THE RENAL PATHOLOGY OF NUTRITIONAL HYPERTENSION IN RATS*

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PLATES 3 TO 6

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In a previous communication (1) it was shown that, in the rat, the feeding of diets deficient in the heat-stable fractions of the vitamin B complex is followed by a persistent rise in blood pressure; that, in the early stages at least, this hypertension can be reversed by restoring these factors to the diet; that partial deficiencies are followed by a higher rise in blood pressure than are complete deficiencies; and that, although the vitamin B_2 complex appears to play the dominant etiologic rôle, other nutritional factors or their interaction with the B_2 complex are also involved. It is the purpose of the present article to report the pathologic changes in the kidneys which accompany this rise in blood pressure. These induced lesions are strikingly similar to those observed in essential hypertension in man.

The pathologic anatomy of essential hypertension in human beings is well known. As reviewed by Hadfield and Garrod (2), the primary and typical change in this disease occurs in the arterial system and consists of a muscular hypertrophy involving chiefly the media, although the intima also shows a reduplication of the internal elastic lamina and a conspicuous development of new musculoelastic layers. In the case of interlobular and glomerular afferent vessels of the kidneys, this muscular hypertrophy is often followed by degenerative changes, which, however, are patchy in distribution. In the interlobular arteries, some of which at least are invariably affected, the hypertrophied muscle in the media undergoes irregular replacement by fibrous tissue, while the newly formed elastic and muscular fibers in the intima are replaced by fibrous, fatty, and fatty-hyaline material. These changes, especially in the smaller vessels, result in narrowing of the lumen and consequent ischemic atrophy of the areas of renal parenchyma which these vessels supply. These vascular alterations are focal, not universal, and the resultant areas of atrophy therefore appear as streak-like or wedge-shaped patches surrounded by secretory tissue which is entirely normal. The same process accounts for the fine granulation of the renal

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surface which is so often seen in this disease. The glomerular afferent arterioles are also the site of a change which is pathognomonic of essential hypertension and which consists of the deposition of refractile eosinophilic hyaline material beneath the endothelium. This hyalinization does not occur uniformly along the course of the vas afferens, nor is it symmetrical circumferentially. Indeed, according to McGregor (3), it may start at an appreciable distance from the glomerulus and, in the early stages at least, these vessels may actually be dilated at their entrance into the glomerular tuft (Loehlein (4); Fishberg (5)). Certainly following and perhaps mediated by these vascular abnormalities, changes occur in the glomeruli. As reported by McGregor, these comprise thickening and wrinkling of the glomerular basement membrane, decrease in the number of loops of the glomerular tuft with consequent simplification of its pattern and diminution in size, thickening of the capsular basement membrane, and an apparent increase in the pericapsular connective tissue, which, because of shrinkage, assumes a lamellated appearance. McGregor has called these structures, altered in this typical fashion, "hypertensive contracted glomeruli." As the arteriolosclerosis progresses, ischemia of the glomerular tuft supervenes, and as a result this structure becomes hyalinized, either partially or completely. Coincidentally with these changes, the tubules draining the involved glomeruli undergo atrophy, either because of interference with their blood supply or from disuse; their epithelium shows fatty and other regressive changes, and finally they are represented only by cords of cells in the midst of replacing connective tissue, until even this disappears.

