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# 25. BIOCHEMICAL AND PATHOLOGICAL ASPECTS OF HEMORRHAGIC PRINCIPLES IN SNAKE VENOMS WITH SPECIAL REFERENCE TO HABU (*TRIMERESURUS FLAVOVIRIDIS*) VENOM

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## Introduction

Hemorrhage is one of the most prominent symptoms following the bites by CROTALINAE or VIPERINAE snakes (1, 2). It was thought that certain proteolytic enzymes in snake venoms cause the hemorrhage (1-7).

We established a quantitative method for determining the hemorrhagic activity of the venom of *Trimeresurus flavoviridis* (8). We initiated systematic studies on the principles responsible for the hemorrhage by this method.

Evidences from our (9-12) and other (13-15) laboratories suggested the presence of more than one hemorrhagic principle in snake venoms. We fractionated the venom of *Trimeresurus flavoviridis* by zone electrophoresis and reported the presence of two hemorrhagic principles, that we designated as HR1 and HR2 (9). They are distinct immunologically from each other (16). Attempts were made to correlate the hemorrhagic activity with proteolytic activity, lethal toxicity, and other pathological activities (9-11, 17, 18).

The purpose of this presentation is to review our data and the data by others on the biochemical and pathological aspects of the hemorrhagic principles in the snake venoms.

## Specification and quantitative determination of the hemorrhagic lesion

In order to approach biochemically the mechanism of the local actions caused by the venom it is essential (1) to specify the experimental conditions to reproduce a separate pathological change involved in the whole local pathological lesion and (2) to establish a method for determining quantitatively the specific principle responsible for each specified pathological change on the basis of the principle of bioassay.

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We wish to express our gratitude to the Division of Public Health, Kagoshima Prefecture, Japan, for their generous gifts of Habu venom.

As for the hemorrhagic principles, these requirements were satisfied since we proposed the quantitative method for determination (8). The method consists of intracutaneous injection of 0.1 ml of venom into the depilated back skin of rabbits, measurement of size of the hemorrhagic spot after 24 hr from the inside of the removed skin and the estimation of the activity by the parallel line assay method.

As shown in Fig. 1, the hemorrhagic spot observed from the inner surface is sharply demarcated. A definite hemorrhagic spot visible from the inner surface was produced with such a small amount of Habu venom as 0.1 to 0.3 $\mu$g; while about 100 to 300 times as much dose was required to produce a feeble reaction, not clearly discernible from the outer surface (Fig. 2). The hemorrhage following the injection with such a large amount of venom as 300 $\mu$g spreads throughout the dermis and muscular layer, and necrosis of the muscle fibers was also observed (9). With such a large amount of venom, so-called "necrosis" (19) or "hemorrhagic necrosis" (20) was observable (8, 18).

When the results obtained by our procedures were analysed statistically, the log-dosage response curves obtained with a large number of crude venoms and their fractions were proved to be linear and parallel to each other (8). Some of the results are shown in Fig. 3. By measuring the potencies relative to that of a standard venom using the parallel line assay method, we succeeded in quantitative determinations of the hemorrhagic activities of various preparations of Habu venom. We concluded, therefore, that the size of the hemorrhagic spot in the skin as determined from the inside, but not from the outside, is an exact measure for the intensity of the hemorrhage under the specified conditions (8). We demonstrated that the method was applicable to venoms of different species of snakes.

## Distribution of hemorrhagic, lethal and proteolytic activities in snakes venoms

We carried out comparative studies on hemorrhagic, lethal and proteolytic activities of venoms from various species. Lethal toxicity (9, 18) was assayed by intravenous injection into mice of an inbred strain weighing 14-17 g with four to five doses graded with 1.25-fold intervals. Death within 4 days was ascribed to lethal toxicity of the venom.

It is noted from Table 1 that hemorrhagic activity is distributed in all the venoms of both Crotalinae and Viperinae, although the ratios of $LD_{50}$ to minimum hemorrhagic dose (MHD) especially for samples No. 5 (*Bothrops atrox*), No. 9 (*Crotalus durissus terrificus*) and No. 15 (*Vipera russellii*) are very small; almost none of the venoms of the Elapidae manifest hemorrhagic activity and therefore the ratios for such venoms are much smaller. The only exception is *Ophiophagus hannah* venom (sample No. 21) whose hemorrhagic activity is as high as that of Crotalinae or Viperinae venoms.

Macroscopic observation indicated the similarity of hemorrhagic lesions caused by venoms from different species of snakes. The common slope ($\bar{b}$) of log-dosage response curves for the hemorrhage is 4.72 with the venom of *Trimeresurus flavoviridis*. Values for b obtained with all the other venoms were approximately the same as that for *Trimeresurus flavoviridis*.

The results (Table 1) may be an indication that the hemorrhagic activity is not directly associated with the proteolytic activity or the lethal toxicity in the crude venoms.

