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STUDIES ON THE CROSS-REACTIVITY OF SNAKE VENOM ANTISERA

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ABSTRACT: Cross-reactivity between antisera and snake venoms which have not been used for immunization is a common phenomenon observed in closely related as well as in quite distinct snake species. A polyvalent antivenom (Behringwerke North-Africa) was found to possess considerable neutralization potency to a North-American crotalid snake *(Crotalus adamanteus)*. By fractionation of *Bitis arietans* and of *Crotalus adamanteus* venom hemorrhagic factors were isolated which were neutralized by the antivenom indicating that there exist commom antigenic properties in venom constituents of even unrelated snake species.
KEY WORDS: Snake venom, antivenom cross-reactivity, hemorrhagic factors.

INTRODUCTION

Snake venoms are complex mixtures of proteins and polypeptides, which have antigenic properties. Immunesera raised against snake venoms contain, therefore, a great number of antibodies, of which some are important to neutralize the venom's lethal effect. Cross-reactivity of antivenoms with venoms of snakes which have not been used in immunization is a common phenomenon[1]. This leads to the assumption that venoms of even unrelated snakes representing different species or genera, or which are geographically separated may have common antigens.

The immunologic analysis of such complex mixtures of antigens and antibodies can be performed by applying immunodiffusion techniques. Precipitation lines, the reaction product between antigen and antibody, permit conclusions about the extent of relationship between venoms. A number of studies exist providing informations on interrelationships between species, on evolutionary and genetic implications[1]. However, the data obtained

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by these methods have limited value, if practical aspects of cross-reactivity, i.e. cross-neutralization, are concerned. The neutralization of the lethal and other harmful effects of snake venoms by antivenoms, which are paraspecific to others not used in immunization can only be evaluated by in vivo test-systems. This includes the neutralization of lethality, LD_{50}, hemorrhagic, necrotizing, defibrinating or coagulant activities in mice or rats. Whereas data on the neutralization capacity of commercial antivenoms to the lethal action of various snake venoms exist, neutralization studies on other venom activities are still rare. The WHO standardization program should certainly contribute to the knowledge on the potential cross-neutralization capacity of antivenoms.[2]

Beside these basic informations: which antivenom reacts with which venom, it appears important to find an answer to the questions: what characterizes common antigens and what is their role in the course of envenomation. It is quite clear that the main approach to this problem should concentrate on those venom components (antigens) only, which are involved in the venom's lethal or pathological actions.

In studies on various commercial antivenoms, their neutralizing capacity to venom activities such as lethal, hemorrhagic, necrotizing, defibrinating and coagulant effects, striking cross-reactivities had been observed[3,4,5] For instance Behringwerke-antivenom North-Africa as well as Wyeth Anticrotalidae-antivenom were both found to be very effective in neutralizing the lethal activity of the venom from the African puff adder (*Bitis arietans*). But it has to be considered that the polyvalent Behringwerke-antivenom only was produced by using *Bitis gabonica* venom as antigen among other venoms such as from *Naja haje, Vipera lebetina* and *Echis carinatus*, whereas in Wyeth-antivenom only North-and Central-American crotalid snake venoms, such as from *Crotalus atrox, C.adamanteus, C.durissus terrificus* and *Bothrops atrox* had been used for immunization. On the other hand, Wyeth-antivenom was much less effective to *Crotalus adamanteus* venom, but Behringwerke-antivenom shows a four times higher activity in the mouse protection test. Therefore, one may conclude that a common antigen (or antigens) responsible for the lethal effect must be present in both venoms.

Bitis arietans as well as *Crotalus adamanteus* venom produce massive hemorrhage which eventually leads to death (at least in mice), if internal bleeding occurs. Consequently the efficacy of both antivenoms to neutralize hemorrhagic activity was tested.