Previous evidence that the vitamin B complex may serve to prevent structural abnormalities of the kidneys is only suggestive, not definitive. Following the pioneer work of Newburgh (6), amply confirmed by others (reviewed by Fishberg (5)), showing that an excessive intake of proteins leads to significant alterations in this organ, it was soon found that these deleterious effects could be prevented by an adequate intake of vitamin B (Reader and Drummond (7); Longwell *et al.* (8)). If the protein was edestin rather than casein, a larger amount of vitamin B was necessary (Hartwell (9)). It was also soon demonstrated that the factors in the vitamin B complex displaying this protective effect were distinguishable from the antineuritic component, for they were able to withstand the action of hot alkalies (Hassan and Drummond (10)) and prolonged autoclaving (Hartwell (9)). A similar protective action of vitamin B was shown in the case of cystine nephrosis (Cox and Hudson (11); Longwell *et al.* (8)). Meanwhile it was demonstrated that if rabbits are fed a high protein diet, albuminuria and cylindruria rapidly occur, but that the addition of green-stuff to the dietary rendered it entirely innocuous; unfortunately, however, when the animals were deprived of greens, they rapidly died, "so that the keeping of such animals over periods which might have produced chronic interstitial changes was found to be impossible" (Maclean *et al.* (12)).

Following the relatively recent discovery of the multiple nature of the vitamin B complex, it has been determined that hemorrhagic degeneration of the kidneys is readily induced by choline deficiencies (Griffith and Wade (13); György and Goldblatt (14)), and that the development of these changes is related to the ratio of cystine and methionine in the diet (György and Goldblatt (14)). Similar lesions in the kidneys have been produced by abnormally low intakes of pantothenic acid (Schaefer, McKibbin, and Elvehjem (15); Supplee *et al.* (16)).

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EXPERIMENTAL

The details of our experimental methods have been presented previously (1). Rats from a breeding colony of the Vanderbilt strain were placed on a diet completely free of vitamin B complex. All received daily supplements of thiamin chloride to prevent cardiac failure. They were divided into four groups of 25 animals each. The various groups received, respectively, autoclaved yeast in the amount of 2.5 per cent of the diet by weight; 5 per cent yeast; yeast as desired; and Valentine's liver extract. The effects of these dietaries on the blood pressure have been reported previously; the first two groups manifested a significant and sustained rise in blood pressure; those permitted to eat yeast *ad libitum* experienced a transient rise in arterial tension which, however, soon fell and by the end of the experimental period was not significantly different from the normal; and the animals receiving liver extract, a potent source of the vitamin B_2 complex, displayed entirely normal pressures throughout the experiment.

These animals were maintained for a period of one year. By the end of that time, all but 3 of the rats receiving 2.5 per cent yeast had died, 15 animals receiving 5 per cent yeast had survived, and the other two groups were practically intact. The survivors were killed, and the kidneys removed, fixed in 10 per cent formalin solution, and embedded in paraffin. Sections of all the material were prepared and sets stained by the usual hematoxylin and eosin method, with van Gieson's stain, with iron-hematoxylin according to Masson's method, and by Weigert's iron-hematoxylin.

Gross Pathology.—Without exception, the kidneys of the animals which had received liver extract and of those permitted to eat yeast as desired were entirely normal on gross examination. The kidneys from the animals receiving 5 per cent yeast presented a fine granularity of the surface. In addition to the gross changes noted in the last group, specimens from the animals which had received 2.5 per cent yeast showed a rather extensive subcapsular accumulation of blood, together with macroscopic areas of hemorrhagic infiltration in the parenchymal tissues.

Microscopic Pathology.—Confirming the gross appearance, microscopic sections of the kidneys from the animals which had received yeast as desired or liver extract were, for the most part, entirely normal. The only detectable change occurred in a few of the large interlobar arteries, some of which showed thickening of their walls and narrowing of their lumens. It is to be emphasized, however, that this process was confined solely to the large arteries and was the counterpart of arteriosclerosis rather than arteriolar sclerosis of human subjects. There appeared to be no interference with blood flow through such vessels, for infarction of large areas of kidney substance was absent. Otherwise, there were no abnormalities; the interlobular and afferent vessels were of normal caliber, the tubules appeared healthy, and the glomerular tufts were of normal complexity.