TABLE 1 — DISTRIBUTION OF THE HEMORRHAGIC, LETHAL AND PROTEOLYTIC ACTIVITIES IN SNAKE VENOMS

| Snake venom | Hemorrhagic activity MHD and its fiducial limits (μg) | Lethal activity $LD_{50}$ and its fiducial limits (μg) | Proteolytic activity (units/mg) | $LD_{50}$/MHD |
|---|---|---|---|---|
| 1. *A. contortrix contortrix* | 1.90 (1.20-2.92) | 200 (163-245) | 33.7 | 105 |
| 2. *A. contortrix mokasen* | 1.20 (0.76-1.90) | 125 (102-153) | 48.1 | 104 |
| 3. *A. piscivorus piscivorus* | 0.80 (0.52-1.20) | 60.0 (48.0-75.0) | 41.5 | 75 |
| 4. *A. halys* | 0.14 (0.09-0.22) | 16.0 (13.0-20.0) | 35.8 | 114 |
| 5. *Bothrops atrox* | 2.11 (1.40-3.20) | 5.6 (4.7-6.7) | 44.5 | 2.7 |
| 6. *Bothrops jararaca* | 0.75 (0.48-1.20) | 18.5 (16.0-22.0) | 74.0 | 25 |
| 7. *C. adamanteus* | 0.04 (0.03-0.06) | 18.5 (16.0-22.0) | 9.76 | 462 |
| 8. *C. atrox* | 0.43 (0.28-0.67) | 45.0 (38.0-54.0) | 83.6 | 105 |
| 9. *C. durissus terrificus* | 18.0 | 3.6 (3.0-4.3) | 39.2 | 0.2 |
| 10. *C. viridis viridis* | 0.56 (0.37-0.85) | 21.0 (17.0-26.0) | 39.5 | 38 |
| 11. *T. flavoviridis* | 0.20 (0.14-0.30) | 54.0 (46.0-63.0) | 33.0 | 270 |
| 12. *T. flavoviridis tokarensis* | 1.15 (0.78-1.80) | 160 (130-196) | 37.8 | 139 |
| 13. *T. elegans* | 0.30 (0.20-0.46) | 71.0 (59.0-85.0) | 13.0 | 237 |
| 14. *T. okinavensis* | 1.38 (0.91-2.10) | 140 (117-167) | 69.0 | 102 |
| 15. *Vipera russellii* | 21.0 | 2.2 (1.8-2.6) | 5.56 | 0.1 |
| 16. *Vipera ammodytes* | 0.47 (0.31-0.72) | 7.4 (6.3-8.7) | 41.7 | 16 |
| 17. *Vipera palestinae* | 0.54 (0.34-0.92) | 7.1 (5.9-8.5) | 4.96 | 13 |
| 18. *Causus rhombeatus* | 0.81 (0.53-1.30) | > 250 | 0.26 | > 300 |
| 19. *Bitis arietans* | 0.15 (0.10-0.22) | 15.0 (13.0-18.0) | 18.7 | 100 |
| 20. *Bitis gabonica* | 0.04 (0.03-0.06) | 13.5 (11.5-16.0) | 11.7 | 338 |
| 21. *Ophiophagus hannah* | 0.84 | 54.0 (46.0-63.0) | 12.0 | 64 |
| 22. *Bungarus fasciatus* | » 100* | 18.5 (16.0-21.0) | 0.06 | 0.18 |
| 23. *Naja melanoleuca* | » 100 | 6.0 (4.8-7.5) | 7.00 | « 0.06 |
| 24. *Naja naja atra* | » 100* | 8.0 (6.5-9.8) | 0.12 | « 0.08 |
| 25. *Naja naja* | » 100 | 5.6 (4.4-7.2) | 2.85 | « 0.06 |

A.: *Agkistrodon* C.: *Crotalus* T.: *Trimeresurus*

* A pinkish macule was observed at the site of injection.

## Evidence for the presence of two hemorrhagic principles in certain snake venoms and for relationships of these principles to proteolytic, lethal and other pathological activities

The presence of more than one hemorrhagic principle in certain snake venoms has been suggested (9-16); attempts have been made to correlate the hemorrhagic activity to proteolytic activity (9-11, 14, 15, 18, 21).

We fractionated the venom of *Trimeresurus flavoviridis* by zone electrophoresis and demonstrated the presence of two hemorrhagic principles, HR1 and HR2 (9, 10), which are distinct immunologically from each other (16). Both of the hemorrhagic principles contained proteolytic activity (9, 10). CM-cellulose chromatography also indicated the presence of two hemorrhagic principles; one was separated from the main part of proteolytic activity, while the other associated with it (11, 14). Iwanaga and his associates (21) purified "proteinase b", one of the three proteinases present in the venom of *Agkistrodon halys* and stated that it is one of the two hemorrhagic principles in this venom.

It would be of much interest to clarify whether or not the principles responsible for hemorrhage also manifest lethal toxicity. We separated one of the hemorrhagic principles (HR2) from the main part of lethal toxicity but failed to separate the other principle (HR1) (9). Immunological studies of HR1 not yet published suggested that separate entities are responsible for the hemorrhagic activity and the main part of lethal toxicity. Separation of hemorrhagic activity from lethal toxicity was indicated also by Gitter and his associates (15) with the venom of *Walterinnesia aegyptia*. On the other hand, Omori and his associates (13) fractionated the venom of *Agkistrodon halys* by DEAE-cellulose chromatography and reported a close association of the major part of the lethal toxicity with the main hemorrhagic fraction.

It would be also of much interest to know whether or not one and the same principle is responsible for necrosis and hemorrhage (7, 18). Histological observations done by us (9) indicated that the muscle degeneration, which led to necrosis or death of the muscle fiber in its severer forms, did not run parallel to either hemorrhagic activity or proteolytic activity.

Further purification of the hemorrhagic principles is needed to correlate hemorrhagic activity with proteolytic, lethal and other pathological activities.

## Purification of the hemorrhagic principles in the venom of *Trimeresurus flavoviridis*

We attempted purification of the hemorrhagic principles of *Trimeresurus flavoviridis* (Batch No. 64-A). The two hemorrhagic principles HR1 and HR2 (Step 1 in Table 2), separated by zone electrophoresis (9), were further purified by different procedures shown in Fig. 4.

