BRADYKININ, A HYPOTENSIVE AND SMOOTH MUSCLE STIMULATING FACTOR RELEASED FROM PLASMA GLOBULIN BY SNAKE VENOMS AND BY TRYPsin

M. ROCHA E SILVA, WILSON T. BERALDO1 AND G. ROSENFELD*

From the Department of Biochemistry and Pharmacodynamics, Instituto Biologico
SÃO PAULO, BRAZIL

In the course of experiments on the physiological action of the venom of Bothrops jararaca, we found that some blood samples taken from a dog after the injection of minute doses of the venom had a stimulating effect upon the isolated gut of the guinea pig. This was not due to direct action of the venom since the gut had been previously made refractory to it; no desensitization could be observed after several additions of the serum to the perfusing bath containing the piece of guinea pig ileum. The stimulating principle disappeared from the blood very quickly after injection and could not be detected in samples taken a few minutes later.

Addition of the venom to defibrinated blood of a normal dog led to the release of a potent stimulating agent very similar to that detected in the blood after injection in vivo. That this substance could not be identified with any of the known pharmacologically active constituents of normal blood was made clear by preliminary tests and by experiments presented below. Among the well defined principles that have been described in the blood are: a) histamine, which is practically absent from dog plasma or serum (1); b) thrombocytin (2, 3) and/or SMC (4), two possibly identical principles extracted from platelets; c) kallikrein, a non-dialysable, heat labile, protein-like material, identified in normal plasma by Kraut, Frey and Werle (5, 6); d) the so-called ‘früh-Gift’ (7) that appears in an active form after the clotting of the blood and was identified by Zipf (8) as adenylic acid; e) acetylcholine, the presence of which in blood serum has been a matter of controversy (9-11).

The principle released in our experiments is dialysable through cellophane paper, is rapidly destroyed by the venom itself and by trypsin, and is not antagonized by antihistaminics and atropine. It could, therefore, be immediately distinguished from kallikrein, histamine, choline or acetylcholine. Since the principle appeared to be derived from plasma or serum, we were able immediately to rule out the possibility of its identity with thrombocytin or the SMC. Moreover, it was shown by Zucker (4) that SMC is resistant to digestion by trypsin, and we have found thrombocytin resistant to destruction by the venom of the B. jararaca.

The new factor, however, has certain analogies with the principle described by Feldberg and Kellaway (12, 13) under the name of slowly reacting substance (SRS). The latter was considered by Trethewie (14) to be released from rabbit spleen by snake venoms and trypsin. Although there is no indication that the new principle is identical with this slowly reacting substance, the contraction produced by it is of a slow type, starting after a short latent interval. We have, thus, given it the name bradykinin, indicating a principle which produces a slow movement of the gut. The globulin fraction (precipitated by a 30 to 45 per cent saturated solution of ammonium sulphate) from which bradykinin is released has been named bradykininogen.

MATERIAL AND METHODS

Defibrinated or oxalated ox blood was used in all large-scale experiments. In preliminary assays, we used dog blood and occasionally the blood of guinea pigs, rabbits and cats. Since red

Received for publication November 10, 1948.

1 From the Department of Physiology, Faculty of Medicine, Sao Paulo.
2 From the Adolfo Lutz Institute, Sao Paulo.
and white cells and platelets were found to contain little or none of the bradykinin precursor, they were discarded. Guinea pig and rabbit serums were used less often since they usually are strongly contaminated with histamine.

**Preparation of Crude Bradykinin.** Ox serum or oxalated plasma was treated with an equal volume of saturated ammonium sulphate at room temperature and left for a few hours. The precipitate, collected by centrifugation, was redissolved in two thirds of the original volume of distilled water and dialysed for 3 or 4 days against running tap water. The dialysed globulin solution (bradykininogen) was then neutralized. Aliquots of 200 cc. of the globulin solution were mixed with 30 cc. of a 0.1 per cent solution of the venom and incubated for 1 to 3 minutes at 37°C. The mixtures were then poured into two volumes of boiling ethyl alcohol, left for 5 minutes in a boiling water bath, and filtered. The filtrate was concentrated in vacuo and dried after washing with anhydrous ethyl ether and acetone. From each liter of the globulin solution, about 450 mg. of the buffy powder (crude bradykinin) was obtained. The activity of this powder is such that it usually produces a moderate contraction of the gut when 0.1 mg. is added to the bath. In terms of potency, the activity of the released material is almost completely recovered as ‘crude bradykinin’.

