4. P. F. Scholander and O. Iversen, *Scand. J. Clin. Lab. Invest.* **10**, 429 (1958).
5. R. J. Callaway, *U.S. Fish Wildlife Serv,. Spec. Sci. Rep. Fish No. 230* (1957).
6. J. A. Ramsay and R. H. J. Brown, *J. Sci. Instrum.* **32**, 372 (1955).
7. E. Cotlove, *Anal. Chem.* **35**, 101 (1963).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
9. M. S. Gordon, unpublished observations.
10. ———, *Handbook of Physiology* (American Physiological Society, Washington, D.C., 1964), section 4, pp. 697–713; G. Parry, *Biol. Rev.* **41**, 392 (1966).
11. Q. Bone, *J. Mar. Biol. Ass. U.K.* **46**, 321 (1966).
12. M. D. Rayner and M. J. Keenan, *Nature* **214**, 392 (1967).
13. J. J. Magnuson and J. H. Prescott, *Anim. Behav.* **14**, 54 (1966).
14. V. Walters and H. L. Fierstine, *Nature* **202**, 208 (1964).
15. C. Wittenberger and I. V. Diaciuc, *J. Fish. Res. Board Canada* **22**, 1397 (1965).
16. M. S. Gordon, unpublished observations.
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Venom of the Scorpion Vejovis spinigerus

Abstract. *The chemical composition of lyophilized venom from* Vejovis spinigerus *is reported. At least 13 distinct bands were obtained on disc electrophoresis; on Sephadex G-50, four major peaks were found. The lethal activity was associated with the second peak. The intravenous lethal dose, median (lethal for 50 percent of inoculated group), of the crude venom was 4.87 milligrams per kilogram of body weight. Neither the crude venom nor its fractions had a deleterious effect on neuromuscular transmission, reflex discharge, or antidromic inhibition. Crude venom evoked simultaneous changes in systemic arterial, venous, and cisternal pressures.*

The scorpion, *Vejovis spinigerus* (Wood), 1863, is found throughout Arizona, in southwestern New Mexico, in parts of Southern California, and in northern Mexico including Baja California (*1*). It is responsible for numerous stingings to humans, although such injuries usually produce little more than transient pain at the site of the sting, and on occasions some localized swelling, paresthesia, and tenderness. On rare occasions a few vesicles may develop around the wound. Systemic manifestations are very rare.

We now report on certain chemical and physiopharmacological properties of the venom of this species.

Approximately 450 *Vejovis spinigerus* were milked (*2*) over a 24-month period. The scorpions were collected from Portal, Tucson, and Wickenburg, Arizona, and from Imperial and Riverside counties in Southern California. An additional lot was supplied by H. L. Stahnke of Tempe, Arizona. All samples were lyophilized immediately after collection and were stored in the dark at 5°C until used. The individual lots were assayed separately and in combinations.

Samples from a pooled lot of 150 mg of venom were assayed for total protein (*3*) [additional determinations were made on several smaller lots (*4*)]; nonprotein nitrogen (NPN) and total nitrogen (*5*); monosaccharides and disaccharides (*6*); sodium (*7*, p. 1140), potassium (*7*, p. 1140), and calcium (*7*, p. 1133). The proteolytic (*8*, p. 167), cholinesterase (*8*, p. 73), amylase (*8*, p. 118), phosphodiesterase (*9*), and L-amino acid oxidase (*10*) activities were also determined.

A sample of venom and serotonin were chromatographed simultaneously at 20°C on Whatman No. 31 paper in a system composed of *n*-butanol, glacial acetic acid, and water (12 : 3 : 5 by volume). The venom (10 mg/ml) was dissolved in deionized water and applied to the paper in spots, the amount of venom increasing by 20-μg increments from 20 to 120 μg. The serotonin was applied in 3*N* acetic acid in spots of 1, 2, 4, and 8 μg. After chromatography, the paper was dried in air, examined under ultraviolet light, dipped in Ehrlich reagent [10 percent *p*-dimethylaminobenzaldehyde in a mixture of 12*N* HCl and acetone (1 : 4 by volume)], and redried; and the developed spots were noted.

The lyophilized venoms of scorpions from Portal (two samples, 27.5 mg and 59.7 mg), Tucson (one sample, 42.6 mg), and southeastern California (one sample, 36.8 mg) were chromatographed separately on Sephadex G-50. The gel was prepared in a 0.1 percent NaCl solution, washed repeatedly, and then added as a slurry to a glass column (2.54 by 50 cm) to a height of 25.5 cm, and equilibrated at 5°C; the venom was then added in 3 ml of solvent. Chromatography was carried out at 5°C, with 0.1 percent NaCl as the eluent; 3-ml samples were collected on an automatic fraction collector.

