AN EXPERIMENTAL STUDY OF THE LATE GLOMERULAR LESIONS CAUSED BY CROTALUS VENOM.*

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PLATE 6.

In 1909 I described acute glomerular lesions produced in the rabbit by the use of rattlesnake venom.[1] These lesions, which followed the intravenous injection of carefully graded doses of venom, were either hemorrhagic or exudative in character and involved either the tuft or the capsular space or both. The tubular epithelium was either normal or showed minor changes only. As it seems probable, in view of the work of Flexner and Noguchi,[2] that the selective action on the glomerulus is to be explained by the action of the endotheliolytic body of venom, the lesion was considered as essentially a vascular nephritis with severe endothelial changes, and therefore as one offering an opportunity for attempting the experimental production of a chronic nephritis. During the past few years this possibility has been kept in mind, and from time to time in connection with various other phases of the studies of experimental nephritis a more or less intermittent study has been made of the lesions occurring after various lengths of time and after single and multiple injections of venom, in the hope of demonstrating chronic glomerular lesions.

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[1] Pearce, R. M., The Production of Edema. An Experimental Study of the Relative Importance of Renal Injury, Vascular Injury and Plethoric Hydremia, *Arch. Int. Med.*, 1909, iii, 422; An Experimental Glomerular Lesion Caused by Venom (*Crotalus adamanteus*), *Jour. Exper. Med.*, 1909, xi, 532.

[2] Flexner, S., and Noguchi, H., The Constitution of Snake Venom and Snake Sera, *Univ. Penn. Med. Bull.*, 1902–03, xv, 345.

The venom used in the experiments was the dried venom of *Crotalus adamanteus.*[3] It was dissolved in salt solution in the proportion of 0.25 of a milligram to one cubic centimeter, which was the usual initial dose. In the first series of animals fresh venom only was used, but as this frequently caused a rapidly fatal result, an old solution of venom which had been kept in the laboratory for a year was substituted. The older solution had lost much of its lethal power, that is, its neurotoxic and hemolytic powers were decreased, but its endotheliolytic power, as shown by the occurrence of albuminuria and the presence of exudation in the capsular space, had not been impaired. It was therefore used in the earlier treatment in doses of 0.25 or 0.5 of a milligram, rising gradually to two milligrams, and then followed by doses of 0.5 of a milligram of fresh venom at various intervals. The intervals between injections depended on the general condition of the animal and the amount of albuminuria. The initial injection was always followed by albuminuria and occasionally by hematuria or hemoglobinuria. When the albumin disappeared or had decreased greatly in amount, another injection was made, and as the periods of albuminuria lessened, the doses were increased. As sometimes a single injection caused a persistent albuminuria for weeks, while in other animals four to eight injections were given, the entire series represents the results of single and repeated injections. Despite the fact that some animals showed a tendency to immunization, it was impossible, even with the greatest care as to food and environment, to carry an experiment over a period of more than six to seven weeks. No cause could be found for this other than a progressive emaciation presumably dependent on the chronic intoxication.[4]

The results are based on the study of twenty-four rabbits, all of which received venom by intravenous injection.

Five animals died within four days after a single injection. Of these, one, which succumbed in six hours, presented the hemorrhagic, and four the typical exudative glomerular lesion of acute

[3] This venom was obtained through the kindness of Dr. Noguchi, of The Rockefeller Institute for Medical Research.

[4] In some instances the loss of weight was equal to 40 to 50 per cent. of the original weight, though usually it was about 25 per cent.

poisoning. One of the latter, dying on the third day, showed also an extreme degree of hyaline degeneration of the epithelium of the convoluted tubules.

Two animals died on the tenth day, after second and third doses respectively, with the usual acute lesions and no evidence of a reparative process. In each instance death followed an increase in the dose. Four died between the fourteenth and twentieth days. These represent one, two (2), and five injections; and all but one (representing two injections) showed evidence of repair of the glomerular lesion. The details of the experiments follow.

