A STUDY OF THE RIBONUCLEASE ACTIVITY
OF SNAKE VENOMS*

BY ARMANDO R. TABORDA,† LAURA C. TABORDA,† J. N. WILLIAMS, JR.,
AND C. A. ELVEHJEM

(From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison, Wisconsin)

(Received for publication, August 31, 1951)

The observation that snake venoms are able to hydrolyze thymus and yeast nucleic acids was first made by Delezenne and Morel (1) in 1919. This discovery remained practically forgotten until 1945 when Gulland and Walsh (2) employed the nuclease in the venom of Vipera russelli in studying the composition of yeast ribonucleic acid. The presence of nucleases in snake venoms assumes a role of great importance if their property can be correlated with the mechanism of poisoning by the venoms, as well as with other physiological actions of snake venoms, particularly the action upon cancer cells (3–5). The connection between cytoplasmic ribonucleic acid (RNA) and protein synthesis in the cell, as emphasized by Caspersson and others (6-8), is highly suggestive of the relation between nucleic acids and the physiological processes involving normal and atypical growth.

Because of the lack of specific knowledge concerning the nuclease activity of snake venoms and because of the possible importance of these enzymes in relation to some of their physiological actions, the present study has been undertaken. In this investigation ribonuclease activities of various snake venoms have been determined, and a detailed study of the properties of one venom in particular, Bothrops jararaca, has been carried out. In the latter case the effects of various inhibitors and activators, some of which are known to influence the toxicity of the venom, have been studied in an attempt to observe whether any correlation between toxicity and ribonuclease activity exists.

EXPERIMENTAL

Methods and Materials—The ribonuclease (RNase) activity of the snake venoms was determined manometrically by the method of Bain and Rusch

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by a grant from the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.
† Permanent address, Instituto Butantan, São Paulo, Brazil.
This involved measuring the evolution of the carbon dioxide liberated from a bicarbonate buffer by the action of free acidic groups produced by the enzyme. The RNA samples employed as substrate were commercial preparations which were further purified in this laboratory by the method of Zittle (10) to remove nucleotides, nucleosides, and inorganic substances which might interfere with the assays.

The snake venoms employed in these experiments were collected at the Instituto Butantan, São Paulo, Brazil, where they were lyophilized or dried at 37° and stored in sealed glass tubes. The antivenom serum used was also prepared at the same institution.

As shown by Kunitz (11) and Bain and Rusch (9), the optimum pH for RNase activity is about 7.5. Therefore, all the components of the test systems were adjusted to pH 7.5, except the venom. The venom was not adjusted with alkali, in order to prevent any possible changes in the venom proteins.

**RNase Activity of Various Snake Venoms**—The venoms employed in these studies were venoms from Bothrops jararaca, Bothrops alternata, Bothrops atrox, Bothrops jararacussu, Bothrops neuwidii, and Crotalus terrificus terrificus. In all cases solutions of the venom and RNA substrate were freshly prepared immediately before experiment. Studies of the optimum substrate concentration for one of the venoms, B. jararaca, revealed that optimum activity was obtained through a broad range of concentrations from 60 to 100 mg. of RNA per 20 mg. of dried venom. Higher concentrations tended to inhibit the activity, while with lower concentrations the maximum activity was not obtained. Therefore, in all the succeeding experiments, 60 mg. of RNA per 20 mg. of dried venom were used as the substrate level.

The venom was weighed and dissolved in 0.15 M sodium chloride. The RNA was first suspended in water and dilute sodium hydroxide added until pH 7.5 was reached. The final test system was as follows: 1.0 ml. of 0.1 M sodium bicarbonate and 2.0 ml. of RNA solution containing 30 mg. per ml. pipetted into the main compartment of the Warburg flasks; 0.5 ml. of the venom solution containing 40 mg. of dried venom per ml. in the side arm. After gassing with 95 per cent nitrogen and 5 per cent carbon dioxide and a 10 minute equilibration period at 37°, the enzyme was tipped into the main compartment cup, the stop-cocks were closed after 3 minutes of shaking, and readings of carbon dioxide evolution were taken at 10 minute intervals for 2 hours.

Control flasks containing all the components of the system except the RNA solution were included in each experiment in order to measure the carbon dioxide evolution due to the action of the venom on the buffer. These values were subtracted from the results obtained by the action of
the venom RNase on the RNA substrate. Other control flasks contained all components of the system except the enzyme, in order to observe whether there was any gas exchange by the substrate. In both types of controls the volume of the substances omitted was replaced by distilled water.

