THE PERIODIC ACID ROUTINE APPLIED TO THE KIDNEY *

J. F. A. McMANUS, M.D.

(From the Department of Pathology, Medical College of Alabama, Birmingham 5, Ala.)

This communication presents results of a study of the kidney with the aid of an original staining method which has been described previously [1] for the demonstration of mucin (Fig. 1).

In brief, microscopic sections are treated with an aqueous solution of periodic acid and then colored with Schiff's reagent for aldehydes. Schiff's reagent or leuko-basic fuchsin is a straw-colored solution which is produced by the action of sulfurous acid on an aqueous solution of basic fuchsin. The reagent takes on a red or violet color when an aldehyde is added. In tissues it forms a red or violet insoluble compound at the sites where aldehyde is present. This property is utilized in various histochemical technics. In the classical Feulgen's test, the aldehyde which is formed from desoxyribose nucleic acid by hydrolysis with weak hydrochloric acid is colored with Schiff's reagent. In similar fashion, Bauer's test for the demonstration of glycogen makes use of Schiff's reagent, aldehyde being produced by the action of chromic acid.

Since periodic acid has been known to produce an aldehyde when acting upon a carbohydrate [2] and when acting upon serine, threonine, or hydroxylysine,[3] it seemed a natural sequence to test it on tissue sections. It has been reported already that the following materials are colored by Schiff's reagent after the action of periodic acid: mucin in the intestinal and respiratory tracts, the colloid of the pituitary stalk and thyroid, mucous salivary glands, certain cells of the anterior hypophysis, and the basement membrane of the renal tubules and glomeruli.[1] The present communication describes the technic in greater detail, including the preparation of the reagents, and reports further results in the application of the periodic acid Schiff's reagent routine to the kidney.

MATERIALS AND METHODS

The sections of kidney which were studied came from young adult males, killed in the European campaign, and from the autopsies of the Jefferson-Hillman Hospital. The material had been handled in a variety of ways, although most of it had been fixed in formol-saline or in Zenker's-formol solution. A cobalt-calcium-formol fixative [4] was found useful, particularly when postchromed by leaving for 24 to 48

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hours in 3 per cent dichromate and washing in running tap water for 12 to 24 hours before dehydration. This is the method of choice when the granular cells of the renal arteriole are to be studied. The tissues were dehydrated in alcohol and embedded in paraffin after either toluene or xylene. Some tissues which had been in formaldehyde solution for a long time gave satisfactory appearances after postchroming. It may be noted here that postchroming appeared to improve the histologic and cytologic detail after nearly all fixatives.

The preparation of the necessary reagents is not a complicated matter.

The routine used to prepare Schiff's reagent is as follows:

1. Weigh out 1 gm. of basic fuchsin
2. Weigh out 1 gm. of anhydrous sodium bisulfite
3. Boil 200 cc. of distilled water
4. Add fuchsin and stir
5. Cool to 50° C.
6. Filter
7. Add 20 cc. of normal HCl (98.3 cc. of HCl (sp. gr., 1.16) made up to 1 liter)
8. Cool to 25° C.
9. Add sodium bisulfite

Keep in the dark. The fluid takes 1 or 2 days to become orange, or straw-colored, when it is ready for use.

Sulfurous acid rinse:

6 cc. of 10 per cent sodium metabisulfite
5 cc. of normal HCl
100 cc. of distilled water

These directions are those of Dr. John R. Baker (personal communication) and derived from Lison.[5]

