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The Influence of *D*-Penicillamine on Enzymatic Activities: Glucose-6-phosphate Dehydrogenase. Correlation with Serum Levels Measured in Humans

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Summary: The influence of *D*-penicillamine on glucose-6-phosphate dehydrogenase of yeast (pure enzyme), human hemolysate, and human skin homogenate were determined. In high concentrations, *D*-penicillamine inhibits glucose-6-phosphate dehydrogenase activity (concentrations above 6.7 mmol/l, i. e. 1 g/l). In low concentrations, *D*-penicillamine exerts an indirect influence by removing some inhibiting metal ions, such as zinc. In human skin homogenates, an activating action of *D*-penicillamine on glucose-6-phosphate dehydrogenase activity occurs due to the chelation of metal ions.

Der Einfluß von D-Penicillamin auf Enzymaktivitäten: Glucose-6-phosphat-Dehydrogenase

Zusammenfassung: Der Einfluß von *D*-Penicillamin auf die Glucose-6-phosphat-Dehydrogenase-Aktivität von Hefe (Reinenzym), von Hämolysat menschlicher Erythrocyten und vom Homogenat menschlicher Haut wurde untersucht. In Konzentrationen ab 6,7 mmol/l (= 1 g/l) entfaltet *D*-Penicillamin eine direkte Hemmwirkung auf die Glucose-6-phosphat-Dehydrogenase-Aktivität der Hefe und des Hämolysates. Niedrigere Konzentrationen beeinflußten die Glucose-6-phosphat-Dehydrogenase-Aktivität durch Entfernung hemmender Metallionen (Zinkionen), was zu einer Aktivierung führte. Im Hauthomogenat ließ sich nur eine derartige Aktivierung nachweisen. Das Zustandekommen der verschiedenen Wirkungen von *D*-Penicillamin auf die Glucose-6-phosphat-Dehydrogenase-Aktivität wird diskutiert.

Introduction

D-Penicillamine (β,β'-dimethylcysteine) is used in human therapy for an increasing number of diseases. The mechanisms whereby this interesting substance exerts its various therapeutic actions, are, however, not always fully understood. There is increasing interest in the changes in enzymatic activities evoked by *D*-penicillamine.

Biochemically, *D*-penicillamine acts as a chelating agent (e. g. chelation of copper ions in *Wilson*'s disease), splits disulfide bonds (exchange reactions), and reacts with aldehyde groups (e. g. with aldehyde groups of pyridoxal-phosphate and tropocollagen). Important enzymes and enzyme systems may be influenced by *D*-penicillamine via all three of the above mechanisms. So far, an anti-collagenase effect of *D*-penicillamine has been reported (1) as well as an inhibitory action on alkaline phosphatase activity (2). The latter effect was attributable to a chelation of important ions. In further investigations, changes in glucose-6-phosphate dehydrogenase activity were encountered under the influence of *D*-penicillamine. As a contribution to the various biochemical actions of *D*-penicillamine, the results of these studies will be reported here.

Methods and Materials

Enzyme sources: Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) activity has been investigated from three sources: from yeast (pure enzyme preparation, commercially available), from human hemolysate, and from human skin homogenates (Blendor: Ultraturrax). Details of preparations have been described elsewhere (3).

D-Penicillamine: *D*-Penicillamine (Biochemie, Vienna) dissolved in saline was added to the solutions with Glucose-6-phosphate dehydrogenase activity. Incubation was performed at 37 °C for 1 h. In every instance, enzymatic activity was compared to a control assay containing saline instead of *D*-penicillamine. Final concentrations of *D*-penicillamine ranged between 6.7 µmol/l (i. e. 1 µg/l) und 335 mmol/l (i. e. 50 g/l).

Zinc ions: As the activity of glucose-6-phosphate dehydrogenase is known to be highly susceptible to the presence of zinc or other metal ions (4, 5), additional investigations were performed with *D*-penicillamine and zinc ions. $Zn(NO_3)_2 \times 4H_2O$ (M = 261.5) was used. The effects of *D*-penicillamine