1. Rosenheim, O., Webster, T. A., *Biochem. J.,* 1941, v35, 920.

2. Carroll, K. K., *J. Lipid Research,* 1960, v1, 171.

3. Carter, H. E., Norris, W. P., Glick, F. J., Phillips, G. E., Harris, R., *J. Biol. Chem.,* 1947, v170, 269.

4. Deichmann, W. B., Radomski, J. L., McDonald, W. E., Kascht, R. L., Erdmann, R. L., *A.M.A. Arch. Ind. Health,* 1958, v18, 483.

5. Baldwin, E., *Dynamic Aspects of Biochemistry,* 3rd Ed. Cambridge Univ. Press, Cambridge, 1957, p271.

6. Blaschko, H., *Pharmacol. Rev.,* 1952, v4, 415.

7. Fouts, J. R., Blanksma, L. A., Carbon, J. A., Zeller, E. A., *J. Biol. Chem.,* 1957, v225, 1025.

8. Bloom, B., Chaikoff, I. L., Reinhardt, W. O., *Am. J. Physiol.,* 1951, v166, 451.

9. Davison, A. N., *Physiol. Rev.,* 1958, v38, 729.

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Some Properties of Rattlesnake Venom Following 26 Years Storage.* (25654)

FINDLAY E. RUSSELL, JERRY A. EMERY AND TRUMAN E. LONG

Lab. of Neurological Research, College of Medical Evangelists, Los Angeles County Hospital

Desiccated extracts of some snake venoms retain their lethality for long periods of time when stored in closed containers in the dark (1,2,3,4). However, certain evidence suggests that some desiccated venoms may deteriorate with time even under optimum conditions of light, vacuum and temperature(3,5). While stability of a venom may be a function somewhat dependent upon the family, genus, or species, lethality and specific modes of action of the venom can be altered by the method of storage.

Studies dealing with toxicity of venoms following periods of long storage are usually confined to determinations of m.l.d. or LD_{50} and give no indication of the changes in zootoxicologic properties. The possibility must be considered that fractions of these complex mixtures may be altered without markedly influencing lethality. The present investigation was initiated to determine the lethal doses of certain rattlesnake venoms stored for 26 to 27 years, and to ascertain the status of several of the known zootoxicologic properties of these venoms(6).

Materials and methods. The venoms were extracted and prepared on the following dates after the methods described by Klauber(6): *Crotalus atrox,* lot 451, July 16, 1932; *C. cerastes,* lot 453, July 16, 1932; *C. mitchelli mitchelli,* lot 510, July 4, 1933; *C. ruber ruber,* lot 496, July 17, 1933; and *C. viridis viridis,* lot 462, Sept. 10, 1932. The intravenous and intraperitoneal m.l.d. and LD_{50} of the dried venoms were determined in 20 g mice as described elsewhere(7). Studies of the effects of the venoms on neuromuscular transmission were made using 15 Bülbring phrenic nerve-diaphragm preparations(8) modified for studies on venoms(9). Studies of cardiovascular, respiratory, and central nervous system effects were made on cats as follows.

In 15 adult cats under pentobarbital hypnosis a common carotid artery and femoral vein were cannulated for measurement of arterial and venous pressures. Bipolar electrodes were placed in the skull over the frontal, parietal, and occipital lobes, bilaterally. Conventional limb, unipolar extremity, and precordial leads were affixed. All electrodes were connected to a Medcraft model D, 8-channel electroencephalograph. Respirations were recorded by means of a cantilever pneumograph(10) fed into the electroencephalograph. In 2 animals intermittent artificial respiration was carried out by positive pressure breathing through a tracheal cannula. Cisternal pressure was measured through a 20-gauge needle fixed in the cisterna cerebellomedullaris, and connected to a modified Brodie tambour in parallel with a water manometer. The tambour activated a wire strain gauge transducer which modulated the ampli-

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TABLE I. Lethality of Crotalus Venoms Following 26 to 27 Years of Storage.

Subspecies	M.L.D. in mg dried venom/20 g mouse			LD_{50}
	Macht	Githens and Wolff	Authors	Authors
C. atrox	.12, .30	.12	.20	.22
C. cerastes	.06	.17	.06	.08
C. mitchelli mitchelli	.045	.0035	.005	.008
C. ruber ruber	.11	.22	.12	.14
C. viridis viridis	.045	.025	.040	.045

tude of a 5-cycle carrier feeding the response into the electroencephalograph. A femoral vein was cannulated for subsequent injection of 2.5 to 10.0 mg of one of the venoms dissolved in 2 ml of saline. All animals were heparinized.