A good stock of the venom of the Bothrops jararaca was used in a 0.1 per cent solution in distilled water for the large-scale preparation. Solutions of the venom for trial experiments were made in saline.

The venoms of Naia naia and Denisonia superba were kindly supplied by Dr. C. H. Kellaway, of the Wellcome Research Foundation, London. The venoms of Bothrops atrox and Crotalus t. terrificus were supplied by the Butantan Institute, São Paulo. The crystalline trypsin, containing about 50 per cent magnesium sulphate, was the commercial preparation of the Worthington Biochemical Laboratory, Freehold, New Jersey. The renin preparation and the angiotonin (hypertensin) were three-year-old preparations kindly supplied by Dr. I. H. Page, of Cleveland, Ohio. The renin, as tested, was in excellent condition and still fairly soluble. The angiotonin preparation was somewhat resinified with a brown, gummy appearance, but still strongly active upon the isolated gut and the blood pressure of the cat and the rabbit.

The determinations on the isolated gut were made in a Dale’s apparatus, in the usual way, using a chamber of 7 cc. capacity.

**RESULTS**

**Release of Bradykinin from Normal Blood by Snake Venoms and Trypsin.** When the venoms of most species of snakes are put into contact with the uterus or the gut of the guinea pig, they cause a sharp increase of tonus that lasts for a long interval of time, even after repeated washings with new Tyrode solution. After relaxation, a second addition of the same dose of venom is either ineffective or produces a lesser effect, followed by a refractory state (desensitization) to any further addition of the venom. This effect, described by several workers (12, 13, 15, 16), was also described for trypsin (17) by one of us. If the guinea pig gut is made refractory to the action of snake venoms and trypsin, it can be used as a suitable test organ for the release of pharmacologically active substances from biological structures. On the other hand, the plasma of certain species (dog, ox and cat) is very poor in histamine and
therefore large doses of the plasma can be added to the perfusing bath without eliciting any response. When defibrinated blood or serum is added to the bath, we can occasionally detect the presence of minute amounts of an unidentified principle. The presence of this principle in normal serum is, however, erratic, as pointed out by Reid (2, 3) and confirmed in our experiments. When, however, a few micrograms of the venom of the Bothrops jararaca is added to a few cc. of blood or serum, a powerful contraction occurs after a short incubation of the serum with the venom. The maximum effect can be observed when the incubation is restricted to 1 to 3 minutes. If incubation is prolonged for 20 or 30 minutes, the active principle (bradykinin) disappears; this indicates that it has been destroyed by the further action of the venom (fig. 1). Crystalline trypsin has the same activity (fig. 2), although doses at least 5 times as great are necessary to produce an effect of similar magnitude. We can follow the generation of this substance by simply adding successively to the bath containing the gut 0.2 cc. of defibrinated blood or serum and 0.1 mg. of the venom of the B. jararaca. After a latency of 25 to 30 seconds the contraction of the gut sets in and proceeds at an increasing rate during the first 1 or 2 minutes (fig. 3).

We have been able to exclude entirely the blood elements as precursors of this stimulating principle. First of all, oxalated or heparinized plasma shows the same effect as whole blood, but even in a more striking manner. If the cells, after removal of the plasma, are resuspended in saline they show a much lesser effect than whole blood or cell-free plasma. Secondly, we could localize in a definite fraction of the plasma or serum proteins the precursor (bradykininogen) of this active principle. The total potency of the blood appeared to be concentrated in the globulin fraction, precipitated by a 30 to 45 per cent saturated solution of ammonium sulphate. As shown in table 1, the supernatants, after precipitation of plasma proteins with increasing concentrations of ammonium sulphate, showed less and less effect. When the concentration reached the 45 per cent level, no activity was found in the supernatant, while the full activity could be recovered from the redissolved and dialysed precipitate.

