JOURNAL OF NUCLEAR MEDICINE 5:304-306, 1964

Intermolecular Reactions between Glycerol and Lactate Dehydrogenase. Influence on the Radiation Sensitivity of the Enzyme[1]

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Recently we have shown (1) that glycerol protects catalase against radiation when added prior to irradiation. A complex involving glycerol and the metal ion present in the enzyme seems to be responsible for the radioprotective effect. As a result of this complex formation, the metal present in the enzyme can no longer function as an energy sink or act as an efficient acceptor for the hydrated electron (e^-_{aq}).

If the protective action of glycerol can be attributed to this mechanism, then it is possible that a modification of the radiation response should also be expected when glycerol is added to other metallo-enzymes. In our present investigations lactate dehydrogenase (LDH) was used. This metallo-enzyme contains zinc bound coordinatively (2).

18 λ of a rabbit muscle LDH stock solution[2] (46.2 mg/ml) were diluted with 100 ml of 0.035 M phosphate buffer, pH 7.4 making a final LDH concentration of 6.15 M Glycerol[3] (spectro-quality reagent) was added to the LDH solution prior to irradiation. The glycerol concentrations used (in λ glycerol/10 ml LDH solution) are mentioned in Figure 1.

One ml samples of LDH solution were irradiated in Lucite containers with different doses (0 to 4.5×10^5 r). The irradiations were done with a beryllium-window X-ray tube[4] (100 KV, 12 mA, HVL 0.064 mm Al.) The dose rate was about 9×10^4 r/min. The X-ray tube was calibrated with an air-wall ionization chamber.

Warburg and Christian have observed (3) that the reduced form of diphosphopyridine nucleotide (DPNH) has an absorption band maximum at 340 mμ where the oxidized form has no absorption at this wave length. The LDH activity was determined spectrophotometrically using the decrease in optical density (of DPNH) at 340 mμ as a function of time after mixing enzyme and substrate. A Cary 14 Spectrophotometer[5] was used for the determination. One ml of 1.14

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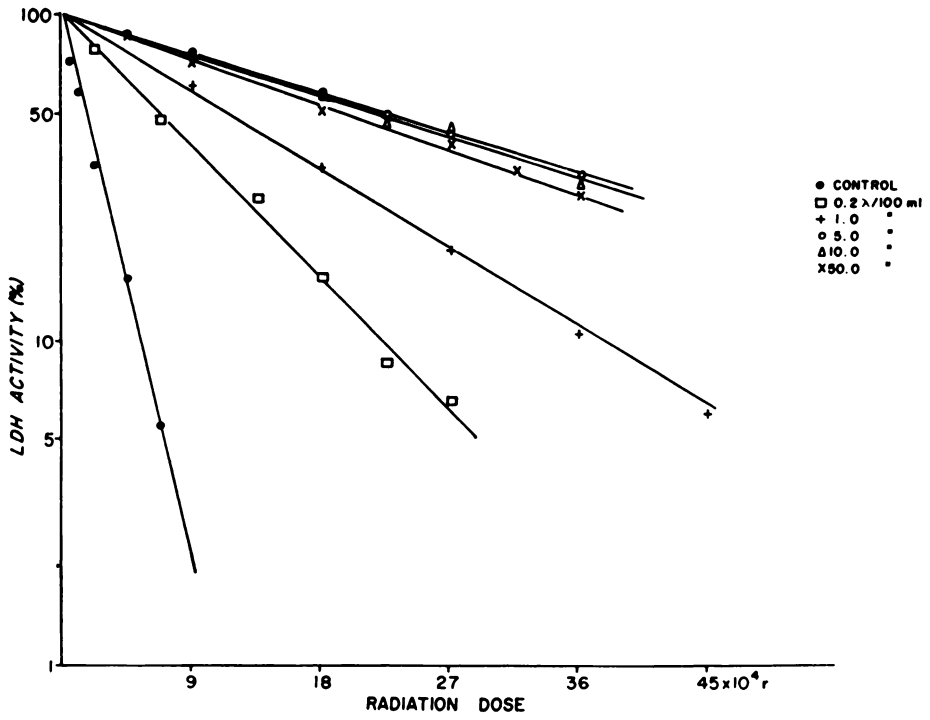

Fig. 1. The influence of different concentrations of glycerol (mg/ml) on the radiation sensitivity of lactate dehydrogenase.

