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21. THE RELEASE OF BIOGENIC AMINES FROM BLOOD PLATELETS UNDER THE INFLUENCE OF *CROTALUS D. TERRIFICUS* VENOM

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In previous studies on the action of certain snake venoms and of bee venom on blood platelets we found, that the venom of the Brazilian rattlesnake (*Crotalus d. terrificus*) causes in even very small concentrations a release of biogenic amines and of ATP from these cells (1). The venom component responsible for this effect is of special interest with regard to an analysis of both the action of the venom and the mechanism of amine release. Therefore, we endeavoured in the isolation and characterization of the amine releasing component of the venom and in clarifying its mechanism of action.

In order to isolate the active amine releasing principle, *Crotalus* venom was disassembled into several components by gelfiltration on columns of Sephadex G 100. The experimental results are summarized in Fig. 1. A separation of the amine releasing component from clotting and phospholipase activities and other ballast proteins could be established. The fractions active on blood platelets were collected and freed from buffer salts by dialysis against destilled water. The specific activity of the solution obtained was 20 times higher than that of the crude venom measured by the serotonin release. The yield of active substance averaged 60-65 per cent. The factor gives the reactions of a high molecular protein.

Furthermore it was studied, whether the amine releasing component posseses other biochemical activities, known to be present in the whole venom, besides its action on blood platelets. It was shown, that the isolated component is related to one of the activities, which could be demonstrated and investigated with whole venom, with the exception of only a small caseinolytic activity. Moreover, pharmacological reactions caused by whole venom as well as general toxicity were found not to be connected with it.

Serotonin release induced by the amine liberating component proceeds with high speed. Under the experimental conditions used more than 50 per cent of the stored amines were released during 5 min by only 0,01 μg of the isolated component per ml. Amine liberation by the venom factor can be inhibited by omitting of calcium and by blocking of the platelet metabolism (Fig. 2). After incubation of platelets with the amine releasing component in the absence of calcium the cells release their serotonin already by resuspending them in a medium containing calcium. Amine liberation by the active venom component is also possible in blood. In the presence of plasma its effectiveness is only slightly diminished. Therefore, we also investigated the action of the isolated component in the circulating blood by injecting it intravenously into rabbits under urethan narcosis. Intravenous injection of the component was followed

