AN EXPERIMENTAL GLOMERULAR LESION CAUSED BY VENOM (CROTALUS ADAMANTEUS).[1]

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PLATE XXIV.

In the course of a recent study of experimental edema (1), rattlesnake venom in small doses was employed, on account of its well-known hemorrhagic action, to bring about the very necessary factor of general vascular injury. For this purpose it proved most satisfactory, without, as a rule, the production of widespread hemorrhages or serious injury to the kidney. The latter, however, in a few instances, presented a very striking and interesting exudative glomerular lesion without evident alteration of the tubular epithelium. It is upon this observation and the experiments to which it led that the present communication is based.

The lesion in question is of double interest: first to those concerned in the experimental study of nephritis; and secondly, to those who have attempted to differentiate the various toxic bodies of snake venom. The recent renewed interest in the study of nephritis by experimental methods centers mainly in an attempt to distinguish between the disturbance due to tubular and to glomerular injury. The important renal poisons affecting only the glomerulus are limited, practically, to cantharidin and arsenic. The addition to this group of venom, which apparently acts through its endotheliotoxic properties is a matter of no small importance to those interested in experimental renal pathology.

On the other hand the lesion throws considerable light on the action of one of the toxic principles of crotalus venom. It may be recalled that in venom poisoning, experimental or otherwise, hemor-

[1] Aided by a grant from the Rockefeller Institute for Medical Research. Received for publication April 5, 1909.

rhagic manifestations are very constant. Mitchell (2), in his first investigation published in 1861, offered no explanation of this lesion, but in his second communication in 1868 (3), as the result of a series of experiments in which venom was applied directly to the exposed mesentery and its action studied under the microscope, he came to the conclusion that hemorrhage is due to the direct action of venom on the vessel wall. "When the venom passes through the peritoneum, it so affects the walls of the capillaries as to allow of their rupture and of the consequent escape of blood." Later, in a third investigation, in association with Reichert (4), it was demonstrable that the hemorrhage occurred not from the arteries and veins but from the capillaries only. At this time they could not find actual breaks in the capillary wall and describe the escape of blood as apparently a filtering of the blood through the capillary wall.

No further important contribution to this subject was made until 1902 when Flexner and Noguchi (5) published the results of an extensive study of the constitution of snake venom. In addition to the hematoxic and neurotoxic principles they recognized as the chief toxic constituent of crotalus venom, an endotheliolytic body to which they gave the name "hemorrhagin" and this they considered responsible for the hemorrhages. They found the hemorrhages limited to the capillaries and small veins. Although the extravasation took place through actual rents in the vessel wall, these breaks in continuity were not simple ruptures for they observed microscopically a disappearance as though by solution, of the parts of the wall at the site of hemorrhage. They therefore considered hemorrhagin as a cytolysin for the endothelial cells of blood vessels.

This observation of Flexner and Noguchi has not, as far as I am aware, been confirmed, and when I used venom as a vascular poison to aid in the production of an experimental edema I had no thought of confirming their observation, as I believe I have done, by demonstrating lesions in such an exquisitely delicate endothelial structure as the capillary tuft of the glomerulus.

The glomerular lesion to be described is essentially a vascular lesion, due apparently to a change in the endothelium of the glome-

rular capillaries. It presents two types: the first, extensive hemorrhage into the tuft leading to enlargement and distortion of the tuft with or without rupture; the second, an extensive exudation of the fluid or cellular elements of the blood into the tuft or the capsular space. The cells lining Bowman's capsule show no change while the tubular epithelium generally is either normal or shows only a moderate granular or vacuolar degeneration.

A thorough search of the available literature dealing with venom intoxication fails to reveal an earlier study of these lesions. Several investigators, as first Mitchell, speak of hemorrhages in the kidney visible to the naked eye, or of bloody urine in the bladder, and Mueller (6) describes hematuria in three individuals bitten by the tiger snake, but histologic studies of the kidney appear to be lacking.

The experiments number twenty-one, in all of which the rabbit was used. For the most part the animals received venom alone but a few experiments are included from the earlier work on edema and in these an excess of water was administered by the stomach or chrome salts were given subcutaneously.

