

from the cuvette containing the 0 μM concentration solution were taken as reagent blanks, and subtracted from the respective absorbance values obtained with respect to the samples from the remaining five cuvettes, which had different acetoacetic acid concentrations of from 50 to 250 μM as mentioned above. Using the absorption data of the samples from the remaining five cuvettes and the reagent blanks, a change in absorbance as between the above two time points was calculated with respect to each of the samples. Results are shown in FIG. 4, which demonstrates the presence of good linearity in the relationship between the change (difference) in absorbance (with respect to the reaction mixture, as between 3 and 8 minutes after the start of the reaction) and the acetoacetic acid concentration.

Industrial Applicability

According to the determination method of the present invention, an error in the quantitative determination of D-3-hydroxybutyric acid and acetoacetic acid can be minimized since two types of coenzymes exhibiting absorbances at different absorption wave-lengths are used. Further, the sensitivity of the determination method can be greatly increased due to the utilization of the enzymatic cycling reaction. Thus, the method of the present invention ensures rapidness and accuracy in the determination of D-3-hydroxybutyric acid and acetoacetic acid, even with the use of a small quantity of a biological sample.

We claim:

1. A method for the quantitative determination of at least one ketone body selected from the group consisting of D-3-hydroxybutyric acid and acetoacetic acid, which consists essentially of:

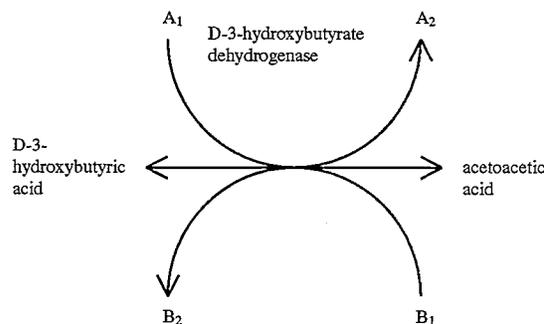
reacting a biological sample containing at least one ketone body selected from the group consisting of D-3-hydroxybutyric acid and acetoacetic acid with a reagent comprising:

- (1) a D-3-hydroxybutyrate dehydrogenase which utilizes the following coenzymes (i) and (ii):
 - (i) a first coenzyme selected from the group consisting of thionicotinamide adenine dinucleotide phosphate or its analog (thio-NADP compound) and thionicotinamide adenine dinucleotide or its analog (thio-NAD compound), and
 - (ii) a second coenzyme selected from the group consisting of nicotinamide adenine dinucleotide phosphate or its analog (NADP compound) and nicotinamide adenine dinucleotide or its analog (NAD compound),

and catalyzes the reversible reaction producing acetoacetic acid from D-3-hydroxybutyric acid as a substrate;

- (2) A_1 ; and
- (3) B_1 ;

said components (1), (2) and (3) participating in the following cycling reaction:



wherein A_1 is a thio-NADP compound, a thio-NAD compound, an NADP compound or an NAD compound; A_2 is a reduced form of A_1 ; B_1 is a reduced NADP compound or a reduced NAD compound when A_1 is a thio-NADP compound or a thio-NAD compound, or a reduced thio-NADP compound or a reduced thio-NAD compound when A_1 is an NADP compound or an NAD compound; and B_2 is an oxidized form of B_1 , wherein the reaction of said biological sample with said reagent is conducted under conditions adapted for the enzymatically catalytic and cycling reaction, thereby effecting said cycling reaction; and

measuring and correlating a change in the amount of A_2 or B_1 to the quantity of said at least one ketone body.

2. The method according to claim 1, wherein said thio-NADP compound is thionicotinamide adenine dinucleotide phosphate (thio-NADP) or thionicotinamide hypoxanthine dinucleotide phosphate.

3. The method according to claim 1, wherein said thio-NAD compound is thionicotinamide adenine dinucleotide (thio-NAD) or thionicotinamide hypoxanthine dinucleotide.

4. The method according to claim 1, wherein said NADP compound is selected from the group consisting of nicotinamide adenine dinucleotide phosphate (NADP), acetylpyridine adenine dinucleotide phosphate (acetyl-NADP), acetylpyridine adenine hypoxanthine dinucleotide phosphate and nicotinamide hypoxanthine dinucleotide phosphate (deamino-NADP).

5. The method according to claim 1, wherein said NAD compound is selected from the group consisting of nicotinamide adenine dinucleotide (NAD), acetylpyridine adenine dinucleotide (acetyl-NAD), acetylpyridine adenine hypoxanthine dinucleotide and nicotinamide hypoxanthine dinucleotide (deamino-NAD).

6. A method for the quantitative determination of at least one ketone body selected from the group consisting of D-3-hydroxybutyric acid and acetoacetic acid, which consists essentially of:

reacting a biological sample containing at least one ketone body selected from the group consisting of D-3-hydroxybutyric acid and acetoacetic acid with a reagent comprising:

- (1) a D-3-hydroxybutyrate dehydrogenase as a first dehydrogenase which utilizes the following coenzymes (i) and (ii):
 - (i) a first coenzyme selected from the group consisting of thionicotinamide adenine dinucleotide phosphate or its analog (thio-NADP compound) and thionicotinamide adenine dinucleotide or its analog (thio-NAD compound), and
 - (ii) a second coenzyme selected from the group consisting of nicotinamide adenine dinucleotide