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48. THE ACTION OF STAPHYLOCOCCAL ALPHA TOXIN AND THE VENOM FROM *BITIS GABONICA* ON RAT STOMACH STRIP

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We are most grateful to be allowed to speak before this most distinguished community concerned with the research of animal venoms. We feel a little embarrassed as poor relatives concerned only with bacterial toxins. In general two types of venoms are treated by entirely different groups with no close relation between them. We hope that our modest contribution might arouse some interest to strenghten the cooperation between workers in the two subjects.

In our laboratory we are interested in the pharmacology of Staphylococcal alpha toxin. From your point of view older findings of the group of North and Doery are very interesting. They demonstrated a protection against Staphylococcal alpha toxin by pretreating the animals with tiger snake venom (1). They suggested that the snake venom and Staphylococcal alpha toxin occupied the same type of receptors. Their experiments point to the probability that the actual protective effect is produced by phospholipase A. Long chain unsaturated fatty acids are liberated and lysophosphatide is formed. Ganglioside has the same protective action against Staphylococcal alpha toxin. The group suggested that phospholipase A plays a role in animal defence against bacterial exotoxins. This points to a possible common mechanisms between animal and bacterial toxins and therefore we looked for suitable models where some similarities and differences could be demonstrated. Originally we planned to use for this demonstration isolated mast cells, however, according to the results we have up to now, the correlations on this model were not so easy to demonstrate. We have therefore taken the liberty to discuss our results on another model, which is not in the original title of our communication.

Material and Methods

Rats of both sexes were fasted over night, killed by decapitation and isolated rat stomach strip preparations using Vane's method (2) were set up. The stomach strips were suspended in 5 ml bath with Krebs solution at 37°C, and bubbled with air.

Two batches of Staphylococcal alpha toxin were used one crude product and one of 70% purity (gift from Dr. Bernheimer). *Clostridium perfringens* toxin was a product of GLAXO laboratories. Venom from *Bitis gabonica* was obtained through generosity of Dr. Kornalík.

Technical assistance: B. Rybová, and O. Fialová.

Results

Crude or purified Staphylococcal alpha toxin elicited on isolated rat stomach strip a contraction which has a two phase character. After the toxin application a rapid contraction developed which started to decline about 60 sec after and then a new slow, long lasting contraction developed. With repeated toxin application the rapid phase of the contraction did not appear any more and the slow reaction was of lower intensity than the first time. Fig. 1 demonstrates the