*Purification of HR1:* Step 2 — A pooled fraction of HR1 (corresponding to 6 g of the crude venom) separated by zone electrophoresis was concentrated by lyophilization and dialysed against 0.005 M Tris-HCl buffer, pH 8.5. The solution at a concentration of 50 mg protein per ml was treated with solid ammonium sulfate to 60% saturation (369 g per liter) (22) and left to stand at 0° for several hours for partial settling. The precipitate was collected by centrifugation at 8,000 r.p.m. for 15 min. The precipitate was dissolved in 30 ml of 0.005 M Tris-HCl buffer, pH 8.5 at a concentration of approximately 65 mg protein per ml. Step 3 — The solution was passed through a Sephadex G-100 column (4 × 97 cm) previously equilibrated with the same buffer. Step 4 — The hemorrhagic fractions from Step 3 were combined and concentrated to about 14 ml by lyophilization. The solution was passed through a Sephadex G-200 column (5 × 115 cm) previously treated with the same buffer. Step 5 — The hemorrhagic fractions from Step 4 were combined, concentrated by lyophilization and

dialysed against 0.005 M Tris-HCl buffer, pH 8.5, the final volume being about 7 ml. The dialysate was placed on a CM-cellulose column (3 × 50 cm). The break-through fractions containing the hemorrhagic activity were combined. Step 6 — The combined fraction was concentrated by lyophilization and dialysed against 0.005 M Tris-HCl buffer pH 8.5. The dialysate of 7 ml containing 120 mg of protein was applied to a DEAE-cellulose column (1.5 × 30 cm). A linear gradient elution with 400 ml of the buffer and 400 ml of the buffer containing 0.5 M NaCl was started.

*Purification of HR2:* Step 2 — A pooled fraction of HR2 (corresponding to 6 g of the crude venom) separated by zone electrophoresis was concentrated by lyophilization and a final volume adjusted to 30 ml. The solution at a concentration of 37.5 mg protein per ml was passed through a Sephadex G-100 column (5 × 100 cm) previously equilibrated with 0.005 M Tris-HCl buffer, pH 8.5, containing 0.15 M NaCl. Step 3 — The hemorrhagic fractions from Step 2 were combined and concentrated to 40 ml by lyophilization. The concentrated solution containing about 15 mg protein per ml was passed through a Sephadex G-75 column (5 × 86 cm) previously equilibrated with 0.005 M Tris-HCl buffer, pH 8.5, containing 0.15 M NaCl. The hemorrhagic activity was eluted in the void volume. Step 4 — The hemorrhagic fractions were combined, concentrated by lyophilization and dialysed against 0.005 M Tris-HCl buffer, pH 8.5 for several hours. Twenty-six ml of the dialysate containing 467 mg of protein was applied to a DEAE-cellulose column (4.6 × 12 cm). After a break-through peak had been collected, elution was carried out with 500 ml of the buffer containing 0.3 M NaCl. The break-through fraction contained the hemorrhagic activity.

TABLE 2 — SUMMARY OF PURIFICATION PROCEDURES FOR HR1 AND HR2

| Step | Protein (mg) | Hemorrhagic activity | | | Proteolytic activity | |
|---|---|---|---|---|---|---|
| | | MHD (μg) | relative specific act. | recovery (%) | specific act. (units/mg of protein) | relative specific act. |
| Starting material | 6,000 | 0.220 | 1.00 | 100 | 24.80 | 1.00 |
| HR1 | | | | | | |
| 1. Electrophoresis | 3,670 | 0.255 | 0.94 | 55.0 | 6.55 | 0.26 |
| 2. Ammonium sulfate (0 - 60%) | 1,890 | 0.259 | 0.86 | 27.2 | 8.26 | 0.33 |
| 3. Sephadex G-100 | 500 | 0.0825 | 2.69 | 22.2 | 14.41 | 0.58 |
| 4. Sephadex G-200 | 162 | 0.0367 | 5.95 | 16.3 | 16.20 | 0.65 |
| 5. CM-cellulose | 120 | — | — | — | — | — |
| 6. DEAE-cellulose | | | | | | |
| a fraction | 14.4 | 0.0193 | 11.41 | 6.6 | 7.66 | 0.31 |
| b fraction | 22.5 | 0.0214 | 10.29 | | 17.65 | 0.71 |
| HR2 | | | | | | |
| 1. Electrophoresis | 1,125 | 0.220 | 1.00 | 18.8 | 74.10 | 2.99 |
| 2. Sephadex G-100 | 590 | 0.311 | 0.71 | 7.0 | 96.20 | 3.88 |
| 3. Sephadex G-75 | 467 | 0.312 | 0.71 | 5.5 | 129.00 | 5.20 |
| 4. DEAE-cellulose | 333 | 0.294 | 0.75 | 4.2 | 128.00 | 5.17 |

The yield and the extent of purification at each step are summarized in Table 2. The hemorrhagic activity of HR1 was eluted from DEAE-cellulose into two peaks. The first peak of hemorrhagic activity (a-fraction in Step 6) containing less proteolytic activity than the second one served as the test sample of HR1 in the following experiment. The specific hemorrhagic activity of HR2 did not increase after Step 2 and the preparation in this step served as the test sample of HR2 in the following experiment.

## The action of the hemorrhagic principles on animal cells cultivated *in vitro*

The crude venom of *Trimeresurus flavoviridis* and of the partially purified hemorrhagic principles, HR1 and HR2, were tested for toxic action on animal cells cultivated *in vitro* (17). The cell strains employed included HeLa cells, MLg cells originated from the lung of new-born mice of ddY strain and T5 cells originated from human embryonic fibroblasts.

