PATHOLOGICAL CHANGES PRODUCED BY THE SUBCUTANEOUS
INJECTION OF RATTLESNAKE (*CROTALUS*) VENOM
INTO MACACA MULATTA MONKEYS *

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Accounts of the gross and microscopic lesions in fatal cases of rattlesnake (*Crotalus*) poisoning are rare. Willson¹ in 1908 collected 48 fatal cases, and of these there had been only 4 post-mortem examinations recorded. Even in these cases the autopsies were incomplete and none included histological examination of the tissues removed.

Complete descriptions of the gross and histopathological changes in tissues following the administration of rattlesnake venom are based almost entirely on observations in experimental animals. Taube and Essex² have reported in detail the pathological changes in dogs which received fatal doses of rattlesnake venom intravenously. The lesions ordinarily found in man follow subcutaneous administration and are not as intense or widespread as those found by Taube and Essex. The dogs used by these investigators died in from 8 minutes to 20 hours following the injection of venom, and showed petechial and massive hemorrhagic lesions which involved practically all the serous and mucous membranes of the body, as well as being distributed throughout many of the parenchymatous organs. Recovery is the general rule, as shown by a mortality rate of 8 per cent in a series of 50 cases of rattlesnake bite reported by Mitchell,³ and 11.7 per cent in a series of 408 cases reported by Willson.¹

Jackson's⁴ observations on dogs which received subcutaneous injection of venom in the thigh are more nearly comparable with those on individuals bitten by rattlesnakes. Postmortem examination revealed a local, spreading hemorrhagic lesion in the tissues about the site of injection. The regional lymph nodes were in-

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tensely hyperemic and occasionally necrotic. The abdominal lymph nodes were involved later.

The pathological lesions produced by subcutaneous injections of rattlesnake venom into the monkey have not been described, as far as we know. The object of this paper is to report the gross and microscopic lesions in the *Macaca mulatta* monkey resulting from the subcutaneous administration of a lethal dose of rattlesnake venom.

MATERIAL AND METHODS

Nine young *Macaca mulatta* monkeys weighing from 2770 to 3950 gm. were given subcutaneous injections of *Crotalus* venom into the lumbar regions. The dry powdered venom was dissolved in physiological saline to make a 1 per cent solution. This toxic solution was divided into two equal parts and injected into both lumbar regions. The dose varied from 7 to 10 mg. per kilo of body weight.

The animals were autopsied on an average of 2 hours after death. Tissues were preserved in Zenker's fluid, formalin and 80 per cent alcohol. Sections were stained with Delafield's hematoxylin and eosin, Heidenhain's aniline blue stain, the scharlach R stain for fat, and by the phosphotungstic acid hematoxylin method.

CLINICAL OBSERVATIONS

Immediately after the injection of venom into the subcutaneous tissues the monkeys became disturbed and excited. After being released they ate, drank and behaved about as usual for several hours. A visible subcutaneous swelling and reddening of the skin about the site of injection gradually developed. After several hours the animals began to appear ill, as evidenced by weakness, torpidity and disinclination to eat. This condition increased in severity until finally death occurred at an average of 36.5 hours after injection. There was never any vomiting or passing of either bloody stools or bloody urine.

AUTOPSY OBSERVATIONS

The skin was discolored dark red in the lumbar region at the site of injection, and this appearance extended from this point into the anterior abdominal wall. When the skin was incised intense

hemorrhagic edema was found to be much more extensive than the discoloration of the overlying epidermis would indicate. This edematous process extended from the midthoracic dorsal region down to the buttocks, and occasionally as far as the knees. On the ventral surface of the body it involved the subcutaneous tissues from the groin up to as high as the middle of the thorax.

The subcutaneous connective and adipose tissues were four to six times their usual thickness. The cut surface was intensely red and thin bloody fluid ran out freely. The advancing edges of the lesion were edematous but the fluid was colorless or pale pink. The lesion was limited in depth by the deep fascia covering the abdominal, thoracic and lumbar muscles.

The regional lymph nodes were swollen and red. This was most marked in the inguinal and iliac nodes, but was quite noticeable in the axillary and preaortic nodes in most of the animals.

On opening the heart the most striking lesion was the presence of small patches of hemorrhage in the left ventricle beneath the endocardium, usually in the interventricular septum near the aortic ring. This was seen in 6 of the 9 animals. Usually slight in extent, in 1 animal it also involved the papillary muscles of the cusps of the mitral valve. In 1 animal also there were subendocardial hemorrhages in both auricles. In 8 of the monkeys the heart's blood was unclotted.