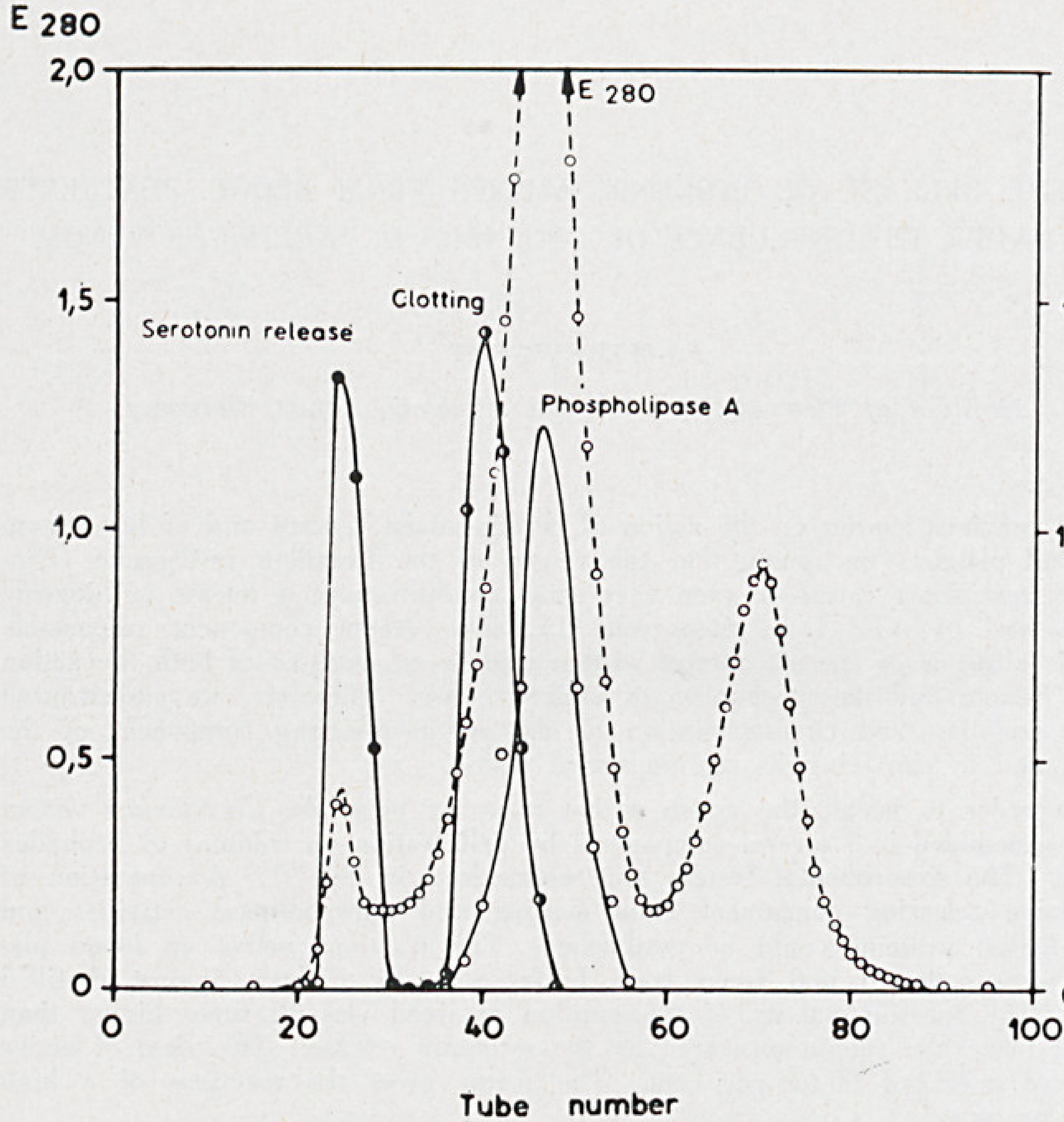

Fig. 1 — Gelfiltration of *C. d. terrificus* venom on Sephadex G 100.

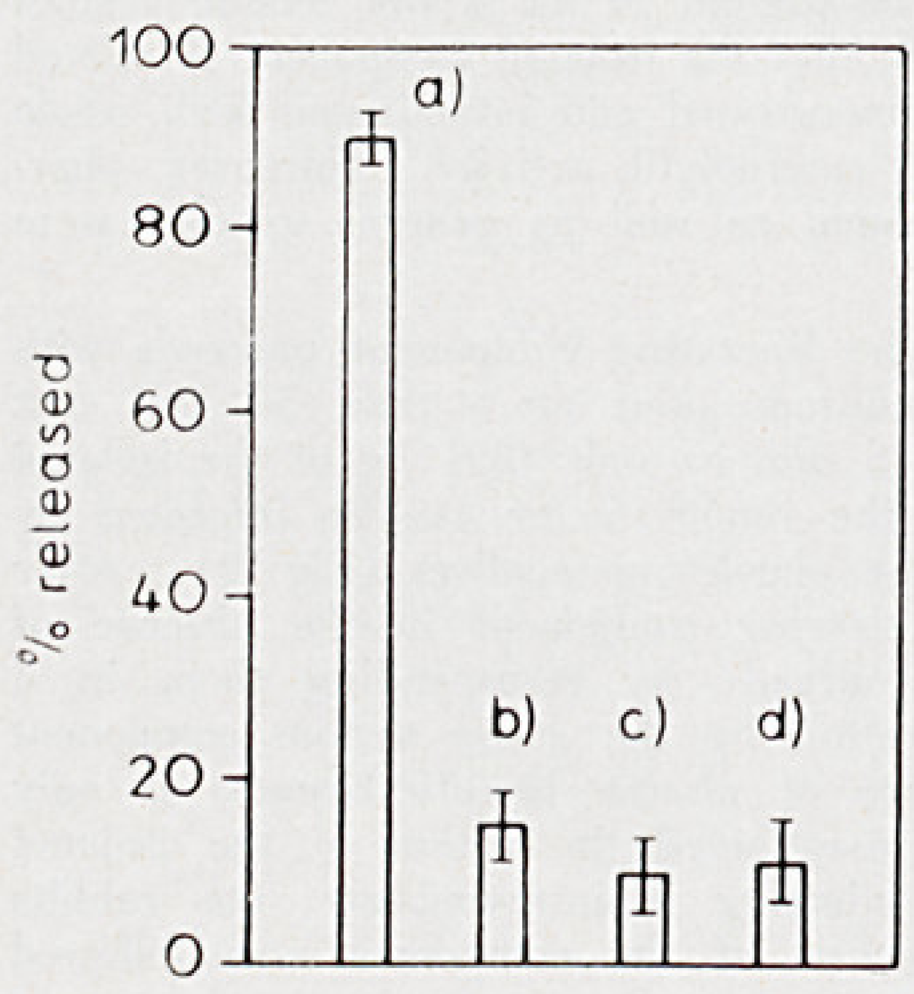

Fig. 2 — Release of serotonin from rabbit platelets by *C. d. terrificus* venom. a) in Tyrode-solution; b) without glucose; c) with 10^{-3} m monoiodoacetic acid; d) with EDTA.

by a fall of the blood pressure, which in the same intensity could be produced also by injection of a serotonin quantity equivalent to the amount contained in blood platelets. A second injection did not result in a blood pressure response (Fig. 3). The number of thrombocytes was found to be lowered to 50 per cent after the injection. Platelets isolated from the blood after administration of the venom factor contained only 20 per cent of its original serotonin level.