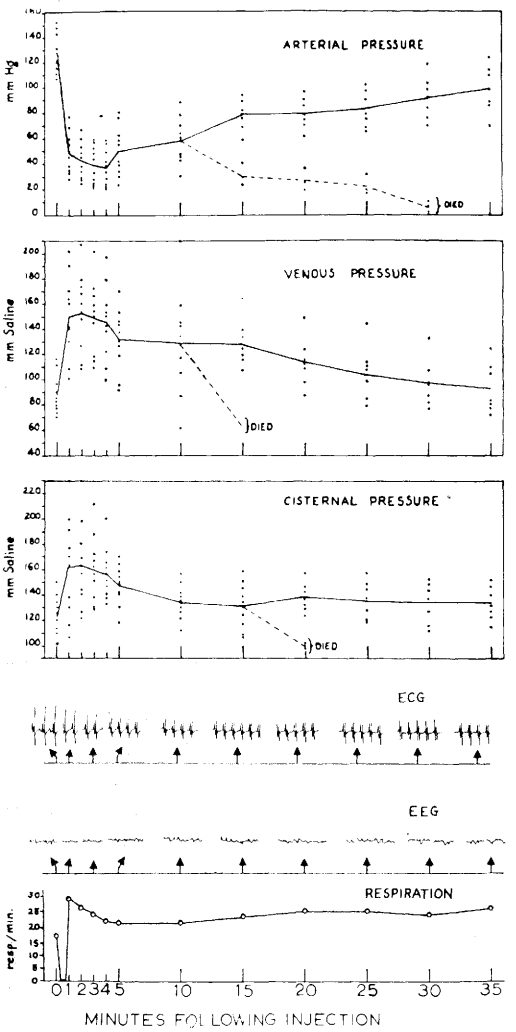

FIG. 1. Effect of stored *Crotalus* venom upon blood and cisternal pressures of 10 cats. A sample ECG (lead II), EEG, and mean respiratory rate for 7 cats are also shown. Inj. at 0 min., 2.5 mg/kg body wt.

Results. Table I shows intraperitoneal m.l.d. in mice of 5 venoms prepared by Klauber in 1932-33, as determined by Macht(11) in 1937, Githens and Wolff in 1939(12), and by us in 1959.

Five mg of *C. mitchelli mitchelli* venom caused complete cessation of the indirectly elicited diaphragmatic contractions in 9 minutes. The blocking capacity in both the stored and fresh toxins decreased in the following order: *C. mitchelli mitchelli, C. cerastes, C. viridis viridis, C. ruber ruber* and *C. atrox,* the last requiring 22 minutes to produce a complete neuromuscular block.

Fig. 1 shows changes produced by the venoms in arterial, venous and cisternal pressures, respiratory rate, and sample electrocardiograms and electroencephalograms. While all venoms produced similar responses, there were individual differences in degree of reaction. These appeared to be dependent upon amount and kind of venom injected. In general, there was a greater variety of responses to these venoms than those freshly prepared and tested in another group of animals(13), and those described by other investigators (14). Following injection of the venom there was an immediate fall in systemic arterial pressure concomitant with an increase in venous and cisternal pressures. Cats receiving artificial respiration displayed similar hypotensive reactions. Apnea of 10 to 45 seconds duration usually occurred after arterial blood pressure had begun to fall. The ensuing respiration was more shallow and rapid, and tended to be abdominal in type. Electrocardiograms showed disturbances in rhythm and ischemic injury patterns. The most common findings were depression of S-T segment, and deviation of T wave from normal. In

some animals no significant changes in electrocardiograms were observed. In 2 cats an ectopic ventricular rhythm developed within 5 seconds following injection and persisted for 10 to 30 seconds. This was followed by sinus bradycardia with pronounced S-T segment shift, and eventually a complete heart block. The electrocardiographic tracings were subject to considerable variation, and while myocardial ischemia, or injury, in some animals may have been due to direct action of the venom on the heart, in the majority of cats the changes appeared to be secondary to the lowered systemic arterial pressure.

Following a sublethal dose of the venom the electroencephalographic tracings flattened; in an occasional animal, random slow waves of varying potentials appeared. The changes occurred during the period when arterial blood pressure was falling. When the point was reached where arterial pressure was beginning to rise and venous pressure to fall, electrical activity over the frontal, parietal and occipital areas began to reappear. Most of the electrical changes could be attributed to changes in blood pressures.

The results indicate that dried rattlesnake venoms stored in closed containers at 42 to 82°F in the dark for 26 to 27 years retained most, if not all, of their lethality, their ability to depress activity at the neuromuscular junction in the phrenic nerve-diaphragm preparation, and the properties responsible for the deleterious changes in arterial, venous and cisternal pressures, respiration, electrocardiogram, and electroencephalogram.

Summary. In mice and cats, dried *Crotalus* venoms stored in closed containers at 42°F to 82°F in the dark for 26 to 27 years retained most of their lethality, their ability to depress neuromuscular junction, and the properties responsible for the deleterious changes in the arterial, venous and cisternal pressures, respiration, electrocardiogram, and electroencephalogram.

1. Mitchell, S. W., Christison, L., Wolmer, E., quoted by M. Phisalix, *Animaux Venimeux et Venins, Masson & Cie, Paris,* 1922, v2, 478.

2. Phisalix, M., *ibid.,* 1922.

3. Schöttler, W. H. A., *J. Immunol.,* 1951, v67, 299.

4. Pollard, C. B., Novak, A. F., Harmon, R. W., Runzler, W. H., *Quart. J. Florida Acad. Sci.,* 1952, v15, 162.

5. Calmette, A., Massel, L., *Compt. rend. Acad. Sci.,* 1914, v159, 152.

6. Klauber, L. M., *Rattlesnakes. Their Habits, Life Histories and Influence on Mankind,* U. Calif. Press, Berkeley, 1956.

7. Russell, F. E., Emery, J. A., *Copeia,* 1959, v1, 73.

8. Bülbring, E., *Brit. J. Pharmacol.,* 1946, v1, 38.

9. Russell, F. E., Long, T. E., in 2nd Internat. Symposium on Myasthenia Gravis, C. C. Thomas, Springfield.

10. Whalen, W. J., De Haan, R. L., *Rev. Sci. Instr.,* 1955, v26, 1073.

11. Macht, D. I., PROC. SOC. EXP. BIOL. AND MED., 1937, v36, 499.

12. Githens, T. S., Wolff, N. O., *J. Immunol.,* 1939, v37, 33.

13. Russell, F. E., Michaelis, B. A., Engelbert, R., in *Fed. Proc.,* 1960, v19, No. 1.

14. Witham, A. C., Remington, J. W., Lombard, E. A., *Am. J. Physiol.,* 1953, v173, 535.

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