 parts of N/10 hydrochloric acid, and 10 parts distilled water, will give a suitable buffer solution of pH ±7.4. Tween 40 (sorbitan monopalmitate, Atlas Powder Co., Wilmington, Delaware) served as substrate. The slides were

incubated from 40 to 48 hours at 37°C. Each slide also contained liver tissue. The latter shows a constant amount of lipase activity (13) and served as a good control for the quality of the preparation.

Phosphatase activity was demonstrated with Gomori's technique as modified by Kabat and Furth (5) with minor alterations (11). No counter stain was used. In order to visualize preformed calcium, which in most cases cannot be distinguished in sections prepared for the demonstration of phosphatase activity, the following technique was used: Slides were covered in the usual way with a 0.25 per cent solution of celloidin in equal amounts of absolute alcohol and ether and placed for 15 minutes in a 2 per cent solution of cobalt nitrate. After washing in tap water, they were immersed for 1 minute in a dilute solution of ammonium sulfide. This stained most of the preformed calcium deposits black. After washing in tap water, the slides were brought into the incubation mixture used for demonstration of alkaline phosphatase activity. A few drops of dilute ammonium sulfide were added to this mixture. The slides were then incubated for 10 to 14 hours at 37°C. They were, after washing, placed for 15 minutes in a 2 per cent cobalt nitrate solution. The formed cobalt phosphate could be demonstrated by covering the slides with a saturated alcoholic solution of dithiooxamide (rubeanic acid—Eastman Kodak Co.) to which a few drops of ammonia water were added. Cobalt forms complex salts of rubeanic acid, which are insoluble in water and dilute mineral acids, and appear as intensely colored yellow-brownish precipitates (15). The preformed calcium which has been stained black contrasts well with the yellowish brown cobalt complex salt of rubeanic acid signifying phosphatase activity. Gomori's method (16) for simultaneous staining of preformed calcium and phosphatase activity could not be used since acridine red Gruebler, essential for this technique, could not be obtained.

Lipase Activity in the Kidneys of Normal Animals

All the species examined showed evidence of lipase activity. It was moderate in the kidneys of guinea pigs, rabbits, hamsters, and mice, irregularly distributed in the kidneys of dogs, and present in large amounts in the kidneys of rats. It was found only in the cortical portion of the kidney and was restricted to the proximal convoluted tubules (Fig. 1). It was present only in the cytoplasm and not in the nuclei. In contrast to the appearance of alkaline phosphatase, it was not concentrated at the brush border but diffusely distributed throughout the cytoplasm of the cells (Fig. 2). The deposits of lead sulfide, signifying lipase activity, were granular but not infrequently formed fine needles. The deposits, in general, seemed to be more distinctly granular when barbital was used instead of the maleate buffer. Occasionally, the epithelium lining of the kidney pelvis showed some activity. The distribution of lipase activity in the cortex of the proximal convoluted tubules corresponded closely to that found for alkaline phosphatase. The latter is also found predominantly in the proximal convoluted tubules. Alkaline phosphatase activity, however, is found not only in the cytoplasm but also, to some extent, in the nuclei of the convoluted tubules. Phosphatase activity also appears in the cytoplasm of the ascending limbs of Henle in the dog (4), mouse, and rabbit (9). In the rat a very extensive reaction is seen in most of the tubules situated in the cortex. Menten, Junge, and Green (17) obtained a similar picture by

using a histochemical azo dye test. They concluded that in the rat kidney the cytoplasmic phosphatase is found not only in the proximal convoluted tubules but also in the loops of Henle and in the distal convoluted tubules. However, in our preparations of normal rat kidneys, even after long incubation no cytoplasmic activity was found in the tubules situated in the medulla where the loops of Henle can easily be identified. There was, however, a varying amount of phosphatase activity in the nuclei of most of the tubules as well as of the capillaries in the medulla. The glomeruli frequently exhibited pronounced activity in the rat (11), mouse (17), and dog.