MATERIAL AND METHODS

Bitis arietans venom was purchased from D. Muller, Johannesburg, South Africa; *Crotalus adamanteus* venom was obtained from F. Kornalik. The following antivenoms were used for neutralization studies: polyvalent Crotalidae antivenom from Wyeth Laboratories, Philadelphia, PA, USA; polyvalent antivenom North-Africa from Behringwerke, Marburg, FRG. LD_{50} was assayed by intravenous injection into mice (20 g, 10 animals per dose), neutralization capacity of antivenoms by preincubating 0.1 ml venom solution (0.9% NaC1) with 0.1 ml antiserum for 30 min at 37° C. Hemorrhagic ac-

tivity was tested in mice according to Taborska[6]. Prior intradermal venom application 0.2 ml of 51_{Cr} labled mice erythrocytes were injected intravenously. In the neutralization tests 50 µg venom in 0.1 ml saline were mixed with 0.1 ml antivenom or normal rabbit serum as control and, after 30 min incubation, injected intradermally in the interscapular region of mice. The animals were sacrificed after 24 hrs and the amount of extravasal blood of a 2.5 cm^3 area at the injection site was calculated from radioactivity. Venom fractionation was performed as described previously[7].

RESULTS AND DISCUSSION

Under the conditions described Behringwerke-antivenom is able to completely neutralize the hemorrhagic activity of *Bitis arietans* and almost completely that of *Crotalus adamanteus* venom (Fig. 1). Wyeth-antivenom is less active towards *Bitis* venom, but provides full protection in the case of *Crotalus adamanteus* venom.

To identify the antigen or antigens involved in this cross-reaction *Crotalus adamanteus* venom was fractionated by gel filtration (Fig. 2). Three main protein-containing fractions were separated. Fraction I contained the hemorrhagic principle of the venom, fraction II several enzymes (proteases, phospholipase A) and fraction III represents a toxin of crotamine-like activity which is rarely found in *Crotalus adamanteus*, but is present in other *Crotalus* venoms[8]. It has myotoxic activity producing local myonecrosis like myotoxin a from *C. viridis viridis* venom. The last two fractions are protein-free and consist of nucleotides or similar compounds. When the LD_{50} of the fractions was determined by i.v. injection, only fraction I and III exhibited marked lethality, whereas fraction II was essentially non-toxic. Since similar toxins like the myotoxin of fraction III are not present in *Bitis* venom, it was assumed that the hemorrhagic factors in both venoms may be responsible for causing cross-reactions. Preliminary experiments demonstrated that fraction III is not neutralized by both antivenoms.

Since fraction II is essentially non-toxic, it may play a minor role in envenomation. Therefore, the hemorrhagic principle present in fraction I was further purified by ion-exchange chromatography on DEAE-cellulose, the hemorrhagin obtained was a semi-pure product which was compared to a hemorrhagin previously isolated from *Bitis arietans* venom[7]. Immunodiffusion tests using the Ouchterlony method revealed that both crude venoms, *Bitis arietans* and *Crotalus adamanteus*, produce several precipitation lines when reacting with Behringwerke-antivenom (Fig. 3). But also the semi-pure hemorrhagins from both venoms show distinct bands. Moreover, the non-toxic fraction II produced much more precipitation lines indicating that criteria for cross-reactivity based only on immunodiffusion tests are less useful.

In preliminary tests using 50 µg of each hemorrhagin mixed with 0.1 ml antivenom Behringwerke-antivenom was more effective (1:8 dilution) in neutralizing *Crotalus adamanteus* than *Bitis arietans* hemorrhagin (1:4 dilution). Wyeth-antivenom exhibited full protection (more than 1:8 dilution) to *Crotalus adamanteus*, but was ineffective to *Bitis arietans* hemorrhagin.