The lungs were well aerated, pink in color, and in 1 of the monkeys there were a few, small, subpleural hemorrhages.

The peritoneal cavity did not contain an excess of fluid. The visceral blood vessels were collapsed and contained very little blood. The intestines were a pale grayish color.

The liver was not unusual, except for a mild passive congestion in 3 animals. In 1 animal there were several small petechial hemorrhages along the anterior border.

The spleen was usually small, firm, and a slate gray in color. The cut surface was red and dry, and very little blood could be scraped from the surface.

The kidneys were pale but showed no other gross changes. In several animals the urinary bladder contained a small quantity of clear, straw colored urine.

The pancreas and adrenals appeared to be normal.

The gastro-intestinal tract showed no constant lesion. One

animal had a small, acute gastric ulcer measuring 0.5 cm. in diameter. Two others showed several small petechial hemorrhages in the submucosa of the colon.

The brain and upper part of the spinal cord were examined in each animal, and the only change of note was a slight congestion of the cerebral vessels.

HISTOLOGICAL OBSERVATIONS

Skin and Subcutaneous Tissues at Site of Injection: There was a marked infiltration of the subcutaneous tissues with erythrocytes and considerable edema. The connective tissue fibers were fragmented and widely separated, and their nuclei stained poorly. Small blood vessels were seen in which the endothelial lining cells were swollen and their cytoplasm was eosinophilic and granular. The nuclei of these cells showed various retrogressive changes. Actual ruptures in the walls of these vessels were seen in places. Many vessels contained thrombi which partially or completely occluded the lumen. Accompanying the necrotic changes in the vessel wall were usually a few, scattered polymorphonuclear leukocytes. The capillaries were greatly distended and in some places appeared to be actually ruptured. Some showed homogeneous thrombi in their lumens. The extravasated erythrocytes were somewhat swollen and spherical, and stained very lightly with eosin. There were scattered collections of polymorphonuclears, lymphocytes and a few mononuclear cells, none of which contained phagocytosed erythrocytes.

The reaction was less severe at the edge of the lesion. There were fewer erythrocytes, but still a considerable quantity of faintly pink staining exudate was seen in the tissues. The fibrous connective tissue strands were not fragmented and nuclei of the fibrocytes stained normally. The capillaries and small blood vessels were greatly dilated but necrotic or thrombotic lesions were not seen.

The overlying epidermis showed no pathological changes except in 1 animal which lived 6 days and which developed a large area of necrosis at the site of injection.

Lymph Nodes: The nodes showing the most advanced and intense reaction were the inguinal, internal iliac and abdominal preaortic nodes. A similar but less intense reaction was found in

the axillary, and occasionally in the tracheobronchial and submaxillary nodes. In its most intense form there was great dilatation of the medullary sinuses which were filled with erythrocytes and large mononuclear phagocytic cells, some of which were distended with as many as fifteen to thirty red blood cells. The lesion was much less marked in the cortex of the nodes. The peripheral sinus, although usually slightly dilated, contained only relatively few monocytes and lymphocytes, and occasionally a few erythrocytes. The medullary blood vessels and lymphatics were dilated but no actual breaks in the vessel walls were found.

In some lymph nodes showing the most intense reaction there was a patchy infiltration with polymorphonuclear leukocytes. In places there was actual suppuration.

Lymph nodes in which the reaction was less severe showed mainly a proliferation of large monocytes in the lymph sinuses of the medulla. Some of these monocytes contained a few brown pigment granules and erythrocytes, but most of the cells contained very little phagocytosed material.

Heart: There was usually a moderate amount of myocardial fragmentation. In 6 animals there were small extravasations of blood into the subendocardial tissues of the left ventricular septum. These collections of erythrocytes elevated the endocardium and separated the superficial myocardial fibers. In 2 animals there was an infiltration with polymorphonuclear leukocytes. In 3 there was a well marked fatty degeneration of the myocardium; this was most marked in the superficial fibers close to the hemorrhagic lesion and was not seen in the more distant fibers.

Aorta: No pathological changes were found except in 1 animal where small deposits of fat were situated in the deeper part of the intima.

Lungs: The alveolar sacs were well distended with air and in 4 of the 9 animals this suggested the presence of a slight degree of emphysema. The alveolar capillaries were not unduly distended.