The change of the cells observed in the earliest stage was rounding. When the rounded cells predominated, the cells became detached from the glass surface resulting in disruption of the cell monolayer. We designated the activity responsible for this change as the cell monolayer-disrupting activity (17).

Table 3 shows the effect of the crude venom and the partially purified hemorrhagic principles on the monolayer of T5 or HeLa cell. It is noted that HR2 with a high proteolytic activity was more potent in the cell monolayer-disrupting activity than HR1 with a low proteolytic activity.

As shown also in Table 3, HR1 at 30 $\mu$g protein per ml did not show any cell monolayer-disrupting effect within 24 hr but did show some effect after 48 hr. The cell monolayer-disrupting activity of HR1 on T5 cells is roughly one sixth that of the crude venom on the basis of protein content (Table 3). The specific hemorrhagic activity of HR1 is about 11 times higher than that of the crude venom (Table 3). Therefore, the cell monolayer-disrupting activity of HR1 per unit hemorrhagic activity is calculated as about one seventieth that of the crude venom. We conclude, therefore, that the hemorrhagic activity of HR1 is virtually independent of the cell monolayer-disrupting (or cytopathic) activity (17).

These results on one hand confirmed and on the other hand contradicted the suggestion made by Gaertner and her associates (23) that there is a close association of cytopathic with hemorrhagic and proteolytic activities.

Fig. 5 demonstrates the absence of parallelism between disruption of the MLg cell monolayer and cytocidal action by the venom preparations. The MLg cell monolayer was disrupted by the crude venom and by the preparation of HR2 but the disruption did not run parallel to the viability of the cells. It is, therefore, very likely that the venom preparations primarily act on the cell surface causing detachment of the cells from the glass surface but not causing serious damage to the vital function of the cells (17).

TABLE 3 — EFFECT OF THE CRUDE VENOM AND THE PARTIALLY PURIFIED HEMORRHAGIC PRINCIPLES ON THE MONOLAYER OF T5 OR HeLa CELL

| Cell strain used | Incubation period(hr) | Venom preparations* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Crude (Hr=1.0 Pr=1.0) | | | HR 1 (Hr=11.4 Pr=0.3) | | | HR 2 (Hr=0.7 Pr=3.9) | | |
| | | 30µg | 10µg | 5µg | 30µg | 10µg | 5µg | 30µg | 10µg | 5µg |
| T5 | 1.5 | +++ | - | - | - | - | - | +++ | - | - |
| | 3.0 | +++ | ± | - | - | - | - | +++ | +++ | - |
| | 4.5 | +++ | + | - | - | - | - | +++ | +++ | - |
| | 24.0 | +++ | ++ | - | - | - | - | +++ | +++ | + |
| | 48.0 | +++ | +++ | ++ | ++ | - | - | +++ | +++ | +++ |
| HeLa | 1.5 | +++ | - | NO | - | - | NO | +++ | - | NO |
| | 3.0 | +++ | ± | NO | - | - | NO | +++ | +++ | NO |
| | 4.5 | +++ | ± | NO | - | - | NO | +++ | +++ | NO |
| | 24.0 | +++ | ++ | NO | - | - | NO | +++ | +++ | NO |
| | 48.0 | +++ | +++ | NO | + | - | NO | +++ | +++ | NO |

−: No change (identical to the control cell culture)
±: Slight morphological changes of the cells without detachment from the glass surface
+: Rounding of the cells without detachment from the surface
++: Partial disruption of the cell monolayer
+++: Complete disruption of the cell monolayer
NO : Not tested
Hr : Specific hemorrhagic activity relative to the crude venom
Pr : Specific proteolytic activity on casein relative to the crude venom
* : Per ml of culture medium

## Conclusion

We succeeded in reproducing experimentally the hemorrhage under our specified conditions. We also succeeded in establishing a quantitative method for determining the hemorrhagic activity. By this method we initiated systematic studies on the principles responsible for the hemorrhage.

We demonstrated that hemorrhagic activity is widely distributed in all the venoms of Crotalinae and Viperinae snakes. Evidences were accumulated to suggest the presence of at least two or more hemorrhagic principles in venoms of certain snakes including *Trimeresurus flavoriridis*. The venom of *Trimeresurus flavoriridis* was fractioned to correlate the hemorrhagic activity with proteolytic activity, lethal toxicity or other pathological activities.

However, further experiments will be necessary to characterize more precisely the principles responsibles for the hemorrhage.

## ADDITIONAL LEGEND TO TABLE 1

Hemorrhagic activity was determined by the method previously described (8). The minimum hemorrhagic dose (MHD) was defined as the least quantity of venom causing a hemorrhagic spot of 10 mm in diameter 24 hr after the intracutaneous injection. Lethal activity was assayed by intravenous injection into mice of an inbred strain weighing 14-17 g with four to five doses graded with 1.25-fold intervals. The $LD_{50}$ was calculated by the Reed-Muench method (24). The standard error of the $LD_{50}$ was calculated according to Pizzi (25). Proteolytic activity was estimated at pH 8.5 with casein as substrate. One unit of the activity was defined as the amount of enzyme hydrolyzing casein at such an initial rate that the amount of TCA-soluble products formed per minute gives the same optical density as that of 1 μg of tyrosine with the Folin reagent.