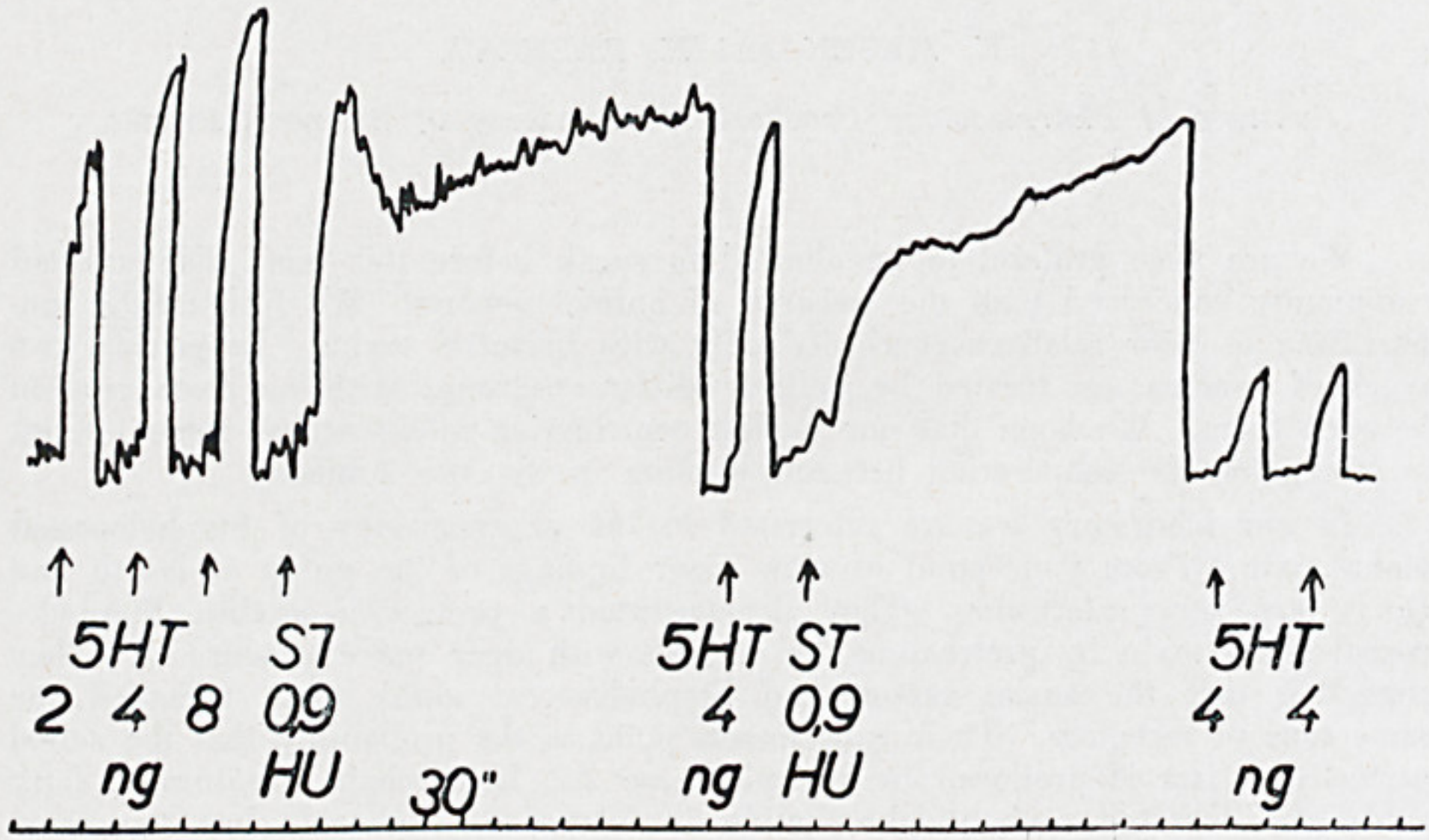

Fig. 1 — Effect of Staphylococcal (ST) alpha toxin on isolated rat stomach preparation. Doses are in nanograms (ng) or hemolytical units (H.U.) per ml of bath.

results. When the repetition of toxin application was continued, the sensitivity of the organ to toxin or 5HT declined and finally vanished. The organ became also unresponsive to 5HT administration. In the next series of experiments the contraction produced by Staphylococcal alpha toxin was compared with the effect of *Bitis gabonica* venom. This venom elicited a rapid contraction which gradually declined even without washing. With repeated doses the organ became