The venom used was the dried venom of *Crotalus adamanteus* obtained through the courtesy of Dr. Hideyo Noguchi of the Rockefeller Institute for Medical Research. A standard solution was made with 0.85 per cent. salt solution of such strength that 1 c.c. contained 0.5 mg. of the dried venom. A single dose of 0.5 mg. given intravenously is frequently sufficient to cause the death of a rabbit weighing 1,500 gm. in from five to six days, but in such animals I have not found frank glomerular lesions. These occur more frequently when this dose is given on each of two or three days and almost constantly follow a single dose of 1 mg. or a total administration of 2 mg. in four doses. With a large single dose the animal usually develops hematuria or hemoglobinuria within twenty-four hours, but this rarely occurs with two or three small doses. With the latter dosage, albuminuria of varying grade develops, though generally not until late in the intoxication. As a rule, the animals have been killed when a frank hematuria or albuminuria appeared. This was done, with few exceptions, in order to avoid the rapid post-mortem decomposition, characteristic of venom poisoning, so inimical to proper histological study. As

a result, eleven of the fourteen rabbits with renal injury represent lesions of the fifth or sixth day; the other three represent periods varying from one to four days. The seven rabbits which showed no lesions were animals receiving a single dose or several small doses, and, with the exception of one dying in twenty-four hours and one killed after thirteen days, fall within the three to five day period.

The lesions in the fourteen animals with glomerular involvement may, in a general way, be divided into two groups, one in which exudative phenomena predominate and one in which the characteristic lesion is a hemorrhage into the glomerular tuft. The two lesions may be associated in the same kidney though one or the other type usually predominates in any one kidney. Several factors, as the dose of venom, the period of intoxication, the age (weight) of the animal and the simultaneous administration of other substances as chrome salts or excess of water have all been considered in an effort to determine the cause of difference in type. None of these factors appear to have a determining influence. It it noteworthy, however, that glomerular hemorrhages were very prominent in four rabbits receiving potassium chromate in addition to venom. This salt given alone produces no change in the glomeruli which can be recognized anatomically but does cause widespread injury, with extensive necrosis, of the tubular epithelium. That it may in these experiments have influenced the lesion due to venom is possible but the manner of so acting is not clear.

The material for histological study was hardened in Zenker's fluid and in alcohol, imbedded in celloidin and stained with hematoxylin and eosin, Weigert's fibrin stain and Mallory's connective tissue stain. Frozen sections of formalin-hardened material were stained by Sudan III and hematoxylin.

The hemorrhagic lesion will be described first. Its chief feature is the amount of hemorrhage which may occur in the substance of the tuft without rupture of the outer wall and the escape of blood into the capsular space (Fig. 1). The hemorrhage which may be single or multiple causes a distention of the tuft to two or three times the normal size. The red cells are closely grouped and form, frequently, more or less hyaline masses in which the individual cells

may not be distinguishable. This appearance may be due in part to the agglutination and fusion of cells, as described by those who have studied the effect of venom on the red cells. Sometimes when the hemorrhage is distinctly central the greatly thinned outer wall of the tuft gives the appearance of a thin-walled cyst containing blood. If the hemorrhage is to one side, the remainder of the glomerulus is compressed into a crescent-like mass and the capillaries of the altered tuft contain little if any blood. Many of the larger hemorrhages give, on account of the compression of the rest of the tuft, the appearance of a hemorrhage into the capsular space, but that this is not the case can always be demonstrated by finding a narrow clear space between the cells of Bowman's capsule and the outer border of the tuft. Small accumulations of fibrin or serum may be associated with the hemorrhage but leucocytes are rarely found.

Frequently associated with this definitely intra-glomerular hemorrhage are other glomeruli with free hemorrhage into Bowman's capsule with (Fig. 2) or without the presence of fibrin. In such instances definite evidence of rupture of the tuft is usually seen, the broken fragment of the destroyed tuft sometimes lying free in the midst of red blood cells or more frequently attached to one side of the capsule. At other times the glomerular space is filled with blood without evidence of the destroyed tuft. The coils of the convoluted tubules in the neighborhood of such glomeruli usually contain a considerable amount of blood.

The extent of the hemorrhagic involvement varies. Sometimes nearly every glomerulus is affected; in other animals the ratio is one to two, three, four or five; rarely it is only one to twelve or twenty according to the severity of the intoxication.

The second type of lesion is that characterized by exudation. This may occur alone or may be associated with intraglomerular hemorrhages. All the elements of the blood may appear in the exudate. Red cells frequently form a large part of the picture, but exudates of serum only (Fig. 3) or of serum and fibrin are frequent. Polymorphonuclear leucocytes are seldom seen except as occasional isolated cells (Fig. 4).

The exudate, no matter what its character, distends the capsular

space to two or three times its normal size; the tuft is usually compressed at one side but may remain in its central position. According to the bulk of the exudate either the latter or the tuft may take a crescentic shape. A common picture is that of a delicate fibrin network in which are enmeshed red blood corpuscles and the granules of coagulated serum. Sometimes the fibrin appears as a dense hyaline mass surrounded by red cells (Fig. 1). Some glomeruli contain red cells only or serum only. The kidneys of one animal showed in practically every capsular space a large crescentic finely granular mass of serum. In another, a closely packed fibrinous exudate, homogeneous in appearance and staining deeply with eosin, occurred in three out of every four glomeruli.