Effect of Poisoning with Mercury Bichloride

Eleven rats varying from 150 to 250 gm. were given one subcutaneous injection of 1 to 2 mg. mercury bichloride in a 4 per cent solution. Five animals were sacrificed or died after 3 to 5 days. These animals showed acute changes while those sacrificed later showed changes which obviously represented a subacute or healing stage. In the acute phase, extensive necrosis of the convoluted tubules was seen. In many of the necrotic tubules, no cellular details could be recognized, while in others the degenerative changes were less severe. Frequently necrotic cells separated from the basal membranes and were expelled into the tubular lumen. In many of the excretory tubules, granular material was seen. In two kidneys frequent mitotic figures were noticed. The glomeruli were essentially normal. In four out of five kidneys calcification was seen which was very extensive in three cases.

Suzuki (18), on the basis of vital staining with carmine, localized the damage in the last third of the convoluted tubules in the rabbit. Simmonds and Hepler (19), using the fat content of the terminal portion of the convoluted tubules as a means of identification, confirmed these findings. Edwards (20) by teasing the macerated kidney of different animals under the microscope, found, however, that the damage was mainly localized in the second and third quarters of the convoluted tubules and that the most distal portion remained frequently undamaged.

The kidneys of three animals sacrificed after 8 to 15 days showed only focal changes. Some of the cells in the convoluted tubules had an irregular appearance with prominent hyperchromatic nuclei. A few necrotic tubules were still present. In the deeper portion of the cortex near the medulla, occasional tubules were dilated and had a somewhat atrophic epithelium. In the kidneys of the three remaining animals, killed after 13 to 18 days, the changes were much more distinct. Focal dilation of the convoluted tubules was seen in many places mostly near to the medulla. In these tubules, the epithelium was flattened and appeared atrophic. Occasionally, a few more normal appearing cells were interspersed in a row of markedly atrophic cells. In one case, interstitial areas of fibroblastic proliferation were observed. Occasionally, tubules still contained frankly necrotic cells. Small deposits of calcium were

seen only in one case. Few of the collecting tubules contained casts. The glomeruli were essentially normal.

Lipase Activity.—In the kidneys of animals showing acute changes, lipase activity was only moderately decreased. Desquamated necrotic cells still contained the enzyme, but took the stain diffusely. There was, however, moderate to marked depletion in some of the damaged convoluted tubules. Occasional lipase activity occurred in the tubular casts.

In the subacute cases depletion of lipase activity was obvious in the atrophic and dilated tubules wherever changes of these sorts were found in hematoxylin-eosin preparations (Figs. 3 and 4). Occasionally, the dilated tubules contained flat cells devoid of lipase activity intermingled with more normal appearing cells showing distinct activity.

Phosphatase Activity.—The changes in alkaline phosphatase activity were quite similar to those in lipase activity. There was moderate depletion of the kidneys showing acute changes. As previously described by Hepler and his coworkers (7, 21, 22), the necrotic tubules were frequently diffusely stained and desquamated cells were still active though often less intensely so than undamaged tubules. The chromosomes of mitotic figures showed activity, as previously described for the dividing cells in other tissues (22–24). Amongst many casts, occasional ones were positive.

By using the technique which permits a simultaneous demonstration of preformed calcium and phosphatase, it could be seen that necrotic, phosphatase-containing cells were the seat of beginning calcification. As the calcification progressed more of the phosphatase disappeared. In segments of tubules that were extensively calcified, stainable phosphatase could no longer be discerned.

In the subacute cases, marked depletion was seen in the atrophic and dilated tubules while the tubules which appeared normal in preparations stained with hematoxylin and eosin showed a normal amount of enzymatic activity.

Effect of Poisoning with Uranium Nitrate

The anatomical changes observed in the kidneys of various animals receiving uranium nitrate including the rat, have been described by Suzuki (18), Oliver (25), and Breedis, Flory, and Furth (8) in connection with the changes in alkaline phosphatase activity.