Experiment 2. Eighteenth Day Lesion; Multiple Injections.—A rabbit weighing 1,560 gm. received 0.5 mg. of venom (old solution) in the ear vein on October 2 and 4, and 1 mg. on October 6. As the injections caused little albuminuria the treatment was continued by using 0.5 mg. of fresh venom on October 11, 13, and 15. The last injections caused a pronounced albuminuria with hematuria. The animal died on October 19. Its weight was 950 gm. No gross lesions were found at autopsy.

Histological Examination.—The kidneys show well marked hemorrhagic and exudative lesions in the glomeruli; hyaline, granular, blood, and hemoglobin casts in both convoluted and collecting tubules; and granular degeneration of the epithelium of the convoluted tubules and loops of Henle.

In addition, however, to these acute lesions, the kidney shows distinct evidence of an attempt to repair the glomerular injury. Here and there it is possible to observe a penetration of the cells of the compressed glomerular tuft into the mass of hemorrhage lying either in the tuft itself or in the capsular space; less rarely is this seen when the capsule contains only serum and fibrin. The cells derived from the endothelium of the tuft are, for the most part, oval or elongated, with large deeply stained nuclei and scanty protoplasm which tends to take the basic stain. Despite the dense staining of the nuclei, a vesicular arrangement is frequently evident. Sometimes the cells, instead of penetrating the mass of hemorrhage, form a capsule about it, suggesting by their appearance, when they surround small masses, a giant cell formation. This proliferation is always from the tuft, never from the capsule (figure 1).

Experiment 7. Sixteenth Day Lesion; Multiple Injections.—A rabbit weighing 1,420 gm. received 1 mg. of venom (old solution) on October 4 and 6. The injections caused only a transient albuminuria, and therefore 0.5 mg. of fresh venom was given on October 11. This caused an albuminuria, increasing daily

in severity, with death on October 20. The weight of the animal after death was 870 gm.

Histological Examination.—The same lesions are found as in experiment 2, but with less marked evidence of glomerular proliferation.

Experiment 17. Eighteenth Day Lesion; Single Injection.—A rabbit received 0.25 mg. of fresh venom on October 26. This caused well marked albuminuria which persisted until death on November 14. Many of the glomerular tufts were compressed by hemorrhage into the capsule or distended by hemorrhage in the center of the tuft itself.

Histological Examination.—The cells of the compressed tufts show marked proliferation with a tendency for the new cells to invade the masses of hemorrhage. No typical mitotic figures are seen, but the character of both the nucleus and protoplasm indicates that these are young cells, and occasionally irregular masses of nuclear material suggest degenerated mitoses.

The period of twenty to thirty days is represented by three animals. Of these one, weighing 1,280 grams, died on the twenty-fourth day after a persistent albuminuria and a loss in weight of 520 grams. The injections were 0.5 of a milligram of venom on October 2 and 4, and one milligram on October 6, with death on October 26.

Microscopic examination shows that the acute lesions had subsided, the only evidence of their earlier presence being the presence of occasional casts and compressed masses of red cells in the glomerular spaces and tufts. About these masses definite proliferation of endothelial cells can be seen, and not infrequently endothelial cells are seen penetrating the hemorrhagic masses. Although no mitotic figures can be clearly distinguished, many of the compressed glomeruli appear to have an increased number of nuclei, and recall the glomeruli of subacute intracapillary glomerular nephritis as seen in man.

Of the other two animals of this group, one, receiving a single injection of 0.25 of a milligram of venom, died after twenty-nine days, and the other, receiving several injections, after twenty-six days. Albuminuria was persistent and marked in each case. Histological examination shows extensive granular degeneration of the convoluted tubules and many casts, but no glomerular lesions.

Seven animals survived for a period of one month or more. Two of these represent a single injection; the others, two to seven injections. The longest period of survival was thirty-eight days.

Albuminuria was persistent in all until death, but although all showed degenerative changes in the tubular epithelium, in none were glomerular changes prominent, and in only two were they even suggestive of reparative proliferation.