The first attempts to isolate bradykinin were very laborious due to the fact that the same agent that released it, after incubation for a few minutes, destroyed it if incubation was prolonged for a few minutes more (15-30 minutes). Both activities, that of release and that of destruction, appeared to be enzymatic, since they depended upon temperature and the actual concentration of the agent used. Release and destruction of bradykinin could be studied quantitatively (fig. 4). If 0.1 mg. of the venom of the B. jararaca was added to each cc. of blood or serum, the peak was observed after 3 minutes incubation, at 37°. If 0.3 mg. was added, the peak was somewhat earlier and destruction was complete after 10 minutes incubation. After complete destruction of the released bradykinin, further addition of the venom had no effect, the precursor being apparently exhausted. If, however, a small dose of the venom is added, say 20 micrograms, to each cc. of a fresh sample of the globulin fraction, a sort of equilibrium is attained in the reacting mixture, and even after one hour incubation, a small quantity of bradykinin can be detected in the system. If now, a larger dose of the venom is added, the release of considerable quantities
Fig. 1. GUINEA PIG ILEUM. After desensitization to 100 µg. of the venom of B. jararaca (B. j.),
the gut responds to the mixture of the globulin and venom (Glob. + ven.) incubated for 30° and
3 min. Further incubation of the mixture for 10 min. destroyed the stimulating principle. H =
histamine 1:2 million.

Fig. 2. GUINEA PIG ILEUM. Upper tracing: The gut desensitized to venom still reacted with
trypsin. Lower tracing: after the gut became refractory to 0.1 cc. trypsin, it reacted powerfully with
a mixture of the globulin + trypsin, incubated for 1 min. After incubation for 45 min. the stimu-
lating principle was completely destroyed; then a new fresh addition of the globulin was added with
venom and a new contraction set in. T = 2.5 mg. crystalline trypsin/cc.

Fig. 3. GUINEA PIG ILEUM desensitized to the venom of B. jararaca: 0.2 cc. of ox serum was
added to the bath, followed by addition of different doses of venom. The latency after addition of
venom is indicated in parentheses. Maximum effect was obtained with 200 µg. of venom. The
shorter contractions were elicited with 0.2 cc. of histamine, 1:2 million.

Fig. 4. GUINEA PIG ILEUM desensitized to the venom of B. jararaca. A, incubation of ox de-
fibrinated serum (for 1 and 4 min.) with 1/10 of its volume with a solution of 0.1% of venom releases
activity, although 10 or 30 min. incubation entirely destroyed it; B, a smaller concentration of
venom (1/50) released less activity, but destruction was also slower; C, a higher concentration of
venom (1/3) released the maximum activity after 1 min. incubation, but destruction was rapid.
After destruction of the principle, a new fresh mixture of serum + venom elicited maximal contrac-
tion.
of the active principle takes place; this shows that the precursor had not been exhausted by the action of the small dose of the venom.

If a large enough dose of trypsin is added to a fresh sample of the globulin, bradykinin is set free and exhaustion can be observed after a while. The venom of Denisonia superba and that of Naia naia were much less potent than that of the Bothrops jararaca in releasing bradykinin. We could not attain a concentration sufficient for exhaustion of the globulin when the latter was tested with the venom.