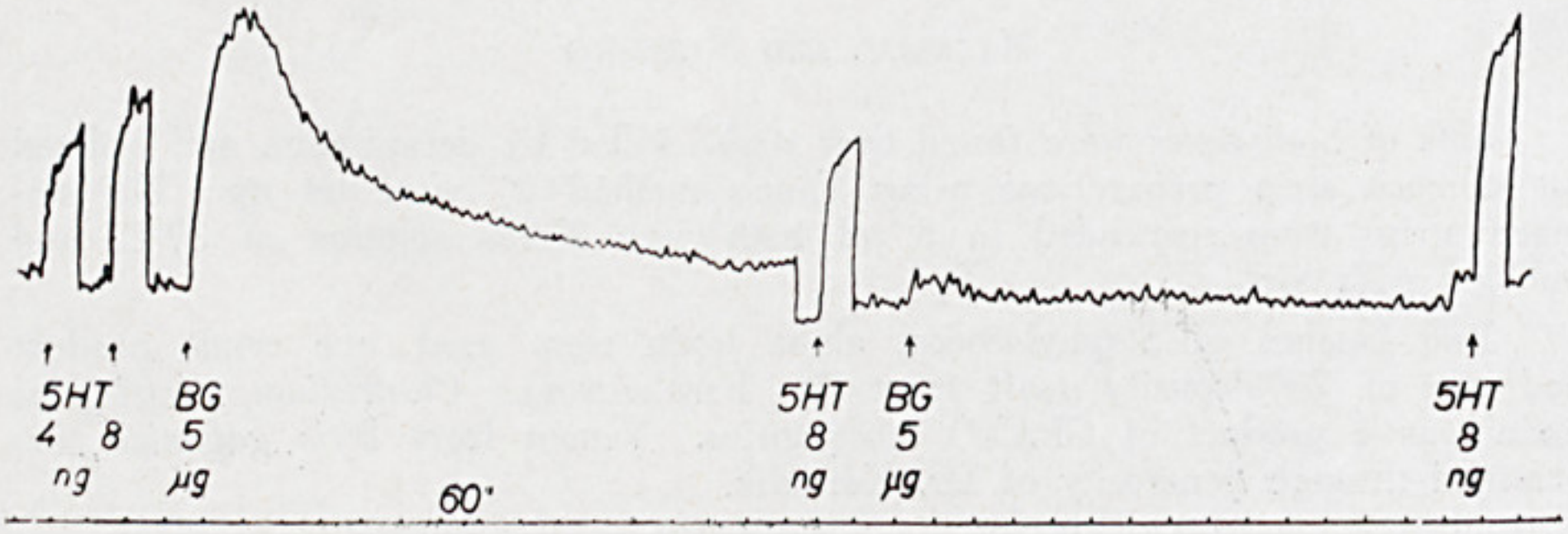

Fig. 2 — Effect of snake venom from *Bitis gabonica* (BG) on isolated rat stomach preparation.

insensitive to further administration of snake venom. On the other hand both serotonin and Staphylococcal alpha toxin elicited a contraction when administered after the snake venom but the character of contraction differs. The first rapid phase is not so evident (Figs. 2 and 3). As may be see from Fig. 4, Staphylococcal alpha toxin administered to the bath rendered the organ almost insensitive to *Bitis gabonica* venom administration.