All fractions were analyzed for absorbance at 260 and 280 mμ in a Beckman DB spectrophotometer. Portions were taken for protein determination (*3*), with bovine serum albumin (Armour) as a standard protein. Both pooled and individual samples of the
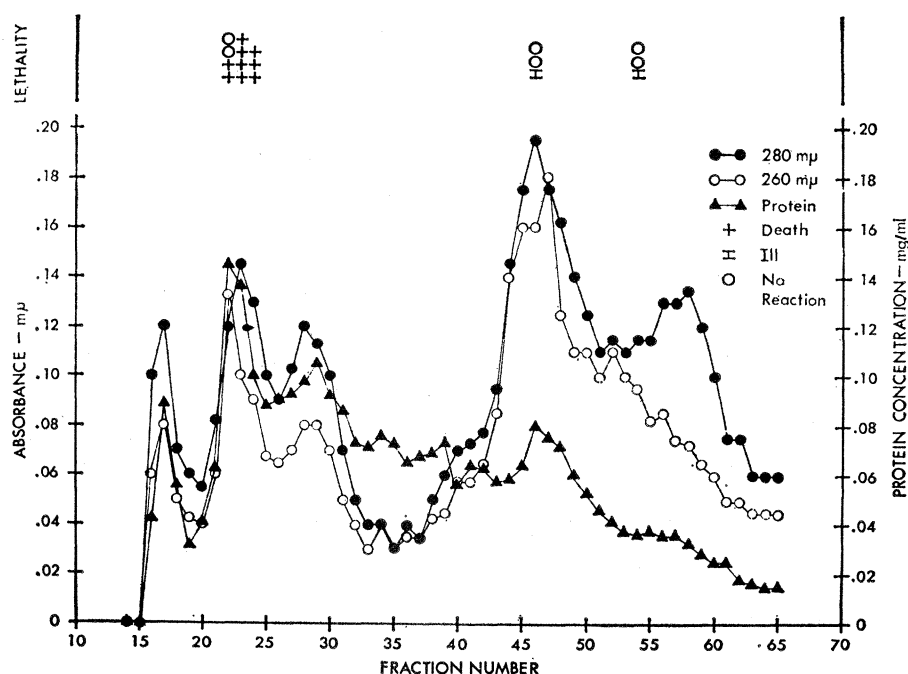

Fig. 1. Elution pattern of *Vejovis spinigerus* venom by gel filtration on Sephadex G-50.

crude venom were studied by disc electrophoresis (11). All fractions and the crude venom were assayed for lethal activity in mice and active fractions were further studied in certain biological assays (12, 13).

The lyophilized venom contained, per 100 mg, 51.4 mg of protein (smaller lots contained 43.5 to 52.6 percent protein); 2.2 mg of nonprotein nitrogen; 7.2 mg of total nitrogen; and 1.5 mg of carbohydrate. Sodium was less than 1 meq per gram of venom; potassium, 2.7 meq per gram; and calcium, 0.2 mg per gram. The venom had no proteolytic, amylase, phosphodiesterase, or L-amino acid oxidase activity; 100 mg of venom in 30 minutes (0.182 μmole per milligram per minute) hydrolyzed 547 μmole of acetylcholine.

On the paper chromatogram, a fluorescent spot with an R_F value of 0.575 and a positive Ehrlich reaction was observed. Serotonin showed fluorescence under the same conditions and had an R_F value of 0.585. Comparison of the intensity of the Ehrlich reactions indicated a serotonin concentration in the venom of approximately 4.0 μg/mg.

The elution pattern for one sample is shown in Fig. 1. The lethal activity of the venom was associated with peak 2 in all samples. This lethal component of the venom appeared to be relatively unstable. It was almost completely destroyed on lyophilization and lost its lethal effect when it was kept at room temperature. However, storage in solution at −60°C did not alter the lethal effect over a 3-month test period. It can be concentrated in a dialysis bag subjected to a draught of air at 5°C.

A second toxic group of fractions was associated with peak 4. Mice receiving injections from samples taken from this peak became ill when given doses equivalent to the lethal dose of peak 2, but recovered within 1 to 3 hours.

Disc-electrophoresis patterns of 25 individual and pooled lots of crude venom showed some variation. Samples from individual specimens within a given geographic area varied as greatly in their electrophoretic pattern as samples from area to area. From 13 to 15 bands were observed in the gels.

