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THE PRODUCTION AND EFFECTIVITY OF RABBIT ANTISERUM AGAINST *VESPA ORIENTALIS* VENOM

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Year after year a number of lethal stings from *Vespa orientalis* occur in Israel (1, 2). It may be safely assumed that many further cases remain unreported throughout the underdeveloped countries of the Mediterranean region, where the presence of the insect is widespread.

Vespa orientalis is a social insect, founding colonies in the springtime (3). The number of members multiplies towards the end of summer, and it is at this period that the majority of cases of stings, producing allergic reactions and in some instances resulting in death, are generally met with. The antigenicity of the venom and the production of a rabbit antiserum and its protective effects against the lethality of venom in mice are described in this report.

METHOD

The venom was obtained by manual "milking" of single adult hornets. It was then lyophilysed. Immunisation was attained by subcutaneous injections of 10 microg. of venom in saline mixed with Freund's adjuvant, at the rate of two injections per week over six weeks. The last injection, a booster dose, contained 100 microg. of venom.

After testing the immunological effect of the antisera by immunodiffusion of a sample obtained by trial bleeding (Fig. 1), the rabbits were bled to death by cannulation of the femoral artery. The blood serum thus obtained was then

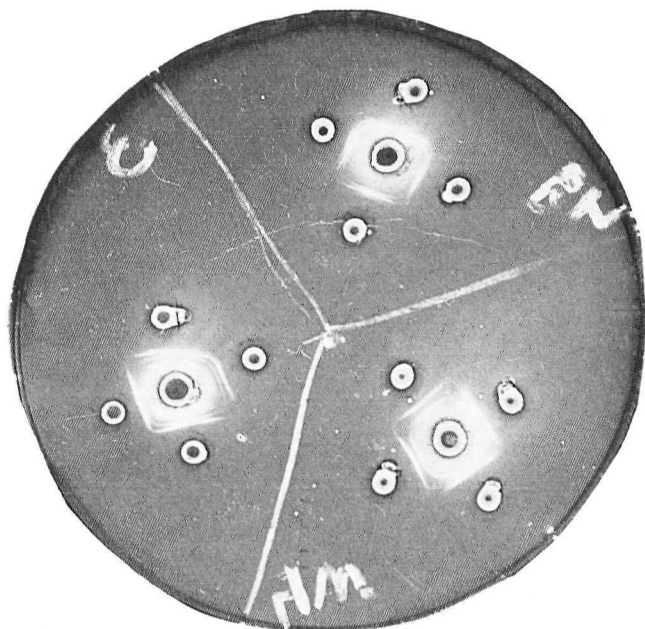


Fig. 1.

Ouchterlony immunodiffusion plate, showing precipitant reaction of three of the six rabbits immunised with *Vespa orientalis* venom. The venom is placed into the central well, and dilutions 1:10, 1:1, 1:2 and 1:4 of the antisera into the outer wells. In all cases two to three fractions can be seen.

collected and pooled by aseptic techniques. The venom sac extract was subcutaneously injected into the dorsum of the neck of random-bred Swiss mice weighing 18 to 22 g. Each consecutive group of animals received a respectively increased dose of S (saline) or A (antiserum). Antiserum was administered by the same route 15 to 20 minutes after the venom injection.

RESULTS

The results obtained may be seen in Table 1 and from the following details: Antiserum in doses of up to 0.6 ml produced no toxic effects. Venom sac extract injected in doses of 2 mg per mouse proved deadly: within 24 hours of administration all the injected mice had succumbed to its lethal powers. On the other hand, protection was assured and death

TABLE
Protection of White Mice against Vespa

Material injected		S	A	V	V	V
Group numbers		1-9*	10-15*	16	17	18
Dose		0.1-0.2 0.9*	0.1-0.2- ...-0.6*	1	2	3
Mortality after:	24 hours	0/18	0/18	0/18	15/18	18/18
	2 days	0/18	0/18	3/18	18/18	/
	7 days	0/18	0/18	3/18	/	/
	10 days	0/18	0/18	3/18	/	/

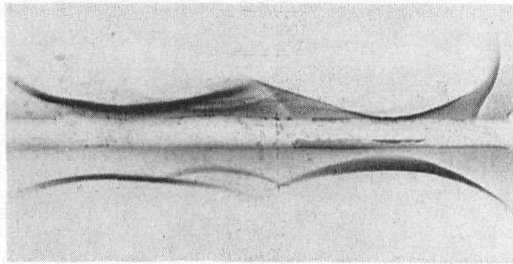


Fig. 2.

Immunoelectrophoresis of *Vespa orientalis* venom. The venom proteins electrophoresed, and the mixed antisera of different rabbits placed in the trough. Four precipitation arches may be distinguished.

prevented in all cases where mice had been injected with venom sac extract but had subsequently been given a 0.5 ml dose of antiserum. It should be noted, however, that this antiserum is not effective in counteracting venom sac extract in doses exceeding 2 mg. Venom sac extract was used in the conduct of these experiments following our vain efforts to

I
orientalis Venom with Rabbit Antiserum.

S = Saline (ml)		A = Antiserum (ml)			V = Venom (ml)	
V ÷ A						
19	20	21	22	23	24	25-30*
V ₁ ÷ A 0.1	V ₂ ÷ A 0.1	V ₂ ÷ A 0.2	V ₂ ÷ A 0.3	V ₂ ÷ A 0.4	V ₂ ÷ A 0.5	V ₃ ÷ A 0.1-0.2-...-0.6*
0/18	7/18	6/18	4/18	1/18	0/18	15/18
0/18	11/18	9/18	7/18	1/18	0/18	16/18
0/18	15/18	13/18	11/18	2/18	0/18	18/18
0/18	17/18	14/18	12/18	3/18	0/18	/

secure the pure substance in a quantity adequate for the purpose.

The immunological reaction between the venom and the antiserum may be observed in Fig. 2. The total number of the immunoprecipitate lines is evident in the immuno-electrophoretic separation of the active principles; the figure shows four distinct lines.

DISCUSSION

Despite the tedious and time-consuming "milking" method of collecting the venom, we selected this procedure so as to avoid the production of antibodies by unspecific proteins of the venom sac wall or other body tissues. In this way the interference experienced by other workers (4) was eliminated.

In a previous paper (5), the LD_{50} of whole hornet venom was found to be 2.5 mg/kg mice. Yet the LD_{50} of venom sac extract is approximately 70 mg/kg mice, which proves that large quantities of tissue protein and other materials making up the extract are obtained from the venom sac. This fact

furthermore emphasises the advantage of using whole venom for immunisation.

It is a well-known fact (6, 7) that a considerable quantity of various low-molecular-weight toxic substances that are not antigenic (mellitin, histamin, etc.) are present in hymenoptera venoms. This may explain why no protection is obtained after the administration of larger doses of venom. However, survival of those animals treated with antiserum in the low lethal range indicates that antigenic, high-molecular-weight toxic proteins can be neutralized by an antiserum.

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