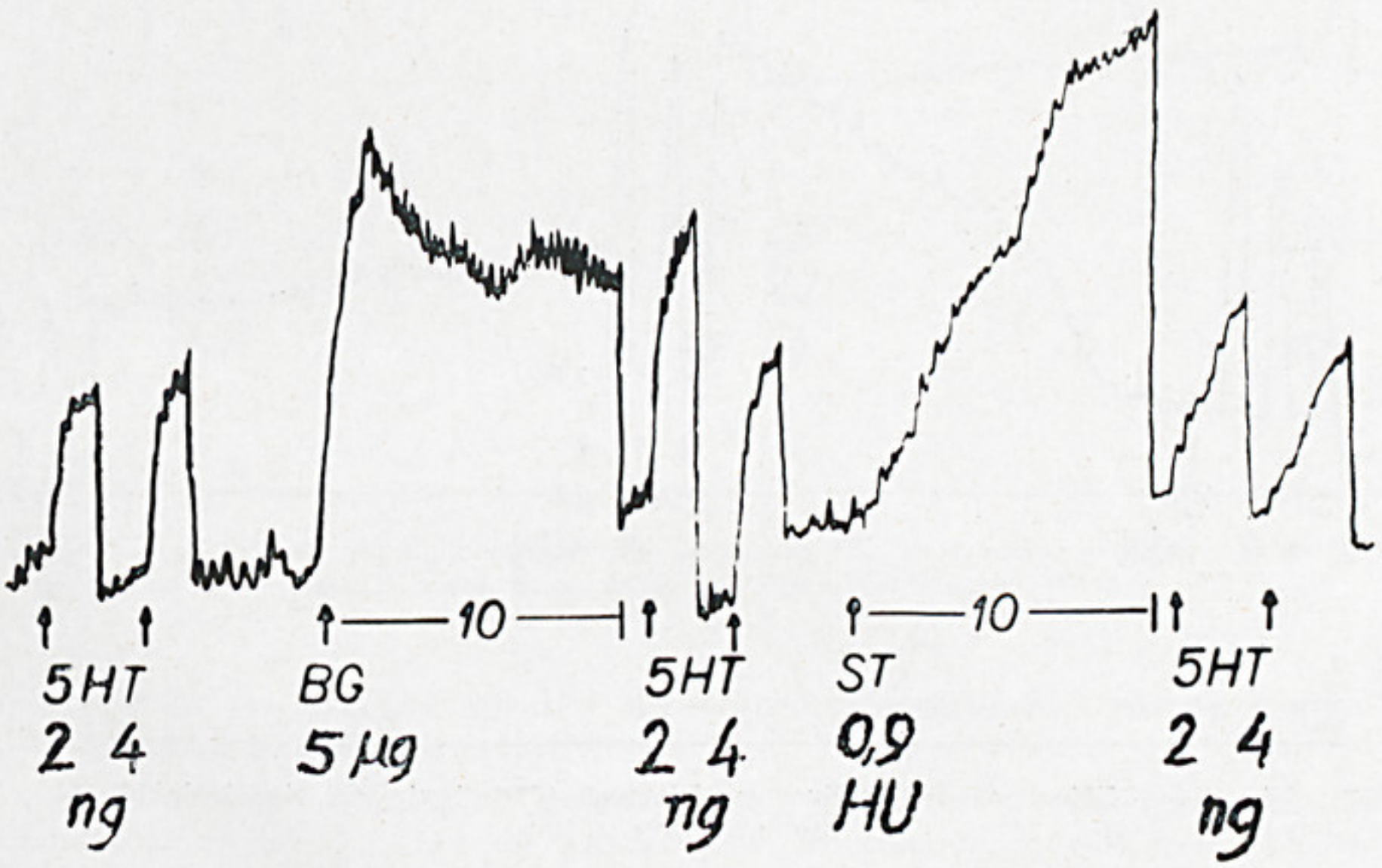

Fig. 3 — Effect of snake venom from *Bitis gabonica* (BG) on the contraction elicited by Staphylococcal alpha toxin (ST) administration.

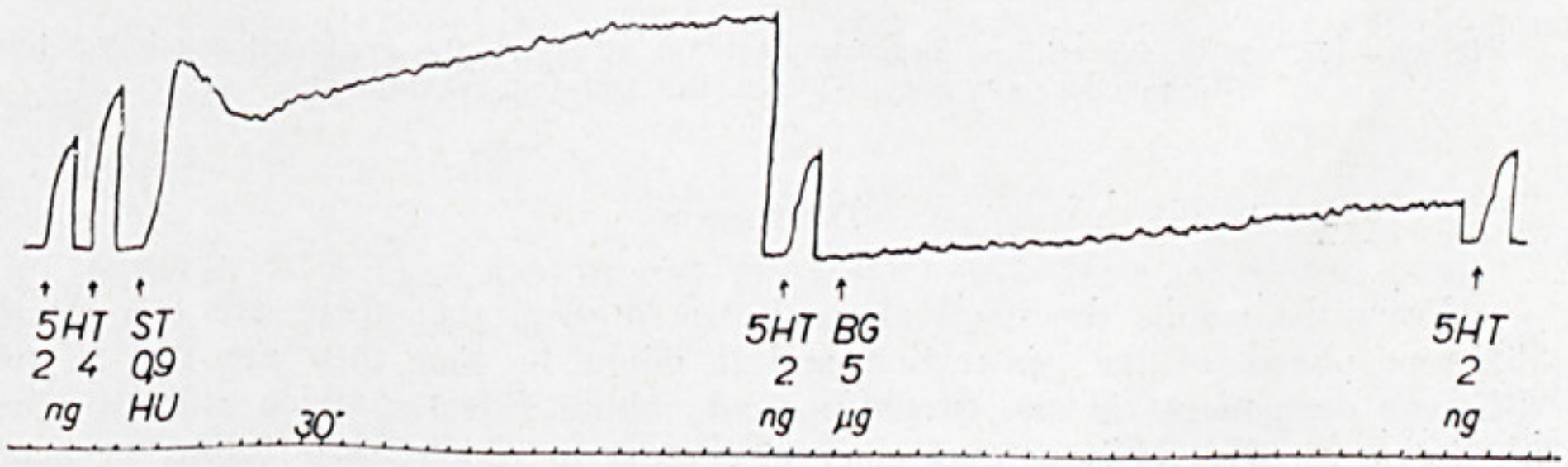

Fig. 4 — Effect of Staphylococcal alpha toxin on the contraction elicited by snake venom from *Bitis gabonica* (BG) on the isolated rat stomach.