The routine used for coloring the basement membrane is as follows: Paraffin sections cut at 3 to 6 μ are brought through xylene and graded alcohol to water. When the tissue has been hardened in a fixative containing mercury, the customary treatment with 0.5 per cent of I_2 in 70 per cent alcohol and 5 per cent sodium thiosulfate is carried out and the sections are washed for several minutes in running tap water. They are placed in a solution of 0.5 per cent periodic acid for 2 to 5 minutes at room temperature and then washed in distilled water briefly, although long washing in tap and distilled water does not appear to influence their appearance. They are placed for 15 minutes in Schiff's reagent at room temperature. After removal, the sections are treated for 2 minutes in each of three changes of sulfurous acid as in the classical Feulgen's test. Departure from the original technic consists of washing the sections, after the last sulfurous acid rinse, in a staining dish through

which a stream of tap water is running, for 5 to 10 minutes. This has been found to enhance considerably the brilliance of the coloration. The sections may then be brought through graded alcohols to xylene and mounted in balsam or, if a counterstain is desired, they may be placed in Harris' hematoxylin for 30 seconds and then washed thoroughly in a stream of tap water for 5 to 10 minutes before being dehydrated and mounted. If a trichrome effect is desired, the sections, after being washed following the hematoxylin nuclear stain, are placed in 0.1 per cent light green for 15 seconds or less and then washed in water before being dehydrated.

Results

The basement membranes of the glomeruli and of the tubules are colored bright red or purple, as are the cell outlines of the smooth muscle cells of the arterioles and capillary walls. Ordinary connective tissue stains little if at all and there is no nuclear coloring without a counterstain. Elastica is not stained nor are the erythrocytes or the cytoplasm of cells apart from the proximal tubule. The "brush" border of the cells of the first convoluted segment is colored constantly and the cytoplasm of these cells colors to a degree which varies from one nephron to another and from case to case. There is some coloring of the cytoplasm of polymorphonuclear leukocytes and of isolated droplets or granules in various parenchymal cells, frequently in the position of the Golgi element.

Counterstaining colors the chromatin blue to black. Light green stains the cytoplasm of the cells and the erythrocytes, and also the basement membrane to some extent, particularly in the glomerulus. The granules of the afibrillar arteriolar cells in the "crush" kidney are colored a bright red, somewhat lighter in shade than that of the basement membranes. When it is desired to study the arteriolar granules, it is necessary to obtain tissue within 1 hour after death, and the cobalt-calcium-formol fixative seems the best, with postchroming essential. Alcohol-toluene or alcohol-xylene methods of dehydration and clearing must be used.

The normal glomerulus shows a single basement membrane by this technic (Fig. 2). It is believed that glomerular structure can be interpreted more thoroughly with the present technic than in sections prepared by the trichrome stain or by modifications of Mallory's aniline blue-orange G mixture.

In the "crush" kidney there is a strong prominence of the granular cells of the renal arterioles, as Goormaghtigh [6] has described. The

fibrin of venous thrombi (Fig. 3) stains strongly by this method. The hyalin-appearing casts in the tubules stain with varying degrees of brilliance. Glycogen and amyloid are colored brightly.

The alteration of the basement membrane of the glomerulus in arteriosclerosis, which was described by McGregor,[7] is made prominent (Fig. 4).

The hyalin of arteriolosclerosis (Fig. 5) shows strong coloring. Hyaline droplets (Fig. 6) in epithelial cells of the proximal tubules color brilliantly. Where tubules have become atrophied in scars, there is persistence of staining of the basement membranes of the tubules except in the oldest scars. It was concluded from the study of many cases that thickening of the basement membrane of the corresponding tubule is an early change which occurs in any progressive glomerular injury.

Discussion

Three features appear to be worthy of discussion at the present time.

The Utility of the Method as a Histologic Aid in the Study of the Kidney

The basement membranes of glomeruli and tubules are so well shown by this technic that it is proposed as the method of choice. Fresh tissues, from autopsies performed within 1 hour after death, are best. When autopsy is delayed, the postchroming in 3 per cent potassium dichromate for 24 hours followed by washing of the tissue in running water for the equivalent time produces satisfactory results.

A particular advantage of the method is that no "differentiation" of the section is necessary. This is in contrast to the aniline blue-orange G and Masson's trichrome technics in which the end result is a personal technical production. With them, differences in results may be produced by minor variations in the length of differentiation or of staining and by minor variations in the quality of the dye. Serial sections cut at quite long intervals of time give identical results with the periodic acid routine.