What are the conclusions from these experiments? Snake venoms con-

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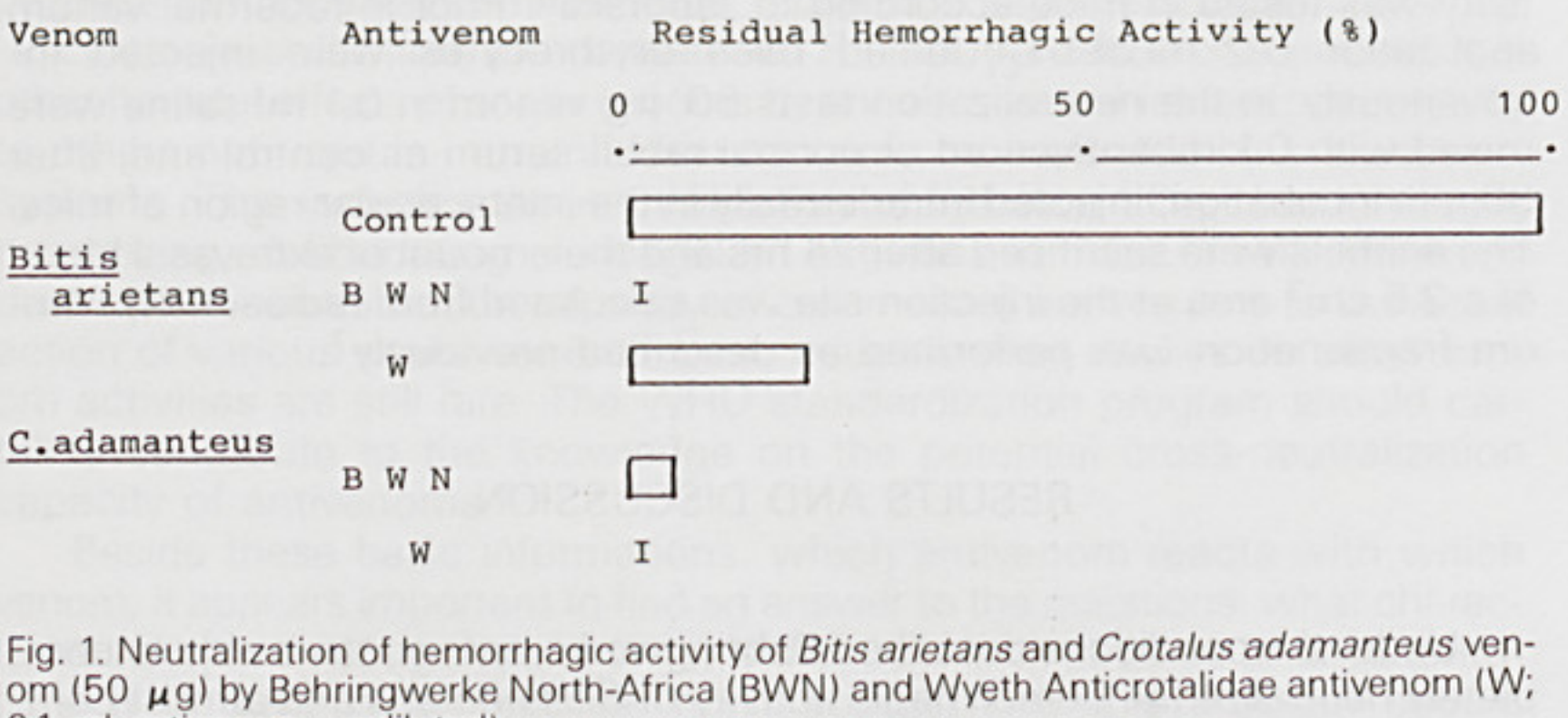

Fig. 1. Neutralization of hemorrhagic activity of *Bitis arietans* and *Crotalus adamanteus* venom (50 μg) by Behringwerke North-Africa (BWN) and Wyeth Anticrotalidae antivenom (W; 0.1 ml antivenom, undiluted).

