Mem. Inst. Butantan
25(1):1-4, 1953



# DECREASED CLOTTING TIME OF RABBIT BLOOD INDUCED BY SNAKE VENOM INJECTION (*)

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The blood clotting action of different snake venoms, especially of those of the *Bothrops* genus, has been well established, but whether injections of diluted solutions of these venoms have any effect on the coagulability of the blood is still a matter of discussion. The increased coagulability of the blood after injections of snake venom solutions has been reported by different authors and has also been recommended as a treatment in hemorrhagic conditions, Stockton and Franklin (1), Peck and Sobotka (2), Hanut (3), Klobusitzky and König (4). Others authors, Kauer, Bird and Reznikoff (5), more recently, deny the increased coagulability of the blood after injections of venom solutions, and also deny the possible usefulness of snake venom products administered parenterally in hemorrhagic conditions. We thought it interesting therefore to reinvestigate this problem by applying the silicone technique, as described by Quick (6) which makes it possible to observe considerably prolonged normal clotting times. The increased coagulability of the blood can be more easily demonstrated by shortening these prolonged clotting times than with the ordinary Lee and White method.

We found the normal blood clotting time of rabbits to be 37.2 minutes, this being the mean of 30 determinations. Then we injected rabbits with increasing doses of *Bothrops* venom (a saline solution containing 0.3% phenol and 0,75 µg/cc *Bothrops atrox* venom, which was previously heated and precipitated at pH 5.0). We found that doses between 12 µg and 240 µg decreas-

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(*) This study was supported by a contract with the Conselho Nacional de Pesquisas.

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Received for publication on February 20, 1953.

ed the clotting time significantly, as shown in Table I. Doses higher than 240 µg. while further shortening clotting times, frequently killed the animals although the toxicity of the heated and precipitated venom is considerably decreased.

TABLE I

*Influence of increased doses of snake venom on clotting time of rabbits, 15 minutes after injection.*

| N.° of rabbits | Dose inj. in µg/kg | Coagulation time (mean in minutes) | Standard deviation |
|---|---|---|---|
| 30 | — | 37.2 | 5.2 |
| 5 | 1 | 37.2 | 6.1 |
| 10 | 4 | 35.6 | 2.8 |
| 15 | 15 | 24.5 | 2.7 |
| 10 | 60 | 20.1 | 2.8 |
| 5 | 240 | 18.0 | 2.6 |

To see how long the decreased clotting times could be observed rabbits were injected, then periodically bled. We found that 5 minutes after the injection of 60 µg a significant decrease in clotting time could be observed and that after 5 hours the clotting time returned to normal as shown in Table II. Rabbits injected with an effective dose showed always normal clotting times after 24 hours.

TABLE II

*Influence of time on increased coagulability of rabbit blood. Dose injected, 60 µg/kg.*

| N.° of rabbits | Time after injection minutes | Coagulation time (mean in minutes) | Standard deviation |
|---|---|---|---|
| 7 | 5 | 20.4 | 3.7 |
| 10 | 15 | 20.1 | 2.8 |
| 5 | 30 | 21.4 | 2.6 |
| 5 | 60 | 22.2 | 2.3 |
| 7 | 120 | 22.1 | 2.8 |
| 4 | 300 | 39.5 | 3.5 |

We also injected rabbits with saline containing the same quantity of phenol as the venom solution, to see whether a simple injection i.e. the trauma could, by itself, cause a decreased clotting time. The mean clotting time taken from 12 animals treated in this way, was 39.6 minutes, showing that these injections had no action whatsoever.

The significantly shorter clotting times observed in more than 250 determinations of 130 rabbits after the injections of the heated and precipitated snake venom solution prove the influence of these venoms on the coagulability of rabbit blood. The positive phase lasted for at least 2 hours. After 5 and after 24 hours the clotting times were normal again.

The mechanism by which snake venoms decreased the clotting time of the blood of rabbits is not clear yet. The power of these venoms to activate prothrombin, or their thrombinomimetic action in vitro, cannot be the explanation, as injections of dilute thrombin or thromboplastin solutions did not result in the shortening of the normal blood clotting time. On the contrary, they resulted, confirming Houssay's findings (7) with snake venom, in the slow precipitation of fibrin and the decreased or complete incoagulability of the blood: Jürgens and Studer (8), Gerendás and Csapó (9). Some other mechanism must therefore be involved, perhaps as suggested by Mauro (10) and Breda, Bernardi, and Sala (11), the thromboplastic content is increased by the action of snake venoms. Nevertheless the mechanism of action of snake venoms on blood clotting still remains a matter of investigation.

## SUMMARY

Snake venom *(Bothrops atrox)* injected intravenously in rabbit in dosis between 12 and 240 µg/k produced a shortening of the clotting time measured in siliconized tubes. The mechanism of such action remains a matter of investigation.

## RESUMO

Veneno botrópico *(Bothrops atrox)* injetado na veia de coelhos em doses de 12 a 240 ug/k, produzem um nitido encurtamento do tempo de coagulação, determinado em tubos siliconizados. O mecanismo de ação dêsse fenômeno continua sendo um tema de investigação.

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