In contrast, significant alterations were readily demonstrable in specimens from the animals which had received 5 per cent or 2.5 per cent yeast. Numerous vessels which by size and location were presumably afferent arterioles

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showed conspicuous degenerative changes, consisting of subendothelial hyaline deposits irregularly disposed circumferentially (Figs. 4 to 8). These deposits occurred at an appreciable distance from the glomerulus itself, that part of the arteriole lying in the tuft stem being spared in every instance. Indeed, in this latter situation, the vessels sometimes appeared to be somewhat dilated (Fig. 9). In their course through the cortex the small interlobular arteries displayed the thickening of their walls and encroachment on vascular lumen characteristic of arteriolosclerosis (Figs. 12 to 14). The changes in these arteries involved both the intima and the media. Subendothelial deposits of hyaline were so extensive as to compromise the lumen and at times obliterate it almost completely. At the same time, degeneration of the media was conspicuous. Resultant, small, streak-like areas of ischemic atrophy were striking features in both cortex and medulla. In the former situation these zones of atrophy were characterized by necrosis and atrophy of the epithelial cells of the uriniferous tubules (Figs. 1 and 2). Fibrous tissue replacement in these areas was not conspicuous. Concomitantly, the surface of the organ was serrated and indented, and at times the atrophy had been so extensive that a glomerulus could be seen lying immediately subjacent to the renal capsule (Fig. 1). Small, rounded areas of ischemic atrophy were exceedingly numerous in the medulla also (Fig. 3). The epithelial cells lining the tubules in these zones were necrotic, and the tubules themselves were represented merely by their fibrous supporting frame-work. Actual replacement by extensive fibrous tissue had not occurred. The lumen of some of these structures was filled with albuminous material. From their number, size, and situation, it appeared that these atrophic areas in the medulla were the consequence of occlusion of the arteriae rectae.

The glomeruli showed the lesions which have been described by McGregor (3) as being typical of essential hypertension. In many of the tufts there was an increase in the thickness of the capillary basement membrane, best shown by Masson's strain (Figs. 4 to 11). In some instances this was manifested only by small areas of dark staining material in some of the loops; in others the process was so extensive that the entire tuft was converted into a shrunken, almost solid, black mass. Accompanying this change in the basement membrane, the component loops of the tuft were reduced in number, with resultant simplification of the glomerular pattern. The glomeruli as a whole were often diminished in size, a 50 per cent reduction in diameter being not uncommon. In addition, there were numerous examples of thickening of the basement membrane of Bowman's capsule, best shown by van Gieson's stain (Fig. 10); while readily detectable, this was not sufficiently marked to cause actual deformity of the capsular structure.

The renal pathology observed in specimens from the rats which had received 2.5 per cent yeast was identical with that just described, except for the fact

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that, in addition, there were numerous areas of hemorrhagic infiltration in the cortical and subcapsular regions.

DISCUSSION

The results of previous studies indicated that when rats are maintained on a diet adequate in thiamin but deficient in the heat-stable portions of the vitamin B complex, they rapidly acquire a persistent hypertension. Examination of the kidneys of animals kept on such a diet for one year, as reported in the present communication, shows well defined structural alterations of this organ. The primary pathological change consists of sclerosis of the afferent arterioles and of the interlobular arteries. Simplification of the glomerular pattern and thickening of the capillary basement membrane of this structure were also noted. Areas of ischemic atrophy of the parenchyma occurred in both cortex and medulla. These histological findings materially enhance the significance of our previous observations on the causal relationship between a restricted intake of the vitamin B_2 complex and the development of the hypertensive state in this animal species.

The possible identity of these renal lesions with those seen in essential hypertension in man is worthy of mention. Although the pathological changes were not so severe as those seen in advanced nephrosclerosis, they were, in most respects, indistinguishable from the findings in human subjects with essential hypertension. The principal difference lay in the occurrence of areas of ischemic atrophy in the renal medulla; this change is not a part of the pathology of essential hypretension as seen in human autopsy material, but may be explainable on the basis of anatomical differences in the two species. That metabolic disturbances incident to nutritional deficiencies may be an important factor in the pathogenesis of essential hypertension in man seems to warrant extensive investigation.