When *Clostridium perfringens* toxin was administered to the stomach strip a slow contraction develops after latency period. A second administration of this toxin was much less effective and the organ lost also its sensitivity to originally active doses serotonin (Fig. 5). Also it was impossible to elicit a contraction with Staphylococcal alpha toxin (Fig. 6). The application of Staphylococcal alpha toxin prior to *Clostridium perfringens* toxin administration caused insensitivity of tissue to *Clostridium perfringens* toxin (Fig. 7).

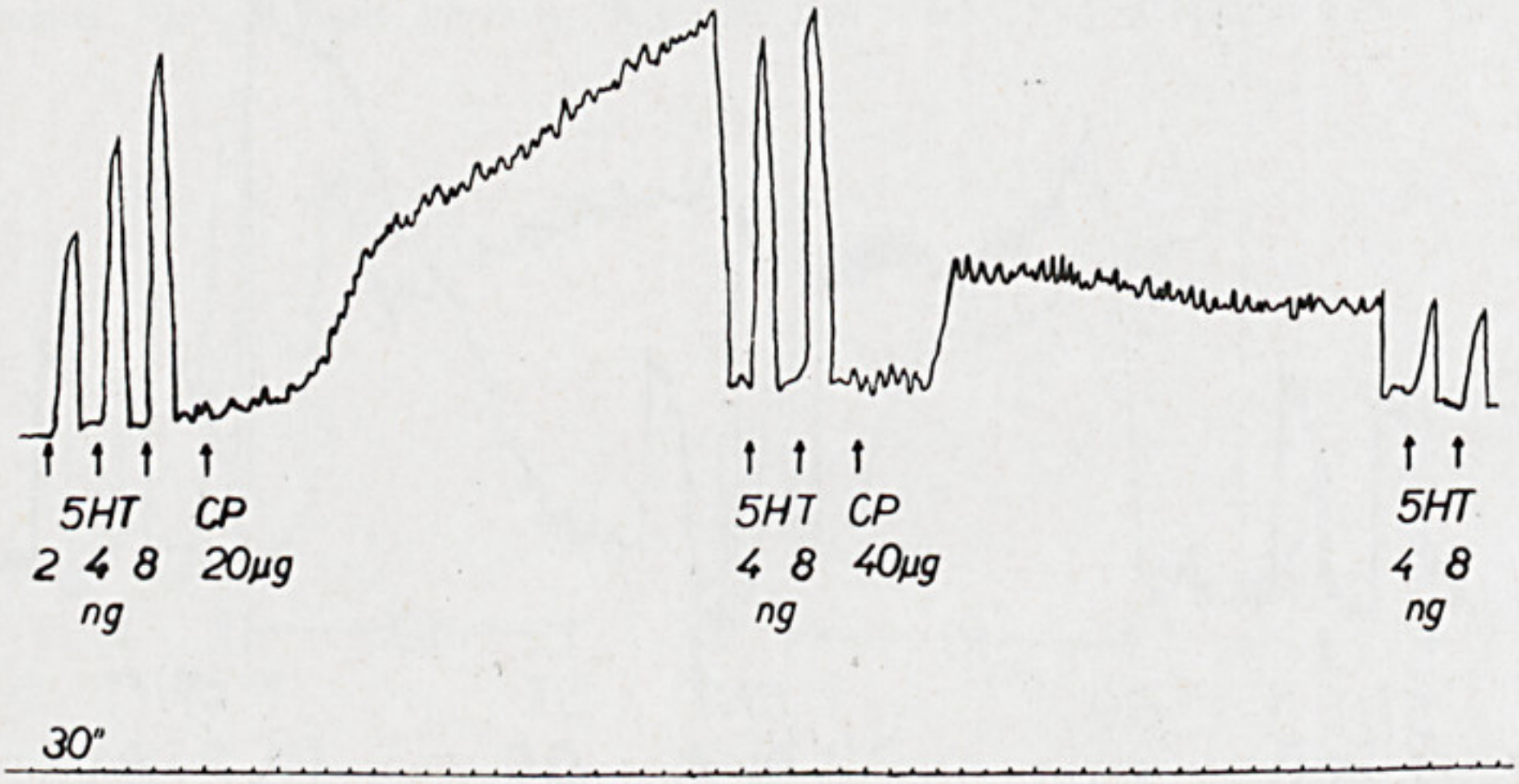

Fig. 5 — Effect of *Clostridium perfringens* (CP) toxin on the isolated rat stomach preparation.