Liver: The venous sinusoids were slightly dilated with blood. In several animals the Kupffer cells seemed somewhat swollen, but this change was not prominent. The liver cells showed a moderate amount of granular degeneration. A small amount of fat was demonstrated in the liver cells of 4 animals and a large amount in 1.

Kidneys: Sections stained with Delafield's hematoxylin and eosin and with Heidenhain's aniline blue stain failed to reveal any pathological changes aside from slight granular degeneration of the tubular epithelium. The endothelial and epithelial cells of the glomerular tuft appeared normal, except for possibly some slight swelling, and were separated in the usual manner by a thin basement membrane. Sections stained with scharlach R showed a moderate increase of fat stored in the form of small globules in the collecting and convoluted tubules in 3 animals.

Gastro-Intestinal Tract: This was essentially normal throughout. In 2 animals small extravasations of blood were present in the submucosa of the colon. A small acute ulcer of the gastric mucosa was found in 1 animal.

Brain and Spinal Cord: There was slight congestion in the small blood vessels of the meninges, gray and white substance of the brain, and of the spinal cord. The cellular changes were not marked but there was evidence of some chromatolysis.

DISCUSSION

It is evident from these observations that rattlesnake venom administered subcutaneously acts as a powerful tissue irritant. In its stronger concentrations it causes actual dissolution of blood vessels and connective tissue. At the advancing edge of the lesion, where the venom is somewhat diluted with tissue fluid, no necrotic vascular lesions are seen and the dilated capillaries and venules show only a somewhat swollen endothelial lining. The altered permeability of these vessels to fluids is evidenced by marked edema in the surrounding tissue. It is probably from this region that maximum absorption of venom-containing fluid takes place.

Drainage from the local lesion is partly by way of the regional inguinal lymph nodes. Here the diluted venom causes great dilatation of the medullary sinuses and proliferation of the reticulo-endothelial cells. The venom is attenuated sufficiently so that necrotic or thrombotic lesions are not found in the blood vessels of these lymph nodes. However, the many free and phagocytosed red blood cells in the medullary lymph sinuses are evidence of the increased permeability of these vessels, which is indicated microscopically by only slight swelling of the vascular endothelium. From the regional lymph nodes the venom-containing lymph

passes along the iliac, preaortic and mediastinal chains of nodes.

Widespread lesions in the organs supplied by the systemic circulation were not striking. There was moderate granular degeneration of cardiac musculature and of the proximal convoluted and collecting tubules of the kidney. None of the exudative or hemorrhagic lesions of glomerular tufts, as described by Pierce⁵ following the intravenous administration of venom into rabbits, were seen. Apart from a rather indefinite swelling of the glomerular endothelial cells, no constant lesion was present.

Mitchell and Reichert⁶ first pointed out that snake venom is a complex material, both as to chemical and as to physiological properties. Flexner and Noguchi^{7,8} established the presence of agglutinins and hemolysins and presented evidence suggesting the presence of a neurotoxin and an anticomplementary body. The cytolytic which causes retrogressive changes in various cells, particularly those of the vascular endothelium, is undoubtedly the hemorrhagin referred to by Flexner and Noguchi. They claimed that the hemorrhages all take place by actual dissolution of the walls of vessels and never by diapedesis of erythrocytes. We have seen these tears in the smaller blood vessels and capillaries, but many vessels show only some degree of damage, as evidenced by nuclear changes and a swollen, indistinct vessel wall infiltrated with a few polymorphonuclear leukocytes. There is no actual rupture although there are erythrocytes in the vessel. It appears, therefore, that *Crotalus* venom may injure vascular endothelium to an extent, varying in proportion to its concentration, from merely a functional change which allows increased permeability to fluids and diapedesis of red blood cells, to actual necrosis, intravascular thrombosis and rupture of the wall.

Mitchell and Reichert⁶ and Rogers⁹ have described a profound fall in blood pressure after administration of crotalin, and Rogers noticed that this was accompanied by marked portal vascular dilatation. Taube and Essex² have redirected attention to this generalized vascular dilatation and point out that the gross and microscopic lesions following intravenous administration of venom are identical with the pathological lesions Moon¹⁰ has suggested are present in the shock syndrome.