Ten albino rats varying from 150 to 325 gm. were used. They were given a single dose of uranium nitrate varying between 1.5 and 3 mg. subcutaneously. Four animals that died or were killed 2 to 3 days after the injection showed extensive necrosis of the tubular epithelium. The damage was located primarily in the distal portion of the proximal convoluted tubules, although it extended into Henle's loops and even into the distal convoluted tubules. Mitotic figures were quite frequent in the epithelium of the proximal convoluted tubules. Many of the distal convoluted tubules and excretory ducts contained granular material derived from the destroyed epithelial cells. Deposition of

calcium mostly occurring in the distal portions of the proximal convoluted tubules was observed in one animal within 2 days after the injection.

Six of the animals were killed after 7 to 25 days. Their kidneys showed various degrees of damage. Necrotic cells were still noticeable. Some tubules showed mitotic figures and in addition, in some of the tubules, multinucleated cells were quite prominent. These cells, according to Oliver (25) are concerned with the regeneration of the necrotic epithelium. Hyaline casts lying in the distal convoluted tubules and excretory ducts were found in several kidneys. Many of the proximal convoluted tubules were markedly dilated. The epithelium was flattened. In other areas, the epithelium of the proximal convoluted tubules appeared atrophic, with a small or even collapsed lumen. Inflammatory changes and proliferation of the connective tissue accompanied the atrophy of these tubules as pointed out by Suzuki and Oliver. The glomeruli showed only little changes.

Lipase Activity.—In the acute cases, there was moderate depletion of lipase in the necrotic tubules. In some the necrotic cells took the stains diffusely. Occasional necrotic cells showed very little enzyme while the undamaged cells contained a normal amount.

In the kidneys of the six rats which were sacrificed after 7 to 25 days, the depletion was quite distinct wherever tubules were dilated and showed atrophic epithelium. Frequently these epithelial cells were free from any activity. The activity in normal appearing cells varied; mostly they showed a normal amount, but occasionally, it was depressed. Many of the casts showed conspicuous lipase activity. The intense staining reaction in these casts was not due to calcium deposits, since calcium stains in control sections were negative.

Phosphatase Activity.—The changes in phosphatase activity resembled closely those described by Breedis, Flory, and Furth (8). In the acute stage, there was diffuse staining of necrotic tubules and moderate depletion in some of them. Casts were frequently positive. In the more chronic cases there was marked depletion in the atrophic and regenerating epithelium. Mitotic figures showed phosphatase activity. Most of the glomeruli showed strong phosphatase activity. As in mercury poisoning, calcium was deposited in necrotic cells which frequently still contained abundant phosphatase.

Effect of Choline Deficiency

The morphological changes occurring in choline deficiency have been described previously (11, 26-28). In the acute stage they consisted mainly of necrosis of the cortical tubules and degenerative changes in tubules not completely necrotic, with accumulation of extravasated blood between the connective tissue of the capsule and the outer surface of the cortex. In addition, intense hyperemia of the capillaries of the cortical portion of the kidney, with blood extravasation, was a very prominent feature in many areas. Acidophilic material in the form of casts was found in the lumen of Henle's loops, distal convoluted tubules, and most extensively in the collecting tubules.

The kidneys of seven animals with acute changes and of six with subacute, were available for study. The diets used for the production of the typical kidney lesions have been reported previously (11). The highest incidence was observed in male weanling rats, given an experimental diet containing 8 to 10 per cent of fibrin and 0.3 per cent cystine as protein source.

Animals which were sacrificed after 50 days on the experimental diet presented changes which were designated as subacute. In this stage, the extravasated blood and the congestion had disappeared. Many of the cortical tubules were atrophic and their cytoplasm was strongly reduced. There was marked condensation and proliferation of the connective tissue stroma around the atrophic tubules and also fibrosis of the renal capsule. Only a few casts had remained. Occasionally the collecting tubules were dilated. In some cases, only small foci of atrophic tubules were observed, while in others most of the cortical tubules were atrophic and only small clusters of normal appearing convoluted tubules remained.

