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Influence of Auxiliary Enzymes on the Spectrophotometric Measurement of Alanine Aminotransferase and Aspartate Aminotransferase Activities

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Summary: We investigated the enzyme activity of the blank in the spectrophotometric determination of alanine aminotransferase activities and aspartate aminotransferase activity. 6 lactate dehydrogenase and 3 malate dehydrogenase preparations from different manufacturers and from different organs showed additional and contaminating activity. The additional activity depends upon the 2-oxoglutarate concentration. The contaminating activity is caused by alanine aminotransferase and aspartate aminotransferase in the auxiliary enzymes. We propose that exact definitions must be given for the auxiliary enzymes in the recommendations of standard determinations for enzyme activities.

Einfluß der Hilfsenzyme auf die spektrophotometrische Bestimmung der Aktivität von Alanin- und Aspartataminotransferase

Zusammenfassung: 6 Lactatdehydrogenase- und 3 Malatdehydrogenase-Präparate verschiedener Hersteller und unterschiedlicher Organherkunft wurden hinsichtlich der Leerwert-Aktivität bei der Alanin- bzw. Aspartataminotransferase-Aktivitätsbestimmung mit dem UV-Test untersucht. Sie zeigten unterschiedlich hohe Neben- und Fremdaktivitätsanteile. Die Nebenaktivität war von der 2-Oxoglutarat-Konzentration abhängig. Die Fremdaktivität war in erster Linie durch die in den Hilfsenzymen enthaltene Alanin- und Aspartataminotransferase bedingt. Es wird gefordert, für Standardvorschriften von Enzymaktivitätsbestimmungen die Qualität der Hilfsenzyme exakt zu definieren.

In determinations of alanine aminotransferase (*L*-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2) and aspartate aminotransferase (*L*-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1) activities the spectrophotometric methods have important advantages over colorimetric procedures. Examining the influence of temperature and other reaction conditions on the measurements of alanine aminotransferase and aspartate aminotransferase activities by the coupled kinetic method we have observed that the sort of auxiliary enzymes used is very important (1, 2). Recently standard conditions for the estimation of enzyme activity were presented (3–6). In these recommendations exact data for the concentration of auxiliary enzymes were given, but nothing was said on the quality of auxiliary enzymes. The aim of this study is to show that it is also necessary to give precise data for the quality of auxiliary enzymes used in spectrophotometric methods for estimation of aminotransferase activities.

Methods and materials

Kinetic measurement of enzyme reaction velocity was performed at 37°C on an LKB Reaction Rate Analyzer 8600. Unless otherwise stated the final concentrations in the test mixture were 100 mmol/l Tris/HCl buffer pH 7.4, 200 mmol/l *L*-aspartate or 400 mmol/l *L*-alanine, 0.18 mmol/l NADH and 12 mmol/l 2-oxoglutarate. The reaction mixture for determination of alanine aminotransferase activity contained, per liter, 2000 U lactate dehydrogenase (*L*-lactate: NAD oxidoreductase, EC 1.1.1.27), for determination of aspartate aminotransferase activity 1000 U malate dehydrogenase (*L*-malate: NAD oxidoreductase, EC 1.1.1.37). In incubations with pyridoxal-5'-phosphate the coenzyme was added in the concentration of 200 µmol/l. The reaction was started by addition of 2-oxoglutarate. The total volume was 1.5 ml including 0.2 ml water in place of serum to achieve the same conditions as for the assay in routine analysis. The activities were measured by continuous monitoring of the reaction rate for 5 minutes. Therefore, the rate of this change per minute if multiplied by the factor 1206 gives the apparent aminotransferase activity in U/l. Duplicate assays were performed and the data given are the means of the duplicates. The standard deviation was estimated by duplicates using the following equation

$s = \sqrt{\frac{R^2}{2\,m}}$ (R = difference between duplicates, m = number of