Mem. Inst. Butantan
Simp. Internac.
33(3):907-912, 1966



# 32. BIOCHEMICAL INVESTIGATIONS ON *HELODERMA* VENOM

D. MEBS and H. W. RAUDONAT

*Institut fuer gerichtliche und soziale Medizin der Universitaet, Frankfurt a.M., Germany*

## Introduction

Beside the snakes the reptiles contain only one venomous lizard family, the *Gila monster*, HELODERMATIDAE. They exist only in two species, the North American *Gila monster,*, *Heloderma suspectum* and its Mexican counterpart, *Heloderma horridum*. In contrary to the snakes their venom glands are located in the lower jaw, the ducts leading to the exterior terminate between the fangs and the lower lip. During the bite, the venom mixed with saliva is conducted into the wound by capillarity. Each fang possesses an indistinct groove along which the venom moves.

The present work deals with the investigation of the venom with modern biochemical and pharmacological methods.

## Methods and Material

For the investigations lyophilized venom of *Heloderma suspectum* and *Heloderma horridum* from the MIAMI SERPENTARIUM, Miami, U.S.A., was used. The $LD_{50}$ was determined in mice by subcutaneous injection. The hyaluronidase activity was estimated after the turbidimetric method of DORFMAN and OTT with hyaluronic acid as substrate and compared with a standard preparation of hyaluronidase from ovine testes (500 IU/mg, SERVA, Heidelberg, Germany), the phospholipase A activity was determined by the Marinetti method, the proteolytic activity, according Kunitz, and the kinin-releasing activity from plasma globulines was tested on the isolated guinea pig ileum.

## Results and Discussion

The venom of *Heloderma suspectum* and *Heloderma horridum* consists, for the most part, of macromolecular proteins, which can be precipitated with trichloracetic acid and ammonium sulfate.

After 48 hours dialysis against destilled water none of the venom had passed the dialysis membrane. By spectroanalysis it was possible to find potassium, sodium, calcium, magnesium as well as traces of aluminium, zinc, cooper, iron and silicon.

The $LD_{50}$ for *Heloderma suspectum* was 0.82 μg/g mouse, for *Heloderma horridum* 1.4 μg/g mouse, s.c. injection. The toxic factor is heat stable; heating the venom at pH 7.2, 100°C for 15 min no loss of toxicity was observed.

A comparative estimation of cobra venom toxicity (*Naja naja*) resulted in a $LD_{50}$ of 0.8 μg/g mouse, thus in effect the toxicity of *Heloderma* venom is comparable with that of cobra venom.

An enzyme analysis of *Heloderma* venom was run for phosphomono- and diesterase, acetylcholinesterase, nucleotidase, ATPase, DNAase and RNAase, aminoacid oxidase, fibrinogenocoagulase, protease, phospholipase A and hyaluronidase. Of all these enzymes we succeeded only in finding phospholipase A, protease and hyaluronidase. A direct hemolytic factor for example as one can find in small amounts in cobra venom could not be proved, washed human erythrocytes were not hemolysed by *Heloderma* venom.

The hyaluronidase in *Heloderma* venom showed a very high activity and was 20-40 fold higher than that of the ovine testes preparation, for 1 mg *Heloderma* venom 10-20.000 IU could be calculated (Fig. 1).

