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DOUBLE DIFFUSION ANALYSIS OF THE VIPERA AMMODYTES A. VENOM.

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The results of double diffusion analysis of the native and dialyzed Ammodytes viper venom are presented. It is found that a group of antigens was lost from the native venom during dialysis procedure, another group being insoluble in distilled water was eliminated from the aqueous venom solution by filtration. The approximative number and characteristics of precipitation lines produced by the native venom or isolated venom fractions with the immune horse serum in agar-gel are reported.

One of the chief aims of immunochemistry is to determine the antigenic composition of complex antigens.

A great deal of information about antigenic composition of snake venom has been acquired from the evidence of cross neutralizing effects between different venoms and their homologous antisera (1).

We succeeded in resolving V. ammodytes crude venom into seven components by free and zone electrophoresis (2). Similar results have been obtained with paper chromatography using buffers as eluents (3).

The Oudin diffusion test offered evidence for the presence of only three antigenic components in the crude ammodytes venom producing the same number of precipitation rings in agar-gel medium (4).

In this preliminary communication we present the results of double diffusion (5) analysis of the crude V. ammodytes venom and some of its isolated fractions. Experiments were carried out with 3 g of crude toxin which was dissolved in 30 ml of 0.15 M sodium chloride, than dialyzed through Visking cellophone tubing (pores, diameter 30–80 Å.) against deionized water in a refrigerator for seven days at 4° C.

During the dialyzing process some water insoluble proteinic antigens were precipitated from the venom solution. The crude venom and its fractions obtained by this procedure were used for double diffusion experiments.

The substances used as antigens were:

1. Native V. ammodytes venom dried at 37° C
   (N 11.21%; S. 3.9%; LD 50 = 18 μg for white mouse weighing 20 g Institute of Immunology, Zagreb.
2. Through a celophane membrane non-diffusible components of the toxin, containing water soluble antigens and a water insoluble precipitate.
3. The water soluble, through the celophane non-diffusible fraction.
4. The water insoluble, non-diffusible antigens of the venom.

The double diffusion experiments were performed with 1 per cent agar gel in the veronal-acetat buffer pH8.6 μ = 0.1. The immune anti-V. ammodytes horse serum (S 23) was poured into the central depression of the agar gel layer. The serum was purified by ammonium sulphate precipitation and peptic digestion and concentrated by ultrafiltration through a collodium membrane. In lateral cups venom fractions as numbered above (2–4) were placed.

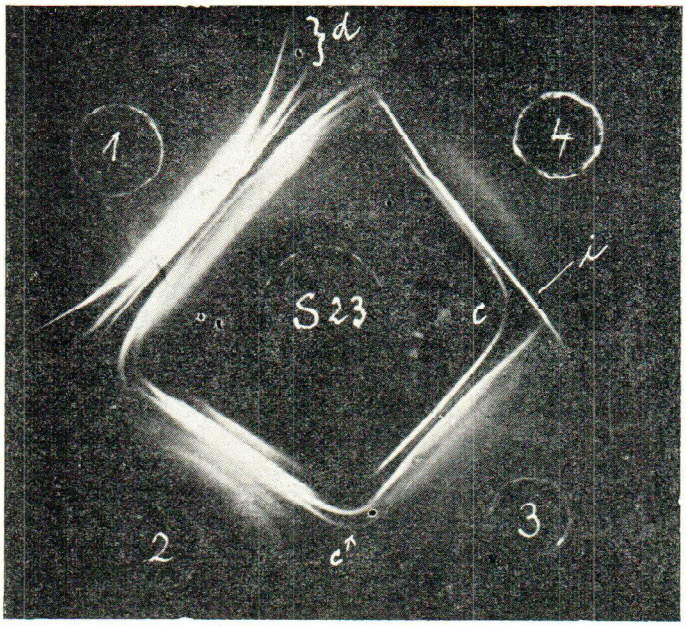

Fig. 1 Double diffusion analysis of V. ammodytes venom in agar gel. – The antitoxin (S 23) was placed in the centre well, four different venom preparations in the peripheral wells. 1. crude venom, 2. dialyzed venom, 3. water soluble antigens from the dialyzed venom, 4. water insoluble antigens. – Experimental conditions: 1.5% of agar in veronal-acetat buffer p_H 8,6 ionic strength 0.05, antigens containing 1% and the antitoxin 6% of proteins. – Precipitation lines were stained with 0.5% of azocarmine B in methanol-acetic acid (9 : 1)

The antitoxin gradually diffused laterally and met antigenic fractions. All those places where antigens and homologous antibodies met at optimal concentrations of the neutralization point precipitation bands appeared. Double diffusion experiments were performed in a humid chamber at 18° C. The results were recorded after 24 hours.

For staining of precipitation bands an 0.5% solution of azocarmine-B in methanol-acetic acid (9 : 1) was used.

The necessary volume and concentration of the antiserum for each antigen-antibody system were determined by experience.

From a typical double diffusion experiment presented in the photograph (Fig. 1) it can be seen that crude V. ammodytes venom (No. 1) with the immune horse serum (S 23), produces at least eight to twelve precipitation lines, most probably corresponding to the same number of antigens.

Other fractions No 2, 3, 4, derived from the crude toxin by the procedures of dialysis or precipitation, showed a lower number of precipitation bands owing to the loss of respective antigens in the course of isolatin.

The group »d« of precipitation lines (the crude venon No. 1) was produced by the antigens diffusible through the celophane membrane. These bands could scarcely be detected with the dialyzed toxin, while with the water soluble fraction (No 3) or water insoluble precipitate (No 4) they could not be detected at all. The dialyzed toxin (No 2) showed six to nine, its water soluble antigens (No 3) six to seven and the water insoluble precipitate (No 4) one to three precipitation lines, containing the corresponding number of antigens.

The water insoluble venom fraction produced only one (i) sharp precipitation line. Other lines were poorly visible and confluent (c) with respective lines of water soluble antigens. They evidently represented adsorbed water soluble antigens as impurities, because the same precipitate investigated by immunoelectrophoresis yielded one precipitation band only. This substance can be isolated from aqueous venom solutions at its isoelectric point pH 5.2 and purified by reprecipitation (2). It is most probably identical with the water insoluble venom substance which washed with 1% sodium chloride and tried by the Oudin diffusion test in tubes produces one precipitation ring only (4).

As we see the double diffusion method permits the control of separation and isolation of distinct venom fractions for a subsequent study of their respective biological activities in comparison with the complex action of the whole venom.

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Sadržaj

ANALIZA OTROVA POSKOKA (V. AMMODYTES A) DVOSTRUKOM DIFUZIJOM

Izvedena je imunoprecipitaciona difusiona analiza toksina poskoka (Vipera ammodytes a.) u agar gelu (Sl. 1). Antigeni upotrebljeni u eksperimentima bili su: (1) sirovi otrov poskoka, antigeni otrova koji ne difundiraju kroz celofansku membranu (2), u vodi topivi dio (3), te u dejoniziranoj vodi netopivi antigeni iz nativnog otrova (4). Kao antivenin (S23) upotrebljen je koncentrirani imuni konjski serum.

Iz rezultata difuzione analize vidi se, da se jedna grupa antigena (d) sirovog otrova gubi iz vodene otopine u toku dijalize, dok se iz dijaliziranog otrova mogu filtracijom odstraniti u vodi netopivi antigeni (i). Određen je približni broj precipitacionih linija odnosno antigenih komponenata u nativnom otrovu i iz njega izoliranim frakcijama.

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