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THE EFFECT OF VITAMIN E ON THE TOXICITY OF CADMIUM IN CADMIUM-SENSITIVE STAPHYLOCOCCUS AUREUS

By

H. Korkeala

KORKEALA, H.: *The effect of vitamin E on the toxicity of cadmium in cadmium-sensitive Staphylococcus aureus.* Acta vet. scand. 1980, *21*, 224—228. — The effect of vitamin E on the toxicity of cadmium (Cd) in Cd-sensitive Staphylococcus aureus 3719— was studied. The addition of vitamin E into the liquid growth medium resulted in a shortened lag phase of growth in Cd-stressed S. aureus 3719—. It is suggested that the decrease observed in the length of the lag phase is due to the antioxidative effect of vitamin E which indicates that Cd together with hydrogen peroxide produced by the bacterium may induce an oxidative damage in staphylococcal cells.

D-alpha tocopherol; antioxidative effect; lag phase; oxidative damage.

Previous work (*Korkeala & Sankari* 1980) showed that the toxicity of cadmium (Cd) with respect to certain bacteria may at least partly be due to its ability to induce oxidative damage when combined with hydrogen peroxide (H_2O_2) produced by the bacterium itself. Vitamin E is an antioxidant and acts in animal cells as a scavenger of short-lived free radicals (*Albert* 1973).

The purpose of the present investigation was to find out whether the toxicity of Cd for the bacteria used in the earlier study is reduced by vitamin E, and thus to throw additional light on the mechanism of the toxicity of Cd with respect to these bacteria.

MATERIAL AND METHODS

The test organism

The microbial strain used in the study was Staphylococcus aureus 3719—. The strain is sensitive to penicillin and Cd ions. The strain was obtained from Dr. K. G. H. Dyke, the Department of Biochemistry, University of Oxford, England.

Chemicals and water

The $CdCl_2 \times 2½\ H_2O$, H_2O_2, $Na_2HPO_4 \times 2\ H_2O$ and $NaH_2PO_4 \times 2\ H_2O$ used were pro analysis grade. The $CdCl_2 \times 2½\ H_2O$ was obtained from J. T. Baker, Phillipsburg, N.J., USA, the H_2O_2 and $Na_2HPO_4 \times 2\ H_2O$ were obtained from E. Merck, Darmstadt, German Federal Republic and the $NaH_2PO_4 \times 2\ H_2O$ was obtained from BDH Chemicals, Poole, England. The D-alpha tocopheryl polyethylene glycol 1000 succinate used was the product of Distillation Products Industries, N.Y., USA. D-alpha tocopheryl polyethylene glycol 1000 succinate contains 260 mg of D-alpha tocopherol per g, equivalent to 387 i.u. of vitamin E. The water used throughout the experiments was double distilled and deionized.

Effect of vitamin E on the growth of S. aureus 3719—

Autoclaved broth containing 10 g of yeast extract (Difco Laboratories, Detroit, Mich., USA) and 1 g of D-glucose (BDH Chemicals) per l distilled water served as the basic medium. The pH of the broth (denoted YG broth below) was 7.0. The filter-sterilized Cd and vitamin E solutions were added to the broth before inoculation. Cells from an overnight culture were used for the inoculations (0.1 ml of staphylococcal suspension to 7 ml of YG broth). The size of the inoculum was determined by plate count agar (Difco). The tubes were incubated in a shaker at 35°C, and the growth was monitored with the Klett-Summerson photoelectric colorimeter (filter no. 42, Klett Manufacturing Co., N.Y., USA). The Cd and vitamin E concentrations used were 0.08 mg of Cd/l and 52 mg of D-alpha tocopherol/l (0.542 i.u. of vitamin E per tube). The study involved four different series of experiments: one without either Cd or vitamin E, one with added vitamin E and one with added Cd as controls, and one with both Cd and vitamin E. For each series five parallel tubes were incubated, and each such experiment was repeated 12 times.

Effect of D-alpha tocopheryl polyethylene glycol 1000 succinate on the decomposition of H_2O_2

A solution containing 52 mg of D-alpha tocopherol/l was prepared in 0.05 M phosphate buffer (pH 7.0). Three ml of the solution was mixed with 0.1 ml of 0.5 M hydrogen peroxide, and the change in absorbance was recorded during 30 min with a spectrophotometer (Perkin-Elmer 550, Norwalk, Conn., USA) at 240 nm.