The Structure of the Normal Glomerular Basement Membrane

The glomerulus in the normal kidney shows a single basement membrane by the present technic. The glomerular alterations in a variety of diseases which have been studied appear to be capable of explanation upon this basis. Nothing is revealed with any certainty as to the nature of the basement membrane of the glomerulus or of the tubules. It is of interest in connection with the pathogenesis of Bright's disease

and other affections of the kidney that a coloring similar to that seen in the basement membrane is observed in the hyalin of arteriolosclerosis, in casts, and in hyaline droplets.

The Histochemical Validity of the Present Method

When the periodic acid technic was described originally, it was pointed out that it was of histologic rather than of histochemical usefulness. That is still the present position. Malaprade [2] had introduced periodic acid into quantitative chemistry, having found that it would produce an aldehyde when it acted upon the connection between two carbon atoms of a chain if each of these two adjoining carbon atoms had a hydroxyl group. Nicolet and Shinn [3] found that the bond between adjoining carbon atoms was broken and that an aldehyde was formed when one carbon atom had a hydroxyl group and the other an amino group. The demonstration of aldehyde in tissue sections by Schiff's reagent after the action of periodic acid was a logical attempt, but histochemical conclusions are difficult to reach because of the following two facts. Lison [5] has shown that a great variety of materials other than aldehydes, notably ketones and unsaturated compounds, will recolor Schiff's reagent. Secondly, many substances in tissues contain one or both of the linkages from which periodic acid can produce aldehyde. Separate identification of glycogen, glycoprotein, glycolipid, and the three amino acids (serine, threonine, hydroxylysine) is necessary if the method described is to give valid histochemical data. It is my opinion that the basement membrane consists of a carbohydrate-protein compound of the mucoprotein type, but decision must be deferred for the reasons given.

Summary and Conclusions

In sections of normal human kidneys the basement membranes, capillary walls, and the outlines of the smooth muscle cells of the arterioles are colored with Schiff's reagent after periodic acid.

Besides these structures, the same routine applied to abnormal kidneys colors the following materials: The hyalin of arteriolosclerosis, hyaline casts, glycogen, amyloid, colloid droplets in tubular epithelium, and the granules of the afibrillar cells of the arterioles.

The material of the basement membrane which takes the stain is believed to be a mucoprotein. Opposed to immediate acceptance of this hypothesis are (1) the nonspecificity of the recoloring of Schiff's reagent, and (2) the production of aldehyde by periodic acid from three amino acids.

