Some Effects of Alloxan on the Canine Kidney, with Special Reference to the Glomerulus.* (18960)

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During a long term study of experimental diabetes mellitus in dogs, 2 animals were encountered which failed to become diabetic after repeated injections of alloxan. In these, the development of renal lesions was followed by serial biopsies. In addition to the tubular damage previously described by others(1-6), we have observed the late appearance of glomerular degeneration, a finding hitherto unreported in the dog.

Method. Young mongrel dogs aged 4-6 months were given alloxan monohydrate (Eastman Kodak Co. or Schuylkill) intravenously in amounts ranging from 50-100 mg per kilo of body weight. Biopsies of the kidney were performed at intervals of from one to several months afterward. All animals were kept in metabolism cages and maintained on constant diets containing ground horse meat, bread, brewer's yeast, and cod liver oil.

Results. Pathological observations. 1. Dog 132. This animal received a total of 3672 mg of alloxan in 4 doses over a period of 5 months (70 mg/kg on 11/19/47 at age 4 months and on 3/11/48; 80 mg/kg on 3/24/48; 100 mg/kg on 4/7/48).

One and one-half months after initial dose of alloxan. The kidney appears normal except for an occasional tiny, pale blue hyaline cast and rare scattered atrophic tubules.

Five and one-half months after initial alloxan. (Fig. 1). The kidney shows moderately severe changes, consisting of some interstitial edema and fibrosis, considerable atrophy of tubules, a few scattered tubules filled with amorphous debris and without tubular epithelium, and a few dilated tubules. The glomeruli are comparatively normal ex-

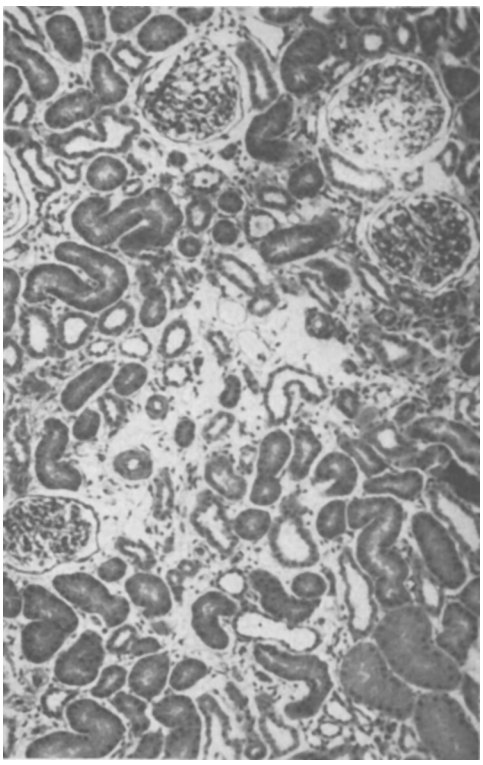

FIG. 1. Photomicrograph (× 110). (Dog 132, 4-29-48). Renal biopsy (Hematoxylin and eosin) taken 22 days after the last of a series of 4 injections of alloxan totalling 3672 mg over a period of 5 months. There is much atrophy of tubules but minimal glomerular damage at this stage. Three relatively normal tubules are seen in the lower right corner.

* This work was performed as part of a project supported by the Division of Research Grants and Fellowships, National Institutes of Health, United States Public Health Service.

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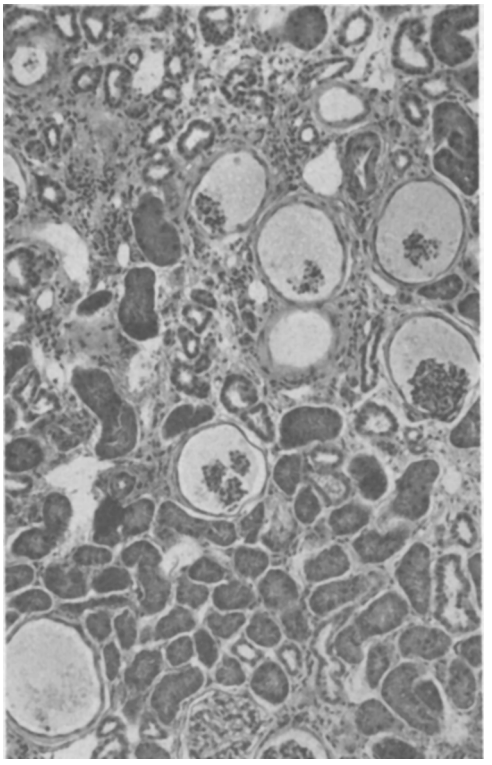

FIG. 2. Photomicrograph (× 110). (Dog 132, 2-3-49). Renal biopsy taken about 9 months later. In addition to extensive tubular atrophy and interstitial fibrosis, glomerular changes are now conspicuous. They consist chiefly of atrophy of some tufts, and dilation of some Bowman's spaces but shrinkage of others.

cept that a few are atrophic and lie in dilated spaces. The arterioles and small arteries are conspicuous but probably not thickened.