| Table 1. Conditions of Extraction of Bradykinin, from Ox Serum or Globulin |

<table>
<thead>
<tr>
<th>Alcohol extraction:</th>
<th>Time of incubation:</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) 5 cc. serum + 0.5 mg. venom, incub. 3 min.</td>
<td>(VIII) Mixture I incubated for:</td>
</tr>
<tr>
<td>(II) Mixture (I) + 2 vol. alcohol; 5 min. boil.; filtrate conc. in vacuo</td>
<td>0' 10</td>
</tr>
<tr>
<td>(III) Mixture (I) + 1 cc. N HCl + 2 vol. alcohol; 5 min. boil.; filtrate conc. in vacuo</td>
<td>1' 60</td>
</tr>
<tr>
<td>(IV) Mixture (I) + 1 cc. 0.5 N NaOH + 2 vol. alcohol; 5 min. boil.; filtrate conc. in vacuo</td>
<td>2' 80</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time of incubation:</th>
<th>Activity of max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3' 50</td>
<td></td>
</tr>
<tr>
<td>4' 50</td>
<td></td>
</tr>
<tr>
<td>10' 15</td>
<td></td>
</tr>
<tr>
<td>30' 0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Thermo resistance:</th>
</tr>
</thead>
<tbody>
<tr>
<td>(V) Extract (II) + 0.2N NaOH heated for 10 min. at 90°</td>
</tr>
<tr>
<td>(VI) Extract (II) + 0.2N HCl heated for 25 min. at 90°</td>
</tr>
<tr>
<td>(VII) Extract (II) + 5 cc. concentr. HCl, heated for 1 hour at 90°</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ppt.</th>
<th>Supernt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>50</td>
</tr>
<tr>
<td>40</td>
<td>70</td>
</tr>
<tr>
<td>45</td>
<td>100</td>
</tr>
<tr>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

Experiments III and IV show that extraction by alcohol is more efficient from the alkaline mixture, than from the acid one, although destruction of the isolated principle is more rapid in alkali (V) than in an acid medium (VI).

of the B. jararaca or trypsin. Crotoxin, a purified lecithinase of the venom of the Crotalus l. terrificus prepared according to Slotta et al. (18), did not release bradykinin nor did a purified preparation of lysolecithin. Therefore, we are bound to assume that the release of bradykinin is unrelated to the hemolytic activity of the venoms studied, but probably dependent upon their proteolytic activity. The capacity of the different agents studied are roughly parallel to their proteolytic and coagulant activity (table 2).

Biological Assay of Bradykinin. Until a pure preparation of bradykinin is available, we must use a temporary standard for comparison of activity. Since the
sensitivity of the gut to histamine or acetylcholine does not necessarily run parallel with that toward bradykinin, we abandoned the idea of comparing the strength of the unknown solutions of bradykinin with a histamine or acetylcholine standard. Moreover, the response of the gut toward these agents is quite different from the response to bradykinin. The latter is definitely slower in its onset and the increase in tonus follows a different course. Therefore, we decided to choose an early homogenized sample of crude bradykinin as a standard. This sample, called Brady I, has been used for comparison in all assays of purification referred to in this paper. For the purpose of comparing two different samples, the assay upon the guinea pig ileum has been most useful. The response of the gut is fairly quantitative (fig. 5), allowing a satisfactory comparison between the unknown and the standard.

Quantitative estimations of the bradykinin precursor (bradykininogen) can be made by determining the maximal potency released by a fixed amount of the venom (e.g., 150 μg/cc. of serum or globulin solution) after incubation with the precursor for increasing intervals of time. It is obvious that this estimation has only comparative value since we cannot be sure that the whole stock of the active material has been released; on the other hand, if the concentration of the venom is increased beyond a certain limit destruction of bradykinin will be very rapid and the maximum peak will not be attained. In consequence, we must fix conditions for a routine comparison of potency of different batches of the globulin preparation. This procedure has been very useful in the large-scale preparations, in which samples of a few cc. of the globulin to be used were submitted to a standard dose of the venom,
for ½ min., 1 min., 2 and 3 mins. incubation and the time for the maximal effect determined.