Lipase Activity.—In the acute stage, lipase activity showed marked reduction which was most evident in the outer portion of the kidney cortex, where necrosis of the tubules was most prominent (Fig. 5). Many of the necrotic tubules showed no activity while others revealed some. Occasionally, necrotic cells showed diffuse brownish staining. Reduction of lipase activity was found not only in completely necrotic tubules but also in tubules that were not so severely damaged as evidenced by their still intact nuclei. The degree of reduction in lipase activity was not uniform and varied in different fields. Most of the casts in collecting tubules were devoid of activity although an occasional one showed faint staining.

In the subacute stage the lipase stain revealed marked depletion in the atrophic tubules (Fig. 6). Most of them were completely devoid of lipase while occasionally some of them showed faint traces of the enzyme. Convoluted tubules showing a normal appearance in hematoxylin and eosin stains also revealed a normal amount of lipase activity.

Phosphatase Activity.—The changes in phosphatase activity have been described previously (11). In the acute stage, marked reduction was found in the necrotic cortical tubules. The degree of reduction varied in different cases and also in different sections of the same kidney. Some of the casts filling the tubular system showed staining.

In the subacute stage, the depletion of phosphatase in the atrophic tubules was even more distinct. Only the convoluted tubules that appeared normal in hematoxylin and eosin preparations showed a normal amount of phosphatase activity. Most of the glomeruli showed very marked activity.

The Changes in Hydronephrosis

In twelve adult white rats, the left ureter was ligated. The animals were killed 10 to 12 days after the operation. Among them, five showed mild to

moderate and seven marked hydronephrosis. In stained sections, there was dilation of the tubular system extending to the outer portion of the cortex in those kidneys in which marked changes were present. The capsular spaces of the glomeruli were frequently distended. The extent of the dilation was not uniform. It was marked in some areas and less in others. The epithelium in dilated tubules was flattened.

Some kidneys showed collapse of some of the proximal convoluted tubules. This phenomenon has been described in detail by Suzuki (18) who distinguished three periods in experimental hydronephrosis of the rat and rabbit. The first period, after ligation of the ureter, is characterized by the general dilatation of the tubular system and the capsular glomerular spaces. This is followed by a second period, characterized by the collapse of the proximal convoluted tubules and the loops of Henle. In the third period, starting about 4 weeks after the ureteral ligation, collapse of the distal convoluted tubules and collecting tubules occurs.

In kidney sections stained with hematoxylin and eosin, many of the cells in dilated as well as collapsed convoluted tubules have lost the eosinophilia of their cytoplasm. In kidneys with only mild changes, the dilatation of the tubules was much less marked and much less extensive. In two kidneys there was subacute inflammation and marked fibroblastic proliferation beneath the epithelium of the kidney pelvis. In all cases, the right kidney was entirely normal.

Lipase Activity.—The kidneys which showed marked hydronephrotic changes revealed considerable depletion of lipase activity (Figs. 7 and 8). This depletion was seen in cells of dilated as well as of collapsed tubules. Occasionally, the epithelium in dilated tubules still showed some lipase activity. Even in kidneys showing conspicuous hydronephrotic changes, the depletion was not evenly distributed and in some fields there were still a number of tubules showing normal amounts of lipase activity. In the kidneys with only mild hydronephrotic changes the loss of enzymatic activity was much less pronounced. It was focal in distribution, mostly in tubules showing some dilatation. The amount of lipase activity in the undamaged right kidney was normal in each case.

