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Intergeneric Relationships among Various Scorpion Venoms and Antivenins

Abstract. Several species of scorpions separated by geographical barriers have certain venom components in common. A study of the comparative venom-antivenin reactions by gel diffusion indicates an approach to the preparation of a polyvalent antivenin against scorpion stings.

The First International Conference on Venoms in 1954 emphasized the importance and complexity of certain harmful substances secreted or ejected by animals (*1*). As indicated by Balozet (*2*), in French North Africa deaths due to scorpion stings are more frequent than deaths due to venomous snakes.

The dissemination of U.S. military personnel over worldwide areas engenders the necessity of investigating antivenin preparations. The final goal is the development of a satisfactory polyvalent scorpion antivenin for therapeutic use.

As a first approach to the problem, this study indicates the prominent comparative venomologic precipitin reactions among 12 scorpion venoms (representing four genera) and five commercially prepared horse antivenins from widely separated world areas.

The venoms were air-dried droplets collected either by electrical stimulation of the living specimens (*2*) or saline extracts of the telsons. Venoms analyzed were from the following areas: São Paulo, Brazil (*3*); Johannesburg, South Africa (*4*); Ankara, Turkey (*5*); Algiers, Algeria (*6*); Camp Bullis, Texas; Tepic and San Blas, Nayarit State, Mexico; and Manzanillo, Colima State, Mexico. The Texas and the Mexican areas provided several species (*7*). Commercially prepared horse antivenins were from São Paulo, Brazil (*8*); Algeria (*9*); Ankara, Turkey (*10*); Mexico City, Mexico (*11*); and Johannesburg, South Africa (*12*).

Immunodiffusion in agar columns was the method of choice for this study, since the venom concentrations were insufficient to give satisfactory resolution by paper electrophoresis or multi-well agar plates. In the agar column method used for these analyses, venom and antivenin-agar mixtures were separated by a clear layer of 0.3-percent saline agar. This clear area formed the reaction arena in which the precipitin systems appeared. Other details of this double diffusion method have been described by Oakley and Fulthorpe (*13*). All of the 60 homologous or heterologous reactions were prepared in duplicate. Where required, the venoms were dissolved in saline at concentrations of 10 mg/ml or 5 mg/ml. The reactions of equal concentrations were compared by making cathetometric measurements of their respective precipitin systems after 68 hours' diffusion at 30° ± .01°C and subsequently computing the diffusivity ratios (*P* values) as described by Preer (*14*). Such values represented the relative diffusion rates of various venom components when reacted with a reference antivenin, or of antivenins when a venom was used as the reference standard.

Since the quantities of venoms were not sufficient for identification of the comparable antigen-antibody systems by the classical absorption method, the identity of comparable precipitin zones was established mathematically by the methods of Oudin (*15*). In brief, finding equal pairs of the 1567 mean *P* value differences permitted simultaneous identification of comparable precipitin systems when one venom was reacted with two or more antivenins and when two or more antivenins were reacted with one venom. The principle of this mathematical identity is based on the diffusion differences of two or more venom mixture components when each will react separately with two or more antivenins (*15*).

Figure 1 diagrammatically shows the only comparable precipitin systems among the various venom-antivenin reactions when all venoms were reacted with the same antivenin (upper row) and when each venom was reacted with the various antivenins (lower row). The results (upper row) show that the venom from *Androctonus australis*, an Algerian scorpion, shares at least one precipitin system in common with two species of *Centruroides* (Mexican) when each venom was reacted with anti-*Tityus spp.* serum from Brazil; two species of Mexican *Centruroides* show comparable systems when reacted with antivenins prepared against venoms of *Parabuthis spp.* from Johannesburg, South Africa; and *Androctonus australis* (Algiers) venom has a precipitin system comparable to that of venom from *Centruroides suffusus* (Mexican) when each was diffused into *Androctonus* antivenin. The situation was more complex when each venom was reacted separately with each antivenin. As shown in the lower row of Fig. 1,

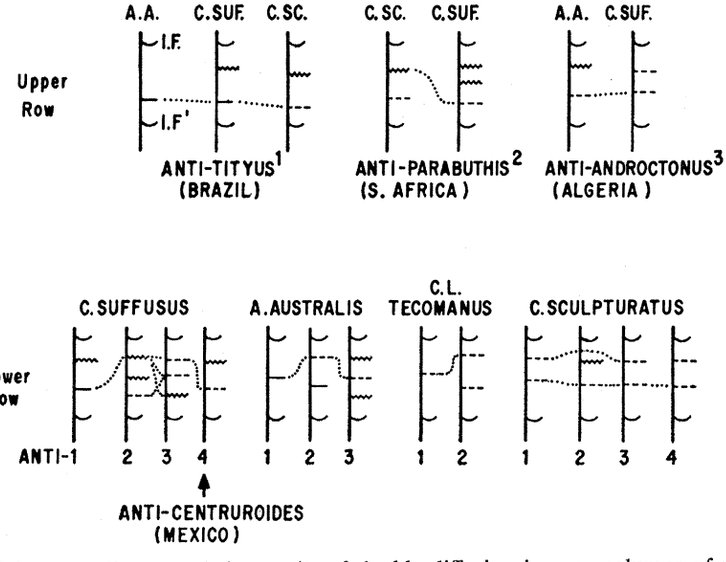

Fig. 1. Schematic diagram of the results of double diffusion in agar columns of various homologous and heterologous scorpion venom–horse antivenin reactions. Dotted lines connect related precipitin systems. IF and IF′ represent the interfaces of venom and clear agar and of clear agar and antivenin-agar mixture, respectively. Precipitin systems are shown as lines between the IF's. Solid lines indicate dense zones; wavy lines, medium density; dashed lines, faint zones. A.A., *Androctonus australis*; C.SUF., *Centruroides suffusus*; C.SC., *C. sculpturatus*. Reaction time, 68 hours at 30° ± .01°C. See text for method of zonal identification.