The venom of *Crotalus adamanteus* was purchased from Ross Allen's Reptile Institute, Silver Springs, Florida, U.S.A. The venom of *Bothrops atrox* was obtained from a supplier in Brazil. *Naja naja atra* venom of Formosan origin and *Bungarus fasciatus* venom were supplied by Dr. T. Suzuki of the Institute for Protein Research, Osaka University, Osaka, Japan and *Naja naja* venom by Dr. B. N. Ghosh of the University College of Science, Calcutta, India. Venoms of *Trimeresurus okinavensis* and *Agkistrodon halys* were obtained from a supplier in Tokyo. *Trimeresurus flavoviridis* venom was supplied by the Division of Public Health, Kagoshima Prefecture, Japan and *Trimeresurus elegans* venom by the Institute of Hygiene of the Ryukyu, Japan. The venom of *Trimeresurus flavoviridis tokarensis* was supplied by Dr. H. Fukushima of Kagoshima University, Kagoshima, Japan. All the other venoms were purchased from the California Corporation for Biochemical Research, Los Angeles, California, U.S.A.

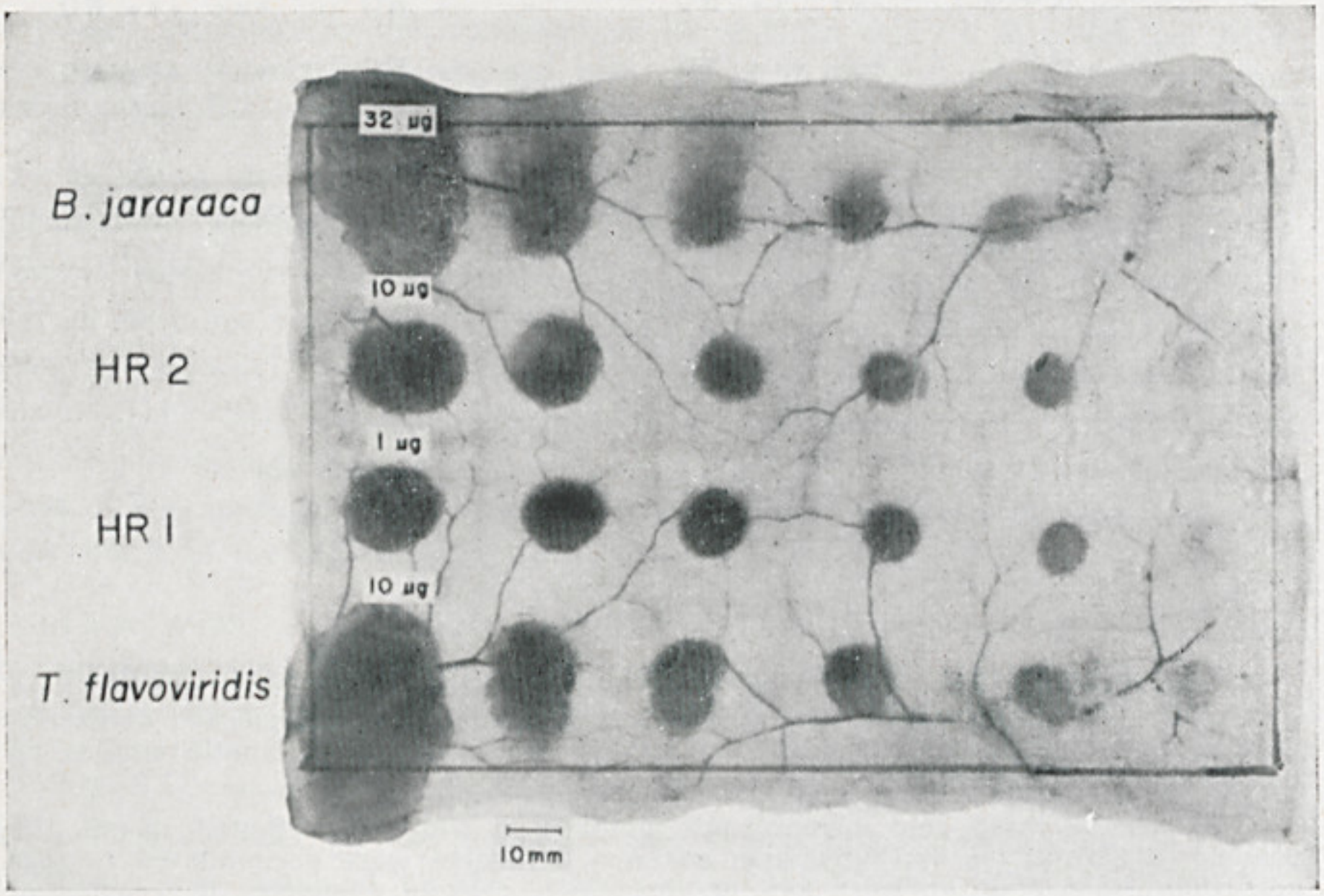


Fig. 1 — Patterns of hemorrhage observed from the inside of the removed skin. An aliquot (0.1 ml) from each of 3-fold dilutions of the venom of *Trimeresurus flavoviridis* or the partially purified hemorrhagic principles (HR1 and HR2) was injected intracutaneously into a rabbit and the reactions were observed after 24 hr. The venom of *Bothrops jararaca* was also injected for comparison.