Not infrequently the exudate is within and limited to the capillary tuft. Under such circumstances the tuft is transformed into a cavity with a thin wall, giving a ring-like appearance; the cavity of the ring being filled with serum and fibrin (Fig. 5). More rarely, two or three small cyst-like cavities in the tuft contain serum and fibrin threads. At other times the capillaries of the tuft are indistinguishable on account of the presence of strands of hyaline fibrin which give the appearance of a fibrinoid degeneration of the tuft.

It is evident that these phenomena constitute a vascular nephritis and that the primary injury is one affecting the glomerular capillaries and analogous therefore to the vascular lesions occurring elsewhere in the body in venom poisoning and leading to hemorrhage. The glomerular lesion differs from hemorrhage elsewhere in that the phenomena of exudation are added to those of extravasation, or at least can be more readily demonstrated. The question naturally arises: Does the study of this lesion of the capillaries of the glomerulus throw any light on the question of the mode of extravasation of blood in venom poisoning? Reference has been made to Mitchell's observation that the hemorrhage occurs from the capillaries and to the demonstration by Flexner and Noguchi of an endotheliolytic body, "hemorrhagin," in crotalus venom. If such a body exists and if it is responsible for the exudation of the fluid elements of the blood and the extravasation of red cells, the glomerular capillaries should show evidence of its action, and

for such evidence I have searched. Readily demonstrable are the coarser changes, as distention or rupture of the tuft by the accumulation of the blood or exudate, all of which point to weakening of the vessel wall. By careful study may be found finer changes indicative of definite injury to the capillary walls. These changes are seen more readily in connection with the exudative type of lesion. The walls of the capillaries frequently appear swollen and granular with pycnotic or swollen, poorly stained nuclei which are apparently reduced in number (Fig. 2). In some glomeruli the outer portion of the tuft has no distinct sharp outline but an irregular indistinct frayed appearance, as if undergoing solution or gradual disintegration (Fig. 3). Several loops of dilated but empty capillaries sometimes may be seen, usually at the periphery of the tuft, entirely devoid of nuclei (Fig. 1). Others containing blood have a distended congested appearance with a similar scarcity or entire absence of nuclei. Occasionally in the midst of a mass of exudate may be seen a tuft with nuclei in its central portion but none at the periphery. Such a picture is suggestive of tissue erosion or solution. These changes, which do not occur in the ordinary forms of experimental nephritis, suggest, in the absence of evidence of definite tissue necrosis, a disintegration process analogous to autolysis and to be explained by the action of Flexner and Noguchi's endotheliolytic body, "hemorrhagin." In support of this view of the selective action of venom on the glomerular endothelium is the almost complete absence of injury to the tubular epithelium. Casts, in which the products of hemolysis are mingled with exudate, were abundant, but the coarse granular and epithelial casts of tubular origin were not present. In most of the animals the tubular epithelium showed no change; in a few, it was granular and swollen and of the general character of a well-marked cloudy swelling; occasionally, a vacuolar degeneration was evident but at no time necrosis or nuclear destruction and only rarely was fat demonstrable by special stain (Sudan III). This holds also for the cells lining Bowman's capsule, which was almost always well preserved and presented properly staining nuclei. This observation in itself, considered in connection with the extensive lesions in the almost contiguous tuft, would appear to be conclusive as to the selective action of venom.

SUMMARY.

The venom of *Crotalus adamanteus* when administered intravenously to rabbits in properly graded doses causes lesions of the glomerulus of the kidney which may be either hemorrhagic or exudative in character. Both types of lesion are usually associated but either one or the other may predominate. The hemorrhagic lesion, which may be confined to the glomerular tuft, or, by rupture of the latter, may involve the capsular space, is a peculiar localization of the hemorrhage so common in other parts of the body in venom intoxication. On the other hand, the exquisite exudative lesion involving usually the capsular space but sometimes limited, as in the hemorrhagic type, to the tuft itself and with little or no tubular injury, constitutes a type of experimental vascular nephritis, hitherto undescribed, which differs widely in its anatomical appearance from that due to arsenic, cantharidin and other vascular poisons.

As the limitation of the lesion to the glomerulus indicates a selective action of the venom, and as the histological changes in the tuft are suggestive of gradual endothelial destruction and solution, the lesion can be explained by the action of the endotheliolytic body of crotalus venom described by Flexner and Noguchi.

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EXPLANATION OF PLATE XXIV.

FIG. 1. The figure illustrates hemorrhage limited to disintegrated portion of tuft without involvement of the capsular space. It shows also compression of remainder of tuft with dilated capillary loop devoid of endothelial cells. From a rabbit weighing 1,070 gm. which received 0.5 mg. of venom and 0.05 gm. potassium chromate on first and fourth days and was killed on fifth day (albuminuria).