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DESCRIPTION OF PLATES

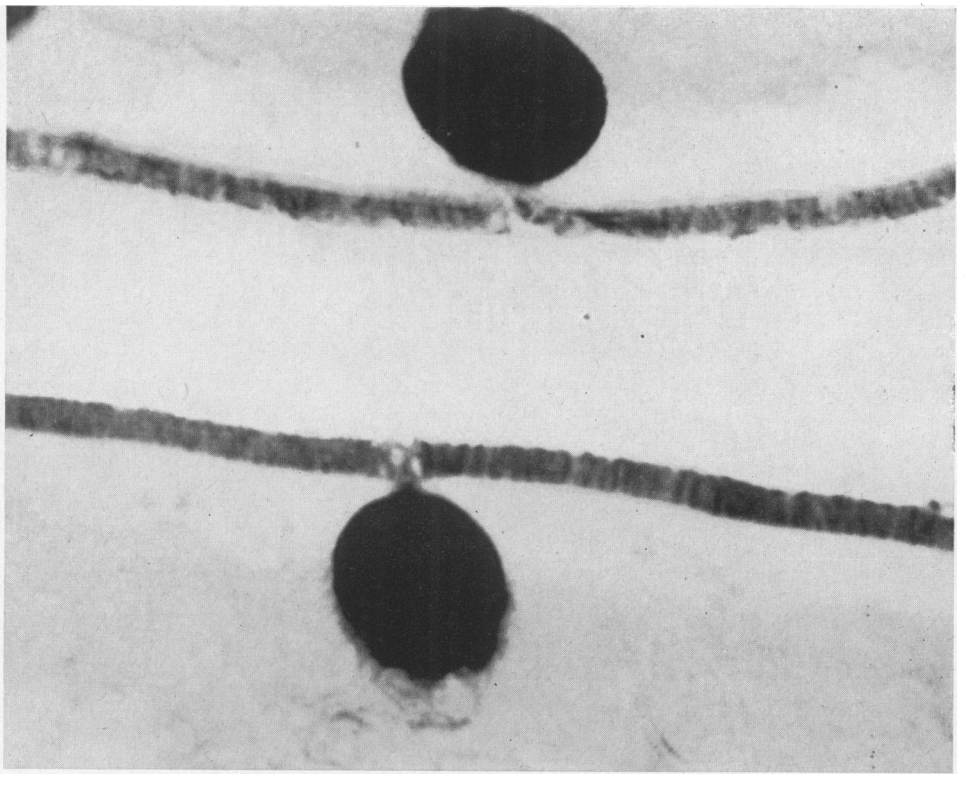
All illustrations were made from sections treated with periodic acid followed by Schiff's reagent.

PLATE 116

FIG. 1. Human jejunum. There is coloring only of mucous goblets and of the free surface ("brush border") of the intestinal epithelium. × 1500.

FIG. 2. Normal glomerulus and arteriole from a white female, 42 years of age. The basement membrane of the glomeruli and tubules is colored as well as the cell outlines in the arteriole. There is slight coloring of the free surface of the proximal convoluted tubules. × 520.