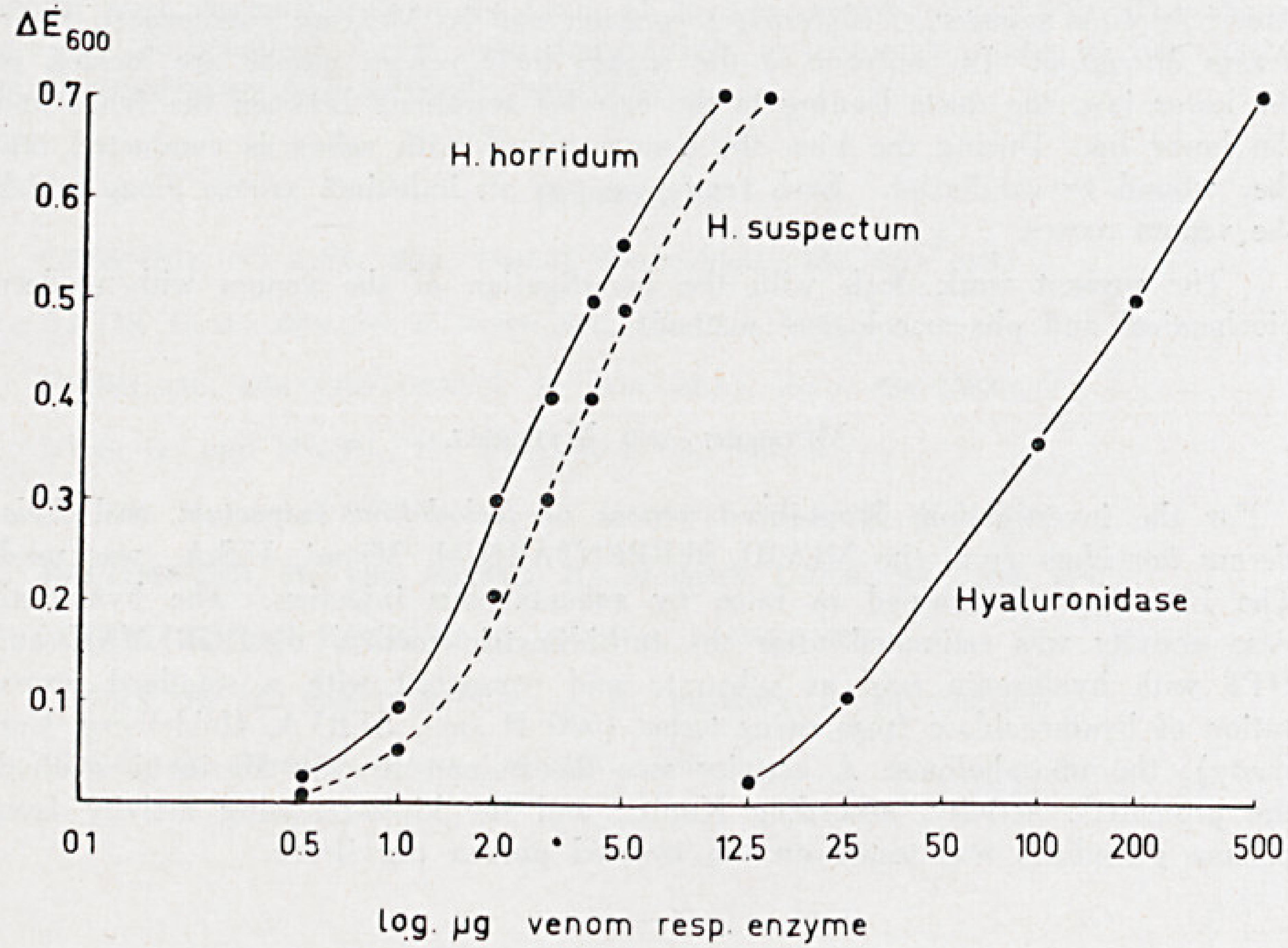


Fig. 1 — Hyaluronidase activity of *Heloderma* venom and Hyaluronidase from ovine testes, in dependence on the turbidity of unaffected hyaluronic acid after incubationtime for 1 h, 37°C.

The phospholipase A which is contained in many animal venoms could be also detected in *Heloderma* venom. Fig. 2 shows the enzyme activity of *Heloderma* venom measured on the rate of clearing of an egg yolk emulsion at pH 7.2, 25°C under the influence of 200 μg venom each, in comparison with cobra venom (*Naja naja*).