FIG. 2. The figure illustrates hemorrhage into capsular space and presence of large mass of compact fibrin. From a rabbit weighing 1,265 gm. which received 0.5 mg. of venom on first day, 1 mg. on second day and 0.5 mg. on fifth day; killed on sixth day (hematuria).

FIG. 3. The figure illustrates extensive pure serous exudate in capsular space and also the common type of disintegrative lesion of the tuft. From a rabbit weighing 1,260 gm. which received 0.5 mg. venom on the first and third day and 75 c.c. of water by stomach tube daily; killed on fourth day.

FIG. 4. The figure illustrates combined capsular and intra-glomerular lesions with compression of tuft. Intra-glomerular lesion shows maximum accumulation of leucocytes observed in any lesion. From a rabbit weighing 2,010 gm. which received 0.5 mg. on first, second and fifth days and 100 c.c. of water by stomach daily; killed on sixth day.

FIG. 5. The figure illustrates exudative lesion (fibrin and red blood corpuscles) limited to the tuft which, except for compressed portion below, is transformed into a cyst-like body. (From same kidney as Fig. 1.)

All drawings are made from sections stained in hematoxylin and eosin after Zenker hardening and celloidin imbedding ($\frac{1}{8}$ obj., 4 oc., Voightlaender).

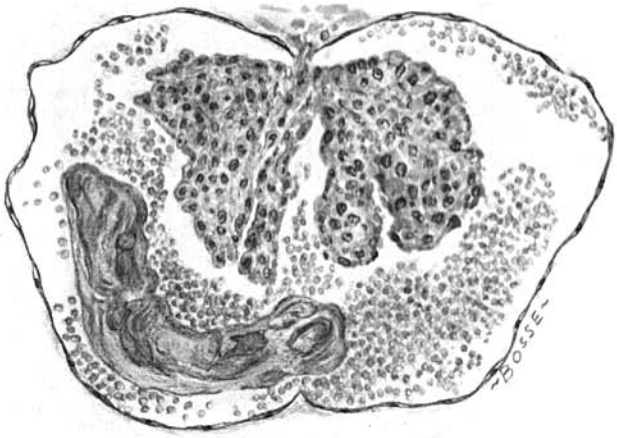
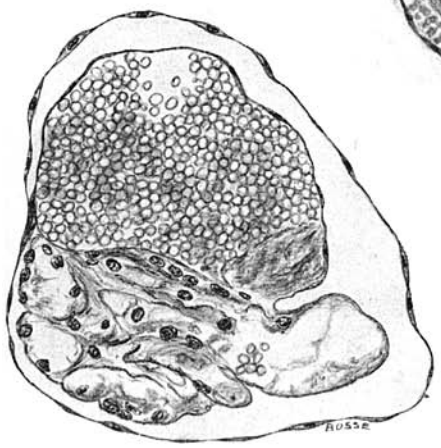


FIG. 2.



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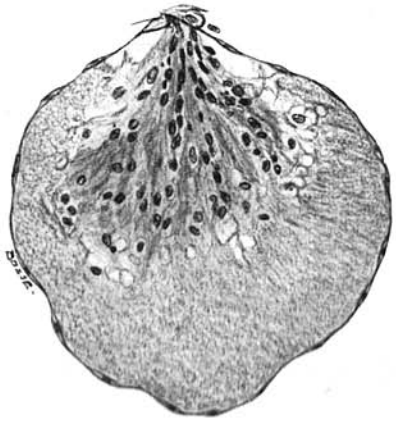

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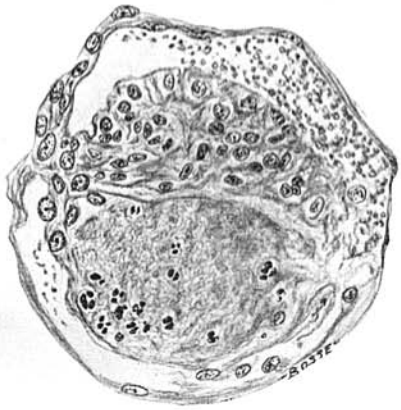

FIG. 4.

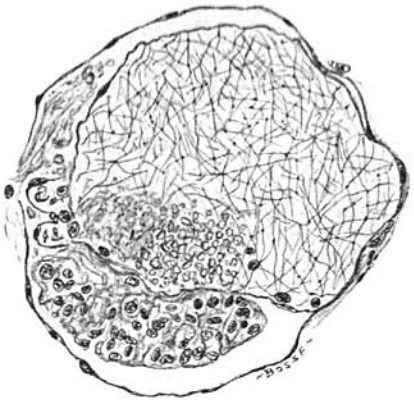

FIG. 5.