Table I shows the data obtained from measurements of the RNase activities of *B. jararaca, B. alternata, B. atrox, B. jararacussu, B. neuwidii,* and *C. terrificus terrificus* venoms. The activities of each of these venoms are expressed as microliters of carbon dioxide evolved per hour per mg. of dried venom. These figures were obtained by calculating the slopes of the curves obtained when the rate of carbon dioxide evolution was plotted. The comparison of the RNase activities of these venoms shows a very important fact, that the most potent venoms in toxicity, *C. ter-

### Table I

<table>
<thead>
<tr>
<th>Snake</th>
<th>CO₂ per hr. per mg. dried venom</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bothrops alternata</em></td>
<td></td>
</tr>
<tr>
<td>&quot; atrox</td>
<td>3.8</td>
</tr>
<tr>
<td>&quot; jararaca</td>
<td>4.4</td>
</tr>
<tr>
<td>&quot; jararacussu</td>
<td>6.6</td>
</tr>
<tr>
<td>&quot; neuwidii</td>
<td>4.0</td>
</tr>
<tr>
<td><em>Crotalus terrificus terrificus</em></td>
<td>7.2</td>
</tr>
</tbody>
</table>

*terricus terrificus* and *B. jararacussu,* are also the venoms exhibiting the highest RNase activities.

**Detailed Study of B. jararaca Venom**

**Action of Heat on RNase Activity**—The well known denaturing effect of heat on proteins has been used quite frequently for the inactivation of toxins and venoms. Several authors have studied the attenuation by heat of the different physiological actions of snake venoms, such as the toxicity, blood clotting, proteolysis, hemolysis, etc. (12-15). Taborda (16) has reported the effect of temperature upon the toxicity, blood clotting, and proteolytic activities of *B. jararaca* venom.

A solution of venom containing 40 mg. per ml. was prepared and aliquots were heated in a water bath until the following respective temperatures were reached: 50°, 60°, 70°, 80°, 90°, and 100°. Maximum coagulation of the venom protein occurred between 60–70°. The RNase activity was measured in the whole coagulated venom, in the supernatant after centrifuging, and in the precipitate as described above. Fig. 1 sum-
marizes the results of a typical experiment of this kind. Only the activity of the whole heated venom and its supernatant is recorded, since the precipitate was found to be completely inactive. Complete inactivation of the RNase activity was observed by heating the venom to 80°, 90°, and 100°. Several remarkable differences in the RNase action of the whole venom and its supernatant were observed. After about 40 per cent inactivation by heating at 50°, a recovery of the original value occurred at 60°, followed by a sudden and sharp inactivation of 80 per cent at 70°. It should be pointed out that between 60–70° (62–63°) the co-

![Figure 1: Effect of heat on RNase activity of B. jararaca venom. Solid line, whole heated venom; broken line, supernatant after removing coagulum of whole heated venom by centrifugation.](http://www.jbc.org/)

agulation of a considerable amount of the venom protein began. It may have been at this point that the RNase enzyme was inactivated. It should be emphasized that the breaks in the curves were not accidentally obtained, since they occurred in all experiments of this type of which the one depicted in Fig. 1 is typical.

Action of Anti-Bothropic Serum on B. jararaca RNase—Since the toxicity of a venom can be neutralized by its antiserum, it appeared important to observe whether the antiserum of a venom has any effect on the RNase activity of this venom, assuming, of course, that the RNase is connected with the toxicity of the venom. In the following experiments the neutralization effect of the anti-Bothropic serum upon the RNase of the B. jararaca (a homologous venom) and C. terrificus terrificus (a heterologous venom) venom was studied.
The same general method of Bain and Rusch (9) for measuring the RNase activity in the other experiments was also employed here except that 0.5 ml. of venom (40 mg. per ml.) plus 1.0 ml. of anti-Bothropic serum (1 ml. neutralizes the toxicity of 1.5 mg. of B. jararaca venom) was placed in the main cup and incubated with closed stoppers for 1 hour at 37°. Control flasks with 0.5 ml. of venom (40 mg. per ml.) plus 1.0 ml. of water instead of serum were treated similarly. Control flasks with venom plus normal horse serum were also used for observing whether there was any neutralization effect of the non-immune serum. The results indicate that the anti-Bothropic serum was able to neutralize 100 per cent of the RNase activity of the B. jararaca venom and 83 per cent of the activity of the C. terrificus terrificus venom. No effect of the non-immune serum was observed.

Inhibitors and Activators of B. jararaca RNase—The purpose of the following experiments was to observe the effect of various substances upon the activity of venom RNase and to compare these results with known effects upon toxicity as far as possible. The anti-Bothropic serum has been shown in a preceding section to neutralize the RNase activity of B. jararaca venom completely and of C. terrificus terrificus almost completely. The effect on B. jararaca RNase of other known inhibitors of toxicity, such as formaldehyde (17-20) and cysteine (21), was also studied. In addition various other substances, listed in Table II, known to affect the activity of certain enzymes were included in these experiments. Because of recent clinical observations that the adrenocorticotropic hormone appears to alleviate the toxic effects of some venoms, the effect of adrenocorticotropic hormone¹ and cortisone¹ upon venom RNase activity in vitro was studied.