Phosphatase Activity.—Slides from each case were incubated for 2 and 12 to 14 hours. In full agreement with Wilmer's findings (9, 10) there was marked depletion in many dilated as well as collapsed proximal convoluted tubules. In the kidneys with mild hydronephrosis, the depletion was much less marked and only focal. The depletion of phosphatase activity was very pronounced in sections incubated for 2 hours. This incubation time was also used by Wilmer. In slides incubated for a longer time the depletion was still quite marked although in tubules in which the enzyme had disappeared from the cytoplasm the nuclei still contained phosphatase. After 2 hours of incubation, the glomeruli in most cases, both in the hydronephrotic as well as in the normal

kidney, showed only faint activity. After longer incubation, however, the glomeruli showed considerable activity in most of the slides. It seems that in the hydronephrotic kidneys there was a somewhat stronger reaction in the glomeruli and the smaller arterioles. This was similar to the previously described findings in choline-deficient kidneys in which the impression was gained that the glomeruli stained more intensely in phosphatase-depleted than in normal kidneys. This difference may, however, be only apparent and due to the visual contrast between the strong staining reaction in the glomeruli and the weak reaction in the depleted tubules. The young fibroblasts found in two cases in the kidney pelvis revealed an intense phosphatase activity. The intense phosphatase activity of young fibroblasts in the rat has been described by Fell and Danielli (29).

COMMENT

In agreement with Gomori (13) the lipase activity in tissue sections was found to be most pronounced in the rat kidney and less in that of the dog and guinea pig, as well as in that of the hamster, rabbit, and mouse. Lipase activity was found only in the proximal convoluted tubules of the cortex in all the animal species examined and very occasionally in the epithelium of the kidney pelvis.

A somewhat different distribution has been found by Weil and Jennings (30). These investigators used the technique of Linderstrøm-Lang (31) and Glick's (32) micromethod for the determination of lipase (kidney esterase) activity in the rabbit kidney. Linderstrøm-Lang's technique consists essentially in estimating the enzymatic activity microchemically and correlating the results statistically with the cells present in alternate microscopic sections. Weil and Jennings found the maximum lipase activity in the cortex but also some activity in the medulla. The cells of the proximal and distal convoluted tubules were about twice as active as the cells of Henle's loops and about 4 times as active as cells composing the collecting tubules.

Lipase activity has been demonstrated by chemical methods not only in liver and pancreas but also in various other organs including the kidneys of various animals and man (33–42). Different substrates have been used for estimating the enzymatic activity. The degree of this activity depends, among other factors, to a large extent upon the substrate used (37). Crandall and Cherry (39), for instance, using olive oil as substrate, found only traces of activity in the dog kidney, but fairly strong activity with ethylbutyrate. Rona and Lasnitzki (36), using tributyrin, found stronger activity in the guinea pig than in the rat kidney. While, in agreement with Gomori, no lipase activity in tissue sections could be demonstrated in the rat spleen with sorbitan monopalmitate, such activity has been demonstrated by chemical estimation with various other substrates (37).

Lipase activity is still present in many of the necrotic cells of kidneys severely

damaged by the application of uranium nitrate and mercuric chloride. As the cells are shed off into the lumen, the tubules become depleted of the enzyme. The regenerating and atrophic epithelium in kidneys of animals surviving the acute intoxication, shows marked depletion in lipase activity. Similar depletion is seen in the acute and subacute phase of choline deficiency as well as in the epithelium of the dilated and collapsed convoluted tubules of the hydronephrotic kidneys. The changes in phosphatase activity are of an essentially identical nature. Both enzymatic systems, therefore, are changed by the experimental damage in a similar manner.

The decrease in enzymatic activity as soon as 10 days after ligation of the ureter, is especially remarkable in view of Suzuki's (18) findings after injection of carmine or trypan blue. These dyes are excreted by the glomeruli and are partially absorbed by the epithelium of the proximal convoluted tubules where they are concentrated in particulate form (43). Suzuki found, even after many weeks of ligation of the ureter, concentrations of dye in the cells of the proximal convoluted tubules, although these cells were changed morphologically. On the other hand, MacNider (44 *a,b*) has shown that after damage with uranium nitrate, the regenerated epithelial tissue of the kidney as well as that of the liver is not only of a changed morphological type but is also resistant to the same, as well as to different toxic substances.

