CHANGES IN SERUM ACID PHOSPHATASE ACTIVITY IN GUINEA PIGS POISONED WITH TOXIN OF Clostridium perfringens TYPE AND WITH A MIXTURE OF TOXIN AND BROTH CULTURE FILTRATE

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Severe lesions can be produced by lysosomal enzymes set free as a result of the action of bacterial toxins of tissue substrates [1, 3]. Previous experiments in vitro showed that an increase in the specific activity of acid phosphatase in the lysosomal fraction of the liver from albino mice takes place under the influence of the toxin of Clostridium perfringens type A and also of the toxin and filtrate of a broth culture of Cl. butyricum [4].

The present investigation was devoted to the study of the changes in acid phosphatase activity in the blood serum of guinea pigs under the influence of Cl. perfringens type A toxin and metabolic products of Cl. butyricum in experiments in vivo.

EXPERIMENTAL METHOD

Experiments were carried out on 64 guinea pigs weighing 280-360 g. Clostridium perfringens type A toxin (1MLD, intramuscularly), obtained from the Khar'kov Research Institute of Vaccines and Sera, was injected into the animals of group 1, the animals of group 2 received filtrate (3 ml, intramuscularly) of a broth culture of Cl. butyricum strain No. 237, the guinea pigs of group 3 received a mixture of toxin and filtrate in the doses mentioned above, the animals of group 4 received toxin with antitoxin (10 antitoxin units intramuscularly) from the bacterial preparations factory of Khar'kov Research Institute of Vaccines and Sera (series 312, No. OBK 1027), and the guinea pigs of group 5 received a mixture of toxin and filtrate together with antitoxin.

Before the experiment, the specific activity of acid phosphatase was determined in 14 animals of the control group. In each experimental group there were ten animals; blood from some of the experimental animals was taken for investigation from the heart 1 and 9 h, and from others 3 and 24 h after injection of the preparation.

Acid phosphatase activity in the blood serum of the guinea pigs was determined by the method described by Bessey et al. [5] in Levitskii's modification [2]. To 0.4 ml sodium p-nitrophenylphosphate in citrate buffer, pH 4.8, 0.1 ml serum was added and the mixture was incubated at 30°C for 2 h. The reaction was stopped by the addition of 5 ml 0.05M NaOH and the optical density was measured on a "Spekol" (East Germany) spectrophotocolorimeter at 410 nm. Each determination was accompanied by a control. The specific acid phosphatase activity was then determined.

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TABLE 1. Changes in Serum Acid Phosphatase Activity of Guinea Pigs Under the Influence of Cl. perfringens Toxin and Filtrate of Broth Culture of Cl. butyricum (M ± m)

Group of animals	Preparation injected	Acid phosphatase activity at different times of investigation of blood serum, i.u.				
		before injection (control)	time after injection			
			1 h	3 h	9 h	24 h
Control	—	7,183±0,718 (14)	—	—	—	—
1-	Toxin	—	5,417±0,450 (5) $P>0,05$	8,021±1,068 (4) $P>0,05$	10,903±1,165 (4) $P<0,02$	17,505±3,408 (3) $P<0,01$
2-	Filtrate	—	6,612±0,943 (5) $P>0,05$	5,917±0,818 (5) $P>0,05$	5,764±0,657 (4) $P>0,05$	8,167±0,533 (5) $P>0,05$
3-	Toxin + filtrate	—	8,389±1,151 (5) $P>0,05$	11,584±1,299 (5) $P<0,01$	21,842±1,397 (4) $P<0,001$	—
4-	Toxin + antitoxin		6,417±1,318 (5) $P>0,05$	8,723±1,225 (5) $P>0,05$	9,167±1,394 (5) $P>0,05$	9,639±0,423 (5) $P<0,001$
5-	Toxin + filtrate + antitoxin	—	11,306±2,353 (5) $P>0,05$	19,307±4,211 $P<0,02$	10,028±1,405 (5) $P>0,05$	5,667±1,109 (5) $P>0,05$

Legend. P determined relative to control group. Number of experiments shown in parentheses.

EXPERIMENTAL RESULTS

In poisoning with Cl. perfringens type A toxin, an increase in the serum acid phosphatase activity of the guinea pigs was observed 3 h after injection of the toxin, and it became significant compared with the control after 9 and 24 h (Table 1). Filtrate of a broth culture of Cl. butyricum caused virtually no change in the serum acid phosphatase activity of the experimental animals at all times of investigation ($P > 0.05$). Meanwhile, filtrate injected together with the Cl. perfringens toxin (animals of group 3) led to an increase in acid phosphatase activity, which became significant as early as 3 h after the injection.

Antitoxic serum was not effective at all times of the investigation when injected both with the toxin and with the mixture of toxin and filtrate of Cl. butyricum (groups 4 and 5 of the animals). For instance, in the group of animals receiving toxin with antitoxic serum, a tendency was observed for activity of the enzyme to increase 3 and 9 h after injection, but after 24 h this increase was significant ($P < 0.01$). The increase in serum acid phosphatase activity after injection of a mixture of toxin with filtrate and antitoxic serum was significant 3 h after injection ($P < 0.02$).

It can thus be concluded from the results of these investigations that under the influence of Cl. perfringens type A toxin the serum acid phosphatase activity of guinea pigs increases. A filtrate of Cl. butyricum causes no appreciable changes in the activity of this enzyme, but leads to an increase in its activity if injected together with Cl. perfringens toxin. Under these circumstances an increase in enzyme activity was observed sooner than if the toxin was injected alone. These results are evidence of potentiation of the action of Cl. perfringens type A toxin under the influence of metabolic products of Cl. butyricum.

It is known that Cl. butyricum may be an associate of Cl. perfringens in gas gangrene [6, 7]. If this microbial association is present in wounds in man, an increase in the serum acid phosphatase activity may be observed.

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