In order to compare directly the effects of formaldehyde upon toxicity and RNase activity of the same venom, solutions of B. jararaca venom were incubated for 2 hours at 37° with 40 per cent formaldehyde in the same proportions and concentrations as those used in the manometric inhibition studies. The incubated material was then used for studying the effects of formaldehyde upon the toxicity of the venom by injecting it into chickens and measuring the minimum lethal dose.²

¹ The authors wish to express their gratitude to Dr. David Klein of The Wilson Laboratories, Chicago, and to Dr. Karl Folkers of Merek and Company, Rahway, for the generous samples of adrenocorticotropic hormone (Corticotropin, Wilson) and cortisone, respectively.

² 1 m.l.d. = 300 γ. The venom was injected into the subwing vein of New Hampshire Red chickens whose average weight was 200 gm. If death occurred in 10 ± 1 minutes, 1 m.l.d. had been injected. The chickens were generously supplied by Mr. Philip Derse of the Wisconsin Alumni Research Foundation.
ordinary minimum lethal dose could be injected without ill effects, demonstrating that the formaldehyde incubation had destroyed toxicity of the venom. Table II shows that the RNase activity of the venom was inhibited 90 per cent.

The same method outlined previously for determining RNase activity was employed in these experiments. Table II summarizes the results obtained. Formaldehyde, cyanide, cupric ions, and cysteine markedly inhibited activity of the enzyme. Manganese and magnesium increased activity of the enzyme, which was depressed by addition of citrate in both cases. Adrenocorticotrophic hormone and cortisone had no effect upon the RNase activity, although the clinical results showing a possible depression of toxicity by ACTH might very well have been indirect effects.

### Table II

**Effect of Various Inhibitors and Activators on RNase Activity of B. jararaca Venom**

<table>
<thead>
<tr>
<th>RNase plus</th>
<th>Concentration</th>
<th>Per cent inhibition</th>
<th>Per cent activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn++</td>
<td>0.003</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Mn++ + citrate.</td>
<td>0.003 + 0.01</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Mg++</td>
<td>0.003</td>
<td></td>
<td>41</td>
</tr>
<tr>
<td>Mg++ + citrate.</td>
<td>0.003 + 0.01</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td></td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Sodium fluoride</td>
<td>1.0</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Ninhydrin</td>
<td>0.1</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Sodium cyanide</td>
<td>1.0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Cu++</td>
<td>0.01</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Cu++</td>
<td>0.1</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Ca++</td>
<td>1.0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Sodium arsenate</td>
<td>0.1</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>0.1</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Ag⁺⁺</td>
<td>0.1</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Iodoacetic acid</td>
<td>0.1</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Hg++</td>
<td>0.01</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.2</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Cortisone</td>
<td>(1 mg. per flask)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Corticotropin, Wilson</td>
<td>(0.1 ml. per flask)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

The results reported in the preceding experiments indicate the importance of the presence of RNase in snake venoms. It is possible that this enzyme implements the toxicity of venom by supplementing the hydrolytic activity of the proteases, peptidases, lipases, and phosphatases, which are also known to be present in snake venoms. It is interesting to note
the striking correlation between toxicity and RNase activity. For example, the most toxic venoms studied, C. terrificus terrificus and B. jararacussu, also showed the highest nuclease activity. Other examples of the correlation between RNase activity and toxicity have been demonstrated in the investigation of the effects of inhibitors on the RNase activity of the venom. Some of the most striking examples in this respect were the inhibition by the antivenom serum, formaldehyde, and cysteine, which are known to inhibit toxicity of snake venoms strongly. The enzyme was also completely inhibited by cyanide and cupric ions.

**SUMMARY**

1. A study has been made of the ribonuclease activity of various snake venoms, particularly those of the genera Bothrops and Crotalus.

2. The effects of various physical and chemical agents upon the ribonuclease activity of Bothrops jararaca venom have been studied in detail. The agents inhibiting the enzymes most markedly are heat, anti-Bothropic serum, formaldehyde, cysteine, cyanide, and cupric ions. The first four are agents known to be able to diminish or even completely neutralize the toxicity of snake venoms.

**BIBLIOGRAPHY**

A STUDY OF THE RIBONUCLEASE ACTIVITY OF SNAKE VENOMS
Armando R. Taborda, Laura C. Taborda, J. N. Williams, Jr. and C. A. Elvehjem


Access the most updated version of this article at http://www.jbc.org/content/194/1/227.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/194/1/227.citation.full.html#ref-list-1