SUMMARY

Rats subsisting on a diet partially deficient in the heat-stable fractions of the vitamin B complex are known to experience a rise in blood pressure. The present study shows that after prolonged administration of this dietary, abnormal structural changes occur in the kidneys. The surface of this organ becomes finely granular. The afferent arterioles show degenerative changes, consisting of irregular subendothelial hyaline deposits which encroach on the lumen. The interlobular arteries undergo the same change, plus degeneration of the media; the lumen of these vessels is likewise compromised. Resultant, small, streak-like areas of ischemic atrophy occur in both cortex and medulla, with necrosis of the epithelial lining of the uriniferous tubules. The glomeruli are reduced in size, the number of their component loops decreased, their pattern simplified, and the capillary basement membrane thickened. In

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addition to these changes, kidneys from animals on a more profoundly deficient diet display numerous areas of hemorrhagic infiltration in the cortical and subcapsular regions.

The possible identity of these lesions with those seen in essential hypertension in man is discussed.

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EXPLANATION OF PLATES

PLATE 3

FIG. 1. Section of kidney from a rat which had received 5 per cent yeast. Note indentation of the surface and tubular atrophy. A glomerulus is seen lying immediately subjacent to the capsule. Van Gieson's stain. × 144.

FIG. 2. Section of kidney from a rat which had received 2.5 per cent yeast. Streak-like areas of ischemic atrophy in the cortex are readily apparent. Van Gieson's stain. × 112.

FIG. 3. Section of kidney from a rat which had received 5 per cent yeast, showing patchy areas of ischemic atrophy in the medulla. Hematoxylin and eosin stain. × 120.

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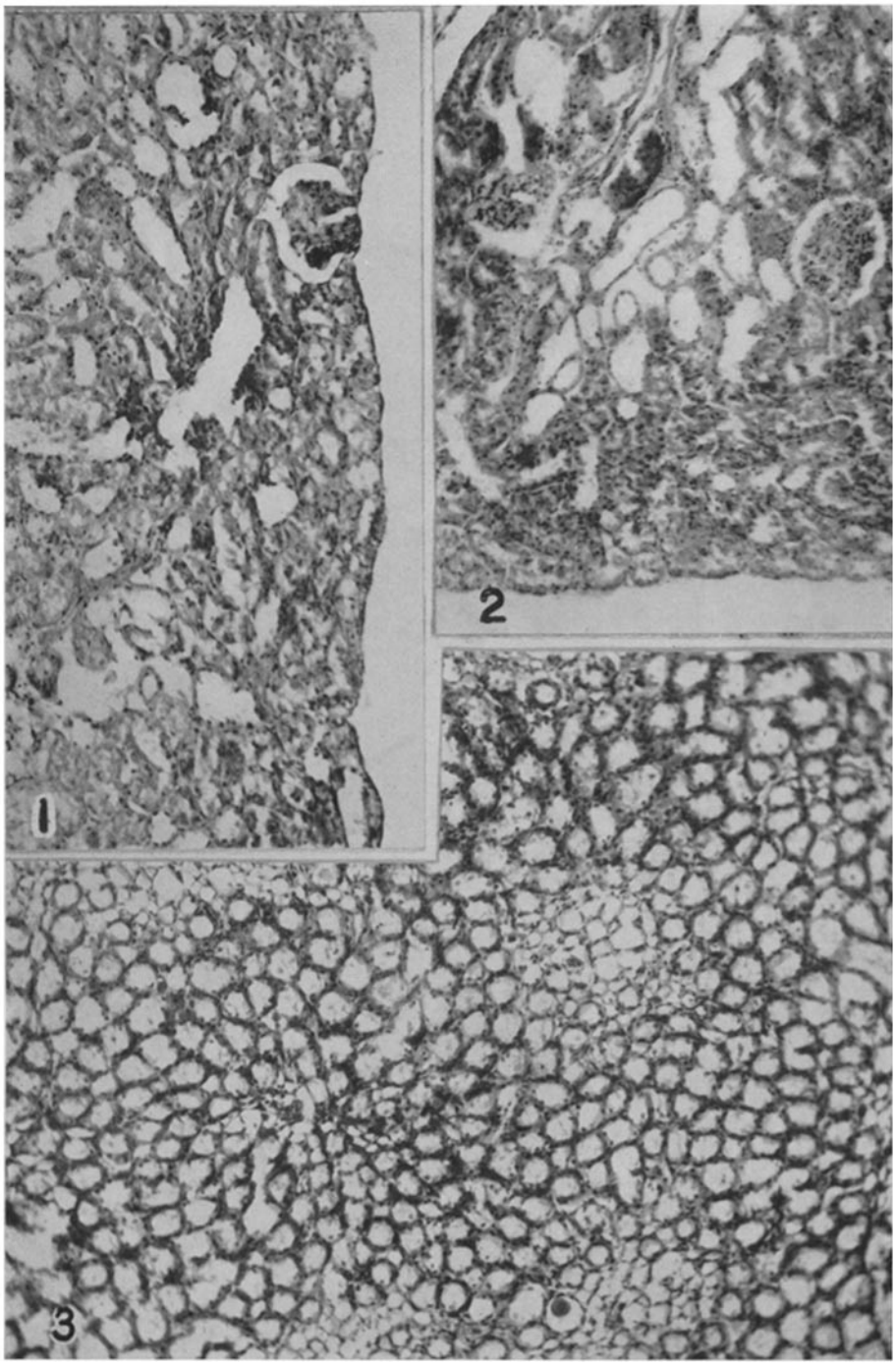