Centruroides suffusus (Mexican) venom reacted with a precipitin produced to other species native to Brazil, South Africa, and Algeria. Venom of the Turkish scorpion, *Androctonus australis*, shares common antigen-antibody reactions with antisera produced in Brazil, South Africa, and Algeria; several *Centruroides spp.* venoms from Mexico also share one or more precipitins with immune sera made in Brazil, South Africa, and Algeria. The antivenins were prepared to the scorpion genus of greatest medical importance in each area. For the purposes of this study intrageneric relationships were not determined.

Extracts of a dried telson of *Androctonus crassicauda* and three post-abdominal segments from the same animal were prepared by trituration in saline followed by centrifugation at 4°C (1000*g*, 30 min). The solutions were then used for fractional absorption of *Androctonus* antivenin (Algeria) at three antigen concentrations. Absorbed antivenins were reacted in double diffusion columns for 91 hours (30° ± .01°C) versus telsons. Of the four precipitin systems in the telson series, two were absorbed with the post-abdominal segment extract. The possibility exists, therefore, that the desiccated solutions used for the immunization of the horses contained post-abdominal segment substance(s), or that such substance(s) or similar ones also occur in the telson.

The complexity of homologous scorpion venom-antivenin reactions was such that some systems, namely, *Parabuthis spp.* venom (10 mg/ml) showed as many as seven precipitin zones at one antiserum concentration when reacted for 68 hours at 30° ± .01°C. There were no homologous reactions that showed fewer than two antigen-antibody systems.

From these results, assuming that the major toxic components of the venoms were reflected in the precipitating systems, the possibility exists that an appropriate combination of certain immune horse sera would be the most feasible approach toward the preparation of a polyvalent antivenin for the genera considered (*16*).

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7. *Centruroides vittatus* (Camp Bullis, Texas); *C. suffusus* (Durango, Mexico); *C. sculpturatus* (Superior, Arizona); *C. noxius* (Tepic, Nayarit State, Mexico); *C. elegans* (San Blas, Nayarit, Mexico); and *C. limpidus tecomanus* (Manzanillo, Colima State, Mexico).
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9. Serum Antiscorpionique, Institut Pasteur d'Algérie.
10. Akrep Serum, Central Institute of Hygiene, Ankara, Turkey.
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Collection and Washout of Airborne Pollens and Spores by Raindrops

Abstract. Data on aerodynamic capture of particles are used to estimate efficiency of collection of pollen grains and spores by falling raindrops. Simple probability arguments then yield the fractional removal of airborne pollens and spores by rain. Pollen grains are generally large enough to be washed out by all but the lightest showers of large thunderstorm-type drops; smaller-sized spores experience lower removal rates. Various specialists concerned with pollen dissemination will find rain-scavenging a significant process.

Whether an airborne pollen grain of density ρ and diameter d (typically of the order of a few tens of microns) will be swept out by an approaching raindrop of diameter D (typically of the order of tenths of a millimeter to a few millimeters) falling with velocity V in air of absolute viscosity μ is dependent upon the magnitude of the dimensionless inertial parameter K given (*1*) by

$$K = (\rho d^2 V)/(9\,\mu D) \qquad (1)$$

It will be acceptable to ignore the terminal velocity of the pollen grain, since it is of the order of only a few percent of V. If K is large, the pollen grain will not easily be pushed aside by the pressure field set up ahead of the falling raindrop, and the probability of collection will be enhanced. The collection efficiency E (defined here as the fraction of all pollen grains lying in the cylindrical region of diameter D swept out by the raindrop which are actually hit by the drop) is, as a result of the recent computer studies, a now-known function (*1–3*) of K and of the Reynolds number at which the raindrop falls. I have here used E values of Fonda and Herne (see *3*), probably the best data now available, interpolating between the viscous and aerodynamic flow regimes after the manner indicated by Langmuir (*1*). Pollen grains may be treated as point-centers in calculating E, with only negligible error because their finite size alters E only for the smallest drizzle drops where removal efficiency is nearly complete anyhow. Values of V and associated Reynolds numbers were taken from the work of Gunn and Kinzer (see *4*). Adequate indication of trends can be displayed by giving results only for raindrop diameters of 0.2, 1.0, and 4.0 mm. A diameter of 0.2 mm is the meteorologically accepted dividing point between large cloud drops and small drizzle drops; 1.0 mm is representative of the bulk of raindrops in most rainfalls; 4.0 mm is near the upper limit of drop diameters occurring in thunderstorm rainfall.

Data on ρ and d for pollen grains were taken from Erdtman (*5*), and data for some spores were taken from Maunsell (*6*). It should be stressed that all one actually needs here is the product, ρd^2. Inspection of Stokes' law for spheres falling at terminal velocity reveals that this product may be computed directly from the pollen or spore terminal velocity, an approach that is actually preferable to separate microscopic measurement of d and pycnometric estimation of ρ inasmuch as any intrinsic departures from Stokes' law (due, say, to grain asphericity or surface roughness) will be automatically incorporated into the ρd^2 product derived from terminal velocity observations, improving precision of estimation of E. The air bladders of pine and certain other conifers depart from the spherical model underlying Stokes' law, but experience with other aspherical particle problems suggests that this will not be serious. Whether every col-