**Purified Bradykinin.** Although experiments are still proceeding in an attempt to further purify bradykinin, we have attained a second step in the procedure of purification that can be outlined now. ‘Crude bradykinin’ is redissolved in distilled water, filtered through a medium porosity sintered glass funnel, and 10 volumes of absolute ethyl alcohol are added with stirring. A flocculent precipitate is formed and removed without any loss of potency. The precipitate contains some inert organic nitrogen and gives a strong reaction with ninhydrin. Bradykinin, however, is fairly soluble in 80 to 90 per cent ethyl alcohol. The filtrate is dried in vacuo, and care is taken to avoid bubbling of air through it. A convenient way to do this is to use a separatory funnel connected with a Kitasato flask to which a strong vacuum pump is connected. The solution contained in the separatory funnel is allowed to fall dropwise to the bottom of the flask; this permits very quick drying in a moderately heated water bath. The material obtained in this way is about 8 to 12 times as active as the original ‘crude bradykinin’ and will be referred to in this paper as purified bradykinin (fig. 6). A highly potent material can be obtained, although in small yield, if the purified bradykinin is redissolved in 85 per cent ethyl alcohol and more absolute alcohol added until a faint precipitate is formed. The mixture left in the ice-box overnight shows a flocculent snow-white precipitate that may be 30 to 50 times as active as the crude preparation. This preparation has been obtained only in very small amounts.

**Some Physical and Chemical Characteristics of Bradykinin.** The active principle is very stable to heat, and resists prolonged boiling in distilled water or dilute HCl (boiling for 1 hour in a 0.1 to 1.0 m HCl solution did not significantly decrease its potency). Boiling with a few cc. of concentrated HCl, as used in the method of Barsoum and Gaddum, as modified by Code (19), destroys its activity. Boiling for a few minutes in a 0.1 m NaOH solution is enough to destroy bradykinin. It is a dialysable substance, insoluble in ether and anhydrous acetone. When absolute ethyl alcohol is used for the extraction of bradykinin, a large quantity of the active material is left behind. As the preparation becomes more purified it does not resist the manipulations to which we can submit the crude material. Especially, bubbling of air through the capillary becomes a very serious cause of loss of potency. This loss of potency by bubbling of air was found to be more important after precipitation of the inert material with 80 to 90 per cent ethyl alcohol, thus suggesting that, through this procedure, a stabilizer is removed.

All bradykinin preparations obtained up to now give a strong ninhydrin reaction and contain considerable organic nitrogen. Since trypsin liberates and destroys bradykinin, it is probable that release of bradykinin is the consequence of the rupture of a peptide linkage and that bradykinin itself is a polypeptide or, at least, contains a peptide linkage, the integrity of which is indispensable to its pharmacological behavior.

**Pharmacology of Bradykinin.** Bradykinin has been found to stimulate all smooth muscle structures so far assayed: the intestine and uterus of the guinea pig, the uterus and intestine of the rat, and the intestine of the rabbit. In our experi-
ments, the guinea pig ileum was the most sensitive, while the intestine of the rat was least so. The purified preparations of bradykinin (P. B.) elicited a strong response of the guinea pig gut, when added to the perfusing bath in doses as low as 2 to 10 micrograms; this corresponds to concentrations in the bath of 1:3 to 1:1 million, approximately. Since there are no indications that we are dealing with highly purified preparations, it appears probable that bradykinin is a very potent pharmacological agent. When 10 or more mg. of the crude bradykinin (Brady I) is injected into the veins of rabbits and cats a sharp fall in arterial blood pressure follows. 'Purified bradykinin', when injected in a dose of 5 to 10 mg., produces a shock-like condition, with a steady fall in arterial blood pressure, followed by a very slow return to the initial level (fig. 7).
That the same principle (bradykinin) is involved in the effects upon both the smooth muscle and the arterial blood pressure is indicated by the fact that the same relationship of potency (1/10) was observed when crude (Brady I) and purified bradykinin (P. B.) were assayed for both actions (figs. 6 and 7).

A few quantitative considerations might help to evaluate the possibility of a release of bradykinin to explain the fall in blood pressure, when the venom or trypsin is injected intravenously. Ten to twenty micrograms of the purified preparation elicit responses in the guinea pig gut that are comparable to those elicited by 0.1 to 0.2 cc. of serum or plasma treated with an optimum amount of the venom. Therefore, 100 to 200 cc. of blood when incubated with the venom for a half minute to 3 minutes would release 10 to 20 mg. of 'purified bradykinin'. Since this amount is sufficient to produce a profound shock when injected intravenously, it becomes quite evident that there is enough available material in the circulating blood to account for most of the hypotensive effect produced by the venom or by trypsin, when given to a normal animal.