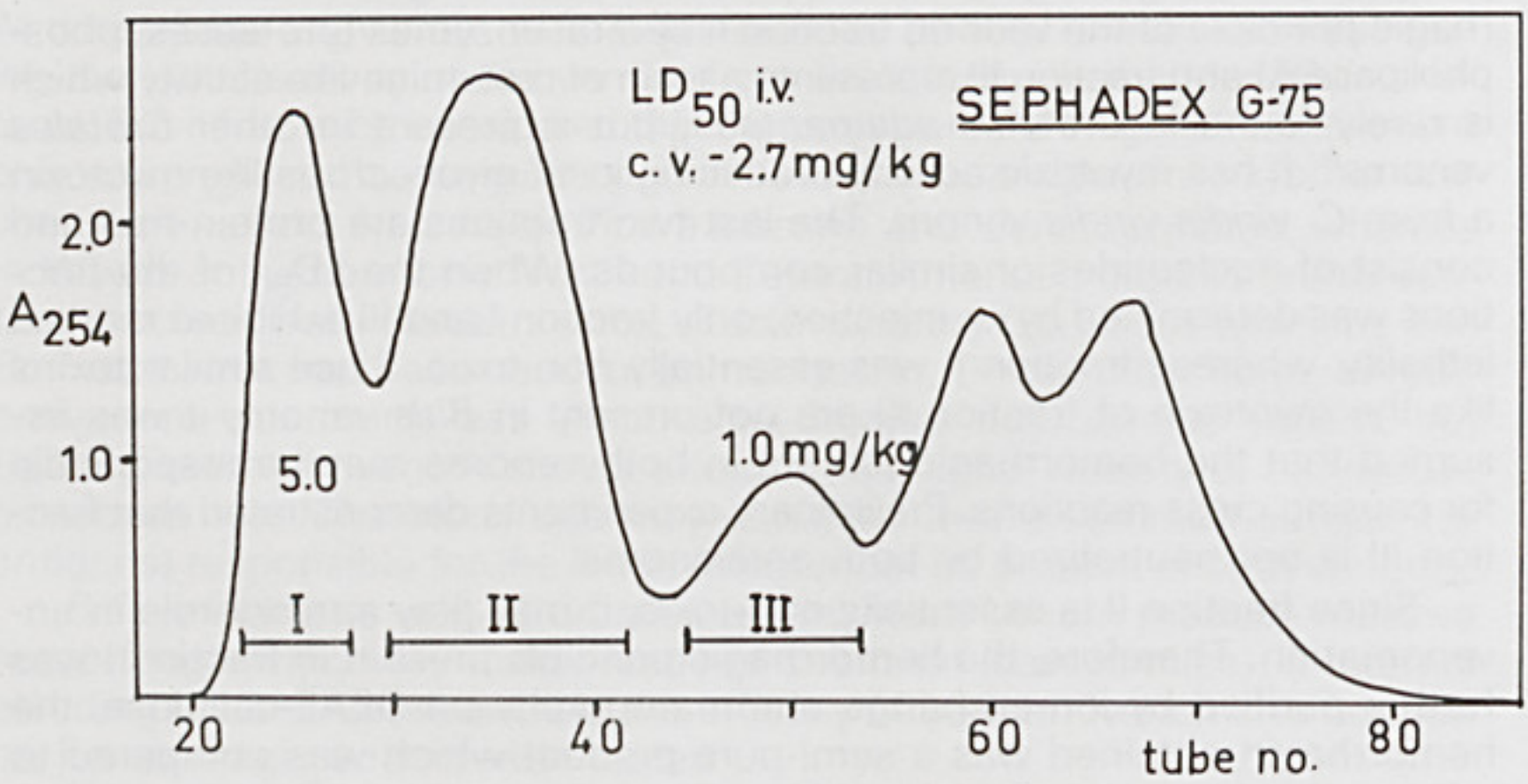

Fig. 2. Gel filtration of 500 mg *Crotalus adamanteus* venom on a Sephadex G-75 column (95 x 2.5cm) eluted with 0.1M ammonium acetate. Fractions of 6 ml were collected at a flow rate of 36ml per hr. LD_{50} values of crude venom (c.v.) and fraction I and III are shown in the elution pattern, fraction II is essentially non-toxic.