Fourteen and one-half months after initial alloxan. (Fig. 2). The tubular changes are more striking than at 5½ months and glomerular changes are now also evident. While some tubules appear comparatively normal, many show severe atrophy and a few are dilated. Interstitial fibrous scars are now more conspicuous due apparently in part to disappearance of some tubules. Such areas seem to have an increased number of glomeruli. About half of the glomerules are relatively normal. The remainder show great atrophy of glomerular tufts; a few are fibrotic. Bowman's spaces are usually dilated, sometimes greatly, but a few are shrunken; there is much fibrous tissue increase around most of the abnormal glomeruli, but their epithelium is thin and flat. They exhibit neither crescents nor synechiae. There is no fatty change and the vessels are not unusual.

Thirty months after initial alloxan. The degenerative changes appear less severe than at 14½ months, possibly because of either a difference in sampling or some degree of recovery. Most of the tubules appear relatively normal as do the majority of the glomeruli. The remainder show atrophy of the glomerular tuft, without epithelial changes, and most of such glomeruli are now shrunken. A few glomerular tufts are fibrosed. A few arteries have thick but probably not thickened walls. There is no fatty change or glycogen deposition.

2. Dog 199. This animal received a total of 2151 mg of alloxan in 3 doses over a period of 6 weeks (75 mg/kg on 6/3/48 at age of 4 months; 60 mg/kg on 7/10/48; 85 mg/kg on 7/20/48).

Two months after initial dose of alloxan. (Fig. 3). This kidney shows severe degenerative change which is chiefly tubular but also glomerular. While a few tubules appear nearly normal, the majority show severe atrophy and many have disappeared. There is, therefore, a relative and probably an absolute increase in interstitial fibrous tissue, together with some edema and an apparent increase in glomeruli. Leukocyte infiltration is minimal. Some atrophic tubules contain small casts and Bowman's spaces show a pinkish precipitate. A few of the atrophic tubular cells show stainable lipid. Practically no normal glomeruli are seen. Most of them exhibit atrophic tufts, some of which lie in dilated and others in contracted Bowman's spaces. Many of the abnormal glomeruli show a marked increase of fibrous tissue in Bowman's capsule but there is no epithelial proliferation. The blood vessels appear normal.

Eight months after initial alloxan. The renal changes are of the same variety as at 2 months, but while they are less extensive they are nevertheless more advanced, possibly owing to a difference in sampling, or in repair, or both. About half of the glomeruli and

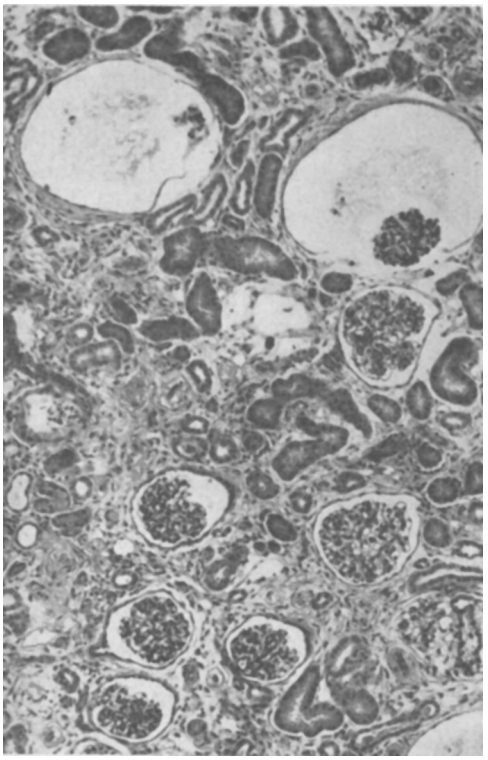

FIG. 3. Photomicrograph (× 110). (Dog 199, 8-12-48). Renal biopsy taken 23 days after the last of a series of 3 injections of alloxan totalling 2151 mg over an interval of 6 weeks. Tubular and glomerular changes are similar to but more severe than those in Dog 132.

many tubules appear relatively normal. Other tubules are atrophic or have disappeared, leaving fibrous scars. A few show a small amount of stainable lipid. Most of the abnormal glomeruli are greatly dilated and enclosed by dense fibrous tissue. They contain tiny atrophic glomerular tufts and a pink-staining deposit. The epithelium is thin and flat, and there are no crescents or synechiae. A few glomeruli are shrunken and in some areas they appear abnormally numerous. A few afferent arterioles appear thickened.