1



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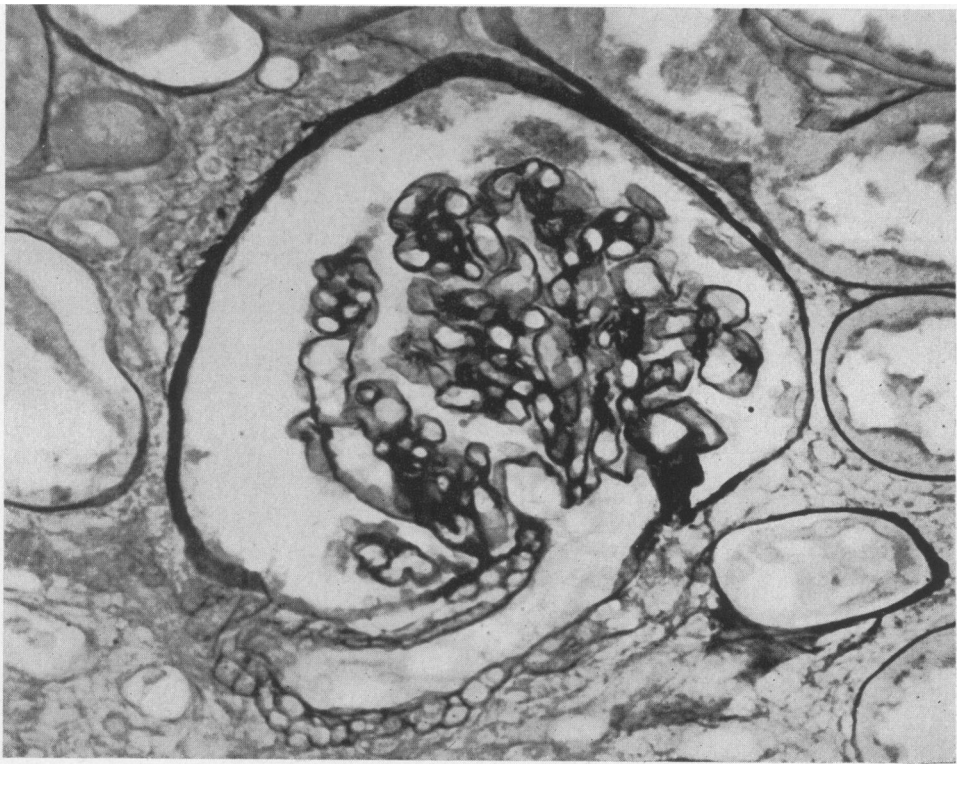
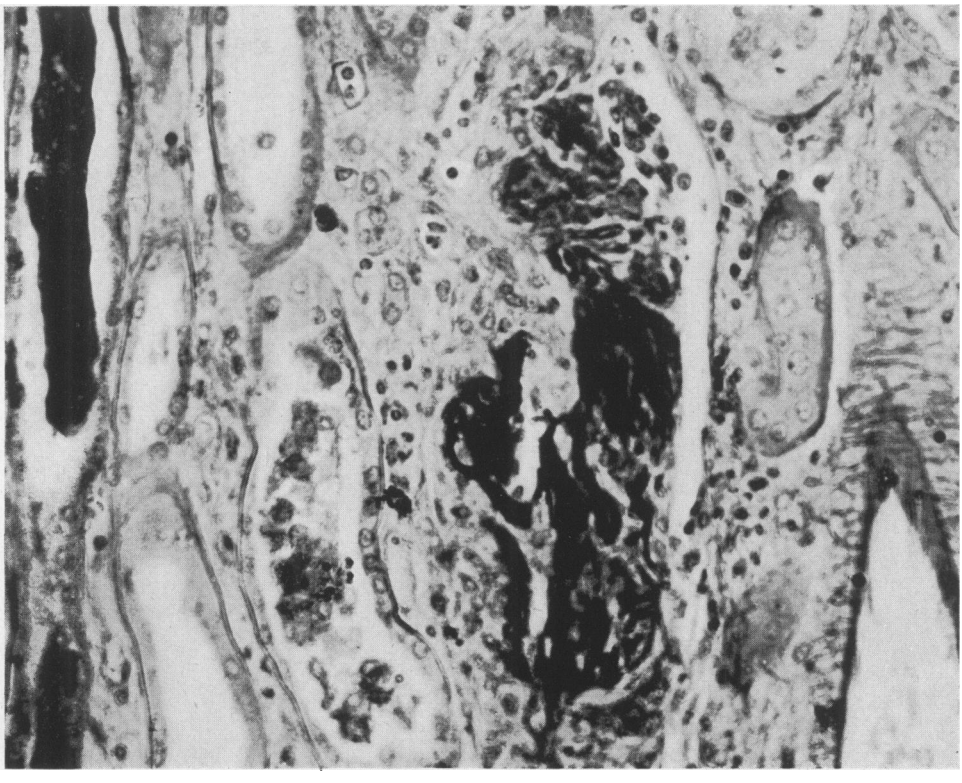


PLATE 117

FIG. 3. Tubular injury and venous thrombosis after a severe wound. A thrombus can be seen protruding into the venule from the inflamed interstitial tissue. There is a hyaline cast in one tubule and some cellular débris in another. Counterstained with hematoxylin. × 320.

FIG. 4. Glomerulus from a case of essential hypertension in a colored female, 36 years old. Death in second cerebral hemorrhage. Most of the glomeruli, like the one of which a corner is shown, appeared normal; a few—one-fourth to one-fifth—showed the wrinkling of the simplified basement membrane which is illustrated. Hyaline arterioles (not shown) were numerous. × 520.