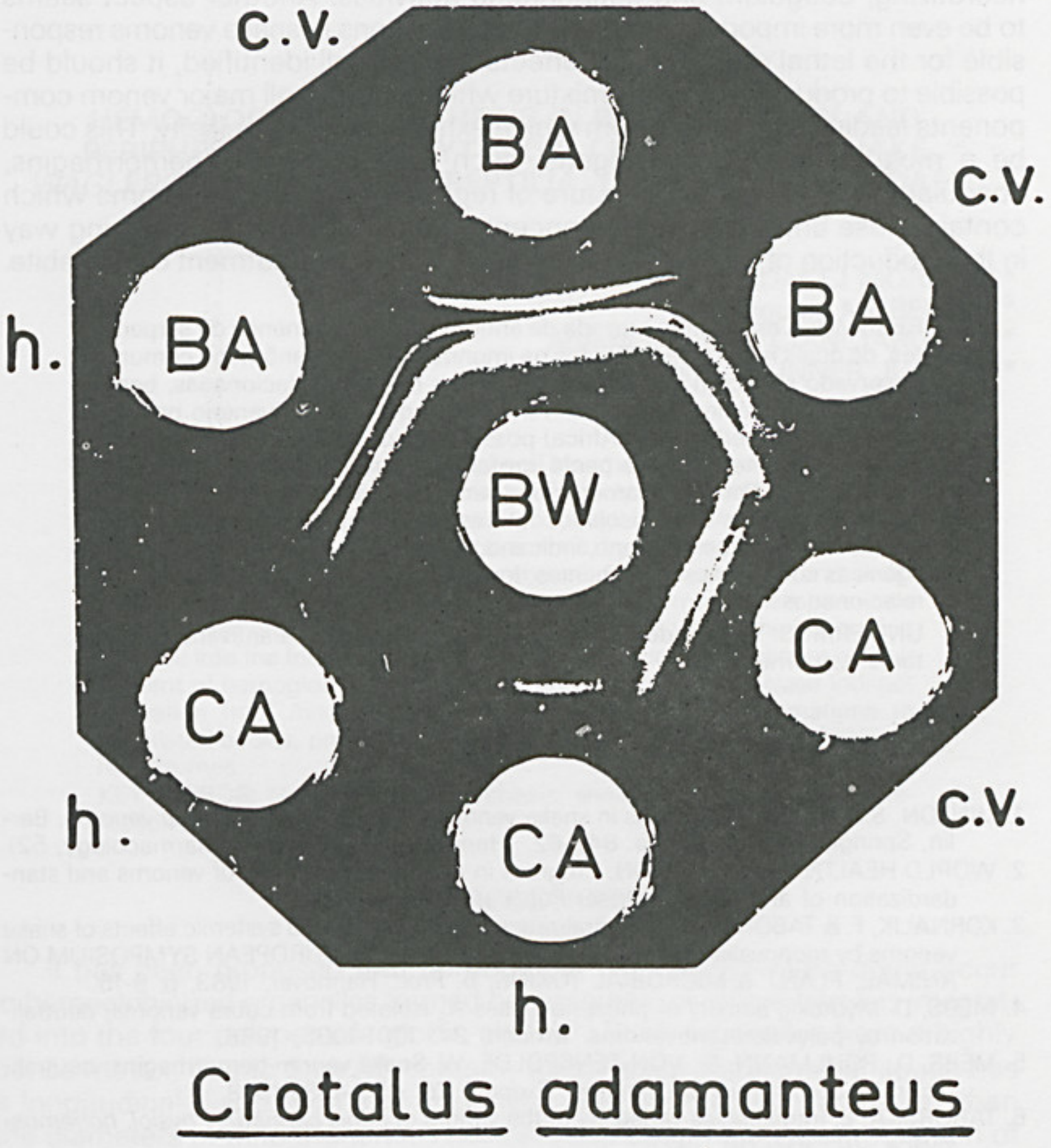

Fig.3. Immunodiffusion-test of *Bitis arietans* (BA), *Crotalus adamanteus* (CA) and of their semi-pure hemorrhagins (h.) to Behringwerke North-Africa (BW) antivenom.