RESULTS

The effect of vitamin E on the growth of S. aureus 3719— in autoclaved YG broth containing 0.08 mg of Cd/l in one experiment is shown in Fig. 1. The figure also shows the effect of vitamin E on the growth of S. aureus 3719— in YG broth without Cd. The size of the inoculum was 14×10^7 cells/ml. Parallel experiments gave more or less similar results.

The D-alpha tocopheryl polyethylene glycol 1000 succinate had no effect on the decomposition of H_2O_2.

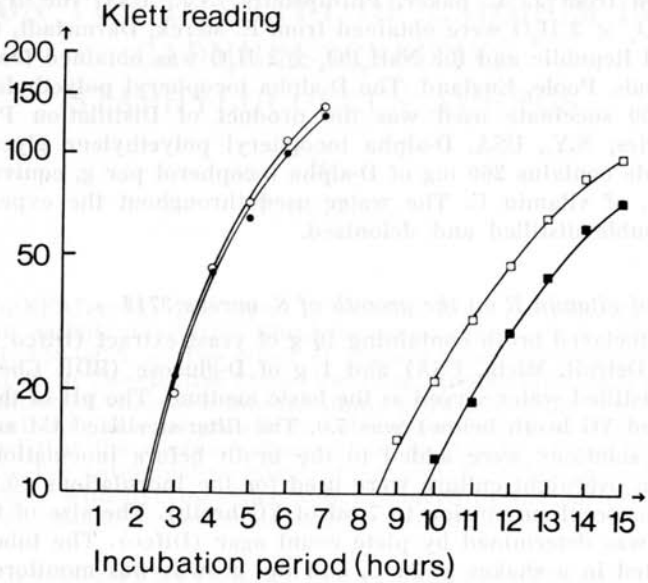

Figure 1. Growth of S. aureus 3719— in autoclaved YG broth with added filter-sterilized Cd solution to 0.08 mg of Cd/l and 52 mg of D-alpha tocopherol/l. Each test tube was inoculated similarly from the same overnight culture. Turbidities were measured with the Klett-Summerson photoelectric colorimeter (filter no. 42). Each point represents the mean of five separate determinations. Symbols: (●) no added Cd and vitamin E, (○) 52 mg of D-alpha tocopherol/l, (■) 0.08 mg of added Cd/l, (□) 0.08 mg of added Cd/l + 52 mg of D-alpha tocopherol/l.

DISCUSSION

The literature includes only few studies concerning the effect of vitamin E on bacteria. *Pamini & Ugolini* (1961) found, using the paper disc diffusion method with Staphylococcus aureus, that tocopherol reduces the activity of kanamycin, tetracyclin,

oxitetracyclin, chloramphenicol and penicillin, while the effect of oleandomycin, erythromycin and dihydrostreptomycin is increased. In the present study, the addition of vitamin E shortened the lag phase of growth caused by Cd in S. aureus 3719— but had no effect on the lag phase of staphylococcal cells incubated without Cd (Fig. 1).

Martius & Müller (1964) observed that tocopherol can replace vitamin K as a growth factor in the vitamin K heterotrophic anaerobic Fusiformis nigrescens. They further observed that tocopheryl quinone is a more active growth factor than tocopherol itself. In the present study, vitamin E apparently did not act as a growth factor, since YG broth cannot be considered to be a minimum growth medium; in addition, vitamin E had no effect on the growth of S. aureus without Cd (Fig. 1).

It is known that pyruvate is spontaneously oxidizable by H_2O_2 (*Stephenson* 1966). Although there are structural differences between pyruvate and succinate, the ability of the vitamin E preparation used (D-alpha tocopheryl polyethylene glycol 1000 succinate) to decompose H_2O_2 was investigated. It was found, as expected, that the preparation did not decompose H_2O_2.

The present results indicate that the decrease observed in the length of the lag phase is due to the antioxidative effect of vitamin E. Thus it is apparent that the oxidative damage caused by Cd and H_2O_2, as suggested by *Korkeala & Sankari* (1980), is one of the toxic mechanisms of Cd in bacteria.

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SAMMANFATTNING

Effekten av E-vitamin på kadmiums toxicitet på kadmiumkänslig Staphylococcus aureus.

Effekten av E-vitamin på kadmiums (Cd) toxicitet på en Cd-känslig Staphylococcus aureus 3719— undersöktes. Tillsatsen av E-vitamin till det flytande näringssubstratet förkortade lagfasen i den Cd-belastade S. aureus 3719—. Det är möjligt att den observerade förminskningen i längden av lagfasen beror på den antioxidativa effekten av E-vitamin. Detta tyder på att Cd tillsammans med väteperoxid producerad av bakterier kunde förorsaka en oxidativ skada på stafylokockceller.

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