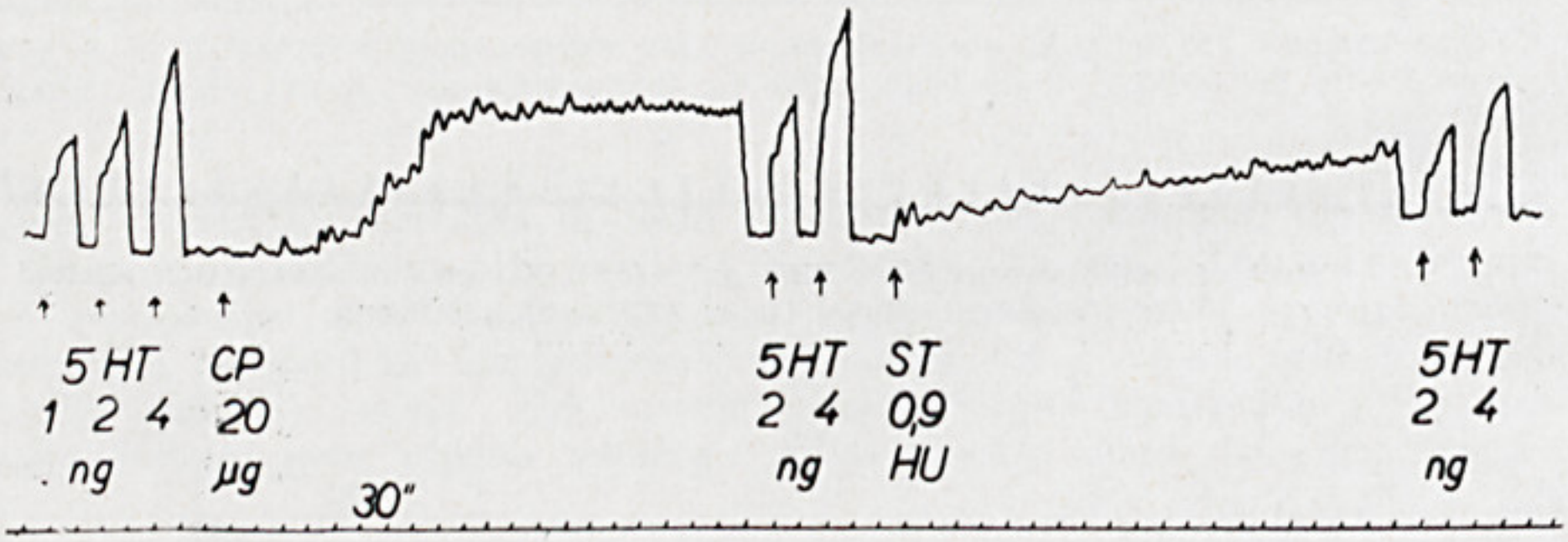

Fig. 6 — Effect of *Clostridium perfringens* toxin (CP) on the contraction elicited by Staphylococcal toxin (ST) on the isolated rat stomach.

Discussion

From the results described above it would seem that there are two clearly different phases of the contraction and it might be that they are due to two different components in the Staphylococcal culture filtrates which contain phospholipases (3, 4). It is not yet clear at present to which toxin (alpha or beta) they belong. We therefore used a snake venom rich in phospholipase A and *Clostridium perfringens* toxin rich in phospholipase C to try to contribute at least