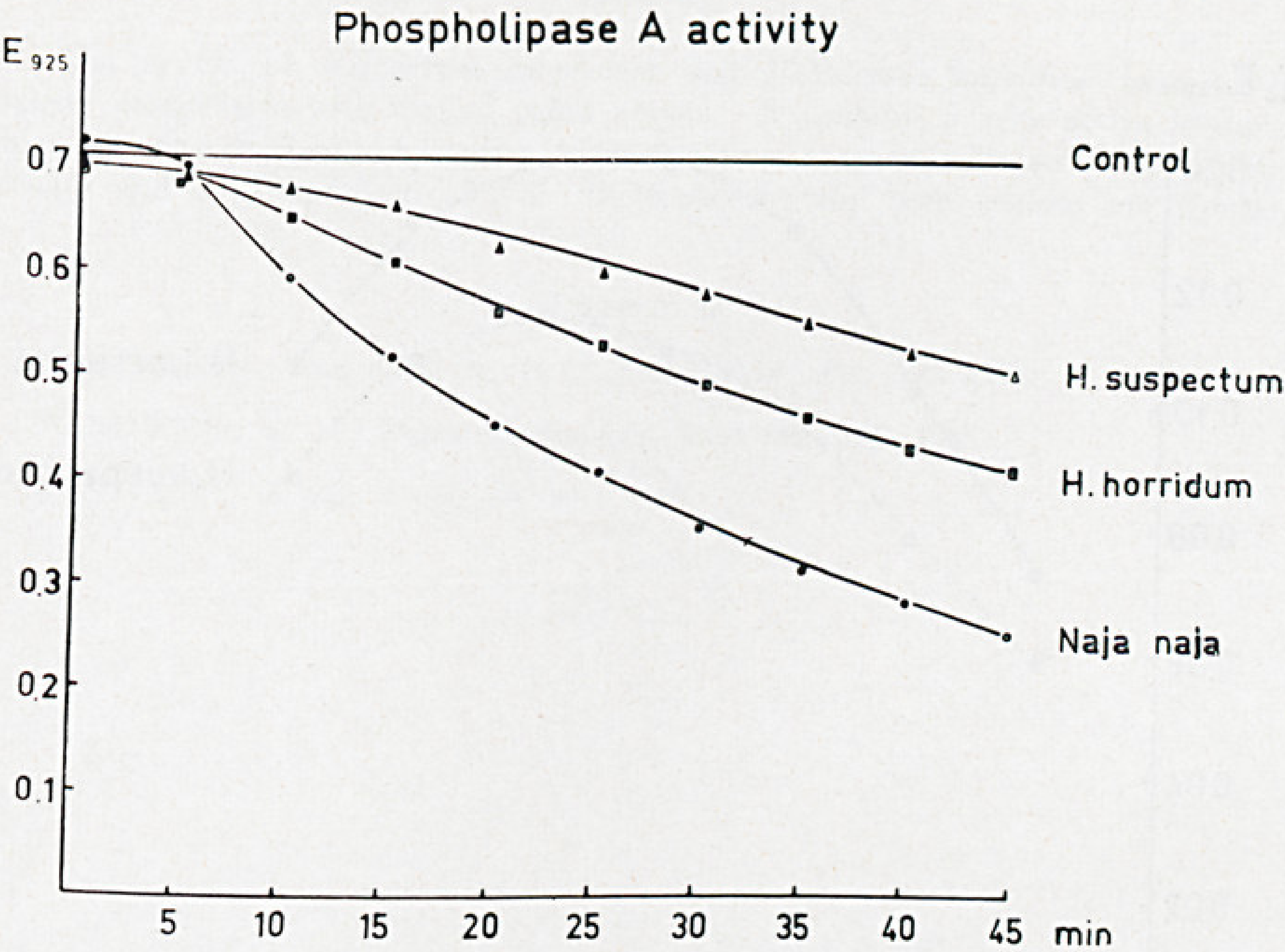


Fig. 2 — Phospholipase A activity of *Heloderma* venom.

*Heloderma* venom showed only a small proteolytic activity with a pH-optimum from 8.0-8.5, tested on casein (Fig. 3). As well as casein, denatured hemoglobin and gelatine were hydrolysed in small rates, likewise benzoyl-arginine-ethyl ester. The proteolytic activity was not affected by soybean trypsin inhibitor.

*Heloderma* venom incubated with bovine plasma globulin releases a substance which causes an atropin resistant contraction on isolated guinea pig ileum. This substance is heat stable and can be extracted from the incubate with hot alcohol and further precipitated with ether from the extract dissolved in acetic acid. Dialysing the alcohol extract against water, even after a relatively short time (3 hours), a part of the ileum contracting activity is found in the dialysate. By the addition of chymotrypsin to the incubate, the ileum contracting factor is rapidly inactivated. A contraction caused by venom-globulin incubate cannot be prevented by antihistaminica.

These facts indicate, that the substance which is released by venom from plasma globulin seems to be bradykinin or a substance like bradykinin. Kinin-release and casein hydrolysis showed the same pH-optimum (8.0-8.5). Paper electrophoresis showed the similarity of the venoms. At pH 6.5, 0.06 M phosphate buffer, seven fractions were observed (Fig. 4), at pH 8.0 only five. Beside the strongly toxic fraction which contains the protease and a part of the hyaluronidase, a further fraction, migrating to the cathode with a lesser toxicity, was separated. The other fractions were nontoxic and without enzymatic activity. *Heloderma* venom, as it is obtained, is a mixture of venom and saliva, so these inactive proteins could be originated from the cavity of the mouth.