Miscellaneous observations. Proteinuria, despite severe renal damage, was only occasional, transitory and usually minimal. The blood urea nitrogen or nonprotein nitrogen rose moderately soon after alloxan administration but declined quickly to normal values. In dog 132 a reducing substance appeared in the urine after 34 months, the glucose tolerance curve being normal. This animal subsequently became frankly diabetic.

Discussion. Recently Mann and Goddard (7,8) have found that male rats made diabetic with alloxan monohydrate developed well-marked progressive lesions in the renal glomeruli as early as 50-90 days. Primarily affected were the walls of the capillary tufts and the parietal layer of Bowman's capsule. Pyelonephritis was a frequently associated condition. Tubular changes were not described. Renal arteriolar lesions were absent. The incidence of glomerular damage could not be correlated with the duration or severity of diabetes, or with the nature of the diet. No lesions were found in non-diabetic, alloxan-injected controls.

Obviously unrelated to alloxan, and possessing their own special significance, are renal changes found in experimental diabetes produced by other means. Thus, Lukens and Dohan(9) described in the kidneys of a dog made permanently diabetic with anterior pituitary extract changes which appeared to be the counterpart of the intercapillary glomerulosclerosis of human diabetes. The development of glomerular degeneration in depancreatized rats has been reported by Foglia and associates(10). The lesions did not appear until more than two months after 95% pancreatectomy. The earliest change was hyaline thickening of the glomerular arteriole and capillaries, and finally sclerosis of the tuft. These lesions were diffuse and severe, but no hyaline masses similar to those described by Kimmelstiel and Wilson(11) were seen.

The observations here reported are, so far as we are aware, the first in which definite glomerular lesions in the dog following administration of alloxan have been described. It is noteworthy that they occurred after mul-

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tiple doses of the drug and that injury of this degree was never seen in many other dogs, including 2 littermates of dog 132, which survived a single dose. Serial biopsies showed, in agreement with earlier reports and with our own observations on other dogs, that the primary effect of alloxan was on the tubules. The glomerular changes lagged behind the tubular degeneration and they appeared both in time and in type to be consequent to the latter. The histologic picture suggests that the glomerular damage originated from a failure of drainage, with resulting dilatation of Bowman's space and atrophy of the tuft. The absence of epithelial and endothelial proliferation and the presence of periglomerular fibrosis are consistent with this interpretation.

Summary. In 2 dogs which did not become diabetic following repeated doses of alloxan, serial biopsies of the kidney showed the late development of glomerular lesions. These lagged behind the usual tubular degeneration and appeared both in time and in type to be consequent to the latter. Similar studies on dogs receiving a single dose of alloxan and surviving for comparable periods of time showed only minimal glomerular changes.

Received June 19, 1951. P.S.E.B.M., 1951, v78.

V. Effect of Epinephrine, ACTH and Cortisone on Citrate, Calcium, Glucose and Phosphate Levels in Rabbits.* (18961)

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The effect of administration of glucose, insulin and epinephrine on serum citrate levels in humans has been studied(1-3). Administration of both glucose and insulin resulted in a fall in citrate levels, and administration of epinephrine resulted in a rise in citrate levels for several hours. That administration of glucose will result in a drop in citrate levels has been confirmed(4,5). Evidence has been presented to suggest that epinephrine stimulates secretion of ACTH and in turn the corticoid hormones(6). For this reason it was decided to investigate the changes in citrate levels occurring in blood under the influence of these hormones.

Serum calcium(7) and inorganic phosphate (8) estimations were carried out in addition to citrate(9,10) and glucose(11). In the case of epinephrine potassium levels(12) were also studied. These additional studies were undertaken because a relationship exists between serum calcium and citrate levels and ionic calcium, citrate and phosphate levels due to the formation of poorly ionized calcium complexes(13). Female rabbits weighing be-

* The fifth study on mechanism controlling serum citrate levels. Supported in part by a grant from the Dazian Foundation.

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