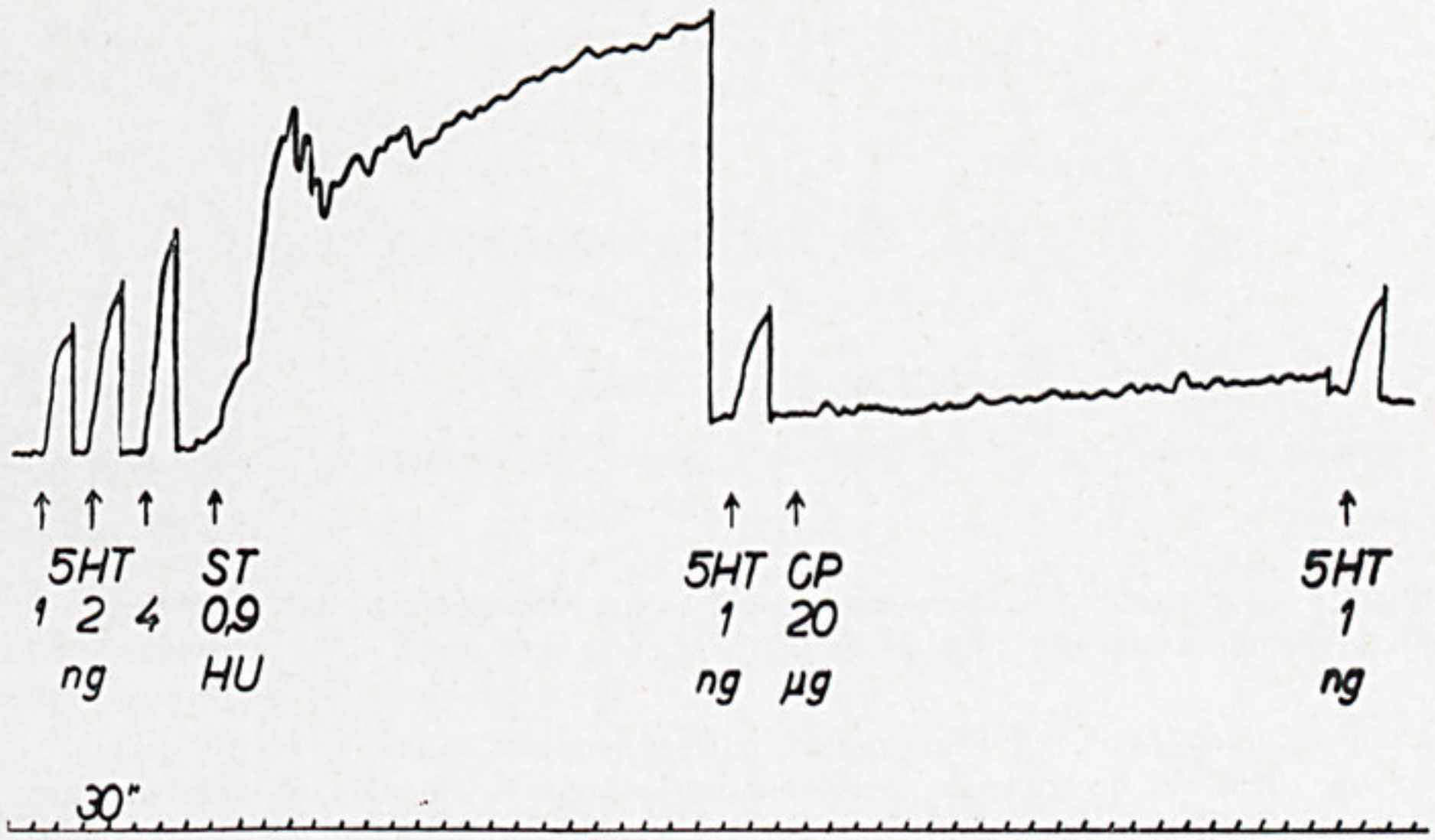

Fig. 7 — Effect of Staphylococcal toxin (ST) on the contraction elicited on the rat stomach preparation by *Clostridium perfringens* toxin.

indirectly to the question whether the observed spasmogenic activity would be attributable to phospholipases activity.

Venom toxin from *Bitis gabonica* does not prevent Staphylococcal alpha toxin from its activity but the character of contraction is different. The first rapid phase is not so evident. On the other hand Staphylococcal alpha toxin does prevent the snake venom from its activity completely. This would mean that part of Staphylococcal alpha toxin action corresponding to the rapid phase on isolated rat stomach are similar to action of venom toxin from *Bitis gabonica* and could be attribute to phospholipase A action. Since *Clostridium perfringens* prevents Staphylococcal alpha toxin from its activity as well the Staphylococcal alpha toxin prevents the action of *Clostridium perfringens* it would be reasonable to assume that both toxins might act on the same place of the phospholipide molecule. Further investigations are in progress.

We hope that this small contribution testifies that combined studies of bacterial and animal venoms might be of considerable interest.

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