## Short communication

## INHIBITION OF THE ACTIVITY OF a - TOXIN OF CLOSTRIDIUM PERFRINGENS BY TOXIC FILTRATES OF CORYNEBACTERIA

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Toxins of Corynebacterium hæmolyticum, C. ulcerans, and C. pseudotuber-culosis are adsorbed onto sheep and rabbit erythrocytes without significantly hæmolyzing them in addition to their similar action in the experimental animal (dermonecrotic and lethal effect in the rabbit and guinea pig). After adsorption of toxins these erythrocytes are not hæmolyzed by  $\alpha$ - and  $\beta$ -toxin of Staphylococcus pyogenes (4, 5, 6, 7, 11).

More recently, phospholipase activity was demonstrated in these corynebacteria [8,9]. Lysolecithin, lecithin, and sphingomyelin are split. The nature of the degradation products suggests that the enzyme activity is due to phosphatidylcholine phosphatidohydrolase. In *C. hæmolyticum* an additional enzyme phosphatide acyl-hydrolase (10) was demonstrated.

Since  $\alpha$ -toxin of Clostridium perfringens is a phosphatidylcholine choline-phosphohydrolase (2, 3) we tested the effect of  $\alpha$ -toxin of C. perfringens on sheep erythrocytes after treatment with toxins of C. hæmolyticum, C. ulcerans, and C. pseudotuberculosis. Figure 1 shows an agar plate with a suspension of sheep erythrocytes. The central well contains  $\alpha$ -toxin of C. perfringens, well 1 a filtrate of a broth culture of C. hæmolyticum, well 2 of C. ulcerans, and well 3 of C. pseudotuberculosis. Well 4 serves as control with culture medium. Zones of inhibition of hæmolysis of sheep erythrocytes by  $\alpha$ -toxin of C. perfringens are apparent around wells 1, 2, and 3. Figure 2 shows a similar device in which corynebacteria toxins were applied 24 hours prior to the addition of  $\alpha$ -toxin of C. perfringens into the central well. The inhibition zones are larger due to the longer diffusion period of the toxins of corynebacteria. The mechanism of the effect of the toxins can be explained by a splitting of sub-

strate in the cell walls of the erythrocytes so that the enzymatic activity of  $\alpha$ -toxin of C. perfringens cannot assert itself, or that the adsorbed toxins interfere with the binding of  $\alpha$ -toxin with the substrate.

From the above described observations it is apparent that  $C.\ hæmolyticum$ ,  $C.\ ulcerans$ , and  $C.\ pseudotuberculosis$  form a group of bacteria similar in their action in experimental animals, their phosphatidylcholine phosphatidohydrolase activity, and their capacity to inhibit the action of  $\alpha$ - and  $\beta$ -toxin of Staphylococcus pyogenes and  $\alpha$ -toxin of  $C.\ perfringens$  in sheep erythrocytes. In our opinion, the phospholipase, which we consider to be phospholipase D, is the most active component of the toxins of the described corynebacteria not only in vitro but also in vivo.

## REFERENCES

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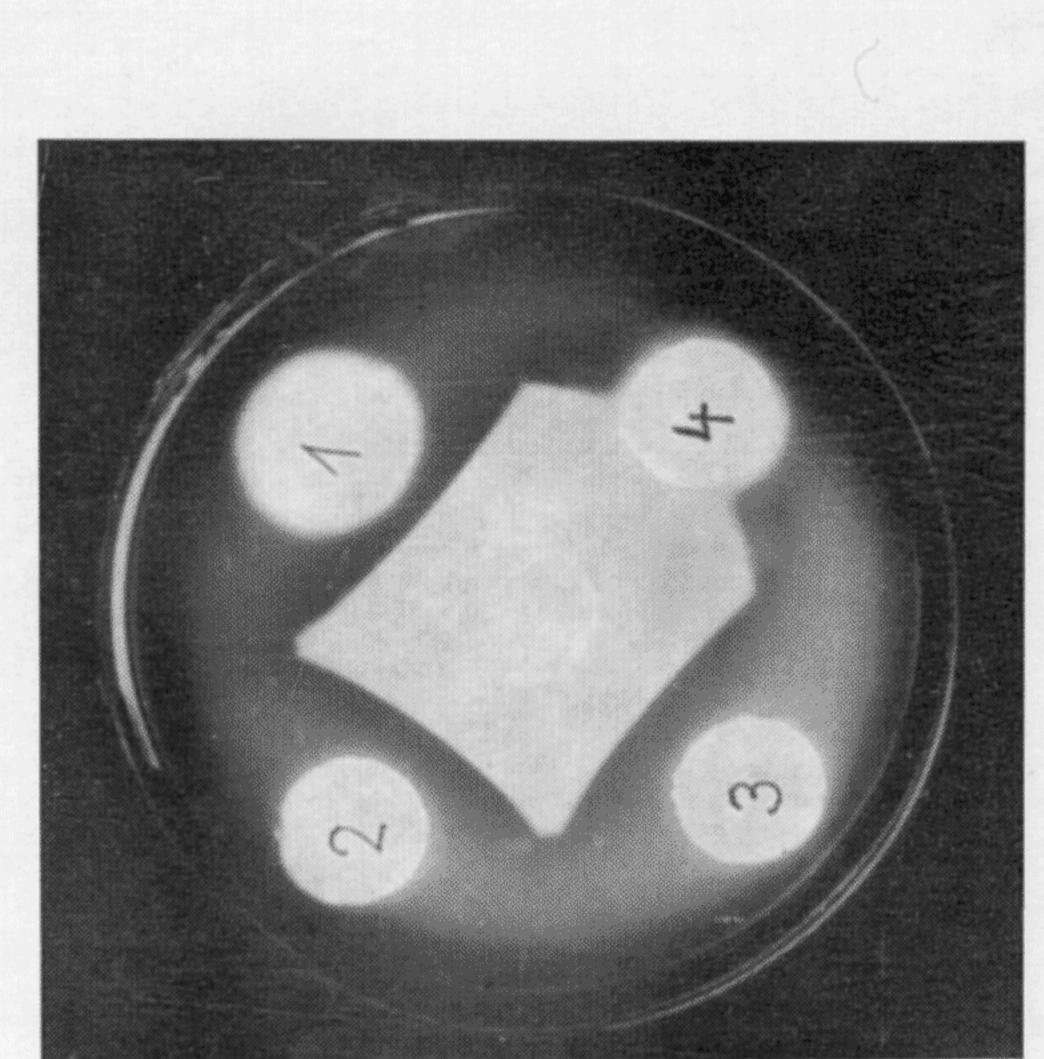
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