These observations indicate that in venom intoxication terminating in death at the end of five to six weeks the early acute glomerular lesion does not tend to become chronic; that is, it does not become transformed through proliferation and reparative processes into a subacute or chronic glomerulonephritis. Proliferation in the presence of an exudative (serum and fibrin) lesion was not seen; in the hemorrhagic lesion, proliferation of the endothelial cells of the tuft and the penetration of these cells into the masses of hemorrhage occurs, but this process is analogous to the organization of a red thrombus and is therefore not similar to the progressive proliferation of tuft or capsule seen in man. The attempt to produce, through the use of venom, a chronic glomerular lesion as the result of repair of acute lesions therefore fails, and with this failure disappears also the possibility of demonstrating experimentally that chronic glomerular lesions in man may have as their origin an injury caused by an endotheliolytic substance.

Several attempts to produce chronic lesions in the dog by repeated injections have likewise been unsatisfactory, owing to the fact that the toxic effect and the degree of hemolysis in this animal are much greater than in the rabbit. As the result of the study of the kidneys of five dogs receiving venom, it may be said that the lesion produced by small doses (0.25 to 0.5 of a milligram per kilo) is a moderate grade of degeneration of the tubular epithelium with cast formation. After the administration of larger amounts this degeneration is more severe, and to it is added the evidence of hemoglobin destruction; but seldom does one see in the glomerular capsule the exudate of serum, and never that of fibrin and red blood corpuscles, which is characteristic of the action of this poison on the rabbit's kidney. On the other hand, a peculiar hyaline change in the capillary loops of the glomerular tuft is a frequent lesion.

In no instance was it found possible to carry a dog for a long period of time. In attempts to accomplish this the smallest dose capable of causing albuminuria was administered, and this dose was

not given again until the albuminuria had disappeared. Despite this, it was not possible to carry an animal longer than two weeks, and at no time could evidence of reparative or subacute glomerular lesions be found.

SUMMARY.

The acute exudative glomerular lesion of the rabbit's kidney caused by crotalus venom does not lead to a subacute or chronic glomerulonephritis. The hemorrhagic lesion of the glomerular tuft may show a process of repair characterized by the ingrowth, into the hemorrhagic masses, of endothelial cells from the uninjured part of the tuft. This process is, however, more analogous to the organization of a red thrombus than it is to any form of glomerular lesion known in man, and can hardly serve as an experimental demonstration of the mode of development of a subacute or chronic glomerular nephritis.

On the other hand, crotalus venom causes a persistent albuminuria and extensive tubular degeneration and cast formation, with death, preceded by great emaciation, after five to six weeks.

EXPLANATION OF PLATE 6.

FIG. 1. Experiment 2. The glomerulus of a rabbit that died on the eighteenth day after repeated injections of crotalus venom. One half of the glomerulus is uninjured and is seen at the left of the figure; in the other half the hyaline area of an early hemorrhage is seen to be invaded by endothelial cells. Leitz oil immersion lens $\frac{1}{12}$; ocular 4.

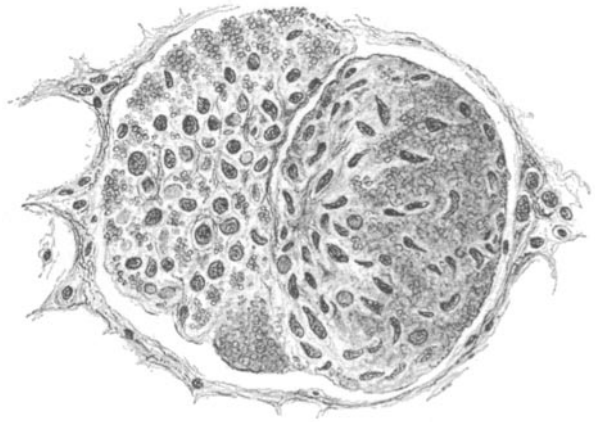

FIG. 1.

(Pearce: Lesions Caused by Crotalus Venom.)