(Calder: Renal lesions in dietary hypertension)

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PLATE 4

FIGS. 4 to 7. Progressive simplification of the glomerular pattern; thickening of the walls of the afferent arterioles, the lumen being completely occluded in Fig. 4. A sclerotic interlobular artery is seen in Fig. 7; immediately adjacent to its right inferior quadrant is an arteriole the lumen of which is almost occluded, and to its left inferior quadrant is another arteriole with a totally occluded lumen. Some of the tubules in Fig. 6 are widely dilated and their cellular lining atrophic. Figs. 4, 5, and 6 are from animals which had received 5 per cent yeast; Fig. 7, 2.5 per cent yeast. Figs. 4 and 5 stained with hematoxylin and eosin ($\times$ 326); Fig. 6 by Masson's method ($\times$ 326); Fig. 7 by Weigert's iron-hematoxylin ($\times$ 417).

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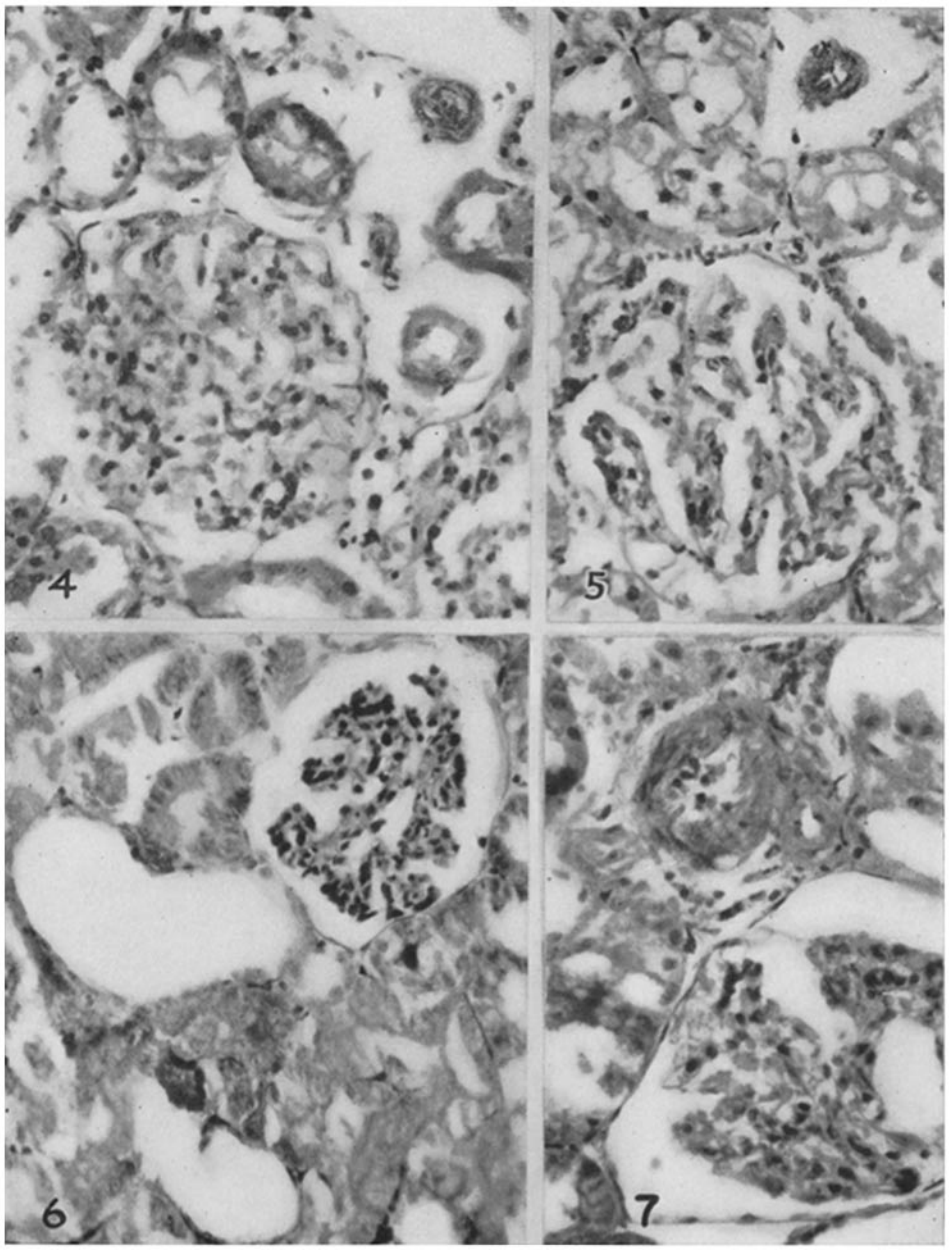

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PLATE 5

FIGS. 8 to 11. Progressive degeneration and contraction of ischemic glomeruli, with advancing thickening of their capillary basement membranes. A sclerotic arteriole is seen in Fig. 8. In Fig. 10, Bowman's capsule is thickened. The vessels of the tuft in Fig. 9 show characteristic lake-like dilatation. Fig. 8 is from an animal which had received 2.5 per cent yeast; the others, 5 per cent yeast. Fig. 8 stained with Weigert's iron-hematoxylin; Figs. 9, 10, and 11, by Masson's method. × 326.