Release of Bradykinin by Renin. It has been reported (20) that hypertensin (or angiot˘n) contracts almost all smooth muscle structures, producing an effect that is strikingly similar to those described above. It has also been shown that renin when put into contact with the pseudo-globulin fraction of normal plasma or serum (hypertensinogen) releases a smooth muscle stimulating agent that has never been chemically separated from hypertensin (21). The following experiments suggest the possibility that this smooth muscle stimulating agent released by renin can be distinguished from hypertensin, and is probably identical with bradykinin.

We could confirm the facts described by previous workers that renin when incubated for 3 to 10 minutes with the globulin fraction releases a factor that contracts the guinea pig ileum. If incubation is prolonged for 60 to 90 minutes, the stimulating factor released by renin is entirely destroyed. If now, the same globulin preparation is treated with the venom, no further release takes place, showing that the bradykinin precursor has been exhausted. Conversely, if the globulin fraction is treated with an appropriate dose of the venom or trypsin, until it is exhausted of its
supply of bradykinin, it no longer releases any smooth muscle stimulating principle, if put into contact with renin.

Differentiation of bradykinin from hypertensin is very easy, since the material obtained from serum globulin by contact with the venom or trypsin has an effect upon the blood pressure of the cat or the rabbit opposite to that obtained by contact of the same material with renin. On no occasion could we observe any hypertensive effect of preparations obtained by treating bradykininogen (or hypertensinogen) with the venom of the *B. jararaca* or trypsin. These facts are clearly shown in figure 8.

Since renin appears to release bradykinin from the pseudo-globulin fraction of the blood, it seems logical to open the question of the identity of hypertensin and the smooth muscle stimulating principle generated by renin when incubated with the globulin preparation. The presumption that a hypotensive, myotropic agent as bradykinin is a common contaminant of the hypertensin preparations available would be analogous to the case of the oxytocic and pressor principles of the posterior hypophysis. These also have a polypeptide nature and are extremely difficult to separate by the usual chemical procedures, although by using more refined methods, evidence has been obtained that they constitute two separate principles (22).

**DISCUSSION**

Very minute doses of the venom of the *B. jararaca* (0.1 mg/kg) render the blood incoagulable, when injected intravenously in a dog. After recovery from the mild shock so produced, the injection of a larger dose of the venom (0.5 mg/kg) causes the animal to develop a severe shock that may lead to death. A quite similar situation is found in the so-called trypsin shock. After the injection of a small dose of crystalline trypsin, the blood becomes incoagulable; if however the dose is again injected, the animal develops a shock-like condition that is irreversible in many instances. The injection of a suitable dose of heparin previous to the injection of trypsin does not prevent death, but may even aggravate the development of the shock. Therefore, intravascular coagulation cannot explain death, but could be responsible for only a minor part of the symptoms produced by these agents. Liberation of histamine, previously thought to constitute an important factor in the production of trypsin shock, can only partially explain its mechanism. The amounts of histamine appearing in the circulating blood are small and by no means enough to explain the severity of the shock. After injection of the venom of *B. jararaca* we have frequently found a drop in blood histamine. There is no question that histamine is released from certain organs, by perfusion with trypsin (23) or the venom of the *B. jararaca*, but the amounts liberated are not of such magnitude as to produce a significant increase in the circulating blood. Moreover, it was shown by Wells *et al.* (24) that benadryl does not prevent trypsin shock in the dog. Although antihistamine substances are rather poor in preventing hypotension due to histamine itself, a significant drop in mortality of dogs treated with benadryl + trypsin should be expected, if the toxicity of the enzyme depended mainly on a liberation of histamine. All these facts would encourage one in looking for a new agent released from blood or tissues in poisoning by trypsin and certain snake venoms.
The presence of a powerful hypotensive and smooth muscle stimulating agent in the pseudo-globulin fraction of normal plasma or serum offers a new possibility for explaining the etiology of many symptoms occurring in the shock states produced by venoms and trypsin. This agent (bradykinin) is present in an inactive form (bradykininogen) and can be released by many agents besides the venom of the *B. jararaca*. Trypsin releases it and there is a certain correlation between the proteolytic and coagulating activity of snake venoms and their capacity for liberating bradykinin. The venoms of *Naia naia* and *Denisonia superba* that show slight proteolytic activity release very small amounts of bradykinin.