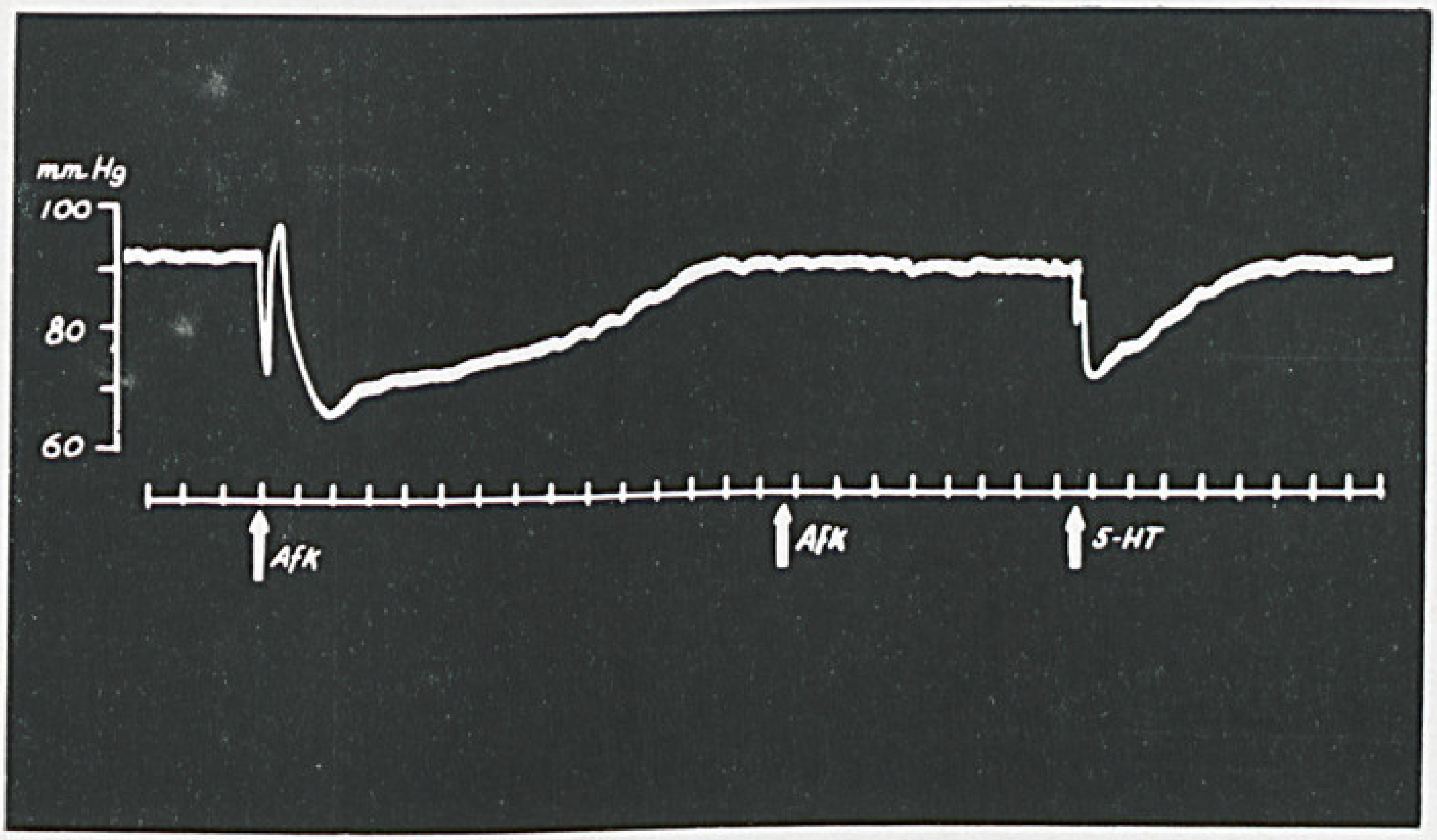

Fig. 3 — Effect of the isolated amine liberating component of *C. d. terrificus* venom (AfK) on blood pressure.

Conclusion — In the venom of the Brazilian rattlesnake (*Crotalus d. terrificus*) there exists a relatively high molecular protein component, which is able to liberate stored biogenic amines from blood platelets. The factor is active in platelet suspensions *in vitro* as well as in the circulating blood. This component is not identical with other biochemical and pharmacological active principles of the whole venom. The induced release-reaction seems to proceed in two steps. In the first step the amine releasing venom factor induces a change in the permeability of the platelet membrane, and in a subsequent reaction amines became liberated.

References

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