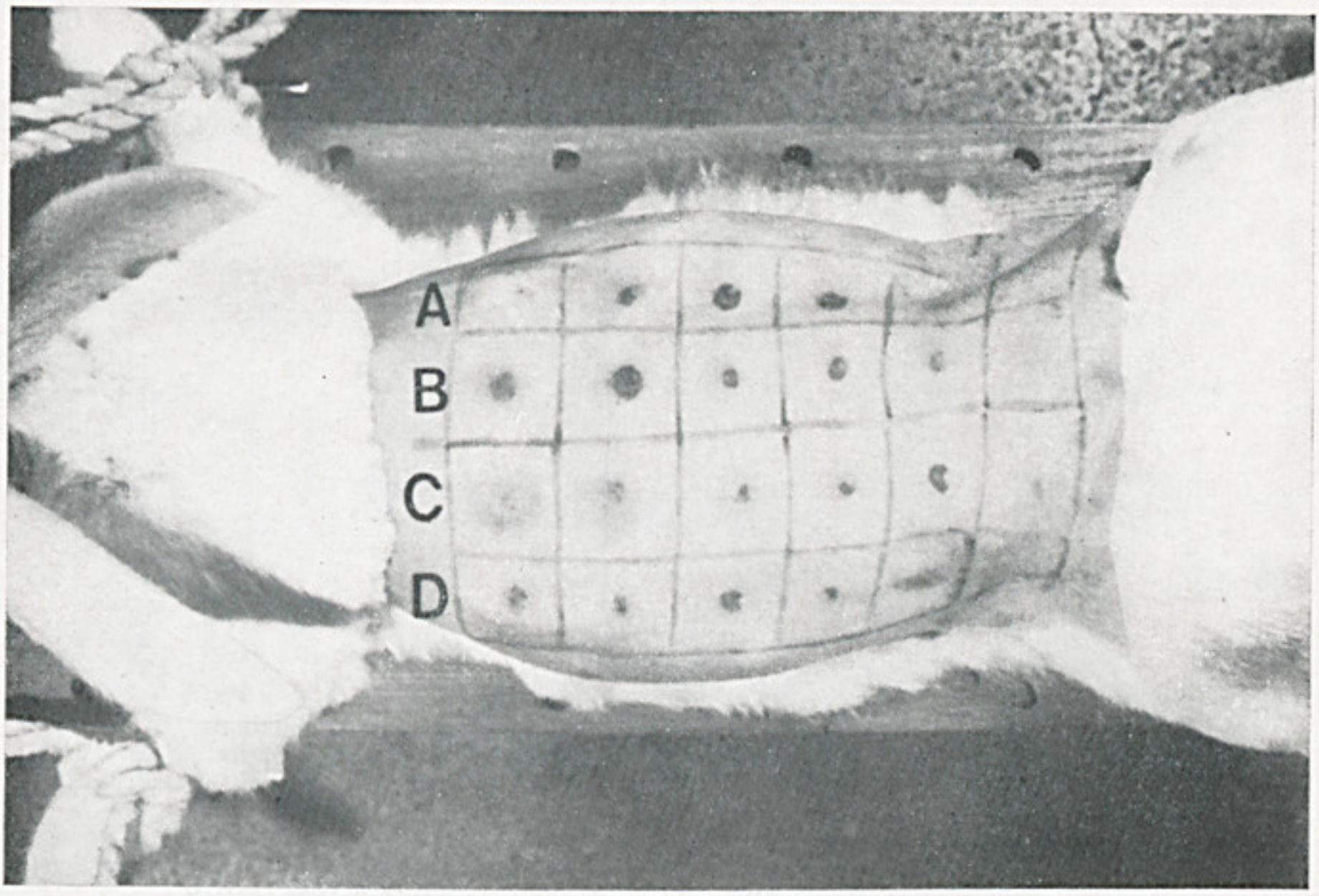


Fig. 2 — Patterns of hemorrhage observed from the outside. The skin is the same which is demonstrated in fig. 1. A: The venom of *Trimeresurus flavoviridis*; B: HR1; C: HR2; D: The venom of *Bothrops jararaca*.