The intravenous LD_{50} (lethal dose, median for 50 percent of inoculated group) of the crude lyophilized venom in mice was 4.87 mg per kilogram of body weight. Even when the venom was stored at 5°C in the dark, the LD_{50}

of the venom slowly changed. In 1 year, the LD_{50} of one sample fell from less than 5.0 mg to approximately 20 mg per kilogram of body weight of the test animal. In a mammalian phrenic nerve-diaphragm preparation the crude venom did not provoke any significant deleterious effect, and none of the fractions separated on Sephadex or by disc electrophoresis impaired neuromuscular transmission. The crude venom had a negligible effect on preparations of deep extensor muscles (medial) of the abdomen of the crayfish. Compared to venom from the scorpion *Centruroides sculpturatus*, approximately 100 times as much was needed to produce a complete neuromuscular block in the same length of time. Neither the crude venom nor its fractions had a significant effect on reflex discharge or on antidromic inhibition in the cat.

When the crude venom was injected into cats, it caused an immediate precipitous fall of approximately 30 percent in the systemic arterial pressure. This fall was followed by a rise, so that within 2 minutes of the injection the arterial pressure was 10 to 30 percent higher than the initial pressure. This elevated arterial pressure persisted, or in some cases increased, during the first several hours after the injection. The specific mode(s) of action for the cardiovascular changes have not yet been determined. The samples associated with peak 2 appeared to produce a similar response on a survey preparation of the mammalian cardiovascular system (13). However, since the quantity of each fraction was very small, a limited number of experiments were completed.

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References and Notes

1. H. L. Stahnke, personal communication.
2. J. Strassberg and F. E. Russell, *Toxicon* 1, 41 (1962).
3. G. R. Kingsley, *J. Lab. Clin. Med.* 27, 840 (1942).
4. O. H. Lowry *et al.*, *J. Biol. Chem.* 193, 265 (1951).
5. F. C. Koch and T. L. McMeeken, *J. Amer. Chem. Soc.* 46, 2066 (1924).
6. O. Folin and H. Wu, *ibid.* 41, 367 (1920).
7. B. L. Oser, *Hawk's Physiological Chemistry* (McGraw-Hill, New York, 1965).
8. J. B. Sumner and G. F. Somers, *Chemistry and Methods of Enzymes* (Academic Press, New York, 1953).
9. J. F. Koerner and R. L. Sinsheimer, *J. Biol. Chem.* 228, 1049 (1957).
10. D. Wellner and A. Meister, *ibid.* 235, 2013 (1960).
11. L. Ornstein, *Ann. N.Y. Acad. Sci.* 121, 321 (1964); B. J. Davis, *ibid.* 121, 404 (1964).
12. F. E. Russell and T. E. Long, in *Myasthenia Gravis*, H. R. Viets, Ed. (Thomas, Springfield, Ill., 1961), p. 101; I. Parnas and F. E. Russell, in *Animal Toxins*, F. E. Russell and P. R. Saunders, Eds. (Pergamon, Oxford, 1967), p. 401; B. Renshaw, *J. Neurophysiol.* 9, 191 (1946).
13. F. E. Russell, F. W. Buess, J. Strassberg, *Toxicon* 1, 5 (1962).
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Collagen Synthesis by Cells Synchronously Replicating DNA

Abstract. *Replication and the performance of a differentiated function have been considered antagonistic processes. When cells in culture are partially synchronized with 5-fluoro-2'-deoxyuridine (FUdR), the synthesis of the specialized protein (collagen) is not reduced during chromosomal replication (S period). Collagen synthesis varies with general protein synthesis through the S period.*

The idea that the production of a specialized protein was precluded in proliferating cells (1) was supported by the work of Holtzer, Marshall, and Fink and by Stockdale and Holtzer (2). These authors showed that dividing muscle cells did not bind fluorescent antibody to myosin, and that cells binding fluorescent antibody to myosin were not dividing. There are many examples, however, showing that the processes of differentiation and proliferation are not mutually exclusive. Wessels (3) found cells in the developing pancreas with mitotic figures and small amounts of zymogen. Mäkelä and Nossal (4) demonstrated that antibody synthesis was occurring in plasma cells before division had ceased. Alpha and beta crystallins were found in proliferating epithelial cells of the developing lens, although mature fibers were not seen until DNA synthesis had ceased (5).

Tissue culture has been a way of determining whether anything specific in cellular division precludes maximum expression of specialized function. Goldberg, Green, and Todaro (6) reported an aneuploid mouse line (3T6) producing about 16 mμmole of hydroxyproline in protein per 10^7 cells per day. Because hydroxyproline is