Experiments indicate that part of the bradykinin is released when blood clots, since there occurs a definite decrease in the bradykininogen potency when measured before and after the coagulation of the blood. This appears to indicate activation of the proteolytic system of the blood during the process of coagulation, as suggested by Ferguson (25). In connection with these findings it appeared important to study the behavior of platelets when disintegrated in contact with the globulin fraction. In some experiments, it appeared quite clear that platelets contain a factor that is able to release bradykinin from the globulin fraction. The experiments were complicated by the fact that platelets themselves contain another factor (SMC or thrombocytin) that contracts smooth muscle and produces a fall in arterial blood pressure. This factor could be definitely distinguished from bradykinin, since as shown by Zucker (4) it is resistant to boiling for one hour with 1.0N NaOH, a concentration of alkali that completely destroys bradykinin in a few minutes. Moreover, bradykinin is rapidly destroyed by incubation with trypsin or the venom of the *B. jararaca* whereas SMC or thrombocytin is entirely insensitive to these treatments.

There are many other questions that should be re-studied on the basis of the present findings. In some preliminary experiments, we have shown that the fibrinolytic enzyme activated by treating the serum with chloroform releases a gut-stimulating principle when incubated with the bradykininogen. Since activation of fibrinolysin occurs in many conditions of shock (26, 27), it is possible that release of bradykinin might explain much about shock of varying etiology. Furthermore, the origin and nature of the so-called anaphylatoxin of Friedberger and Bordet (28) has been an open question. Experiments by Dale and Kellaway (29) have shown that a smooth-muscle-stimulating principle is generated in normal guinea pig serum, by contact with starch or bacterial suspensions. At the same time, activation of proteolytic and fibrinolytic activity was shown in similar conditions (30). Activation of the proteolytic and fibrinolytic power of the blood during anaphylactic shock has been demonstrated (26) and, recently, Ungar (31) has also shown activation of a fibrinolytic enzyme when organs of a guinea pig are put into contact with peptone and the antigen. All these findings point to a 'proteolytic crisis' occurring in these kinds of shock and it is quite conceivable that release of bradykinin constitutes an aggravating factor in many conditions.

**SUMMARY**

The pseudo-globulin fraction (precipitated by 30 to 45% saturation with ammonium sulphate) of normal plasma contains the precursor (bradykininogen) of
a hypotensive and smooth muscle stimulating factor (bradykinin) that can be released by proteolytic and coagulating venoms of the Bothrops genus and by trypsin. If an appropriate dose of the enzyme or the venom is used, the maximum release is observed after 1 to 3 minutes incubation, at 37°. If incubation of the globulin with the venom of B. jararaca or trypsin is prolonged for 10 to 20 minutes the released factor is destroyed; this suggests that it has a polypeptide nature or at least a peptide linkage, the integrity of which is necessary for its pharmacological activity.

Bradykinin is thermostable, dialysable through cellophane, resistant to prolonged boiling in 0.1 to 1.0~HCl solution, but rapidly destroyed if heated in an alkaline solution.

The guinea pig gut is the most sensitive smooth muscle organ assayed and the rat intestine the least so. Doses of bradykinin equivalent to those released from 100 to 200 cc. of plasma produce a steady fall in arterial blood pressure of the cat, the rabbit and the dog. The possibility that bradykinin is a mediator in several kinds of shock is discussed.

We are indebted to Dr. Sylvia O. Andrade for the estimation of the proteolytic and coagulating activity of the venoms used. All experiments were done with the technical assistance of Mr. Jayme Ferraz. The venom of the B. jararaca used was the stock venom for preparing immune-serums, at the Butantan Institute.

REFERENCES