Some Preliminary Studies of "Stress" in the Western Diamondback Rattlesnake (Crotalus atrox)

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A total of 12 animals *(Crotalus atrox)* were used, 4 serving as control specimens and 8 as experimentals. The control animals consisted of 2 males and 2 females which were captured in Roman Nose State Park, Blaine Co., Oklahoma, on April 5, 1959. They were brought to the Medical School immediately following capture and sacrificed. The 8 experimental animals were received at various times from the Lincoln Park Zoo Reptile Garden in Oklahoma City through the courtesy of Mr. Robert Jenni, Curator of Reptiles. These were selected from new shipments from the Rio Grande valley of southern Texas. Blood was drawn by cardiac puncture, and the organs were dissected free, weighed, and preserved in 10% neutral formalin. The experimental animals were kept in a special box in the animal house at the Medical School. They were given water but no food. No record was kept of their water consumption.

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The experimental animals were "stressed" each day at approximately the same time until death ensued. The "stress" consisted of lifting each animal from the box with a handling "hook," placing it on the floor, pinning its head with the hook and picking it up by hand. This was done by grasping it at the posterior portion of the head and keeping the quadrate and mandibular bones firmly under pressure. Each animal was held in this fashion for 3 to 5 minutes during which time the mouth was inspected for any signs of injury. This procedure was repeated daily until the animal began to show signs of incoordination. These consisted of incoordinate head movements when crawling and also following manipulation with the hook.

These signs would usually begin to appear at 24 to 48 hours prior to death. From this point the animals were kept under close observation and sacrificed when judged to be very near death. At least one specimen was lost to the record when it died during a brief interval when it was not being observed. Post mortem changes rendered the tissues valueless for histological studies.

At autopsy 5 cc of blood were drawn from each animal, allowed to clot at room temperature for 30 minutes and refrigerated at 4° C. The serum proteins were studied by electrophoretic separation using a Spinco Model R Paper Electrophoresis System.

Tissue sections were cut at 7-10μ following paraffin embedding and stained with Hematoxylin and Eosin.

RESULTS

The histological changes observed in the tissues investigated were not as marked as might have been expected. A certain irregularity of the cord structure in the adrenal cortex was noted along with a marked degree of vacuolization of the cortical cells indicating the presence of an alcohol soluble material. The normal tissue exhibited a regular cord-like arrangement of cortical cells with a less-marked degree of vacuolization. The thyroid tissue of the experimental animals showed a definite decrease in the height of the follicular epithelium with small flattened nuclei while that of the normal controls was a fairly high cuboidal epithelium with well rounded nuclei. No detectable differences were noted in the thymuses of the experimentals over those of the controls.

In comparing the serum electrophoretic patterns the loss of one particular peak was noted in the patterns of the experimentals. This same fraction appeared albumin-like in that it was soluble in a 27.7% saturated Na_2SO_4 solution.

Changes were also observed in the weights of the adrenals and thyroids. (Table 1)

Table 1. Body and organ weights of stressed and unstressed rattlesnakes.

	Controls	Range	Experimentals	Range
Body Wt. (gms)	1117.7	963-1282	939.8	210-2116
Adrenals (mgs)	258.7	170-344	349.1	81.6-564
% of Body Weight	.022		.037	
Thyroid (mgs)	68.7	48-94	91.1	12-268
% of Body Weight	.006		.009	

The per cent weight of the adrenals of the experimental animals represents a relative increase of 68% over that of the controls. These increases as well as the histological changes of this organ described above, are

considered to be significant, and are indicative of changes which might be expected in the exhaustion stage of the General Adaptation Syndrome (1,2). Interestingly enough, no gastrointestinal lesions or hemorrhages were observed in any of the experimental animals.

The weight increase noted in the thyroid of the experimental animals as well as the histological changes in this organ described above probably represent a normal physiological response to temperature differences between the hibernating and active states.

SUMMARY AND CONCLUSIONS

Histological changes and increases in weight were noted in the adrenal glands of all experimental animals. Changes were also noted in the serum electrophoretic patterns of these animals. It is suggested that these changes are due to the stress mechanisms.

The changes observed in the histological structure and weight of the thyroid glands of the experimental animals are interpreted as normal physiological responses of that gland to higher temperatures.

LITERATURE CITED

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