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A New Enzymatic Determination of Cholesterol

The Use of Aldehyde Dehydrogenase to Measure H_2O_2 Producing Reactions, II.

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Summary: Cholesterol oxidase, coupled with the catalase and aldehyde dehydrogenase, is proposed for the determination of cholesterol. The main advantages of this procedure over comparable methods employing cholesterol oxidase are its short reaction time and the use of NADP which permits the direct calculation of the cholesterol concentration from the absorbance value.

Neue enzymatische Methode zur Bestimmung der Cholesterinkonzentration. Verwendung von Aldehyddehydrogenase zur Indikation H_2O_2-bildender Reaktionen, 2. Mitteilung

Zusammenfassung: Die mit Katalase und Aldehydrogenase gekoppelte Cholesterinoxidase-Reaktion wird zum Nachweis der Cholesterinkonzentration vorgeschlagen. Die wichtigsten Vorteile dieses Verfahrens gegenüber vergleichbaren Cholesterinoxidase-Methoden sind eine kurze Reaktionszeit und der Einsatz von NADP, der eine direkte Berechnung der Konzentration aus den Absorptionswerten ermöglicht.

Introduction

Recently we have proposed the use of catalase coupled with aldehyde dehydrogenase to measure the action of H_2O_2 producing oxidoreductases (1, 2). Meanwhile this principle has been extensively evaluated for the determination of uric acid in human serum and urine samples (3). The main advantages over comparable methods are: the reaction is completed in a few minutes and the use of NAD^+ or $NADP^+$ (3) permits the direct calculation of the substrate concentration from the absorbance value without reference to a standard solution. This is especially relevant if the preparation of primary standard solutions is problematical.

In the following study the cholesterol concentration was determined with cholesterol oxidase (4) using the aldehyde dehydrogenase principle:

$$\text{cholesterol ester} + H_2O \xrightarrow[\text{esterase}]{\text{cholesterol}} \text{cholesterol} + \text{fatty acid}$$

$$\text{cholesterol} + O_2 \xrightarrow[\text{oxidase}]{\text{cholesterol}} \text{cholest-4-en-3-one} + H_2O_2$$

$$H_2O_2 + \text{ethanol} \xrightarrow{\text{catalase}} \text{acetaldehyde} + 2\,H_2O$$

$$\text{acetaldehyde} + NAD(P)^+ \xrightarrow[\text{dehydrogenase}]{\text{aldehyde}} \text{acetate} + NAD(P)H + H^+$$