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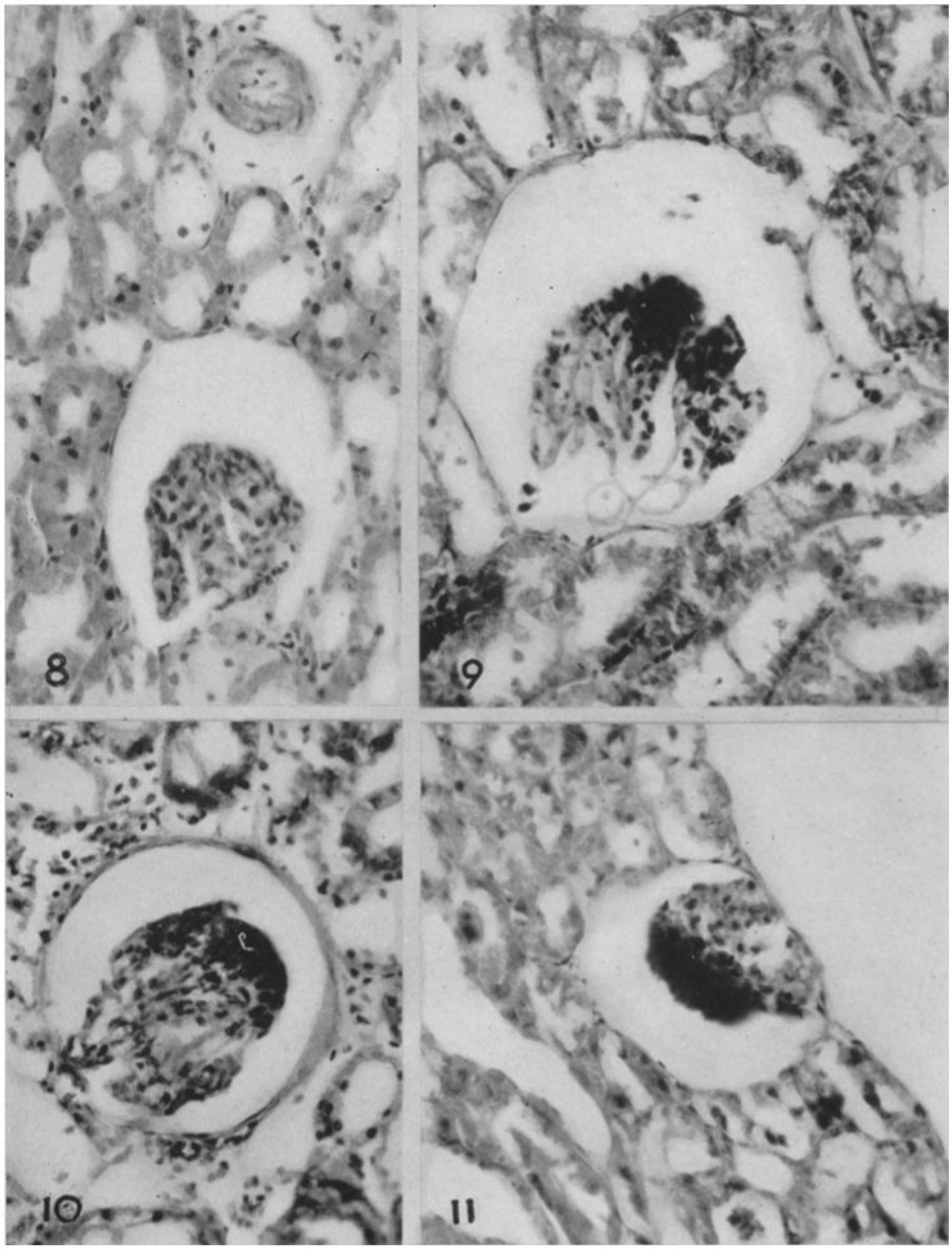

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PLATE 6

FIG. 12. At the left, an interlobar artery, the walls of which are moderately thickened. The other vessel is an interlobular artery, the lumen of which is practically occluded. A hypertensive contracted glomerulus is seen at the right. From an animal that had received 2.5 per cent yeast. Weigert's iron-hematoxylin. × 220.

FIG. 13. Higher power view of the interlobular artery shown in Fig. 12. × 330.

FIG. 14. Sclerotic interlobular artery in the kidney of a rat which had received 5 per cent yeast. The lumen is almost closed. Masson's stain. × 330.