tain common antigens which are of minor importance in envenomation, but others which may also be involved in the venom's lethal or noxious action. From a practical point of view, demonstration of cross-neutralization seems to be of great value, if a wider application of polyspecific antivenoms in snakebite cases is concerned. On the other hand, our present knowledge on cross-reactivity and neutralization is rather poor, if one considers the number of commercial antivenoms available and the tests which could be done with snake venoms involved in human envenomations. Beside lethality-neutralization this should include other test systems such as hemorrhagic, necrotizing, coagulant and defibrinating activities. Another aspect seems to be even more important. Since common antigens in snake venoms responsible for the lethal or pathologic effects can be well identified, it should be possible to produce an antigen mixture which includes all major venom components leading to an antivenom which exhibits broad specificity. This could be a mixture of purified antigens, such as neurotoxins, hemorrhagins, coagulant factors etc., or a mixture of representative snake venoms which contain these antigens in high concentrations. It may be a promising way in the production of more effective antivenoms for the treatment of snakebite.

RESUMO: A reatividade cruzada de antivenenos com venenos de serpentes, os quais não foram utilizados na imunização, é um fenômeno comum observado seja com espécies de serpentes bastante relacionadas, bem como com espécies diferentes. Verificou-se que um antiveneno polivalente (Behringwerke North-Africa) possui considerável potência neutralizante para veneno de serpente crotalidea norte-americana *(Crotalus adamanteus)*. Por fracionamento dos venenos de *Bitis arietans* e de *Crotalus adamanteus* foram isolados fatores hemorrágicos, os quais foram neutralizados pelo antiveneno, indicando a existência de propriedades antigênicas comuns nos constituintes dos venenos, mesmo de espécies não relacionadas.

UNITERMOS: Veneno de serpente, reatividade cruzada de antiveneno, fatores hemorrágicos.

REFERENCES

1. MINTON, S.A. Common antigens in snake venoms. In: LEE, C.Y. ed. *Snake venoms*. Berlin, Springer Verlag, 1979 p. 847-62. (Handbook Experimental Pharmacology, 52).
2. WORLD HEALTH ORGANIZATION. Progress in the characterization of venoms and standardization of antivenoms. *Offset Publication* (58), 1981.
3. KORNALIK, F. & TABORSKA, E. Neutralization of lethal, local and systemic effects of snake venoms by monovalent and polyvalent antivenoms. In: EUROPEAN SYMPOSIUM ON ANIMAL, PLANT & MICROBIAL TOXINS, 5. *Proc.* Hannover, 1983. p. 9-15.
4. MEBS, D. Myotoxic activity of phospholipases A_2 isolated from cobra venoms; neutralization by polyvalent antivenoms. *Toxicon, 24*: 1001-1008, 1986.
5. MEBS, D.; POHLMANN, S.; VON TENSPOLDE, W. Snake venom hemorrhagins; neutralization by commercial antivenoms. *Toxicon, 26*: 453-458, 1988.
6. TABORSKA, E. Intraspecies variability of the venom of *Echis carinatus Physiol. bohemoslovaca, 20*: 307-318, 1971.
7. MEBS, D. & PANHOLZER, F. Isolation of hemorrhagic principle from *Bitis arietans* (puff adder) snake venom. *Toxicon, 20*:509-512, 1982.
8. MEBS, D.; EHRENFELD, M.; SAMEJIMA, Y. Local necrotizing effect of snake venoms on skin and muscle; relationship to serum creatine kinase. *Toxicon, 21*: 393-404, 1983.