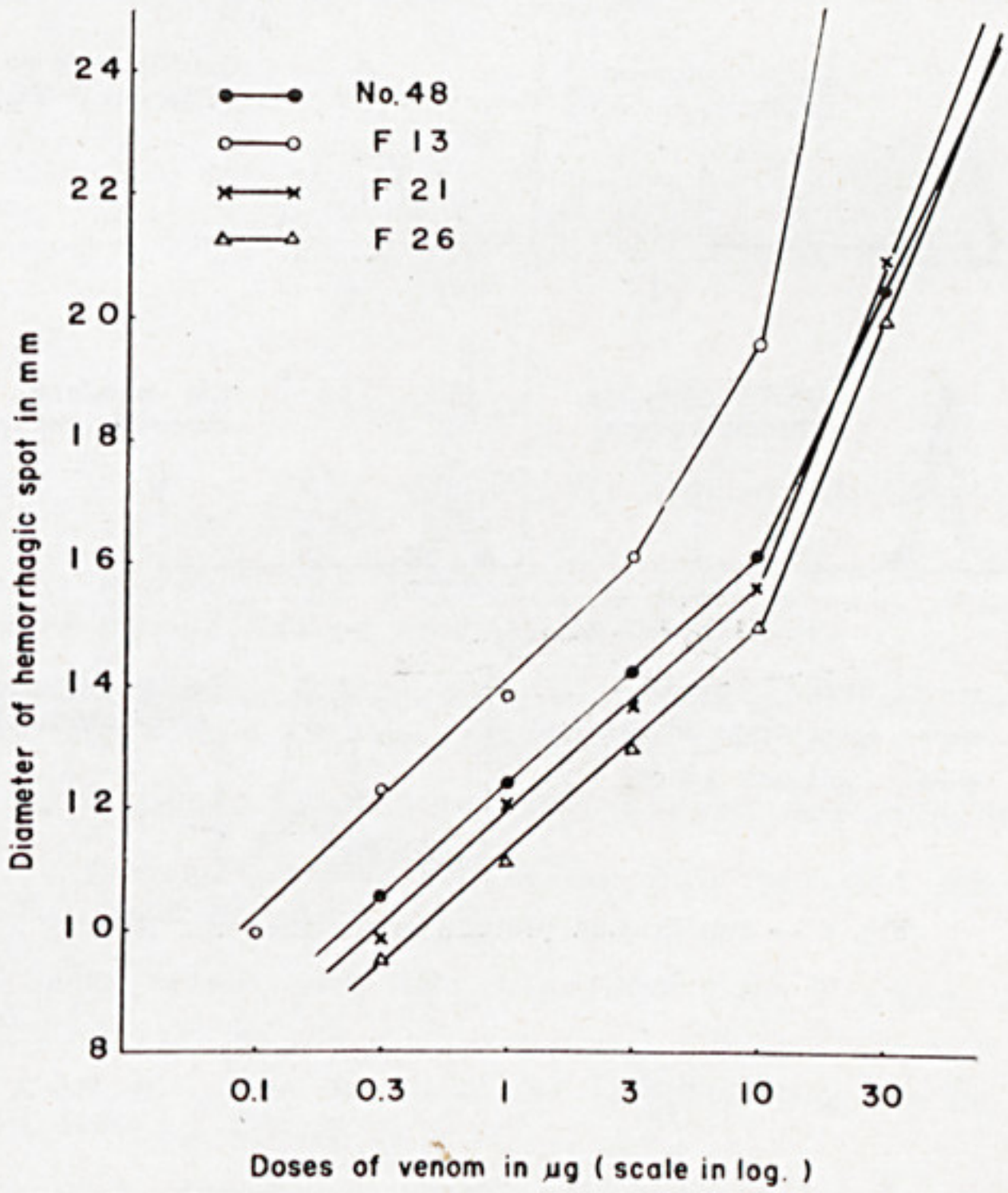


Fig. 3 — Dosage response curve for a crude venom (Batch nº 48) and its electrophoretic fractions (F 13 and F 26).

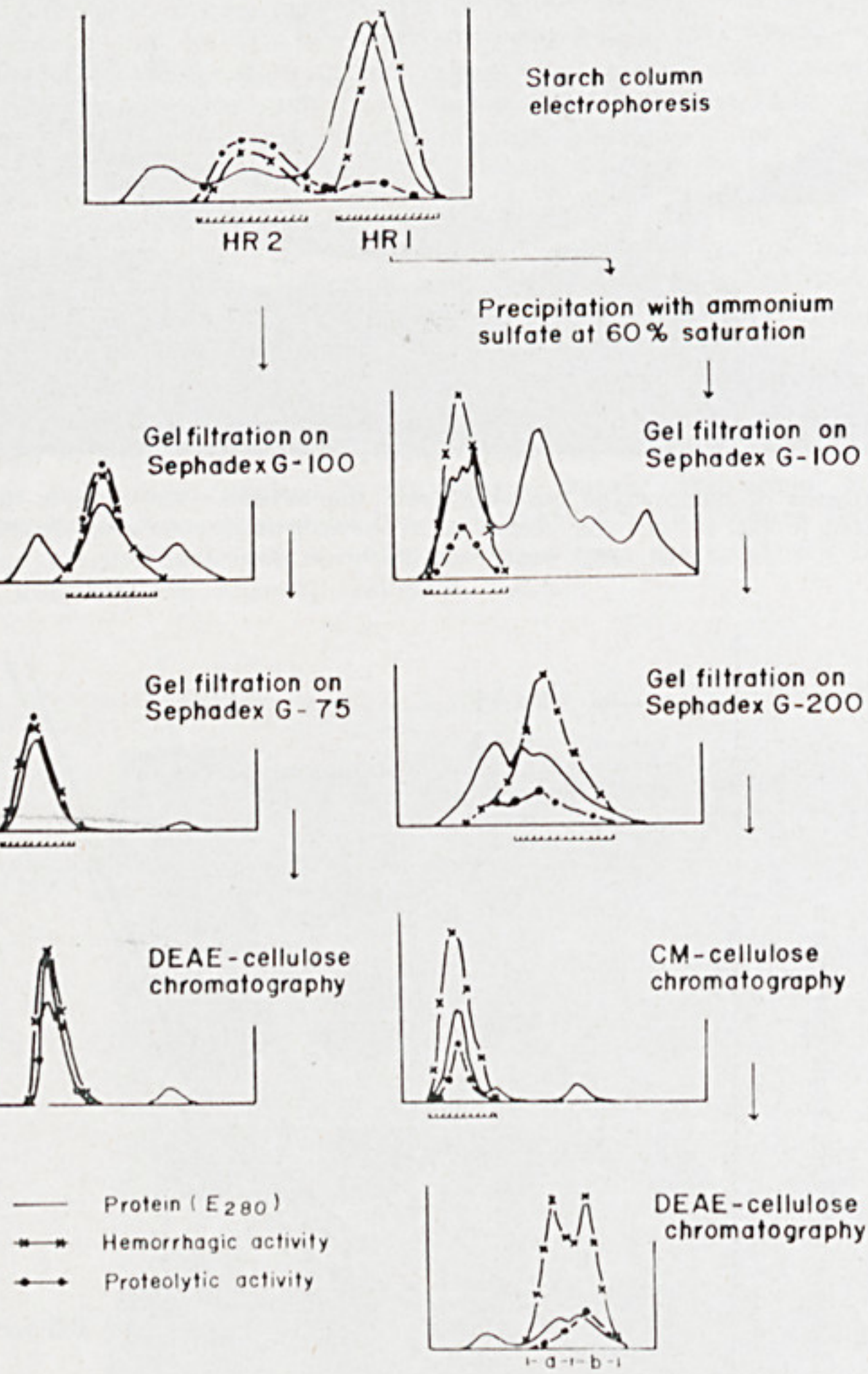


Fig. 4 — Purification procedures for HR1 and HR2.