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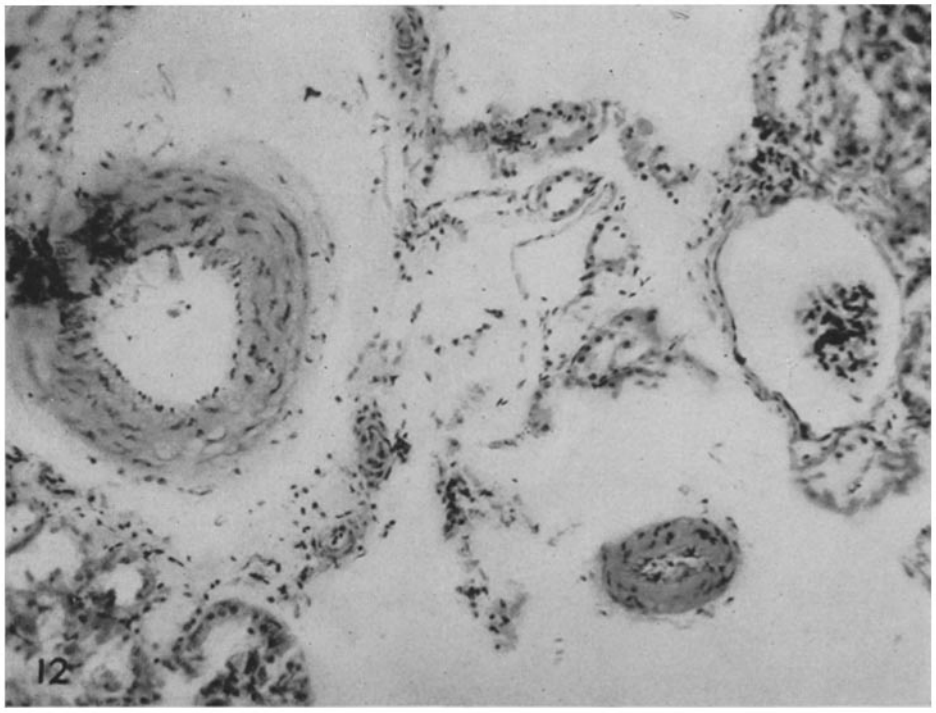

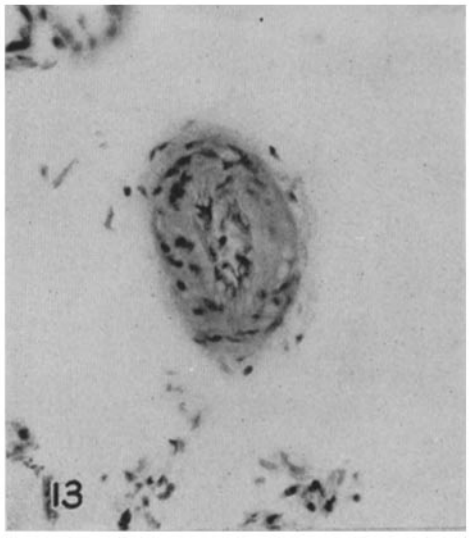

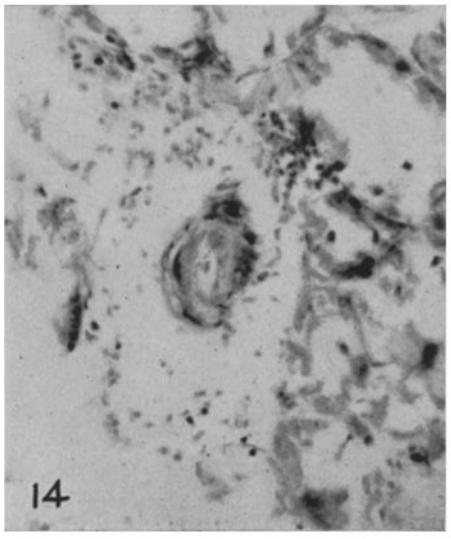

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