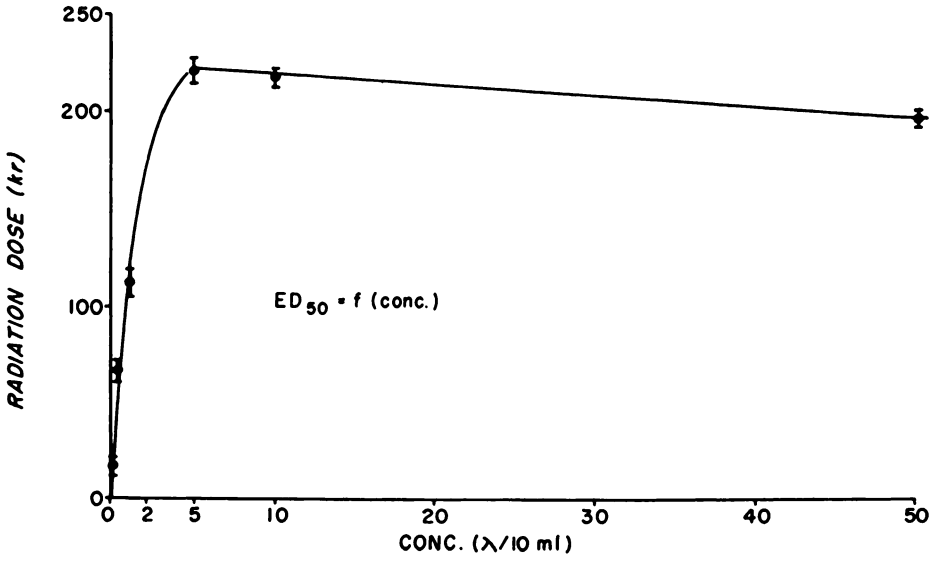

Fig. 2. 50%-dose values as function of the glycerol concentration (values from Fig. 1).

$\times 10^{-3}$ M pyruvic acid[1] was rapidly injected with a syringe into 1 ml of each LDH solution and DPNH solution[2] (3.45×10^{-4} M). All solutions were prepared fresh daily and all measurements and irradiations were done at room temperature.

The radioprotective effect exhibited by glycerol with LDH is shown in Figure 1. Unirradiated samples were used for control values and their activity was taken as 100 per cent. The activities of the LDH solutions irradiated with varying X-ray doses are shown relative to the control value. As can be seen from Figure 1, the addition of small amounts of glycerol exerts a considerable protective effect. A maximum of the protective effect seems to be present for glycerol concentrations much less than the highest ones used. Five λ of glycerol in 10 ml of LDH solution (0.0005 vol.%) gave the greatest protective effect. A similar result was obtained for the maximal protective effect in the case of catalase.

The radiation dose which was required to reduce the LDH activity to 50 per cent is plotted in Figure 2 as a function of glycerol concentration using the results from Figure 1. At maximal protection (5λ/10 ml), when compared to the control, a ca. 13 times greater dose is required to reduce the enzyme activity to 50 per cent. The protective factor for the higher concentration of 50λ/10 ml is ca. 10 per cent less than that observed for 5λ/10ml.

All of the results obtained, including the maximum for the protective effect (ca. 5λ/10ml), are similar to the results obtained using catalase. It, therefore, may be concluded that the glycerol-metal complex mechanism might also be applicable for the protection of LDH. The best protection seems to be obtained with chelating agents complexing with metal ions present in biological systems.

SUMMARY

The change in radiosensitivity of lactate dehydrogenase by glycerol was investigated spectrophotometrically. The irradiation doses varied between 0 to 4.5×10^5 r. Small amounts of glycerol (0.0005 vol.%) gave a maximum protective effect. This protective effect can be explained by a complex formation between glycerol and the metal ions present in the enzyme molecule.

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