IMMUNOLOGICAL STUDIES ON SCORPION
(B. QUINQUESTRIATUS) ANTIVENIN

A. H. MOHAMED, MEDHAT A. DARWISH and M. HANI-AYOB
Faculty of Medicine, Ain Shams University, Cairo, Egypt

(Accepted for publication 6 August 1974)

The scorpion Buthus quinquestriatus is the most prevalent and most toxic in Egypt; its stings may be followed by death especially in children and debilitated persons. Treatment should be instituted early with the specific antiserum. Trials of anti-scorpion sera for their paraspecific action have shown variable degrees of success (Balozet, 1971). The aim of the present work was to prepare a potent antivenin against the Egyptian scorpion B. quinquestriatus and to study its specific and paraspecific actions.

Venoms used were those of Egyptian scorpion B. quinquestriatus, Libyan scorpions Buthus occitanus and Androctonus aeneas and the Liberian giant scorpion Pandinus sp. Scorpions were electrically milked and their venoms lyophilized. Lethality of the different venoms was determined in albino Swiss mice (15 g each). Results (survival or death) were recorded at 24 hr and the LD₆₀ for each venom (µg per 15 g mouse) was calculated according to the method of Reed and Muench (1938).

Preparation of antiscorpion serum was carried out in 4-yr old horses, passively immunized against tetanus. Because of the poor immunogenicity of the scorpion venom, a prolonged hyperimmunization course was given using the chemically purified picrate venom prepared from dried telsons of B. quinquestriatus according to the method published by Mohamed (1944). The venom was freshly dissolved in saline as a 1 per cent solution. Prior to injection, the venom solution was mixed with the contents of two vials of penicillin-streptomycin (Panbiotic, Biochemie, Austria) and the mixture was administered subcutaneously. The initial venom dose was 0.5 mg, and the injection was repeated every week with the dose of venom gradually increased over 10 months' duration until the animal received 80 mg per dose. At that dose the serum showed satisfactory potency. The animal was thereafter maintained on 80 mg venom per month. Bleedings of 21. at a time were made monthly, and the serum was separated and seitz filtered. Reference antiscorpion sera from Agouza laboratories (Egypt), Pasteur Institute (Algeria) and Lister Institute laboratories (England) were used for comparison.

For carrying out neutralization tests, various doses of venom were prepared and adjusted to a volume of 0.25 ml. Each dose was mixed with 0.25 ml serum and the mixtures were incubated for 30 min at room temperature (25°C). The mixtures were injected intraperitoneally into albino Swiss mice (15 g each) with 5 animals used per dose. The survival rate was recorded at 24 hr, and the LD₆₀ calculated according to the method of Reed and
Muench (1938). The neutralizing capacity of the serum was expressed as the number of LD₅₀'s of venom neutralized by 1 ml of serum.

Ouchterlony’s immuno-diffusion technique was performed in plates of agar (Noble-agar, Difco). Venoms were used as 1 per cent solution in distilled water against the different antiscorpion sera (Ouchterlony, 1962).

The neutralization capacity of the antiscorpion sera against the venom of B. quinquestriatus were as follows: 56, 28, 12 and 12 LD₅₀ were neutralized by 1 ml of serum prepared by Ain Shams (Egypt), Agouza (Egypt), Pasteur Institute (Algeria) and Lister Institute (England), respectively. One LD₅₀ was 23 μg per 15 g mouse and control horse serum showed zero neutralization. We also observed weak neutralization by the Egyptian serum against the two Libyan scorpion venoms B. occitanus and Androctonus aeneas (1 ml neutralized 14 and 6 LD₅₀'s, respectively). The LD₅₀ values of B. occitanus and A. aeneas venoms were 63 and 72 μg per 15 g mouse, respectively.

Immuno-diffusion patterns showed six precipitation lines between the Egyptian scorpion venom B. quinquestriatus and its homologous serum. However, the venom exhibited only one or two bands with the other sera (Fig. 1). While the Egyptian antiscorpion serum showed six bands with its homologous venom B. quinquestriatus, weaker reactions with fewer bands were elicited with the two Libyan venoms B. occitanus and A. aeneas and no reaction with the Liberian venom Pandinus sp. (Fig. 2).

Our data indicate that antiscorpion serum is highly specific, offering weak or no paraspécific activity. Other investigators have also studied the specificity and paraspéricity of antiscorpion sera against the Turkish venom B. quinquestriatus and the North African venom A. australis which showed high specificity against their homologous venoms and no or weak action on heterologous venoms (Tulga, 1960; Balozet, 1971). Among five sera studied by Whittmore et al. (1961) four sera gave slight protection, while only one antiserum, that against A. crassicauda, had wide paraspéric activity. We may therefore conclude that antiscorpion sera possesses a low paraspéric action and this can be attributed to the low immunogenicity of scorpion venoms. Although the use of antiscorpion sera for their paraspéric activity is not recommended, it may be resorted to when the specific antivenin is unavailable.

Acknowledgements—This work was supported by a grant no. 03.006-1 from the National Institute of Health, Bethesda, Md., U.S.A. The authors thank Dr. Moftah El Osta Omar, Minister of Health in Libya, and the local authorities in Sebha and Kufra for their help, and Mr. S. A. Ghouzlan and Mr. A. Khasara for their technical assistance.

REFERENCES


Fig. 1. Patterns of double diffusion in agar (Ouchterlony technique) between scorpion antivenins and scorpion venoms.
1—Egyptian scorpion antiserum; 2—Pasteur Institute scorpion antivenin; 3—Lister Institute scorpion antivenin; 4—Agouza laboratories scorpion antivenin. In the central well was placed Egyptian scorpion venom *B. quinquestriatus*.

Fig. 2. Patterns of double diffusion in agar between scorpion venoms and antivenins.
1—Egyptian scorpion venom *B. quinquestriatus*; 2—Libyan Kufra scorpion venom *A. aeneas*; 3—Libyan Sabha scorpion venom *B. occitanus*; 4—Liberian scorpion venom *Pandinus* sp. In the central well (B) was placed Egyptian scorpion antiserum.