While the results obtained seem to point to the conclusion that shock is the major factor in the death of an animal injected with

crotalin intravenously, the evidence is not so clear for this assumption following subcutaneous administration. Moon¹⁰ says: "The evidence does not support the idea that local loss of blood and/or fluid at the site of injury is an adequate explanation for shock." In our monkeys this local loss of blood and fluid was the most marked feature present. Any hemorrhage and edema appearing elsewhere was negligible in amount. The abdominal and thoracic viscera, instead of showing the marked engorgement, edema and hemorrhage associated with shock, were pale, their cut surfaces dry, and the blood vessels were collapsed. The local lesion, consisting of extensive subcutaneous hemorrhagic edema, may however, as Moon suggests, "contribute to the circulatory deficiency in proportion to the volume of blood and fluid lost." In our animals the subcutaneous hemorrhagic lesions were very extensive and involved about 50 per cent of the body surface.¹¹ It is possible that this local lesion may have caused enough disparity between the blood volume and the volume capacity of the vascular system to produce at least some degree of shock.

Before the primary cause of death can be ascribed to shock, however, it would seem essential to eliminate the possibility that some other fraction of the venom, such as the neurotoxin, may have a major or at least an important contributory part in fatal termination.

SUMMARY AND CONCLUSIONS

Lethal doses of rattlesnake (*Crotalus atrox*) venom were administered subcutaneously into the lumbar regions of 9 *Macaca mulatta* monkeys. Death occurred on an average of 36 hours after injection.

The outstanding lesion at autopsy was a marked hemorrhagic edema at the site of injection which spread extensively throughout the adjacent subcutaneous tissues. The regional lymph nodes were greatly swollen and hemorrhagic; the more distant nodes were less affected. In 6 of the 9 animals petechial hemorrhages into the subendocardial tissues of the left ventricle occurred. These were the only constant gross lesions. In 1 animal, small subpleural hemorrhages and subcapsular hemorrhages of the liver were found, and in 2 others there were similar lesions in the cecum.

Microscopically, in the region of the hemorrhagic edema necrotic

changes were found in the walls of the small blood vessels and capillaries. Many of these vessels contained ante mortem thrombi. The exudate in the surrounding tissues consisted mainly of erythrocytes and fluid but there were also scattered collections of polymorphonuclear leukocytes. The regional lymph nodes showed marked hyperplasia of the reticuloendothelial cells, which was most marked in the medullary portion. Here, erythrocytes lay free in the dilated sinuses and appeared to be phagocytosed by the reticuloendothelial cells. The hemorrhagic lesion in the left ventricle showed, in addition to the capillary dilatation and extravasation of blood into the tissues, fatty degeneration of the myocardium in the immediate region of the lesion. There was slight granular degeneration of the parenchymatous cells of the liver and kidney, and in 3 animals there was fatty degeneration of the kidney tubules.

Rattlesnake venom is a powerful tissue irritant which causes necrosis and actual dissolution of blood vessels with subsequent extravasation of erythrocytes and serum into the tissues.

Absorption seems to be mainly from the region of the advancing edge of the subcutaneous hemorrhagic lesion. At this point the venom-containing edematous fluid seems to be absorbed by the lymphatics and passes up the inguinal, iliac and mediastinal chains of nodes.

Shock seems the most probable major factor in the death of animals injected with venom intravenously, but the evidence is not so clear for this assumption following the subcutaneous administration. Our animals showed no generalized vascular lesions, only a subcutaneous loss of blood and fluid, so extensive however that it might be considered capable of at least producing some degree of shock.

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DESCRIPTION OF PLATES

PLATE 73

- FIG. 1. Small blood vessel in the subcutaneous adipose tissue near the site of crotalin injection. The wall of the vessel is swollen and indistinct and erythrocytes are seen passing through by the process of diapedesis. $\times 850$.
- FIG. 2. Blood vessel in the region of subcutaneous hemorrhagic edema. An ante mortem thrombus is attached to the endothelium and partially occludes the lumen. The wall is ruptured in one place and erythrocytes are infiltrating the adjacent tissues. $\times 600$.

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Fidler, Glasgow and Carmichael

Injection of Rattlesnake (*Crotalus*) Venom

PLATE 74

FIG. 3. Medullary portion of an inguinal lymph node. The lymph sinuses are dilated and contain many erythrocytes, some of which are seen within large phagocytic mononuclear cells. $\times 400$.

FIG. 4. Myocardium of the left ventricle in the region of the intraventricular septum. The endocardium is elevated by a collection of erythrocytes. Similar collections are seen separating the underlying bundles of myocardial fibers. $\times 400$.

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4

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Injection of Rattlesnake (*Crotalus*) Venom