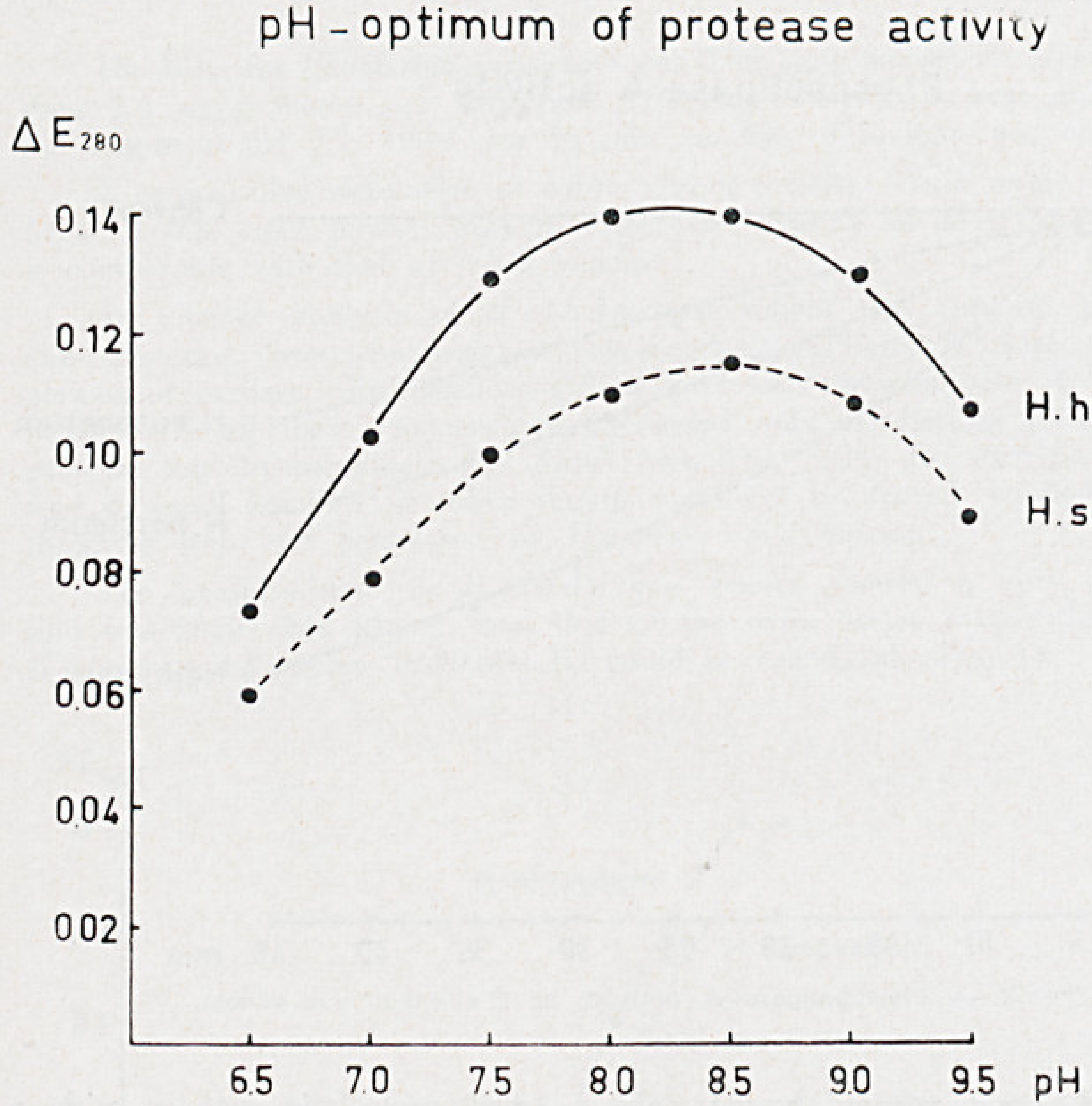


Fig. 3 — pH-optimum of the proteolytic activity of *Heloderma* venom, tested on casein splitting, incubation of 1% casein solution for 5 h, 37°C, with 250 $\mu$g venom each.