Calcification is frequently seen in necrotic cells still containing phosphatase after poisoning with mercuric chloride and uranium nitrate. In contrast, Hepler and Simmonds (22) maintained that calcium is deposited only in tubules in which the epithelium has been so severely damaged that the phosphatase has been rendered inactive, and concluded that phosphatase does not play any part in the process of calcification. The findings reported here are in agreement with Gomori's experience (16, 45) that calcification of recently necrosed tissue is dependent upon the presence of alkaline phosphatase.

While the abundance of alkaline phosphatase has been explained by the importance of this enzyme for the reabsorption of sugar from the glomerular filtrate (10, 46), apparently no special function has thus far been inferred from the presence of lipase in the kidney. However, certain clinical and experimental facts point to an essential rôle of the kidney in fat metabolism. Cholesterol, lipid phosphorus, as well as neutral fat, are frequently elevated in the serum of patients with renal disease (47). Increase in serum lipids has been reported in rats after partial nephrectomy (48, 49), in acute nephritis induced by anti-kidney serum (50), and after poisoning with mercury bichloride (51, 52). A rise in serum lipids has been observed in dogs, after unilateral nephrectomy (51–53), bilateral nephrectomy (51–54), bilateral ureteral ligation (54), and after poisoning with potassium dichromate, uranium nitrate, and mercury bichloride (51–53). Rise in serum lipids after nephrectomy has also been noticed in cats (55) and monkeys (54). Furthermore a considerable turn-

over of phospholipids can take place in the intact kidney even of the hepatectomized dog (56). It seems conceivable that enzymes concerned with fat metabolism in the kidney may be significant for the regulation of lipid metabolism of the whole organism.

SUMMARY

Lipase activity was found in the cytoplasm of the proximal convoluted tubules in tissue sections of rat, rabbit, dog, mouse, hamster, and guinea pig, stained according to Gomori's method. Uranium and mercury poisoning do not inactivate the enzyme in necrotic cells of the proximal convoluted tubules. Its activity diminished in the atrophic and regenerating cells of the kidneys of rats, surviving the acute phase of the intoxication. In the acute stage of choline deficiency marked reduction in enzymatic activity was seen in the necrotic tubules, and in the atrophied and regenerating tubules in the subacute stage. Lipase activity was markedly diminished in hydronephrotic kidneys 10 to 12 days after ligation of the ureter. In sections stained for alkaline phosphatase activity nearly identical alterations were found. Experimental damage influences both histochemically demonstrable enzymes in a similar manner.