3



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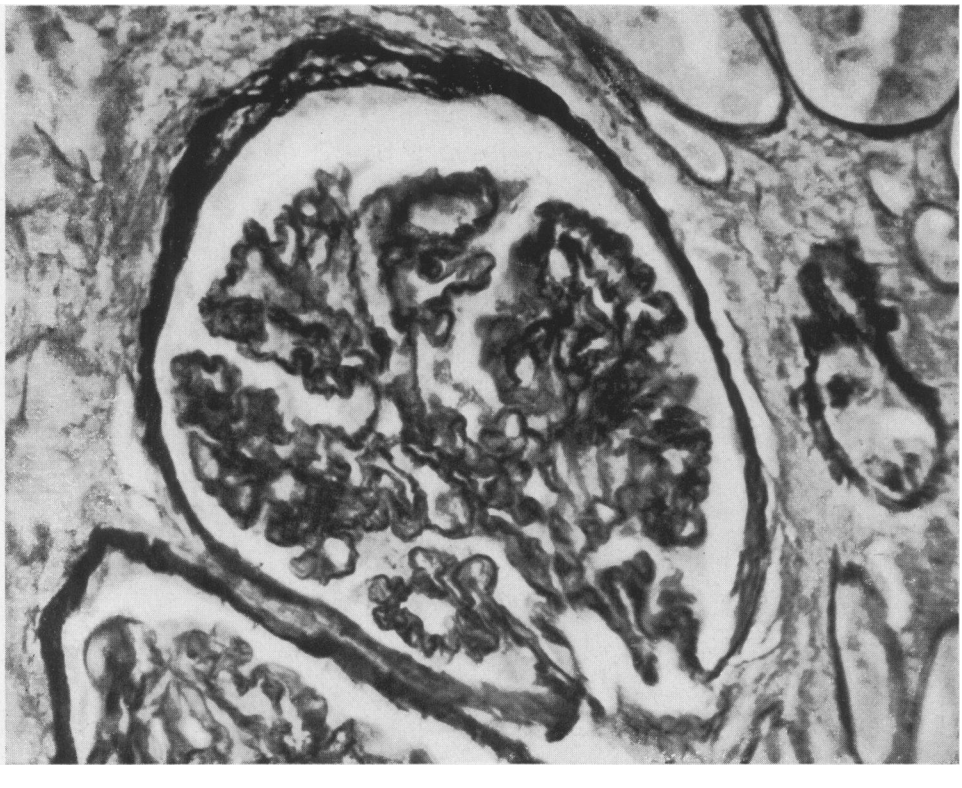
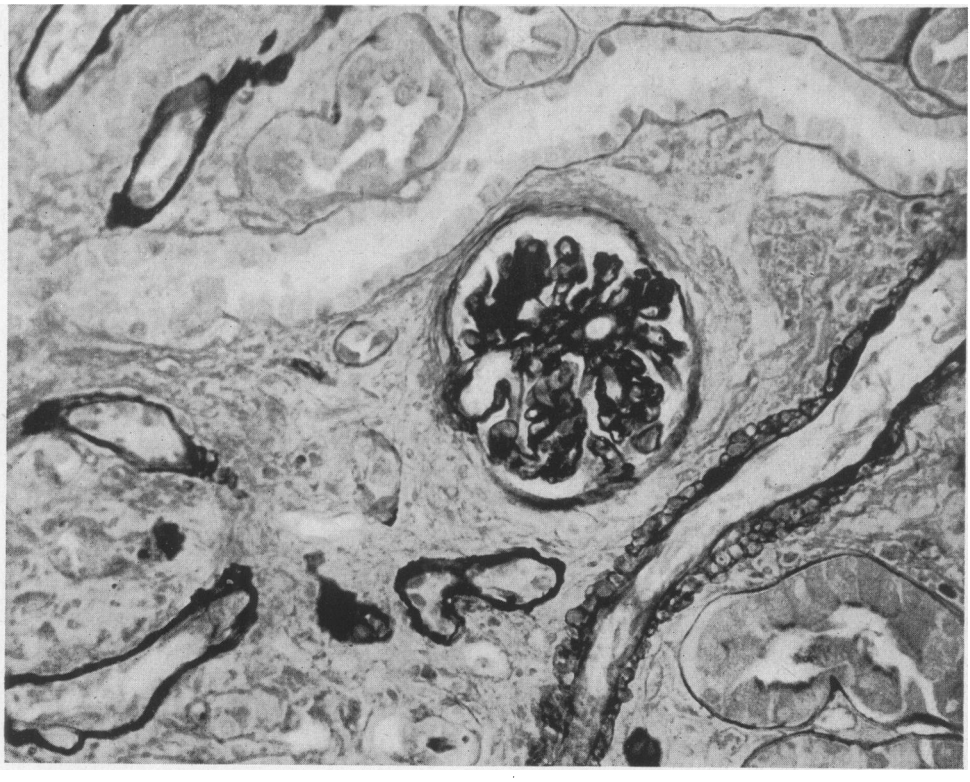