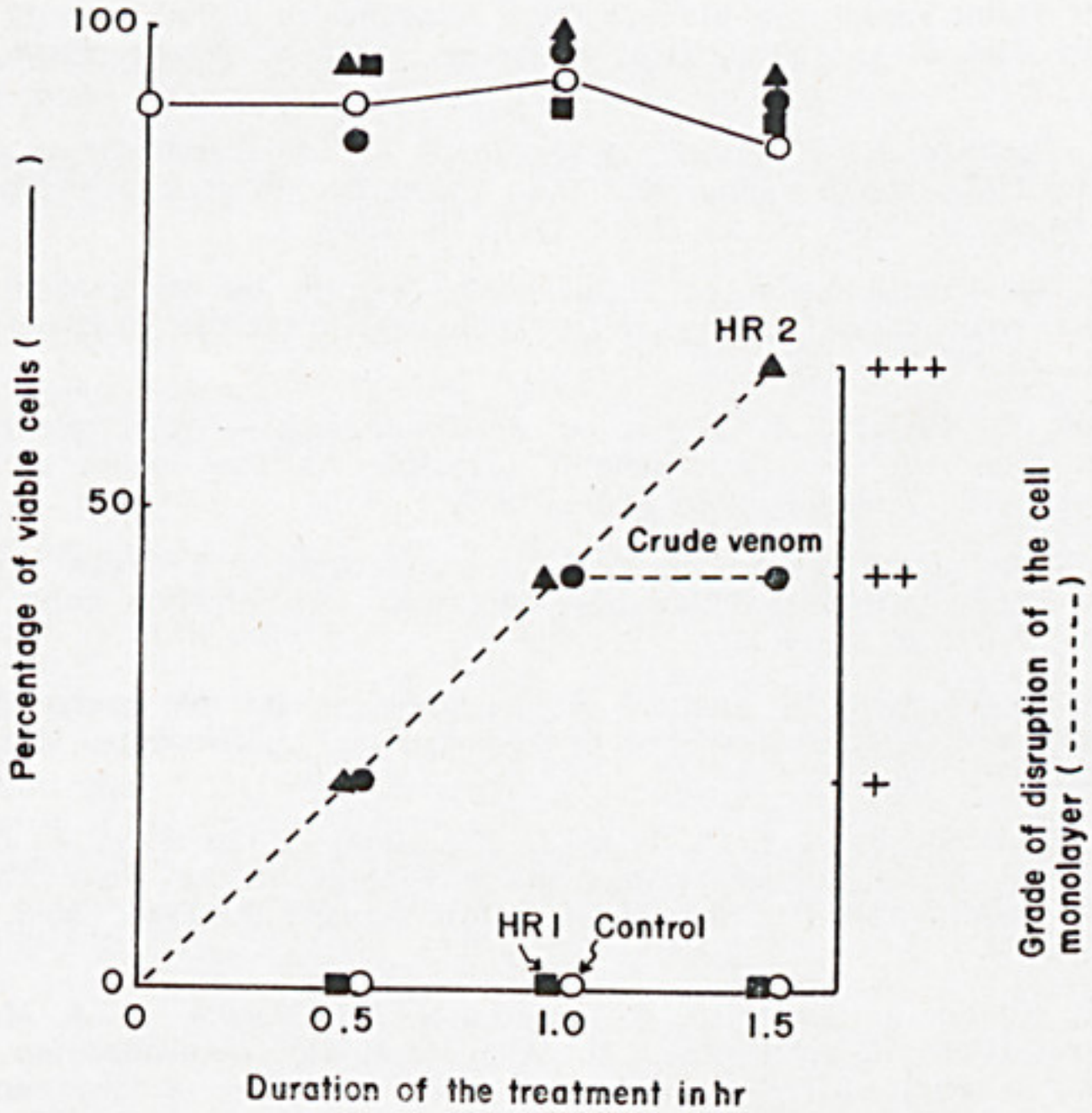


Fig. 5 — Disruption of the MLg cell monolayer without cytocidal action by the venom preparations. (The concentration of the venom preparations was 20 microgram/ml. See also the legend to Table 3 for the grades of disruption of the cell monolayer).

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## Discussion

*C. Purananda:* "The haemorrhage principles $HR_1$ and $HR_2$ once introduced into the body, how long will they remain inside the body? Did you follow up?

*A. Ohsaka:* I don't know how long these principles remain in living animals because I haven't followed up."

*P. Krag:* "Were the neutralizing curves for $HR_1$ and $HR_2$ with same slope when treated quantitavely?"

*A. Ohsaka:* "Slopes of neutralization curve for $HR_1$ and $HR_2$ were statistically the same, showing a value around one."

*A. do Amaral:* "1. Have you done with the Mamushi venom the same large series of experiments (tests) as with the Habu venom? 2. Have you used, in the good series of venoms you tested, always and consistently the same process in: a) extraction; b) preparation; c) preservation — for every one of those venoms?"

*A. Ohsaka:* No, we haven't done any experiments with Mamushi venom. But Dr. Suzuki and his associates have worked on the separation of hemorrhagic principles from the crude Mamushi venom. 2. The venom of *Trimeresurus flavoviridis* was a pool of specimens collected throughout one year, being processed under the same conditions of extraction and drying and stored under the same conditions. All the other venoms we used were commercial preparations or gifts from other institutions."