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EXPLANATION OF PLATES

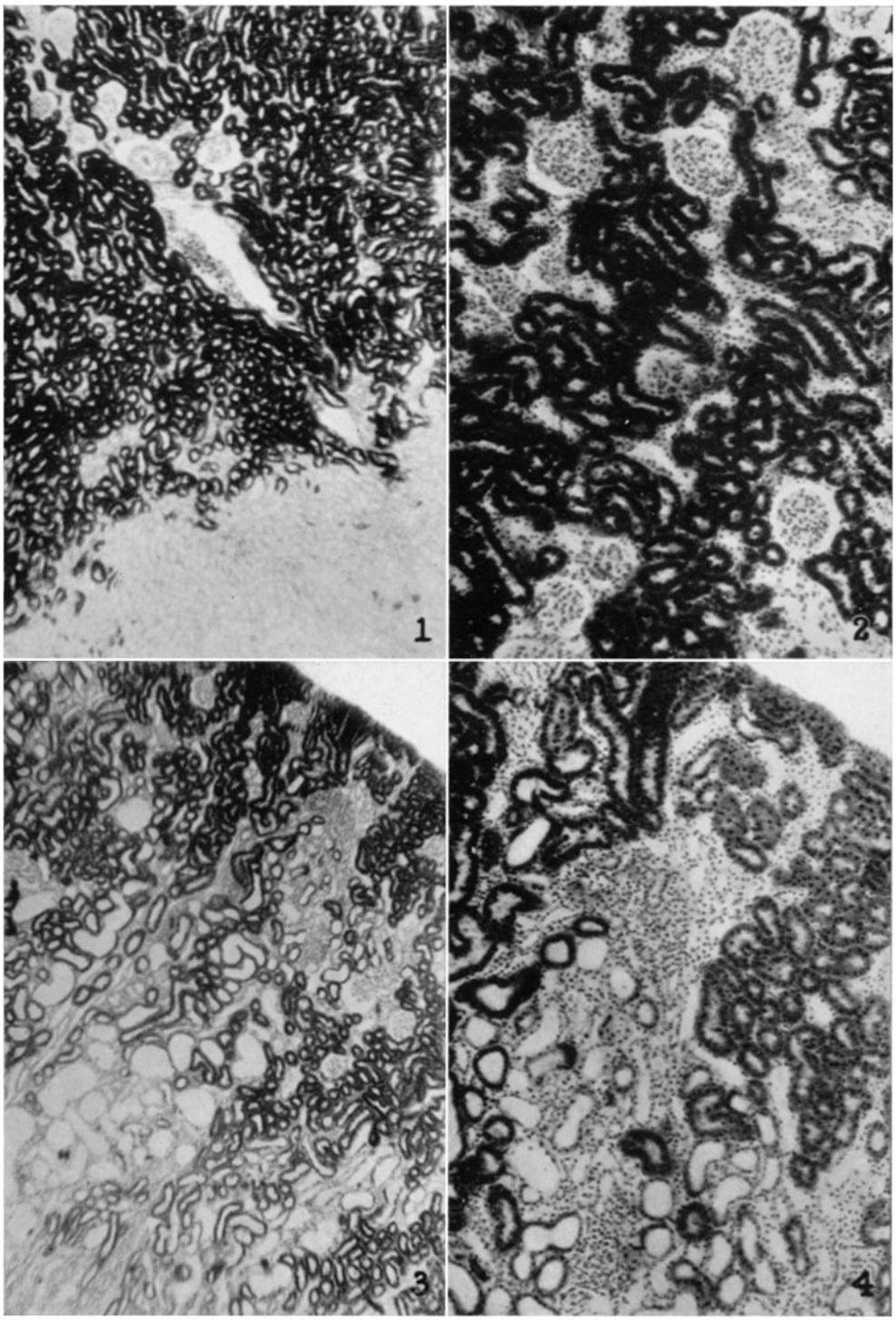
PLATE 1

FIG. 1. A histotechnical preparation for the demonstration of lipase in the normal rat kidney. The sites of enzymatic activity stain dark. There is intense activity in the proximal convoluted tubules. × 35.

FIG. 2. A similar preparation of the normal rat kidney at a higher magnification. × 100.

FIG. 3. Preparation of the kidney of a rat killed 18 days after injection with mercury bichloride. There is depletion of lipase in the epithelium of the dilated and atrophic tubules. × 35.

FIG. 4. A similar preparation of the kidney of a rat killed 18 days after injection with mercury bichloride. × 100.



(Wachstein: Influence of kidney damage on lipase activity)