PLATE 118

FIG. 5. Area from the generally normal kidney of a white female, 46 years old, dying with metabolic craniopathy. An obsolescent glomerulus is seen. There are several segments of a tubule in process of disappearance and showing thickening of the basement membranes. The arteriole shows several patches of hyalin. × 260.

FIG. 6. From the same case as used for Figure 5. Several tubules, the cells of which contain hyaline droplets, are seen. One other tubule is in process of disappearance. It shows a greatly thickened basement membrane and a diminished or absent lumen. × 950.

5



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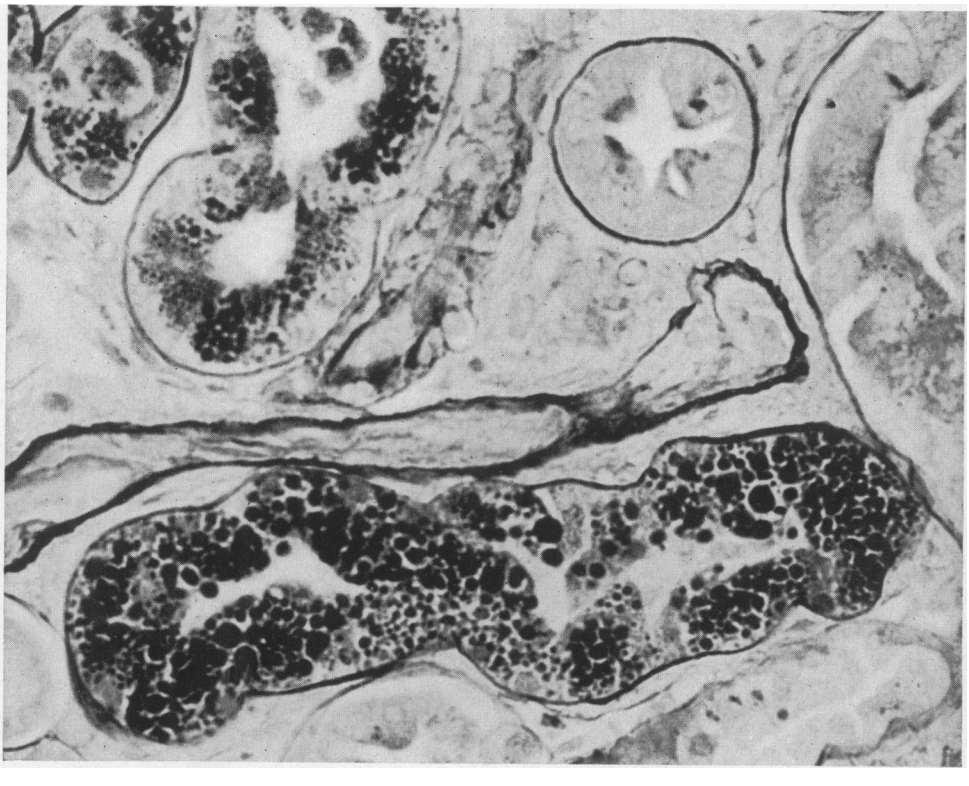

McManus Periodic Acid Routine Applied to Kidney