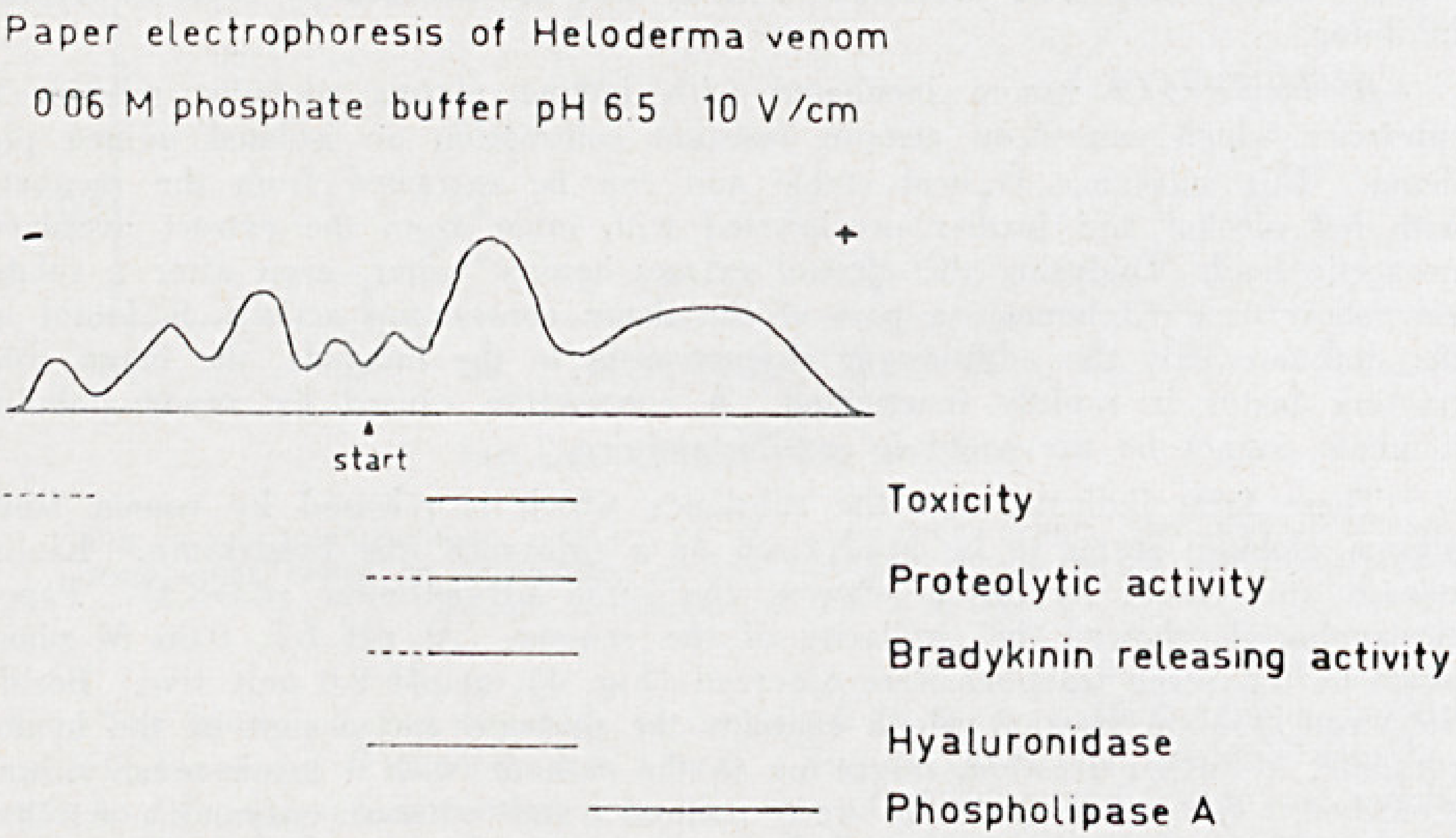


Fig. 4 — Paper electrophoresis of *Heloderma* venom, 0.06 M phosphate buffer pH 6.5, 10 V/cm.

## Summary

The venom of *Heloderma suspectum* and *Heloderma horridum* possesses a toxicity comparable with that of cobra venom. It contains a very active hyaluronidase, a phospholipase A and a kinin-releasing enzyme with small proteolytic activity and a pH-optimum of 8.5. Electrophoretically both venoms are similar.

## References

1. DORFMAN, A., and OTT, M. L., *J. biol. Chem.*, **172**, 367, 1948.

2. MARINETTI, G. V., *Biochim. Biophys. Acta,* **98**, 554, 1965.

3. KUNITZ, M., *J. gen. Phys.*, **30**, 291, 1947.