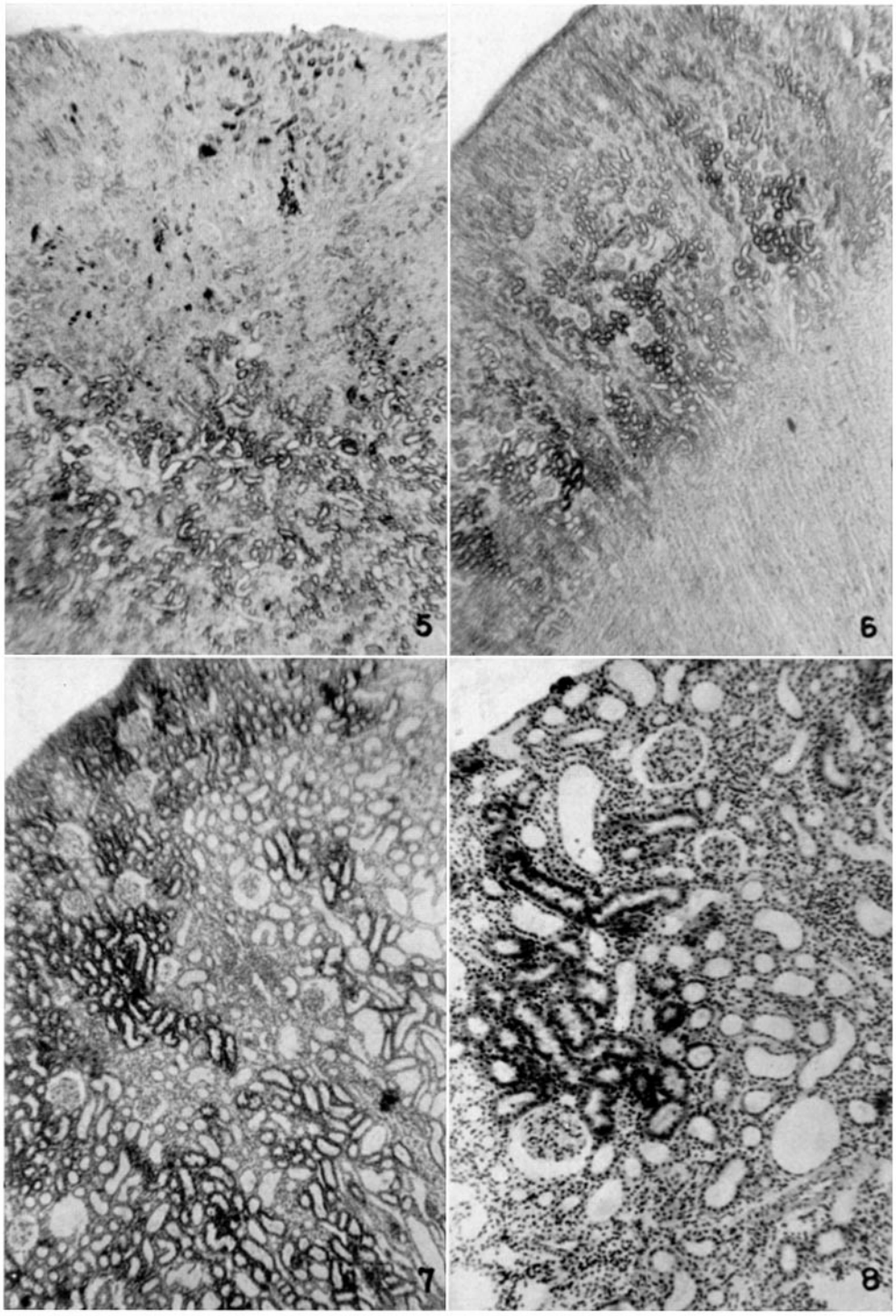
PLATE 2

FIG. 5. The kidney of a young rat with acute choline deficiency. There is extensive depletion of the enzymatic activity in the necrotic tubules of the outer parts of the cortex. × 35.

FIG. 6. The kidney of a young rat with subacute choline deficiency. The lessened enzymatic activity in the atrophic tubules of the cortex is conspicuous. × 35.

FIG. 7. Hydronephrotic left kidney from the animal furnishing Figs. 1 and 2. Many of the dilated and collapsed tubules show loss of enzymatic activity. × 35.

FIG. 8. Higher magnification of a part of the same organ. × 100.



(Wachstein: Influence of kidney damage on lipase activity)