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Influence of Sugars on the Radiation Sensitivity of Catalase[1]

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INTRODUCTION

Recently we have shown (1) that glycerol forms a complex with metal ions in the catalase molecule and, therefore, protects the enzyme against reactive radicals. When these metal ions are complexed, there is no longer catalytic destruction of hydrogen peroxide into OOH and OH radicals which are responsible for the inactivation of the catalase molecule.

Diglycyl-glycine (2) was found to protect catalase by acting as a radical scavenger. The protective effect, however, was less than that obtained by glycerol. It, therefore, seems probable that the best protection will be obtained using chelating agents, because the radical scavenging substances are less efficient.

Bacq and Alexander (3) have pointed out that sugars and other compounds containing hydroxyl groups are weak radiation protectors. The mechanism of this protective effect may be explained by a radical scavenging reaction. It is interesting to note that no protective effect has been observed using glucose. It is the purpose of this communication to show the effects of fructose, sucrose, and glucose on the radiation response of catalase.

MATERIALS AND METHOD

Two ml of lyophilized beef liver catalase solution (8×10^{-8} M, 0.05 M phosphate buffer, pH 7.0; Worthington Biochemical Corp., Freehold, New Jersey) were irradiated in a Lucite container with doses varying from 0 to 8.1×10^6 r. The dose rate of the beryllium-window x-ray tube (100 kV, 12 mA, HVL 0.065 mm Al; Philips Electronics Inc., Mt. Vernon, New York) was about 9×10^4 r/min.

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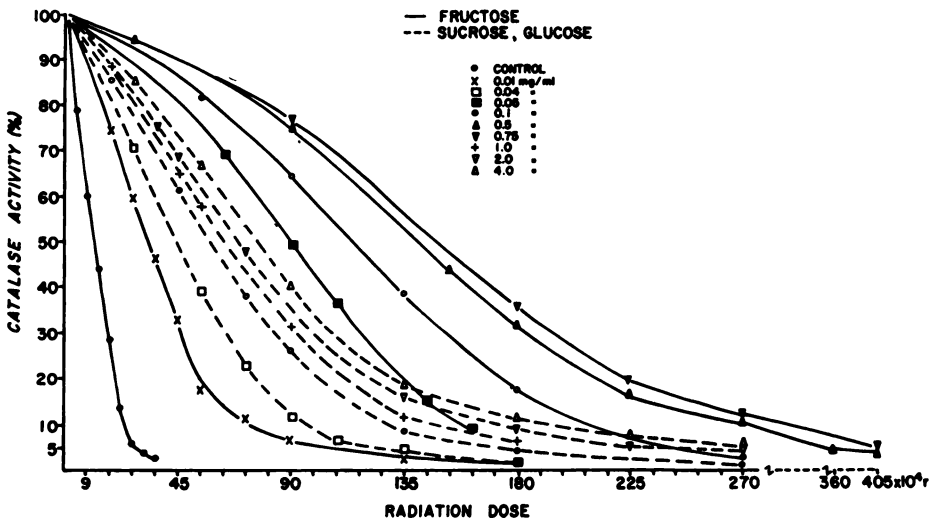

Fig. 1. The Influence of Different Concentrations (mg/ml) of Several Kinds of Sugar on the Radiation Sensitivity of Catalase (Broken Lines for Sucrose and Glucose).

Different amounts of the several sugars (sucrose and glucose, Fisher Scientific Co., Fair Lawn, New Jersey; fructose, Pfanstiehl Laboratories Inc., Waukegan, Ill.) investigated were added to the catalase solution before irradiation. The sugar concentrations used are shown in Figure 1. The catalase activity was determined spectrophotometrically using the method of Beers and Sizer (4). Details of the procedure were described previously (1). All measurements and irradiations were done at room temperature.

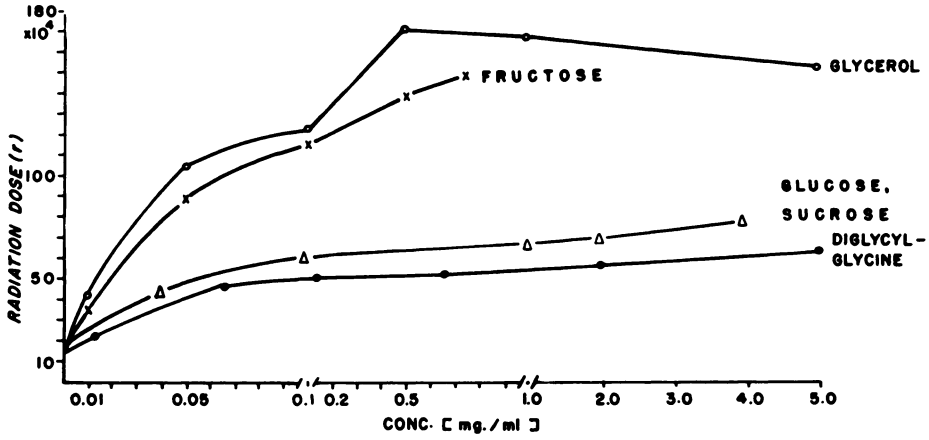

Fig. 2. 50%-Dose Values as a Function of the Sugar Concentration (Values from Fig. 1; Note the Interruption in the Concentration Scale). Glycerol Values (1) and Diglycyl-Glycine Values (2) for Comparison.

RESULTS

In Figure 1, the catalase activity is plotted against the radiation dose. As can be seen, a protective effect is observed for the sugars which were investigated. Fructose was found to have the greatest radiation protective effect of the three sugars. Experimental results obtained with glucose and sucrose agreed within error limitations.

The radiation dose which was required to reduce the catalase activity to 50 per cent is plotted in Figure 2 as a function of sugar concentration. Results from Figure 1 were used in obtaining these values. The ED_{50}-values for glycerol (1) and diglycyl-glycine (2) are also shown for comparison. The protective effect observed with fructose is almost comparable to that found for glycerol. Since glycerol protects through its chelating ability, the question of whether it might be suspected that fructose acts as a chelating rather than a radical scavenging compound arises. Experiments were performed in order to determine the mode of fructose protection. A small amount of $CuCl_2$ was added to the fructose-enzyme solution. After the addition of $CuCl_2$, the fructose should complex with the copper ions and consequently there should be a reduction in the protective effect obtained by fructose if chelating is responsible for the radio-protective mechanism. However, the same protective effect was found for solutions containing fructose as well as with solutions containing both fructose and copper ions.

Copper ions in the concentrations used in this experiment have been found to exert a considerable protective effect on catalase (5). However, it was found that the protective effect of fructose and copper ions respectively was not additive. Moreover, the effect observed with the sugars is based on a radical scavenging mechanism.

SUMMARY

The change in radiosensitivity of catalase by sucrose, fructose, and glucose was investigated spectrophotometrically. The irradiation doses varied between 0 to 8.1×10^6 r. The radiation protective effect is considerably greater for fructose than for sucrose and glucose. Radiobiological implications are discussed.

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